Different roads lead to venous thrombosis after lower-leg injury and knee arthroscopy: mechanistic insights in the development of venous thrombosis

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Part I

Impact of lower-leg injury and knee arthroscopy on the coagulation system

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Lower-leg injury and knee arthroscopy have distinct effects on coagulation

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ABSTRACT

Background: Lower-leg injury and knee arthroscopy are associated with increased venous thromboembolism (VTE) risk. It is unknown how these conditions affect coagulation.

Objective: To study the effect of 1) lower-leg trauma and 2) knee arthroscopy on coagulation.

Methods: Plasma samples of the POT-(K)CAST trials were used, which were collected shortly after lower-leg trauma (POT-CAST) and before/after (<4 hours) knee arthroscopy (POT-KAST). For aim 1, 1204 lower-leg injury patients were compared with preoperative samples of 1001 controls. Mean differences/ratios (if ln-retransformed due to skewedness) were adjusted for sex, age, body mass index, comorbidity, malignancy, oral contraceptives using linear regression. For aim 2, pre- and postoperative samples of 715 arthroscopy patients were compared resulting in paired mean changes. Plasma levels of fibrinogen, factor (F)VIII, IX, XI, von Willebrand Factor (VWF), D-dimer were measured in all individuals. Parameters of underlying mechanisms (including tissue factor, interleukin-6 [IL-6], myeloperoxidase-DNA, cell-free DNA) were measured in random subsets.

Results: In patients with lower-leg injury, plasma levels of coagulation parameters increased, especially FVIII, VWF and D-dimer, i.e., adjusted mean differences: FVIII 26.8% (95%CI 23.7;29.9), FIX 13.8% (95%CI 11.9;15.6), FXI 5.1% (95%CI 3.3;7.0), VWF 29.8% (95%CI 26.0;33.6), fibrinogen 32.5 mg/dL (95%CI 25.8;39.2), D-dimer [mean ratio] 3.3 (95%CI 3.1;3.6). Levels of remaining parameters were unchanged, except for (somewhat) increased IL-6 levels. After knee arthroscopy, plasma levels of all parameters decreased.

Conclusion: Lower-leg trauma is associated with increased procoagulant factor levels, in contrast to knee arthroscopy. This finding suggests that in both situations, different pathways are involved in development of VTE.

INTRODUCTION

Lower-leg injury and knee arthroscopic surgery are conditions known to be associated with venous thromboembolism (VTE), mostly located in the deep veins of the leg (i.e., deep vein thrombosis) or in pulmonary arteries (i.e., pulmonary embolism). According to a recent meta-analysis, the risk of symptomatic VTE is 2% in patients with lower-leg injury and 0.6% in patients who underwent knee arthroscopy in the following 3 months.[1] Adequate thromboprophylaxis is important given the considerable mortality and chronic morbidity associated with VTE.[2-4] However, the POT-(K)CAST trials showed that the current thromboprophylaxis strategy, i.e., low-molecular-weight heparin (LMWH), is not optimal.[5]

In order to improve prevention of VTE in patients undergoing lower-leg cast application or knee arthroscopy, specific knowledge regarding the mechanism of VTE in both patient groups is necessary.[1] Considering Virchow's triad it can be assumed that vessel wall damage, due to injury, and venous stasis, due to reduced mobility, play a role in (pathological) thrombus formation following lower-leg cast immobilisation.[6] This differs from knee arthroscopy, which is characterised as a minimally invasive procedure and is therefore associated with slight tissue damage and limited postoperative immobilisation. Furthermore, these patients are mostly operated with use of thigh-tourniquet application (in order to obtain a dry surgical field), in which the induced hypoxia could play a role in pathological thrombus formation.[7] It is unknown whether lower-leg trauma and/or knee arthroscopy induce abnormalities in blood composition, such as increased levels of procoagulant factors, which constitutes the third element of Virchow's triad.

For this study, we aimed to explore the impact of (1) lower-leg injury and (2) knee arthroscopy on the coagulation system. For this purpose, several haemostatic components were assessed as well as markers for underlying mechanisms (inflammation, cell damage and neutrophil extracellular traps formation).

