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ORIGINAL ARTICLE

The influence of lower-leg injury and knee arthroscopy on natural anticoagulants and fibrinolysis

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Abstract

Background: Patients with lower-leg injuries and those undergoing knee arthroscopy are at increased risk of developing venous thromboembolism. The mechanism is unknown, including the influence of lower-leg injury and knee arthroscopy on natural anticoagulant factors and fibrinolysis.

Objectives: To study the effect of lower-leg injury and knee arthroscopy on plasma levels of anticoagulant and fibrinolytic factors.

Methods: We applied the following 2 designs to investigate this effect: a cross-sectional study for lower-leg trauma and a before-and-after study for knee arthroscopy. Plasma samples of POT-CAST– and POT-KAST–randomized clinical trial participants (collected shortly after lower-leg trauma or before or after arthroscopy) were analyzed for clot lysis time and levels of antithrombin, tissue factor pathway inhibitor, protein C, free protein S, plasminogen, tissue plasminogen activator, plasminogen activator inhibitor 1, antiplasmin, thrombin activatable fibrinolysis inhibitor, plasmin-antiplasmin, and D-dimer. For the effect of lower-leg injury, samples of 289 patients were compared with preoperative samples of 293 arthroscopy patients, acting as controls using linear regression and adjusting for age, sex, body mass index, comorbidities, and diurnal variation. For the effect of knee arthroscopy, mean changes were calculated for 277 patients using linear mixed models adjusted for diurnal variation. Parameters other than CLT and D-dimer were measured in smaller subsets.

Results: In lower-leg injury patients, most parameters were stable, whereas D-dimer increased. After arthroscopy, most parameters decreased (especially clot lysis time, D-dimer, plasminogen, and anticoagulant factors), whereas tissue plasminogen activator and thrombin activatable fibrinolysis inhibitor slightly increased.

Conclusion: In contrast to lower-leg injury, knee arthroscopy was associated with decreased natural anticoagulant factor levels. Neither lower-leg injury nor knee arthroscopy affected *in vivo* fibrinolysis.

KEYWORDS
arthroscopy, blood coagulation factor, fibrinolysis, knee injury, leg injury

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1 | INTRODUCTION

Venous thromboembolism (VTE) involves the occurrence of pathological clotting in veins, most commonly in the deep veins of the legs (deep vein thrombosis) and pulmonary arteries (pulmonary embolism) [1]. Adequate thromboprophylaxis has become a priority, given the considerable burden that can be caused by VTE, including long-term morbidity or even death [2–4]. Lower-leg cast application and knee arthroscopic surgery are conditions that are associated with VTE [5,6]. Worldwide, lower-leg fractures are the most common type of fractures, with an incidence rate of 420 per 100,000 persons in the period of 1990 to 2019, whereas knee arthroscopy is one of the most frequently performed orthopedic procedures with 2 million procedures each year [7,8]. Therefore, adequate thromboprophylaxis in both patient populations is essential. However, according to the Prevention of Thrombosis following (Knee Arthroscopy) CAST immobilization [POT-(K)CAST]–randomized controlled trials, current prophylactic treatment with low-molecular-weight heparin (LMWH) does not sufficiently reduce VTE risk in these patients, because 1.0% to 1.5% of the patients receiving LMWH still develop VTE [9].

Improvement of this thromboprophylaxis strategy is therefore necessary to reduce VTE risk. Knowledge of the pathophysiological mechanism of thrombus formation following lower-leg trauma and knee arthroscopy may help identify new targets for prevention [10]. In our previous studies, we observed different effects of lower-leg injury and knee arthroscopy on the coagulation system. Activity levels of factor VIII (FVIII), FIX, and FXI, von Willebrand factor antigen and fibrinogen plasma levels, and thrombin generation increased after lower-leg injury, whereas these changes did not occur after knee arthroscopy [11]. Enhanced in vivo and ex vivo thrombin generation were noted after lower-leg trauma, but not after knee arthroscopy. It has, however, not yet been established whether changes in the fibrinolytic system, which may contribute to or counteract the changes in the coagulation system, occur [12]. In addition, it has not yet been established whether increase in plasma levels of procoagulants are accompanied by simultaneous increases in plasma levels of anticoagulant proteins [13]. In patients with major trauma and those undergoing major surgery, a severe inhibition of fibrinolytic capacity has been described [14–16]. This trauma–related or postoperative fibrinolytic "shutdown" is likely related to substantial increase in plasma levels of plasminogen activator inhibitor type 1 (PAI-1) [17]. It is unknown whether less severe injuries, such as of the lower-leg (caused by trauma or knee arthroscopy), are also associated with impairment of fibrinolytic capacity.

The aim of our study was, therefore, to assess the impact of (1) lower-leg trauma and (2) knee arthroscopy on plasma levels of natural anticoagulant factors and on fibrinolysis.

Essentials

- The mechanism of venous thromboembolism (VTE) after lower-leg injury and knee arthroscopy is unknown.
- The effect of both conditions on natural anticoagulants and fibrinolysis was explored in this study.
- Natural anticoagulant factor levels decreased after knee arthroscopy, unlike after lower-leg injury.
- *In vivo* fibrinolysis was neither affected by lower-leg injury nor by knee arthroscopy.

