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Routine haemostasis testing before transplanted kidney biopsy: a cohort study

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SUMMARY

Kidney biopsy can result in bleeding complications. Prebiopsy testing using bleeding time (BT) is controversial. New whole blood haemostasis tests, such as platelet function analyser-100 (PFA-100) and multiple electrode aggregometry (MEA), might perform better. We postulated that PFA-100 would be suitable to replace BT prebiopsy. In 154 patients, transplanted kidney biopsies were performed after measurement of bleeding time, PFA-100, MEA and mean platelet volume (MPV). Bleeding outcome (haemoglobin (Hb) drop, haematuria (±bladder catheterization), ultrasound finding of a bleeding, need for (non)surgical intervention and/or transfusion) after the biopsy was correlated to each test. Male–female ratio was 2:1. 50% had a surveillance biopsy at either three or 12 months. Around 17% (had) used acetylsalicylic acid (ASA) prebiopsy. Of 17 bleeding events, one subject needed a transfusion. Most bleeding events were Hb reductions over 1 mmol/l and all resolved uneventful. BT, PFA-100, MEA and MPV did not predict a bleeding outcome; prior ASA use however could (odds ratio 3.19; 95%-CI 1.06 to 9.61). Diagnostic performance data and Bland–Altman analysis showed that BT could not be substituted by PFA-100. ASA use was the best determinant of bleeding after kidney biopsy. Routine haemostasis testing prebiopsy has no added value.

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Introduction

In up to 90% of all kidney biopsies, the procedure is uneventful. Incidences of major bleeding complications requiring surgical intervention or resulting in death are low (<0.5%), making it a safe diagnostic procedure [1]. Biopsies of transplanted kidneys have a lower overall complication risk (6% has only minor complications) compared to native kidneys, a finding that so far remains unexplained [2]. In a large registry of over 8500 biopsies, macroscopic haematuria was seen most frequently, in around 2% of all native kidney biopsies, while the need for transfusion was seen in less than 1% [3]. Risk factors contributing to these bleeding complications are well described [3–9]. In spite of the apparent overall safety, many nephrologists are reluctant to proceed to biopsy without any laboratory testing. At the same time, controversy remains about the added value of laboratory testing and in particular about the utility of the bleeding time [10–14], a rather poor performing test that remains persistently in use, despite its accepted shortcomings.

From a traditional perspective, it is still often routine practice [15] to assess a bleeding time prior to kidney biopsy as an overall haemostasis test. The common rationale is that in chronic kidney disease, uremic patients have an acquired platelet dysfunction as well as deranged coagulation system [12, 16]. Prolonged bleeding times trigger physicians to administer 1-desamino-8-D-arginine vasopressin (DDAVP) to enhance platelet function during kidney biopsy [1, 17]; it has been proven to reduce haematoma formation after this procedure [1, 16, 18]. In the Netherlands, consensus on the use of specific laboratory tests is lacking, in spite of a previous study from this country comparing a historical cohort using the bleeding time with a prospective cohort of the platelet function analyzer 100 (PFA-100) [19].

Nowadays an array of *in vitro* platelet function tests is available on the market that could at least supersede the *in vivo* bleeding time. Multiple electrode aggregometry (MEA, Multiplate) is an *in vitro* whole blood point-of-care test device of platelet function in which platelet activators (i.e. COL (collagen), ADP (adenosine diphosphate), ASPI (arachidonic acid) or TRAP (thrombin receptor activating peptide-6)) are added, and platelet aggregation is measured using an impedance method. PFA-100 also measures activated platelets but it incorporates flow, making it more sensitive to the effects of von Willebrand factor. Both tests depend on platelet count, while PFA-100 results need to be corrected for haematocrit as well [20]. PFA-100 is more sensitive in comparison with the bleeding time for bleeding disorders, however, could still produce false-negatives in mild bleeding disorders [21, 22], for which MEA might perform better. Additionally, mean platelet volume (MPV) is an indirect measure of platelet turnover and is related to platelet reactivity [23]. A high MPV is seen in states of high megakaryocyte stimulation due to thrombocytopenia [24]. MPV could be helpful in predicting bleeding.

Therefore, in this study, we have investigated which of these tests (Ivy bleeding time, MEA, PFA-100 or MPV), if any, could predict bleeding after kidney biopsy, assuming that the PFA-100 would be superior to the bleeding time, due to its potential higher sensitivity [22].