METHODS

Study population

We used data from the POT-CAST (Prevention of Thrombosis following CAST immobilisation) and POT-KAST (Prevention Of Thrombosis following Knee Arthroscopy) trials, which are described elsewhere in detail.[5] In short, the aim of these multicenter randomised controlled trials was to assess the effectiveness of LMWH on the occurrence of symptomatic VTE in the first three months after lower-leg cast application (POT-CAST) and knee arthroscopic surgery (POT-KAST). Between March 2012 and January 2016, individuals who were admitted to the emergency department with a lower-leg injury and individuals scheduled for elective knee arthroscopy were included in the trials. Participants were

randomised to receive either prophylactic LMWH or no thromboprophylaxis (control group). LMWH was administered during the complete period of cast immobilisation and for 8 days following knee arthroscopy. The occurrence of symptomatic VTE was assessed by a blinded independent outcome adjudication committee. Individuals aged 18 years or older were eligible for inclusion. Specific indications for knee arthroscopy included, but were not limited to, meniscectomy, diagnostic arthroscopy, and removal of loose bodies. Individuals complying to one of the following criteria were excluded: history of VTE, current use of anticoagulant therapy (except antiplatelet medication), contra-indications for use of LMWH, pregnancy, mental or physical disability to fulfil study requirements or insufficient knowledge of the Dutch language. In both trials, participants completed a questionnaire (digital [online] or postal) on putative thrombotic risk factors (such as age, sex, comorbidities, use of oral contraceptives). Injury-specific and knee arthroscopy-specific data were collected upon inclusion and completed from medical records or clinical research forms. POT-CAST participants provided a blood sample at the emergency department shortly after trauma. POT-KAST participants provided a preoperative blood sample (within four hours before knee arthroscopic surgery started) and a postoperative blood sample (within four hours after the surgery ended). In case of randomisation to LMWH, the first dose was administered after (postoperative) blood sampling in all participants. Written informed consent was received from all participants. The Medical Ethics Committee of the Leiden University Medical Center approved both trials. The ClinicalTrials.gov Identifiers of both trials are NCT01542762 (POT-CAST) and NCT01542723 (POT-KAST).

Current study

Aim 1: The effect of lower-leg trauma on the coagulation system

Main analysis

For the first aim, we compared plasma samples of individuals with lower-leg injury with control samples. Individuals with lower-leg injury were included from the POT-CAST trial (further referred to as 'patients with lower-leg injury'). As control samples, we used the preoperative plasma samples of individuals who participated in the POT-KAST trial (further referred to as 'controls'). These samples were considered to be suitable as control samples, as at the point of blood sampling coagulation was not influenced by an acute trauma caused by knee arthroscopy or by its indication. The predominant indication for knee arthroscopy was a meniscectomy, which is performed in patients with persisting complaints after minor knee trauma in the past. Furthermore, samples from both groups were processed and stored in a similar way, and the laboratory measurements were done in both groups in the same batches.

Subgroup analyses

1) Severity of injury

To study a possible association between severity of injury and changes in coagulation factor levels, we classified the severity of injury in a systematic manner by using the Abbreviated Injury Scale (AIS). This scale is commonly used in trauma surgery and gives a comprehensive ranking of injury types by severity, in which severity is defined as the probability of death caused by the injury. The lowest score (i.e. 1) means 'minor injury', while the highest score (i.e. 6) stands for 'fatal injury'.[8] Patients with a contusion, ankle distortion or phalanx fracture had an AIS score of 1. The remainder of lower-leg injuries had an AIS score of 2. We further subdivided the injuries a priori, after consulting two trauma and orthopaedic surgeons. This resulted in a severity ranking of lower-leg injuries with six categories. Category 1 consisted of contusion, ankle distortion (including ankle luxation without fracture) or phalanx fracture, category 2A included metatarsal fractures and category 2B Achilles' tendon ruptures. Category 2C involved tarsal fractures or infrasyndesmotic ankle fractures. Category 2D consisted of suprasyndesmotic and transsyndesmotic ankle fractures and, finally, category 2E involved tibia and/or fibula shaft fractures. Patients with fractures or Achilles' tendon ruptures who were operated, were grouped separately (both Category 2F), assuming that the severity of these injuries was such that it necessitated surgical treatment.

2) Time between trauma and blood sampling

Not all patients with lower-leg injury were admitted to the hospital on the same day as trauma occurrence. We studied the effect of time between injury and blood sampling in two ways, i.e., by studying the correlation between coagulation factor levels and timing of blood sampling after trauma, and by performing a sensitivity analysis in which the main analysis was restricted to patients with blood sampling on the same day as trauma occurrence.