2 | METHODS

2.1 | Study population

Data of participants of the POT-CAST and POT-KAST trials were used for the current analysis. These patients had been treated with lower-leg cast immobilization (POT-CAST) or have undergone knee arthroscopy (POT-KAST). Details of these randomized controlled trials have been published previously [9]. In short, the effectiveness of LMWH as thromboprophylaxis, compared with no therapy, was studied in both study populations by evaluating 90-day incidences of symptomatic VTE. In both trials, participants reporting lower-leg trauma or undergoing knee arthroscopy between March 2012 and January 2016 were included. Patients with a traumatic injury below the knee, which required lower-leg cast immobilization (for at least 1 week), and patients scheduled for elective knee arthroscopic surgery were eligible for inclusion. The study included individuals who were aged 18 years or older and did not meet any of the following exclusion criteria: history of VTE, current use of anticoagulant therapy (except antiplatelet medication), contraindications for the use of LMWH, pregnancy, mental or physical disability to fulfill study requirements, or insufficient knowledge of the Dutch language. Participants in both trials were asked to complete a questionnaire on putative thrombotic risk factors for VTE. In addition, all patients provided blood samples. For POT-CAST participants, blood samples were collected shortly after lower-leg trauma, mostly on the same day when the trauma occurred. Furthermore, for injuries that needed surgery, blood samples were always collected before the surgery was performed. For POT-KAST participants, the following 2 blood samples were provided: 1 sample before surgery (≤4 hours preoperatively, T0) and one sample after surgery (≤4 hours postoperatively, T1). In both patient groups, all samples were collected before thromboprophylaxis (LMWH) was administered, which also applied to the postoperative samples in the POT-KAST participants. Both trials were approved by the Medical Ethics Committee of Leiden University Medical Center. The ClinicalTrials.gov Identifiers of the trials are NCT01542762 (POT-CAST) and NCT01542723 (POT-KAST).

2.2 | Current study

In total, 1435 individuals participated in the POT-CAST trial, of whom 23 developed VTE in the first 3 months after lower-leg trauma (a risk of 1.6%). Of the total 1451 POT-KAST participants, 8 developed VTE in the first 3 months following knee arthroscopy (a risk of 0.6%). Participants who did not develop VTE and whose blood sample(s)

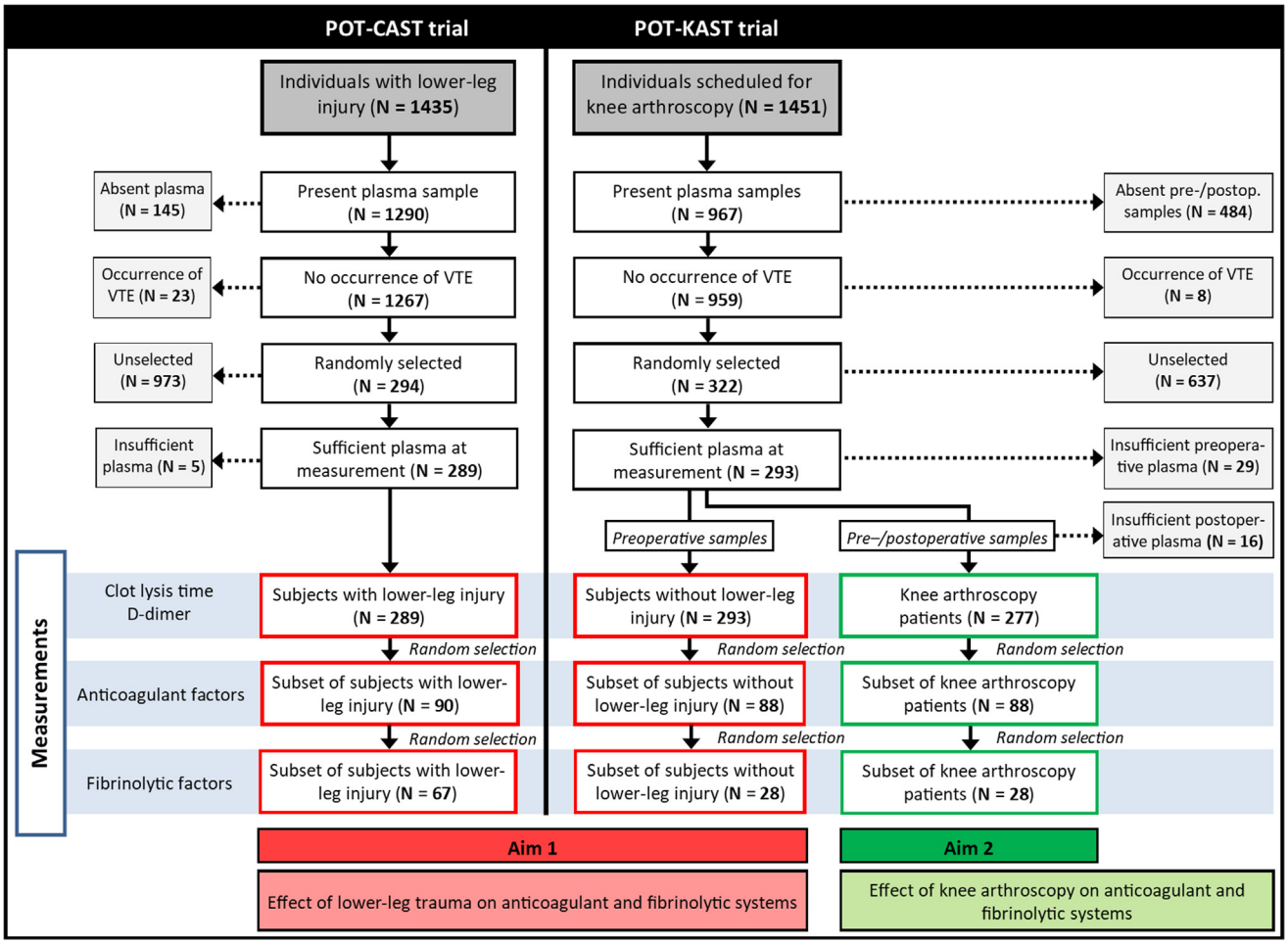

FIGURE 1 Flowchart presenting patient selection from POT-CAST and POT-KAST trials

were available were eligible for random selection and inclusion into the current study. Details of these selections are provided in the flowchart (Figure 1).