Patients and methods

Study design and in- and exclusion criteria

We organized a prospective observational study in patients with chronic kidney disease, who were scheduled to undergo a biopsy of a previously retroperitoneal transplanted kidney in the iliac fossa. Subjects were included at three or 12 months check-up for surveillance kidney biopsy, or outside these scheduled check-ups for other various reasons (nonsurveillance biopsies). Minors (age <18 years) were excluded from this study. Other exclusion criteria were known hereditary bleeding disorders, platelet count $<80*10^9$/l or haematocrit <0.25 l/l. Subjects on antithrombotic medication use were managed as follows. Acetylsalicylic acid (ASA) was stopped at least 5 days prebiopsy. All low-molecular-weight heparins (LMWH) were stopped, and anti-Xa testing was performed to rule out residual heparin activity before biopsy. The use of vitamin K antagonists was stopped, and INR had to be <1.6 prebiopsy. All subjects gave informed consent prior to enrolment in this study for extra blood sampling and analysis. This study was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). Our local institutional review board approved the conductance of this study.

Routine tests and reference intervals

According to routine care, the following tests were performed prior to the biopsy: haemoglobin level (Hb), thrombocyte count (PLT) and mean platelet volume (MPV) in ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood (collected in 5 ml K2-EDTA tubes,

Becton Dickinson BV, Breda, The Netherlands) on a Sysmex XE-5000 machine (Sysmex Nederland B.V., Etten-Leur, The Netherlands). Anaemia was defined as an Hb below 8.2 mmol/l for men and below 7.3 mmol/l for women. Thrombocytopenia was defined by a PLT lower than $130*10^9$/l. For MPV, results were interpreted according to our previously established reference intervals of 9.2–12.7 fl [23]. The activated partial thromboplastin time (aPTT) and prothrombin time corrected by the internationalized ratio (PT-INR) were determined in citrate-anticoagulated plasma (collected in 4.5 ml 3.2% (w/v) citrate tubes, Becton Dickinson BV, Breda, The Netherlands). An aPTT over 32 s and a PT-INR higher than 1.2 were both considered abnormal. Urea and creatinine levels were measured in serum plasma tubes (Becton Dickinson BV, Breda, The Netherlands). Estimated glomerular filtration rate (eGFR) was calculated according to the MDRD formula [25]. Subjects were grouped by disease state according to the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines [26]. Elevated urea was defined as a urea greater than the upper limit of normality (>8 mmol/l). High urea was defined as a urea greater than twice the upper limit of normality (>16 mmol/l).

Index tests and reference intervals

Bleeding time was performed according to the Ivy method [27]. Three consecutive cuts were made by a trained technician, and the average bleeding time was noted. Bleeding times over 4 min were considered to be abnormal.

Multiplate was performed in hirudin-anticoagulated whole blood according to the manufacturer's guidelines. Platelet aggregation was initiated by adding one of the following reagents: ADP (final concentration, f.c. 6.5 μM), ASPI (f.c. 0.5 mM), COL (f.c. 3.2 μg/ml) and TRAP (f.c. 32 μM). Area under the curve (AUC in U; 1 U equals 10 Arbitrary Units*min)) of the multiplate results was calculated. AUC was corrected for thrombocyte count according to Kuiper *et al.* [20] to determine whether an AUC was within normal range or reduced.

PFA-100 testing was performed in citrate-anticoagulated whole blood using both the COL/ADP and the COL/EPI cartridge according to the manufacturer's instructions. Closure time (CT) in seconds was used for analysis. CT was corrected for thrombocyte count and haematocrit level according to Kuiper *et al.* [20] to determine whether a CT was within normal range or prolonged.

Procedure

Although DDAVP could be given at 0.3 μg/kg iv prior to the biopsy, if deemed necessary, none of the subjects actually received DDAVP during this study. For all biopsies, a 16-gauge ultrasound-guided spring-loaded biopsy gun was used, after administration of local anaesthetics at the intended puncture site. Biopsies were performed by radiologists experienced in doing so or radiologists in training under supervision of experienced radiologist. A minimum of three samples were taken and investigated directly after biopsy for adequacy with the aid of a magnifying glass for the presence of glomeruli. Following biopsy, all subjects were kept bedridden for 6 h with a one kg bag of sand resting on the puncture site. Subjects were monitored with a follow-up of the puncture site for the first 2 h and a urine analysis with complete blood count at four hours postbiopsy. After the 6 h bed rest, subjects were allowed to mobilize and were discharged if all checks were normal. A control ultrasound was performed within 24 h after biopsy in case of clinical suspicion of a bleeding complication (Hb drop, pain, macroscopic haematuria and/or hypotension).

Endpoint definitions

A haemoglobin drop of one mmol/l or more (equal to 1.61 g/dl) was considered to have clinical consequences and thus be a bleeding outcome. A positive ultrasound finding of a perinephric and/or subcapsular haematoma after biopsy was also deemed a bleeding endpoint. Haematuria detected with microscopic urine analysis (defined in our study as >200 red blood cells per high power field (RBC/HPF)), which was not present prebiopsy and lasted longer than 24 h, was also defined as a bleeding outcome. Only subjects needing a postbiopsy (surgical) intervention (e.g. embolization, coiling, explantation, transfusion of packed cells, bladder catheterization or other) were marked having a major bleeding complication. All bleeding complications without need for an intervention were considered to be nonmajor (i.e. minor) bleeding endpoints.