3) Synergistic effects

Possible synergistic effects of lower-leg injury and known risk factors for VTE on coagulant factor levels were assessed. For this purpose, we split the participants into the following subgroups: (1) risk factor absent and lower-leg injury absent, (2) risk factor present and lower-leg injury absent, (3) risk factor absent and lower-leg injury present, (4) risk factor present and lower-leg injury present. Risk factors included male sex, age >55 years, obesity (Body Mass Index [BMI] >25 kg m^{-2}), comorbidity, use of oral contraceptives (in women), Factor V Leiden mutation, Factor II 20210A mutation and non-O blood group.

Validation in population controls

To verify our main results, patients with lower-leg injury were compared to a second control group. For this purpose, we used data of the MEGA (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) study, of which details were published previously and described in the **Supplement**.[9] In short, the MEGA study is a population-based case-control study of which the controls involved persons with no current or history of VTE. All controls who provided a blood sample were included in current control group and considered as population controls. Pregnant controls were excluded in order to obtain a comparable control group for lower-leg injury patients (since pregnancy was an exclusion criterion in the POT-CAST trial). Likewise, lower-leg injury patients aged over 70 years were excluded for validation analysis since controls in the MEGA study were aged up to 70 years. Different laboratory devices were used for the measurements in the MEGA and POT-CAST study (i.e., STA-R and ACL-TOP 700 analyser, respectively), inducing possible misclassification bias when comparing both measurements. Therefore, a sensitivity analysis was employed in order to assess the risk of misclassification. Details of this analysis are included in the **Supplement**.

Aim 2: The effect of knee arthroscopy on the coagulation system

For the second aim, we compared the postoperative plasma sample of individuals who had undergone knee arthroscopy (i.e. the POT-KAST participants) to their preoperative plasma sample, for which we excluded individuals with a missing postoperative sample. The included patients for this aim are further referred as 'knee arthroscopy patients' (**Fig 1**).

Outcomes

Plasma levels of fibrinogen, factor (F)VIII, IX, XI, von Willebrand factor (VWF) and D-dimer were measured. In addition, the amount of soluble tissue factor (TF) in plasma was quantified. In order to obtain more insight in underlying mechanisms of coagulation activation, biomarkers of inflammation (i.e., interleukin-6, IL-6), cell damage (i.e., cell-free DNA, cfDNA) and neutrophil extracellular traps formation (i.e., myeloperoxidase-DNA complexes, MPO-DNA) were assessed. TF, IL-6, cDNA and MPO-DNA were measured in randomly selected subsets of both POT-CAST and POT-KAST populations who did not develop symptomatic VTE in first three months, as depicted in **Fig 1** and **Fig S2**.

Laboratory measurements

Blood of every participant in the POT-(K)CAST trials was collected in vacuum citrate tubes (0.105 M, 3.2%). These tubes were centrifuged during 10 minutes at 2500G at 18°C. The resulting plasma was aliquoted and stored at -80°C within 4 hours after vena puncture. All coagulation factors in plasma were measured using ACL-TOP 700 analyser with HemosIL reagents (Werfen Instrumentation Laboratory, Barcelona, Spain). FVIII, FIX, and FXI were

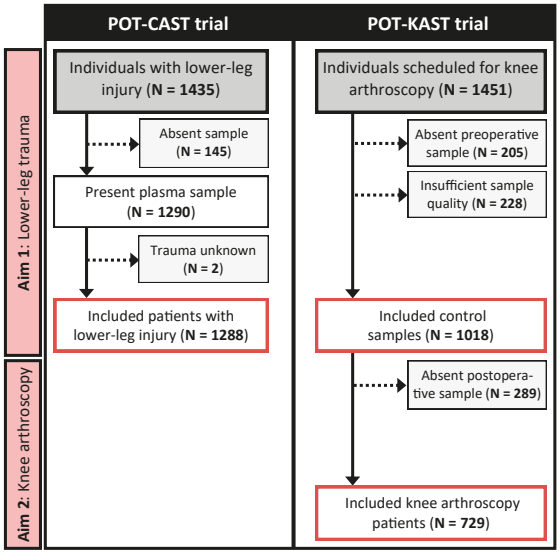

Fig 1 – Flowchart of participant inclusion from the POT-CAST and POT-KAST trials for measurements of coagulation factors.