For addressing the first aim, ie, to study the impact of lower-leg trauma on natural anticoagulant factors and fibrinolysis, blood samples of 289 randomly selected individuals with lower-leg trauma (POT-CAST trial), referred to as "patients with lower-leg injury," were used for measurements. As control samples, we used the preoperative blood samples (baseline) of 293 randomly selected knee arthroscopy patients (POT-KAST trial) who had not sustained a recent lower-leg trauma. The latter group is referred to as "controls." These individuals were deemed suitable as a control group, because the control samples were processed and stored in a similar manner as samples of lower-leg injury patients. Moreover, the predominant indication for knee arthroscopy was a meniscal lesion for which an elective meniscectomy was planned. These surgeries were performed in patients with persisting complaints after minor knee trauma in the distant past, for which physical therapy proved inadequate. Hence, at the time of blood sampling, no acute or recent trauma was present, which could have affected plasma levels of anticoagulant and fibrinolytic factors. For addressing the second aim, ie, to study the impact of knee arthroscopy on natural anticoagulant and fibrinolytic systems, pre- and postoperative blood samples were compared for 277 of the 293 randomly selected knee arthroscopy patients, hence they acted as their own controls. These individuals are further referred to as "knee arthroscopy patients." The remaining 16 patients had to be excluded from these analyses because of the lack of sufficient postoperative plasma.

2.3 | Outcomes

Plasma levels of the following natural anticoagulant factors were measured: antithrombin activity, free protein S antigen, protein C activity, and tissue factor pathway inhibitor (TFPI) antigen. Fibrinolytic activation was quantified both *in vitro* (*ex vivo*) and *in vivo*. *In vitro* fibrinolysis involved the overall fibrinolytic tendency, expressed as clot lysis time (CLT). Plasma levels of plasmin-alpha-2-antiplasmin (PAP) complexes were measured as an estimate of *in vivo* fibrinolysis. Furthermore, plasma levels of plasminogen, tissue plasminogen activator (tPA), PAI-1, thrombin activatable fibrinolysis inhibitor (TAFI), and antiplasmin were measured. Finally, we estimated the amount of degraded fibrin resulting from fibrinolysis by measuring plasma levels of the degradation product D-dimer.

For addressing both aims, CLT and D-dimer were measured in all included individuals. Anticoagulant and fibrinolytic factors were measured in smaller random subsets, as depicted in Figure 1.

2.4 | Laboratory measurements

Details of the performed laboratory measurements are included in the Supplementary Material.

2.5 | Statistical analysis

For addressing both aims, means with standard deviations (SDs) were used to describe the data. For the first aim (comparing lower-leg injury patients with controls), mean differences with 95% confidence intervals (CIs) were obtained using linear regression and adjusted for sex, age, body mass index (BMI), comorbidities, and malignancy (diagnosed in the past year). Comorbidities included chronic obstructive pulmonary disease, liver disease, kidney disease, rheumatoid arthritis, multiple sclerosis, heart failure, hemorrhagic stroke, and arterial thrombosis. Mean differences were additionally adjusted for time of blood sampling (on a continuous scale in minutes counted from midnight) to correct for diurnal variation. For addressing the second aim (comparing pre- and postoperative samples of knee arthroscopy patients), paired mean changes with their corresponding 95% CIs were calculated. To correct the mean changes for diurnal variation, linear mixed models were employed, in which time of blood sampling both pre- and postoperative was included. If data were not normally distributed, they were transformed using natural logarithms (*ln*), resulting in geometric means (with 95% CIs) instead of means (with SDs) and mean ratios instead of mean differences [18]. Details of these calculations are included in the Supplementary Material.

For addressing the first aim, CLT was stratified for type of lower-leg injury, which meant that injuries were grouped according to severity, which was ranked by the abbreviated injury scale and consultation of 2 trauma and orthopedic surgeons [19]. Not all patients with lower-leg injury presented on the same day as the trauma occurred. To screen what the effect of time between trauma and blood sampling was, we employed sensitivity analyses in which the main analyses were restricted to patients with blood sampling within the first 24 hours after lower-leg trauma. Moreover, we plotted time between trauma and blood sampling against levels of antithrombin, CLT, and PAP as scatterplots. For addressing the second aim, we performed a subgroup analysis in which the (geometric) means and mean changes of CLT and D-dimer were stratified for anesthetic technique. Finally, for both aims, (geometric) means of tPA, PAI-1, and PAP were plotted against time of blood sampling to assess the role of diurnal variation. Selection of these 3 factors was based on their known association with diurnal variations from the literature [20].

All analyses were performed in Statistical Package for the Social Sciences version 25 (SPSS, IBM) and Stata version 16.0 (http://www.stata.com). SPSS was also used to perform automatic random selections of the individuals for inclusion in this study. Figures were generated using GraphPad Prism version 9.0.1 (GraphPad Software, http://www.graphpad.com).