Statistical analysis

All the statistic testing was performed using SPSS v23 (IBM Corp, Armonk NY USA). Categorical and ordinal data are expressed as number with percentage. Interval data are expressed as median with interquartile range (IQR). One-way ANOVA with post hoc correction for

multiple comparisons was performed for the occurrence of differences between variables among the different chronic kidney disease (CKD) stadia. For equal variances a Hochberg's GT2 and for nonequal variance a Games-Howell correction were used. Odds ratios (OR) of binomial data were calculated for relevant (risk) factors. PFA-100 and MEA results of all subjects were plotted in GraphPad Prism (GraphPad Prism version 5.0a for Windows, GraphPad Software, San Diego, California, USA) with the previously researched reference intervals [20]. Diagnostic performance data (sensitivity, specificity, area under the receiver operating characteristic curve (ROC-AUC), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLHR) and negative likelihood ratio (NLHR) for each test and an endpoint; kappa between BT and each test in correctly predicting a bleeding endpoint) were computed using the pooled data after a multiple imputation procedure ($N = 5$) because of data missing at random. To see whether the bleeding time could be substituted by PFA-100, Bland–Altman analysis reporting bias and 95% limits of agreement was performed.

For all analyses, a P-value below 0.05 was considered statistically significant.

Results

Baseline characteristics

A total of 154 subjects were included. 64% were of male gender and the overall median age was 59 years (interquartile range (IQR) was 48.8–66). Around half of the subjects had a surveillance biopsy at either three or 12 months post-transplantation. ASA use was reported in 26 subjects and stopped adequately 5 days prebiopsy in 25 of the subjects. One subject had a protocol violation by not having stopped its use 5 days prebiopsy. Anaemia was present in 63% of all subjects, while 5.8% had a thrombocytopenia (thrombocyte counts $<130*10^9$/l). MPV data were available in 134 of all studied subjects, which revealed an abnormally low MPV in around 79% of all subjects. Other baseline characteristics can be found in Table 1.

Endpoint characteristics

Seventeen subjects (11%) had a clinically relevant bleeding endpoint (Table 2). In three subjects, a positive ultrasound for haematoma formation was found. Two of these subjects had a nonrelevant drop in haemoglobin (<1 mmol/l), while the other subject had an accompanying haemoglobin

Table 1. Baseline patient, clinical and test characteristics.

Baseline Demographics	All biopsies (n = 154)
Patient characteristics	
Male gender – no. (%)	98 (63.6)
Age – years (IQR)	59.0 (48.8–66)
Surveillance post-transplant biopsy – no. (%)	83 (53.9)
After 3 months – no. (%)	48 (31.2)
After 12 months – no. (%)	35 (22.7)
Had previous biopsy in study – no. (%)	21 (13.6)
Acetylsalicylic acid use – no. (%)	26 (16.9)
Clinical characteristics	
Systolic blood pressure – mmHg (IQR)	140 (127–150)
Diastolic blood pressure – mmHg (IQR)	77 (70–85)
Haemoglobin – mmol/l (IQR)	7.0 (6.2–8.3)
Anaemia (male: <8.2; female: <7.3 mmol/l) – no. (%)	97 (63.0)
Haematocrit – l/l (IQR)	0.34 (0.30–0.40)
Thrombocyte count – $\times 10^9$/l (IQR)	222 (177–284)
Thrombocytopenia ($<130 \times 10^9$/l) – no. (%)	9 (5.8)
Mean platelet volume* – fl (IQR)	8.2 (7.5–9.0)
Low (<9.2 fl) – no. (%)	106 (79.1)
Normal – no. (%)	27 (20.1)
High (>12.7) – no. (%)	1 (0.7)
PT-INR** – s/s (IQR)	1.0 (1.0–1.1)
PT-INR normal – no. (%)	145 (96.7)
PT-INR prolonged (>1.2) – no. (%)	5 (3.3)
aPTT*** – s (IQR)	28 (27–30)
aPTT normal – no. (%)	129 (86.6)
aPTT prolonged (>32 s) – no. (%)	20 (13.4)
Urea**** – mmol/l (IQR)	11.1 (7.8–17.4)
Normal – no. (%)	41 (27.0)
Elevated (>8 to 16 mmol/l) – no. (%)	67 (44.1)
High (>16 mmol/l) – no. (%)	44 (28.9)
Creatinine**** – µmol/l (IQR)	212 (132–434)
eGFR**** – ml/min/1.73 m^2 (IQR)	29.0 (12.3–46.0)
CKD Stage I–II – no. (%)	13 (8.4)
CKD Stage III – no. (%)	61 (39.6)
CKD Stage IV – no. (%)	32 (20.8)
CKD Stage V – no. (%)	46 (29.9)
Test characteristics	
Performed Ivy bleeding time – no. (%)	150 (97.4)
Performed PFA-100 – no. (%)	151 (98.1)
Performed MEA – no. (%)	148 (96.1)

Measured in amount of biopsies: *N = 134; **N = 150; ***N = 149; ****N = 152.

aPTT, activated partial thromboplastin time; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MEA, multiple electrode aggregometry; PFA-100, platelet function analyser-100; PT-INR, prothrombin time correct by the internationalized ratio.

Table 2. Bleeding endpoint characteristics.

Variable – no. (%)	Biopsies with endpoint
Composite clinically relevant bleeding complication*	17 (11.0)†
Stadium I–II CKD	1 (7.7)‡
Stadium III CKD	8 (13.1)‡
Stadium IV CKD	4 (12.5)‡
Stadium V CKD	4 (8.7)‡
Major complication	1 (0.6)†
Intervention needed	1 (100)§
Hb drop ≥1 mmol/l	1 (100)§
Ultrasound finding	1 (100)§
New haematuria	0 (0)§
Minor complication	16 (10.4)†
Intervention needed	0 (0)§
Hb drop ≥1 mmol/l	13 (81.3)§
Ultrasound finding	2 (12.5)§
New haematuria	4 (25)§

*Proportions are not significantly different among the CKD stadia ($P > 0.05$).

†% of all biopsies.

‡% of subjects within CKD stadium.

§% of complication group.

Table gives an overview of bleeding endpoint characteristics. *Composite endpoint of clinically relevant bleeding complication* was defined as haemoglobin drop ≥1 mmol/l, need for transfusion or intervention, positive ultrasound postbiopsy or postbiopsy haematuria (>200RBC/HPF on microscopy) without prebiopsy haematuria on microscopy. A *major complication* was defined as subjects needing a intervention (e.g. embolization, coiling, explantation, transfusion, bladder catheterization). A *minor complication* was defined as all clinically relevant nonmajor bleeding complications.

CKD, chronic kidney disease; Hb, haemoglobin; RBC/HPF, red blood cells per high power field.

drop of 1.8 mmol/l and needed transfusion of packed cells at a haemoglobin of 3.9 mmol/l; otherwise, the patient recovered uneventful. Thirteen subjects with a relevant Hb drop of ≥1 mmol/l had a postbiopsy haemoglobin level of 5.6 mmol/l or greater and needed no interventions and were managed by observation only.

Of all subjects, 35.1% had haematuria prebiopsy. The majority of those (87.0%) were scheduled for a non-surveillance biopsy. Four subjects had newly developed haematuria of >200 RBC/HPF on urine analysis but needed no bladder catheterization.

One of all subjects had a positive bleeding endpoint (haemoglobin drop of 1.0 mmol/l) after a previously uneventful biopsy in this study.

Besides the one transfusion, none of the subjects needed an intervention (e.g. embolization, coiling or explantation of the kidney), had graft loss, or died within 30 days.

Medication effects

ASA use was reported in six of the subjects with a bleeding endpoint. The patient not having stopped ASA did not have a bleeding endpoint. Subjects reporting the use of ASA had a higher risk of at least one bleeding endpoint compared to those not using ASA (OR, 3.19; 95% confidence interval (95% CI), 1.06 to 9.61).

ASA use had an OR of 8.11 (95% CI, 1.28 to 51.4) for prolonged PFA-100 COL/EPI using the newly defined reference intervals [20] and an OR of 4.84 (95% CI, 1.60 to 14.63) using the original reference intervals.

ASA users did have a 2.41 OR for a reduced collagen activated on newly corrected MEA (95% CI, 1.00 to 5.82) and a 4.05 OR for a reduced ASPI activated AUC on MEA when using the original reference intervals (95% CI, 1.51 to 10.83).

In five subjects, PT-INR was elevated (>1.2). In these subjects, median PT-INR was 1.4 (IQR 1.28–1.60). None of these subjects experienced a bleeding endpoint.

Point-of-care testing of thrombocyte function and bleeding prediction

The bleeding time was prolonged in eight subjects (bleeding time range: 243–476 s), while in only one of these subjects, a positive bleeding endpoint occurred. Bleeding time did not differ among different CKD stadia (Table 3).

For the PFA-100, six subjects had a prolonged COL/ADP test and four a prolonged COL/EPI test. Figure 1 has the PFA-100 results plotted for either correction of thrombocyte count or of haematocrit. One bleeding subject had a prolonged COL/ADP test, while none of the bleeding subjects had a prolonged COL/EPI test.