2.6 | Sample size considerations

We aimed to achieve a power of 90% and a level of significance of 0.05 (2-sided). For CLT, we anticipated an observed mean difference or change of 9 minutes with an SD of 6, based on previous studies [21]. This resulted in a sample size of at least 18 patients for aim 1 (9 in each group) and 8 patients for paired measurements for aim 2. For protein C (and other anticoagulant factors), an observed mean difference or change of 19% with a SD of 18 was anticipated, resulting in a sample size of at least 30 patients for aim 1 (15 for each group) and 13 patients for paired measurements for aim 2. For PAI-1 (and other fibrinolytic factors), an observed mean difference or change of 14 ng/mL with a SD of 12 ng/mL was anticipated, resulting in a sample size of at least 32 patients for aim 1 (16 for each group) and 11 patients for paired measurements for aim 2. Numbers for protein C and PAI-1 were obtained from a previous study including patients with major trauma because there were no studies performed in patient groups more similar to those in our study [22]. As depicted in Figure 1, CLT and D-dimer were measured in all included individuals for both aims, whereas the *in vivo* measured anticoagulant and fibrinolytic factors were measured in smaller (randomly selected) subsets.

3 | RESULTS

3.1 | Aim 1: Impact of lower-leg injury on anticoagulant and fibrinolytic systems

3.1.1 | Study population

In Table 1, general characteristics of all individuals are shown. Both patients with lower-leg injury and controls had a median age of approximately 50 years and similar proportions of men (59% vs 52%). Controls were more often obese (24% vs 15%). Lower-leg injuries mostly involved metatarsal (37%), tarsal (17%), and transsyndesmotic ankle fractures (17%). In 61% of patients with lower-leg injury, blood was drawn within the first 24 hours of trauma. General characteristics were similar in the smaller subsets of both groups in which anticoagulant and fibrinolytic factor levels were measured, of which the smallest subsets are shown in Supplementary Table S1.

3.1.2 | Outcomes

Of the anticoagulant factors, only antithrombin and, to lesser extent, TFPI were higher in patients with lower-leg injury compared with controls, which remained after adjusting for age, sex, BMI, comorbidities, and malignancy (Table 2). However, after additional adjustment for time of sampling, all mean differences for anticoagulant

TABLE 1 Aim 1: General characteristics of individuals with and without lower-leg injury.

Patient groups		Patients with lower-leg injury (*N* = 289)[a]	Controls (*N* = 293)[b]
Sex	Male, *n* (%)	150 (51.9)	173 (59.0)
Age	*Median in years (IQR)*	48.9 (35.6-60.2)	51.0 (43.0-58.0)
Body mass index (BMI)	<20 kg m^{-2}, *n* (%)	8 (3.0)	4 (1.4)
	20-25 kg m^{-2}, *n* (%)	108 (40.2)	98 (34.1)
	25-30 kg m^{-2}, *n* (%)	112 (41.6)	115 (40.1)
	>30 kg m^{-2}, *n* (%)	41 (15.2)	70 (24.4)
At least one comorbidity	Yes, *n* (%)	38 (13.9)	28 (9.8)
Infection in last 2 months	Yes, *n* (%)	39 (14.5)	43 (15.0)
Smoking	Yes: currently, *n* (%) Yes: formerly, *n* (%)	64 (23.9) 83 (31.0)	53 (18.5) 89 (31.0)
Current use of oral contraceptives[c]	Yes, *n* (%) of women	20 (15.7)	14 (12.0)
Malignancy in last year	Yes, *n* (%)	7 (15.7)	1 (0.3)
ABO blood type	Homozygote non–O, *n* (%)	25 (9.2)	29 (10.3)
	Heterozygote O, *n* (%)	127 (46.7)	121 (43.1)
	Homozygote O, *n* (%)	120 (44.1)	131 (46.6)
Factor V Leiden	Heterozygote, *n* (%)	9 (3.3)	13 (4.7)
	No polymorphism, *n* (%)	264 (96.7)	266 (95.3)
Indication for lower-leg cast	Ankle distortion, *n* (%)	4 (1.4)	NA
	Contusion, *n* (%)	4 (1.4)	
	Achilles' tendon rupture, *n* (%)	19 (6.6)	
	Phalanx fracture, *n* (%)	5 (1.7)	
	Metatarsal fracture, *n* (%)	108 (37.4)	
	Tarsal fracture, *n* (%)	49 (17.0)	
	Infrasyndesmotic ankle fracture, *n* (%)	26 (9.0)	
	Transsyndesmotic ankle fracture, *n* (%)	48 (16.6)	
	Suprasyndesmotic ankle fracture, *n* (%)	10 (3.4)	
	Other ankle fracture, *n* (%)[d]	16 (5.5)	
Surgical treatment of injury	Yes, *n* (%)	33 (11.4)	NA
Timing blood sampling after lower-leg trauma	Within 24 h, *n* (%)	173 (61.1)	NA
	Within 7 days, *n* (%)	96 (33.9)	
	After 7 days, *n* (%)	14 (5.0)	
Randomization to prophylaxis with LMWH[e]	Yes, *n* (%)	148 (51.2)	152 (51.9)

IQR, interquartile range (25th-75th percentile).

Percentages indicate the proportions in non-missing data only.

[a] Numbers of missing data for lower-leg injury patients: BMI (*N* = 20), comorbidity (*N* = 15), infection (*N* = 20), smoking (*N* = 21), oral contraceptives (*N* = 12), malignancy (*N* = 15), ABO blood type (*N* = 17), factor V Leiden (*N* = 16), and timing of blood sampling (*N* = 6).

[b] Numbers of missing data for controls: BMI (*N* = 6), comorbidity (*N* = 6), infection (*N* = 6), smoking (*N* = 6), oral contraceptives (*N* = 3), malignancy (*N* = 6), ABO blood type (*N* = 12), and factor V Leiden (*N* = 14).

[c] Oral contraceptives included any type of hormonal therapy, including oral contraceptives and intrauterine devices.

[d] Including torus fracture of distal tibia (*N* = 1).

[e] LMWH was administered after blood sampling.

TABLE 2 Aim 1: Anticoagulant and fibrinolytic parameters in patients with lower-leg injury compared to controls.