MEA was reduced in 23% of the subjects for ADP, 30% of the subjects for ASPI, 30% of the subjects for COL and 14% of the subjects for TRAP (Fig. 2) for the newly defined reference intervals [20]. The distribution among the CKD stadia did not differ for the number of subjects with reduced MEA results (Table 3). Of all subjects, almost half had a low AUC on at least one of the four MEA tests. Eight bleeding subjects had no test on MEA reduced, two had one test reduced, three had two tests reduced, four had three tests reduced and none had all four tests reduced. This distribution was not significantly different among the five combinations

Table 3. Study parameters classified according to CKD stadium.

Variable – no. (% of stadium)	Overall	Stadium I-II CKD	Stadium III CKD	Stadium IV CKD	Stadium V CKD
Low MPV (<9.2 fl)[b]	105 (78.9)	9 (90.0)	50 (89.3)[†]	23 (85.2)	23 (57.5)[†]
Anaemia (male: <8.2; female: <7.3 mmol/l)[c]	96 (63.2)	2 (15.4)*	23 (37.7)*	25 (78.1)[#]	46 (100)[#]
Thrombocytopenia (<130 × 10^9/l)[a]	8 (5.3)	0 (0)	2 (3.3)	1 (3.1)	5 (10.9)
Elevated urea (>8–16 mmol/l)[b]	67 (44.4)	1 (7.7)[†,‡]	37 (60.7)[†, #]	14 (45.2)[‡]	15 (32.6)[#]
High urea (>16 mmol/l)[c]	43 (28.5)	0 (0)*	0 (0)*	14 (45.2)**	29 (63.0)**
Prolonged Ivy bleeding time (>4 min)[a]	8 (5.4)	0 (0)	2 (3.3)	1 (3.2)	5 (11.4)
Prolonged PFA-100 (at least one)[a]	10 (6.8)	0 (0)	4 (6.9)	0 (0)	6 (13.6)
Prolonged COL/ADP[a]	6 (4.0)	0 (0)	1 (1.7)	0 (0)	5 (10.9)
Prolonged COL/EPI[a]	4 (2.8)	0 (0)	3 (5.1)	0 (0)	1 (2.4)
Reduced MEA (at least one)[a]	72 (49.3)	6 (50.0)	29 (48.3)	13 (41.9)	24 (55.8)
Reduced ADP[a]	34 (23.3)	4 (33.3)	9 (15.0)	7 (22.6)	14 (32.6)
Reduced ASPI[a]	44 (30.1)	4 (33.3)	16 (26.7)	8 (25.8)	16 (37.2)
Reduced COL[a]	44 (30.1)	4 (33.3)	21 (35.0)	8 (25.8)	11 (25.6)
Reduced TRAP[a]	20 (13.7)	2 (16.7)	5 (8.3)	7 (22.6)	6 (14.0)

[a]Proportions are not significantly different among groups ($P > 0.05$).

[b]Significance levels for coupled proportions between CKD stadia: [†]$P < 0.01$, [‡] or [#]$P < 0.05$.

[c]All significantly different at $P < 0.01$ (except coupled proportions [#]$P < 0.05$, * or **$P > 0.05$).

Table shows the study parameters classified according to CKD stadium before multiple imputation for missing data. Prolonged PFA-100 and reduced MEA were classified according to Kuiper *et al.* [20].

ADP, adenosine diphosphate; ASPI, arachidonic acid; CKD, chronic kidney disease; COL, collagen; COL/ADP, collagen/adenosine diphosphate cartridge; COL/EPI, collagen/epinephrine cartridge; MEA, multiple electrode aggregometry; MPV, mean platelet volume; PFA-100, platelet function analyser-100; TRAP, thrombin receptor activating peptide-6.

possible. None of the four MEA tests had a significant OR for the prediction of a bleeding endpoint.

Other predictors of bleeding complications

Nonsurveillance kidney biopsy had similar odds for a bleeding endpoint compared to surveillance biopsies (OR, 0.46; 95% CI, 0.15 to 1.38).

Thrombocytopenia was evenly distributed among the CKD stadia (Table 3). None of the bleeding subjects had thrombocytopenia.

A low MPV was seen less frequent in stadium V CKD in comparison with the other stadia (Table 3). Reduced MPV did not give higher odds for a bleeding endpoint (OR, 1.82; 95% CI, 0.39 to 8.60).

Bleeding complications did not differ significantly among the different CKD stadia (Table 3). As could be expected, urea levels were inversely related with eGFR (Pearson correlation coefficient, $r = -0.69$; $P < 0.001$). Elevated (OR, 0.66; 95% CI, 0.23 to 1.89) or high urea (OR, 0.93; 95% CI, 0.28 to 3.10) did not prove to give higher odds ratio for a positive bleeding endpoint (OR, 0.87; 95% CI, 0.29 to 2.65 when elevated and high urea were combined in comparison with normal urea level).

Diagnostic performance of endpoint prediction

Table 4 shows the data after multiple imputation on diagnostic performance of bleeding endpoint prediction when using bleeding time, the two PFA-100 cartridges (comparing the standard and newly defined reference intervals), all four MEA agonists (comparing the standard and newly defined reference intervals) and the MPV. PPV is low for all tests in our population, ranging from 0% to 17%. NPV is between 88 and 93% for all tests.

Substitution of bleeding time by PFA-100

Bland–Altman analysis between bleeding time and PFA-100 COL/ADP revealed a mean bias of 35.8 s (95% limits of agreement, −95.7 to 167.3 s) with more dispersion at higher means. The same was apparent for the bleeding time and the PFA-100 COL/EPI cartridge (bias, 11.3 s; 95% limits of agreement, −122.7 to 145.3 s).

Because of higher dispersion at larger means, bias and 95% limits of agreement were corrected for the mean. This gave for ADP/COL a bias of 27% (95% limits of agreement, −71% to 125%) and for ADP/EPI a bias of 5% (95% limits of agreement, −92% to 102%).

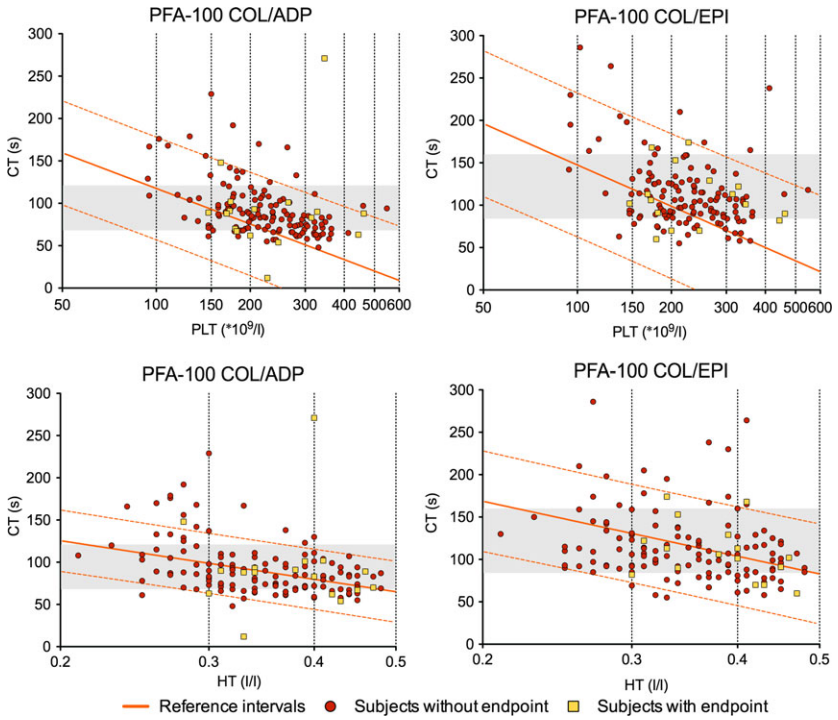

Figure 1 Prebiopsy PFA-100 data corrected for thrombocyte count and haematocrit levels. The grey areas are the originally defined reference intervals as advised by the manufacturer of PFA-100. The orange dotted lines are the newly defined reference intervals by Kuiper *et al.* [20] Red dots depict results of the subjects without a bleeding endpoint. Yellow squares represent subjects with a bleeding endpoint. COL/ADP, collagen/adenosine diphosphate cartridge; COL/EPI, collagen/epinephrine cartridge; CT, closure time; HT, haematocrit; PFA-100, platelet function analyser-100; PLT, thrombocyte count.

Kappa values between bleeding time and both PFA-100 tests showed no agreement in correctly predicting a combined endpoint, except for the COL/EPI cartridge with the original reference intervals, which has moderate agreement (Table 4).

Discussion

In this study, we have investigated whether bleeding complications after transplanted kidney biopsy could be predicted using Ivy bleeding time, MEA, PFA-100 and/or MPV. Furthermore, we assumed that the PFA-100 would be superior to the bleeding time in predicting bleeding. None of our assumptions were true. We could not reliably predict bleeding complications after transplanted kidney biopsies by any of the tests. Prior ASA use, even after having stopped it for 5 days, was the only determinant of a bleeding complication.

Postbiopsy major bleeding complications with severe consequences are infrequent in patients after kidney transplantation [1–3]. In our study, 17 of 154 subjects (11.0%) had a clinically relevant bleeding event of which only one subject (0.65%) had a severe haemoglobin drop and needed a transfusion. Most of the subjects (9.1%) had a haemoglobin drop of more than 1 mmol/l without clinical consequences. Previously, 'macrohaematuria' was defined as more than 50 RBC/HPF [28, 29], but in our laboratory, this cut off value is not reported and more than 200 RBC/HPF was used instead. New haematuria was seen in 2.6% of our subjects, which is slightly higher in comparison with other studies [1, 3]. Our conclusion is that these bleeding endpoint data are coherent with previous studies [1–3, 7, 9, 30].