	Mean (SD)[b]				
	Patients with lower-leg injury	Controls	Mean difference/ ratio (95% CI)[b]	Adj. mean difference/ ratio (95% CI)[a,b]	Adj. mean difference/ ratio (95% CI)[b,d]
Anticoagulant factors	(*N* = 90)	(*N* = 88)			
Antithrombin (%)	113.6 (13.0)	109.6 (10.6)	4.0 (0.5-7.5)	4.7 (1.1-8.3)	2.0 (−2.4 to 6.4)
Free protein S (%)	102.5 (20.4)	103.6 (17.9)	−1.1 (−6.8 to 4.6)	−2.2 (−7.5 to 3.2)	−5.6 (−12.4 to 1.1)
Protein C (%)	123.9 (24.0)	125.6 (21.4)	−1.7 (−8.4 to 5.1)	−1.0 (−7.4 to 5.5)	−5.0 (−13.1 to 3.1)
TFPI (ng/mL)[c]	38.8 (13.7)	35.9 (13.2)	2.8 (−1.5 to 7.2)	0.9 (−3.2 to 4.9)	−1.1 (−6.3 to 4.1)
Overall fibrinolytic potential	(*N*> = 289)	(*N* = 293)			
Clot lysis time (min)	70.0 (15.7)	77.8 (23.7)	−7.7 (−11.0 to −4.5)	−5.3 (−8.5 to −2.2)	−2.4 (−6.6 to 1.8)
Fibrinolytic factors	(*N* = 67)	(*N* = 28)			
Plasminogen (%)	105.4 (15.4)	107.7 (13.3)	−2.3 (−8.9 to 4.4)	−0.5 (−6.9 to 5.9)	−0.4 (−7.7 to 7.0)
tPA (ng/mL)	6.2 (2.9)	7.3 (5.4)	−1.1 (−2.8 to 0.6)	−1.4 (−3.1 to 0.2)	−0.5 (−2.3 to 1.4)
PAI-1 (ng/mL)	*1.1 (0.9-1.3)*[b]	*1.1 (0.8-1.6)*[b]	*1.0 (0.7-1.4)*[b]	*0.9 (0.6-1.2)*[b]	*1.1 (0.7-1.5)*[b]
Antiplasmin (%)	98.3 (9.1)	100.7 (8.7)	−2.4 (−6.4 to 1.6)	−1.8 (−5.6 to 2.0)	−2.4 (−6.7 to 1.9)
TAFI (%)	104.2 (16.3)	106.3 (24.2)	−2.1 (−10.6 to 6.4)	−0.6 (−9.1 to 7.9)	1.8 (−8.0 to 11.6)
PAP complexes (ng/mL)	*296.1 (269.5-325.4)*[b]	*254.0 (203.0-317.9)*[b]	*1.2 (0.9-1.4)*[b]	*1.1 (0.9-1.4)*[b]	*1.1 (0.9-1.4)*[b]
Degradation product	(*N* = 289)	(*N* = 293)			
D-dimer (ng/mL)	*714.5 (631.8-807.9)*[b]	*234.4 (211.9-259.4)*[b]	*3.0 (2.6-3.6)*[b]	*3.0 (2.5-3.5)*[b]	*2.9 (2.3-3.5)*[b]

BMI, body mass index; LMWH, low-molecular-weight heparin; PAI-1, plasminogen activator inhibitor-1; PAP, plasmin-alpha-2-antiplasmin; TAFI, thrombin activatable fibrinolysis inhibitor; TFPI, tissue factor pathway inhibitor; tPA, tissue plasminogen activator.

[a] Adjusted for age, sex, BMI, comorbidities, and malignancy.

[b] If not normally distributed, data were log-retransformed, resulting in geometric means with 95% confidence intervals and mean ratios (indicated by this symbol and italics).

[c] TFPI was measured in *N* = 67 subjects with lower-leg injury and *N* = 82 subjects without lower-leg injury after exclusion of those with plasma of inadequate quality.

[d] Adjusted for age, sex, BMI, comorbidities, malignancy, and time of blood sampling (diurnal variation).

factors decreased, including antithrombin (2.0% [95% CI −2.4 to 6.4]) and TFPI (−1.1 ng/mL [95% CI −6.3 to 4.1]). CLT was lower in patients with lower-leg injury compared with controls, with an adjusted mean difference of −5.3 minutes (95% CI −8.5 to −2.2). This decreased after additional adjustment for diurnal variation to −2.4 minutes (95% CI −6.6 to 1.8). Plasma levels of fibrinolytic factors were comparable for both groups. D-dimer levels were 3-fold higher in patients with lower-leg injury with an adjusted mean ratio of 2.9 (95% CI 2.3-3.5).

Analyses restricted to patients with blood sampling within 24 hours after lower-leg trauma (sensitivity analyses) yielded similar results (Supplementary Table S2), except for CLT, for which the difference between patients and controls became less pronounced; adjusted mean difference of −0.6 minutes (95% CI −4.8 to 6.1). In line with this, Supplementary Figure S1 shows that antithrombin, CLT, and PAP were not influenced by time between trauma and blood sampling. Furthermore, CLT was similar among all injury types (Figure 2). Finally, diurnal variation was observed for tPA and PAI-1 levels with the highest levels measured in the morning (Supplementary Figure S2 and Supplementary Table S4). PAP levels were highest in the evening. At all timepoints, all 3 parameter levels were slightly higher in patients with lower-leg injury as opposed to controls.

3.2 | Aim 2: Impact of knee arthroscopy on anticoagulant and fibrinolytic systems

3.2.1 | Study population

Knee arthroscopy patients (*N* = 277) had a median age of 51 years. Of these patients, 61% were men, 25% were obese, and 10% had a comorbidity. The arthroscopic procedures involved mostly meniscectomies (66%) and were performed under general anesthesia (63%). Virtually, all procedures were performed using a thigh-tourniquet (99%) (Table 3). General characteristics were comparable in the smaller subsets of patients in whom anticoagulant and fibrinolytic factor levels were measured, of which the smallest subset is presented in Supplementary Table S3.

3.2.2 | Outcomes

As shown in Table 4, all anticoagulant factor levels decreased after arthroscopy compared with preoperative levels. This decrease in anticoagulant factor levels enhanced after adjustment for time of

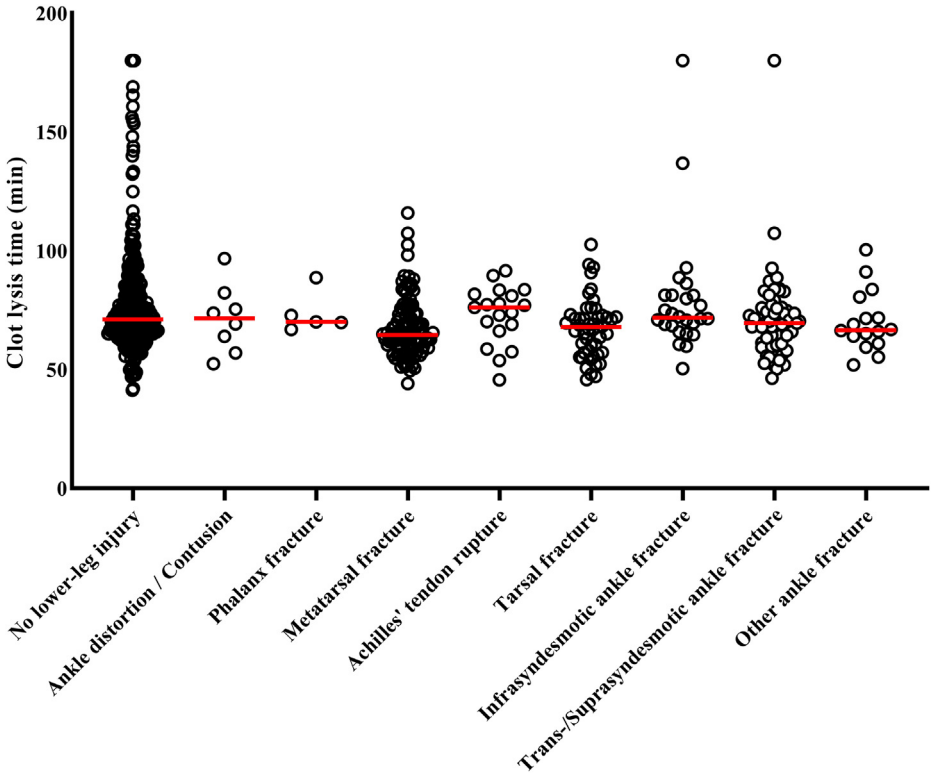

FIGURE 2 Aim 1: Clot lysis time according to lower-leg injury type based on the severity of injury with medians (lines)

blood sampling, resulting in following mean changes: antithrombin −9.6% (95% CI −12.3 to −6.9), free protein S −4.5% (95% CI −7.4 to −1.7), protein C −9.0% (95% CI −12.0 to −6.0), and TFPI −3.8 ng/mL (95% CI −6.0 to −1.6). Additionally, CLT decreased postoperatively, which was slightly less pronounced after adjusting for diurnal variation (−9.0 minutes [95% CI −11.7 to −6.2]). Plasma levels of all fibrinolytic factors decreased postoperatively. This decrease in fibrinolytic factor levels attenuated for all factors after adjusting for time of sampling, although plasminogen levels still decreased postoperatively (−5.8% [95% CI −9.8 to −1.7]). In fact, tPA and TAFI increased postoperatively to 1.3 ng/mL (95% CI −1.8 to 4.5) and 1.6% (−6.5 to 9.8), respectively. Furthermore, D-dimer decreased postoperatively, which became stronger after correcting for diurnal variation (−72.9 ng/mL [95% CI −143.1 to −2.6]). As shown in Supplementary Table S5, CLT was similar for patients who underwent general or spinal anesthesia. D-dimer, in contrast, decreased substantially after arthroscopy was performed under spinal anesthesia; paired mean change corrected for diurnal variation was −134.7 ng/mL (95% CI −298.8 to 29.5). However, given the wide CI, this is probably mostly because of chance.

As shown in Supplementary Figure S2 and Supplementary Table S4, modest diurnal variation was observed for tPA and PAI-1 (slightly higher levels in the morning than in afternoon). PAP levels were stable over the day. PAI-1 and PAP levels were comparable pre- and postoperatively. Preoperative tPA levels were higher in the morning than postoperative levels, whereas opposite was the case for mid-day levels. For both measurements, PAP levels did not vary over the day.

4 | DISCUSSION

This study was set up to improve our understanding of the mechanism of thrombus formation after lower-leg injury and knee arthroscopy. Following lower-leg injury, we found a 3-fold increase in D-dimer levels, although there was no sign of fibrinolytic activation based on *in vivo* analysis of fibrinolytic factors. Following arthroscopy, there did not seem to be *in vivo* fibrinolytic activation considering the decreased levels of D-dimer, PAP, and plasminogen. Moreover, tPA and TAFI (both known as inhibitors of fibrinolysis) were slightly increased after arthroscopy. At the same time, *in vitro* fibrinolytic potential, as reflected by CLT, was enhanced after arthroscopy. Anticoagulant factors were stable or somewhat decreased in lower-leg injury patients, whereas they all clearly decreased after knee arthroscopy.