Previous research has shown that antiplatelet and/or anticoagulant drugs should be stopped prebiopsy [31] and that a thinner needle (i.e. a larger gauge) is important in reduction of complications [7]. This and more research has led to evidence-based recommendations for kidney biopsies to minimize bleeding complications [1]. In our study, previously reported use of ASA was a risk factor (three times higher odds) for having a bleeding endpoint. An ASA effect could still be identified using MEA (reduced AUC for the COL reagent) and PFA-100 (prolonged CT in the COL/EPI cartridge); stopping ASA 5 days before biopsy might be too short; however, the one patient not having stopped its use did not have a bleeding event. Similarly, Mackinnon *et al.* [31] found that, if antiplatelet medication was continued, patients have a bigger drop in haemoglobin than when it was stopped 5 days before biopsy. Rates of major complications were not statistically different in their study. Interestingly, in the systematic review and meta-analysis of Corapi *et al.*, [7] the withdrawal of antiplatelet medication shorter than 7 days did not result in more

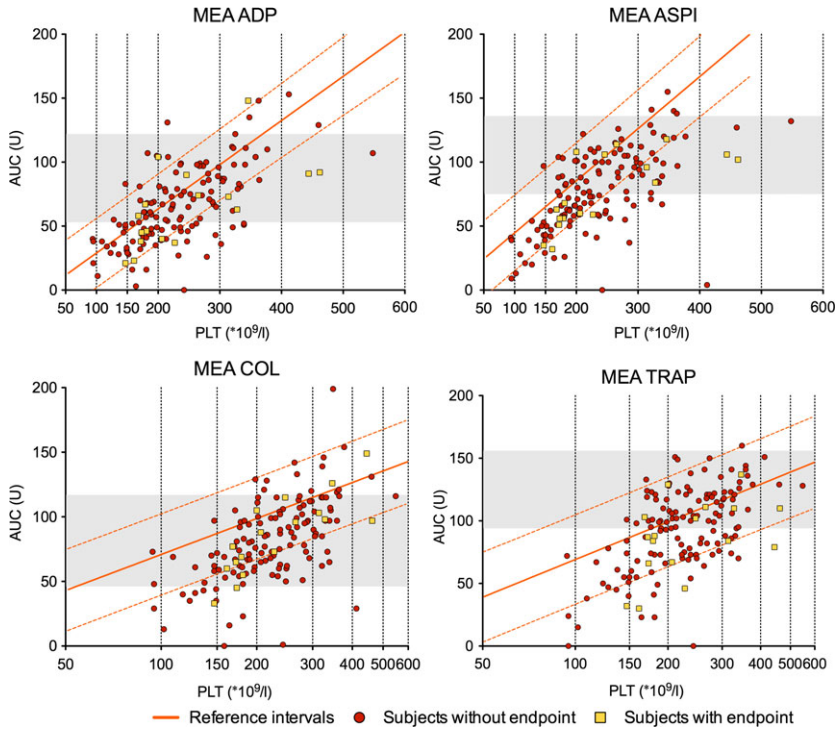

Figure 2 Prebiopsy MEA data corrected for thrombocyte count. The grey areas are the originally defined reference intervals as advised by the manufacturer of multiplate. The orange dotted lines are the newly defined reference intervals by Kuiper *et al.* [20] Red dots depict results of subjects without a bleeding endpoint. Yellow squares represent subjects with a bleeding endpoint. ADP, adenosine diphosphate; ASPI, arachidonic acid; AUC, area under the curve; COL, collagen; MEA, multiple electrode aggregometry; PLT, thrombocyte count; TRAP, thrombin receptor activating peptide-6.

erythrocyte transfusion or macroscopic haematuria rates. As is discussed by Nayak-Rao, the cessation of antiplatelet medication should be balanced between bleeding complications if continued and thrombotic complications when stopped [32]. And it may be the case that the bleeding risk outweighs the thrombotic risk in our study.

When looking at PFA-100 results, only few subjects had a prolonged result when comparing our newly defined reference intervals. This would indicate that hardly any of the subjects have a bleeding tendency, due to a platelet dysfunction. On the contrary, many subjects showed reduced MEA results (Fig. 2), which would indicate the presence of platelet dysfunction. Indeed, the stadium V CKD patients seem to be more prone to bleeding (Table 3). They have significantly more anaemia due to renal dysfunction, higher grades of uraemia, and, although not significantly, more thrombocytopenia and more prolonged bleeding times. Renal capillary wall incompetence due to renal fibrosis is thought to be the main culprit causing haematuria in deteriorating CKD [16, 33]. Similarly is bleeding caused by anaemia, uremic toxins or higher fibrinolytic activity in the urinary tract due to urokinase [16, 33]. However, in our study, stadium V CKD did not result in higher rates of bleeding complications. Importantly, this is the first large study to date to report platelet dysfunction in patients with CKD using whole blood impedance aggregometry such as MEA. Other researchers have drawn similar conclusions using a far less number of subjects and different measuring devices [34].