To our knowledge, the effect of a minor trauma of the lower-leg on anticoagulant and fibrinolytic factors has not been studied before. In our previous analyses, we found that patients with lower-leg injury had elevated levels of procoagulant factors (such as FVIII and FXI) and enhanced thrombin generation [11] . The fact that the anticoagulant factor levels did not increase simultaneously with procoagulant factor levels indicates a net prothrombotic tendency after lower-leg injury, which is in line with the enhanced thrombin generation that we found previously. Although we earlier found indications for endothelial cell activation after lower-leg injury (such as elevated plasma levels of von Willebrand factor) [11], levels of endothelial-derived TFPI did not appear to be increased. In addition, for fibrinolysis, we did not observe a clear effect. *In vitro* fibrinolytic capacity was minimally elevated in

TABLE 3 Aim 2: Characteristics of knee arthroscopy patients.

Patient group		Knee arthroscopy patients (*N* = 277)[a]
Sex	Male, *n* (%)	169 (61.0)
Age	*Median in years (IQR)*	51.0 (43.0-58.0)
Body mass index (BMI)	<20 kg m^{-2}, *n* (%)	4 (1.5)
	20-25 kg m^{-2}, *n* (%)	91 (33.6)
	25-30 kg m^{-2}, *n* (%)	109 (40.2)
	>30 kg m^{-2}, *n* (%)	67 (24.7)
At least one comorbidity	Yes, *n* (%)	26 (9.6)
Type of procedure	Meniscectomy, *n* (%)	184 (66.4)
	Diagnostic arthroscopy, *n* (%)	18 (6.5)
	Removal of loose bodies, *n* (%)	5 (1.8)
	Other, *n* (%)	30 (10.8)
	Multiple procedures, *n* (%)	40 (14.4)
ASA classification	ASA I, *n* (%)	169 (63.3)
	ASA II, *n* (%)	96 (36.0)
	ASA III, *n* (%)	2 (0.7)
Type of anesthesia	General, *n* (%)	173 (63.4)
	Spinal, *n* (%)	99 (36.3)
	Epidural, *n* (%)	1 (0.4)
Use of thigh-tourniquet	Yes, *n* (%)	267 (98.9)
Total duration of knee arthroscopy[b]	*Median in minutes (IQR)*	21.0 (16.0-30.0)
Duration of surgery[c]	*Median in minutes (IQR)*	13.0 (10.0-18.0)
Randomization to prophylaxis with LMWH[d]	Yes, *n* (%)	141 (50.9)

ASA, American Society of Anesthesiologists classification; IQR, interquartile range (25th-75th percentile); LMWH, low-molecular-weight heparin.
Percentages indicate proportions in non-missing data only.
[a] Numbers of missing data: body mass index (*N* = 6), comorbidity (*N* = 6), ASA classification (*N* = 10), anesthesia (*N* = 4), and tourniquet (*N* = 7).
[b] Total duration was from the time patient received anesthesia to the time patient left the operating department.
[c] Duration of surgery was defined from the time of incision to the time of wound closure.
[d] LMWH was administered after postoperative blood sampling.

patients with lower-leg injury. There was no *in vivo* fibrinolytic activation, as reflected by unchanged levels of PAP and profibrinolytic factors (including plasminogen and tPA). Moreover, for the antifibrinolytic factors, there was no unanimity; TAFI was elevated, whereas antiplasmin was decreased. Based on the elevated D-dimer levels, it can be suggested that fibrin, generated following lower-leg injury, is cleared by basal fibrinolytic activity. In other words, fibrin generated after lower-leg injury does not appear to be accompanied by additional activation of the fibrinolytic system. Of note, we measured D-dimer and PAP levels only at a single timepoint, and hence, it is possible that we have missed transient elevations in PAP complexes because the half-life of PAP complexes is shorter than the half-life of D-dimer [23]. Alternatively, the fibrin generated may be cleared by proteases other than plasmin, for example, by neutrophil elastase or cathepsin G [24,25].

Knee arthroscopy did not lead to increased plasma levels of procoagulant factors and thrombin generation in our previous studies. In fact, plasma levels of procoagulant factors decreased postoperatively [11]. Our present study showed that plasma levels of anticoagulant factors also markedly decreased after arthroscopy [20]. Although this could indicate a change toward a more prothrombotic state, it may also be that the concurrent decrease in procoagulant factors leads to a net unchanged situation, which is also indicated by our previous findings on thrombin generation. For fibrinolysis, we observed a postoperative hyperfibrinolytic potential *in vitro*, based on shorter postoperative CLT. However, *in vivo* levels of fibrinolytic proteins were mostly stable with the exception of a decrease in plasminogen, known as a profibrinolytic factor, and increase in tPA and TAFI, known as antifibrinolytic factors. Based on these findings, it can be concluded that a substantial activation of fibrinolysis *in vivo* is absent in these patients. In fact, postoperative D-dimer levels were clearly decreased, in parallel with the decrease in pro- and anticoagulant factor levels. This was especially pronounced in patients operated under spinal anesthesia, of which it should be noted that the CI was wide. In literature, only slight increase has been linked to spinal anesthesia [26]. A possible explanation for the decrease we observed could be hemodilution because of routine administration of up to 0.5 L of intravenous saline during the procedure. Based on an average plasma volume of 3 L [27], this would have resulted in a maximal reduction of 14% in circulating factor levels (which would be less because of concurrent fluid excretion by kidneys).

TABLE 4 Aim 2: Pre- and postoperative levels of anticoagulant and fibrinolytic parameters in knee arthroscopy patients.

	Mean (SD)			
	Preoperative	Postoperative	Paired mean change (95% CI)	Paired mean change (95% CI)[a]
Anticoagulant factors (*N* = 88)				
Antithrombin (%)	109.6 (10.6)	101.7 (10.9)	−7.8 (−9.5 to −6.0)	−9.6 (−12.3 to −6.9)
Free protein S (%)	103.6 (17.9)	99.4 (17.8)	−4.3 (−5.8 to −2.7)	−4.5 (−7.4 to −1.7)
Protein C (%)	125.6 (21.4)	117.3 (20.3)	−7.9 (−9.4 to −6.4)	−9.0 (−12.0 to −6.0)
TFPI (ng/mL)[b]	35.8 (12.9)	33.0 (12.5)	−2.8 (−4.3 to −1.3)	−3.8 (−6.0 to −1.6)
Overall fibrinolytic potential (N=277)[c]				
Clot lysis time (min)	77.8 (23.7)	65.1 (14.1)	−13.3 (−15.4 to −11.2)	−9.0 (−11.7 to −6.2)
Fibrinolytic factors (*N* = 28)				
Plasminogen (%)	107.7 (13.3)	101.3 (16.1)	−6.8 (−9.4 to −4.2)	−5.8 (−9.8 to −1.7)
tPA (ng/mL)	7.3 (5.4)	6.8 (5.6)	−0.4 (−3.3 to 2.4)	1.3 (−1.8 to 4.5)
PAI-1 (ng/mL)	*1.1 (0.8-1.6)*[d]	*0.8 (0.5-1.1)*[d]	−0.7 (−1.0 to −0.3)	−0.3 (−0.7 to 0.0)
Antiplasmin (%)	100.7 (8.7)	96.2 (8.1)	−4.6 (−7.0 to −2.3)	−2.1 (−5.3 to 1.1)
TAFI (%)	106.3 (24.2)	103.4 (18.3)	−3.4 (−9.3 to 2.6)	1.6 (−6.5 to 9.8)
PAP complexes (ng/mL)	*254.0 (203.0-317.9)*[d]	*247.9 (201.4-305.3)*[d]	−32.3 (−276.6 to 211.9)	−3.3 (−271.5 to 264.9)
Degradation product (*N* = 277)				
D-dimer (ng/mL)	*228.7 (206.4-253.5)*[d]	*212.0 (193.5-232.3)*[d]	−56.1 (−101.3 to −11.0)	−72.9 (−143.1 to −2.6)

TFPI, tissue factor pathway inhibitor; Tpa, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin activatable fibrinolysis inhibitor; and PAP, plasmin-alpha-2-antiplasmin.

[a] Paired mean changes corrected for the time of blood sampling (diurnal variation); calculated using linear mixed model.

[b] TFPI was measured in *N* = 80 patients only, whereas blood samples of other patients had inadequate quality.

[c] Fibrinolysis was not measured in the postoperative samples of 16 patients because of the lack of plasma (*N* = 13) or inadequate quality of plasma (*N* = 3), resulting in *N* = 277.

[d] D-dimer, PAP complexes, and PAI-1 were log-retransformed, resulting in geometric means with 95% CIs.

Strengths of our study were that plasma samples were collected shortly after exposure to either lower-leg trauma (in the acute phase) or knee arthroscopy (within 4 hours postoperatively) and before administration of thromboprophylaxis (LMWH) in a large, unselected population. Therefore, we were able to study the effects on natural anticoagulant and fibrinolytic systems in the acute phase without interference of exogenous anticoagulants. However, there were also a couple of limitations we must address. First, blood sampling after trauma depended on the timing of presentation at the emergency department. Hence, approximately 39% of patients with lower-leg injury presented at the emergency department more than 24 hours after the trauma. Based on our finding that antithrombin, CLT, and PAP levels did not correlate with time between trauma and blood sampling, it was unlikely that delayed blood sampling affected our results. Second, as multiple comparisons were made, type I errors might have occurred. However, because we focused on biologically plausible and consistent changes in marker levels, statistical significance only played a minor role in the interpretation of the results. Finally, our measurements were not performed locally (in the injured or operated leg), and therefore, we could have missed local effects. However, it can be assumed that such effects were probably of limited clinical relevance if they were not detected systemically.

Because lower-leg fractures are the most common type of fractures worldwide and knee arthroscopy is one of the most frequently performed orthopedic procedures in the world [7,8], adequate prevention of VTE under these conditions is important. Knowledge about mechanistic pathways underlying thrombus formation in these patients is therefore relevant to improve the current thromboprophylaxis strategy. Not only did population–based administration of LMWH prophylaxis appear to lower VTE risk insufficiently [9], but routine administration to all patients is also not desirable, given the disadvantages of LMWH such as risk of (major) bleeding and uncomfortable administration [9,28]. Moreover, it is costly to treat all patients with LMWH. Hence, research for new and safer prevention targets is necessary. However, based on our findings, natural anticoagulant factors and fibrinolysis do not seem to qualify as potential prevention targets, as because these controlling mechanisms were not impaired following lower-leg injury and knee arthroscopy.

In conclusion, lower-leg injury was found to be associated with stable or somewhat decreased anticoagulant factor levels, whereas *in vitro* and *in vivo* fibrinolysis was not affected. Knee arthroscopy was associated with strong decrease in anticoagulant factor levels, which might be clinically relevant. Although *in vitro* fibrinolytic potential was enhanced after arthroscopy, *in vivo* fibrinolytic activity was

unaffected. At this point, fibrinolytic abnormalities do not seem to play a role in the mechanism of thrombus formation in both situations.

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DECLARATION OF COMPETING INTERESTS

All authors declare no competing financial or other interests.

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