No test in our study arsenal could adequately predict postbiopsy bleeding. The low PPV of all tests we found reflects that no test seems suitable for screening purposes. PFA-100 has properties similar to the bleeding time by incorporating flow into the analysis. However, Bland–Altman analysis showed that PFA-100 had bias in comparison with the bleeding time and could not give a meaningful verdict of the bleeding time because of large 95% limits of agreement in our population. Valeri and Ragno reported a similar finding, calculating correlation coefficients between the two tests [35]. Prediction of bleeding complications seems difficult for kidney biopsies. Bleeding time (having a high specificity) could help in excluding bleeding complications. The PFA-100 has a similar high specificity of 96% and 95% for the COL/ADP and COL/EPI cartridge, respectively. The kappa value of 0.48 showed moderate agreement between the two tests in correctly predicting a bleeding outcome (COL/EPI original reference intervals versus the bleeding time). However, Islam and colleagues stated based on their results that PFA-100 is unlikely to be of benefit to patient care in routine kidney biopsies [36]. Although similarities can be drawn

Table 4. Diagnostic performance of all tests in predicting a bleeding endpoint.

Investigated test	Sensitivity	Specificity	ROC-AUC	PPV	NPV	PLHR	NLHR	Kappa*
BT	0.07	0.95	0.496	0.14	0.89	1.35	0.98	NA
PFA-100 standard RI								
COL/ADP	0.12	0.87	0.490	0.10	0.89	0.89	1.02	0.04
COL/EPI	0.15	0.88	0.474	0.13	0.89	1.25	0.97	0.48
PFA-100 new RI								
COL/ADP	0.06	0.96	0.494	0.16	0.89	1.55	0.98	−0.07
COL/EPI	0.00	0.95	0.529	0.00	0.88	0.00	1.05	0.00
MEA standard RI								
ADP	0.41	0.61	0.507	0.11	0.89	1.04	0.97	0.20
ASPI	0.53	0.51	0.504	0.12	0.90	1.07	0.93	0.13
COL	0.12	0.90	0.501	0.13	0.89	1.17	0.98	−0.10
TRAP	0.59	0.56	0.552	0.14	0.92	1.33	0.74	0.10
MEA new RI								
ADP	0.35	0.78	0.436	0.17	0.91	1.60	0.83	0.24
ASPI	0.35	0.70	0.480	0.13	0.90	1.17	0.93	0.24
COL	0.24	0.68	0.531	0.08	0.88	0.74	1.12	−0.12
TRAP	0.24	0.86	0.440	0.17	0.90	1.68	0.89	0.40
MPV	0.87	0.22	0.532	0.12	0.93	1.12	0.59	0.02

*Between BT and study tests in correctly predicting a positive endpoint.

Table portraits the diagnostic performance of all tests in predicting a bleeding endpoint after multiple imputation of missing data. New reference intervals were used from Kuiper *et al.* [20].

ADP, adenosine diphosphate; ASPI, arachidonic acid; BT, bleeding time; COL, collagen; COL/ADP, collagen/adenosine diphosphate cartridge; COL/EPI, collagen/epinephrine cartridge; MEA, multiple electrode aggregometry; MPV, mean platelet volume; NLHR, negative likelihood ratio; NPV, negative predictive value; PFA-100, platelet function analyser 100; PLHR, positive likelihood ratio; PPV, positive predictive value; RI, reference intervals; ROC-AUC, area under the receiver operating characteristic curve; TRAP, thrombin receptor activating peptide-6.

between the bleeding time and the PFA-100, they are essentially different [37]. The PFA-100 cannot be entitled as an *in vitro* bleeding time.

As a final conclusion, we like to state that severe bleeding complications after kidney biopsy on transplanted kidneys are low; that laboratory tests (especially MEA) show that patients with CKD have an *in vitro* platelet dysfunction on top of ASA use; that this acquired *in vitro* platelet dysfunction does not result in an increased bleeding risk after kidney biopsy, except possibly in ASA use; that the bleeding time could not be substituted by any other test we used; and that no test seems to able to adequately predict bleeding after kidney biopsy.

We recommend that routine haemostasis assessment before performing a transplanted kidney biopsy be abandoned after exclusion of patients with contraindications.

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Conflict of Interest

The authors declare that they have no competing interests.

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Authorship

GK: contributed to the interpretation of data; contributed to the critical writing and revising the intellectual content; approved the final version to be published. MC: contributed to the concept and design; contributed to the inclusion of patients; contributed to the interpretation of data; contributed to the revising the intellectual

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