quantified employing specific APTT assays and fibrinogen was determined with the Clauss method.[10] Automated latex enhanced immunoassays were used to measure VWF and D-dimer with vWF:Ag reagent and D-dimer HS 500 reagent, respectively. ELISA assays were employed to quantify TF (Human Coagulation Factor III/Tissue factor Quantikine ELISA, R&D Systems, Minneapolis, MN, USA) and IL-6 (Human IL-6 ELISA MAX, BioLegend, San Diego, CA, USA); IL-6 levels <8.0 pg/mL deviated too much from the calibration line and were therefore undetectable. MPO-DNA was assessed using monoclonal antibody to MPO (Clone 4A4, Sanbio, Uden, the Netherlands) and an anti-DNA antibody from the cell death detection ELISA (Sigma-Aldrich, Zwijndrecht, the Netherlands).[11] cfDNA concentrations were assessed employing a double strand DNA assay kit (Quant-iT PicoGreen, Fisher Scientific, Landsmeer, the Netherlands).

Laboratory reference ranges were derived from protocols of the employed laboratory tests and the Dutch blood bank (Sanquin).[12]

Statistical analysis

Aim 1: The effect of lower-leg trauma on the coagulation system

Plasma levels were expressed as means ± standard deviation. Mean plasma levels and mean differences with their 95% confidence intervals (95%CI) were calculated using linear regression. If not normally distributed, data were ln-transformed and ln-retransformed after analyses resulting in *geometric means* instead of means and *mean ratios* instead

of mean differences. Details of these calculations are included in the **Supplement**. As IL-6 levels <8.0 pg/mL were not detectable, we performed censored linear regression resulting in *left-censored* mean differences. All obtained mean differences/ratios were adjusted for sex, age, BMI, comorbidity, infections in the last two months, malignancy in last five years. Mean differences/ratios obtained for FVIII, FIX, FXI, VWF, fibrinogen and D-dimer were also adjusted for use of oral contraceptives.[13-17] Comorbidities included Chronic Obstructive Pulmonary Disease (COPD), liver disease, kidney disease, rheumatoid arthritis, multiple sclerosis, heart failure, haemorrhagic stroke, arterial thrombosis.

As mentioned above, three subgroup analyses were performed. First, the main results were stratified according to severity of injury. Severity of injury was correlated to coagulation factor levels using linear regression (adjusted for the addressed confounders). This resulted in regression coefficients, which reflected the increase/decrease in factor levels per step increase of injury severity. If data were ln-retransformed because of skewedness, the result should be interpreted as a relative increase. Second, time between trauma and blood sampling was correlated to coagulation factor levels in scatterplots with regression lines. Furthermore, a sensitivity analysis was performed in which the main analysis was restricted to subjects with blood draw on the day of trauma occurrence. Third, we assessed possible synergistic effects of lower-leg injury and known risk factors for VTE on coagulation factor levels by composing subgroups as mentioned above and using linear regression, resulting in mean differences (adjusted for the same confounders as mentioned previously) compared with the reference group consisting of subjects with neither the risk factor or lower leg injury. Finally, as a validation of our main results we performed the same analyses with a second control group consisting of population controls.

Aim 2: The effect of knee arthroscopy on the coagulation system

Preoperative and postoperative plasma levels of knee arthroscopy patients were also described as means ± standard deviation. Non-normally distributed data were analysed in the same way as for the first aim of this study. Paired mean changes with corresponding 95%CIs were calculated. IL-6 was dichotomized based on detectable (>8.0 pg/mL) and undetectable (≤8.0 pg/mL) levels. Possible switches between these two categories within patients were described and paired mean change was calculated for patients with detectable pre- and postoperative IL-6 levels (>8.0 pg/mL). Finally, subgroup analyses were performed in which coagulation factor levels were stratified for age, sex, BMI, comorbidity and aspects of the surgery, such as duration of surgery.

All analyses were performed in Statistical Package for the Social Sciences version 25 (SPSS, IBM, Armonk, NY, USA) and Stata 16.0 (http://www.stata.com). Figures were generated using GraphPad Prism version 9.0.1 (GraphPad Software, San Diego, California USA, http://www.graphpad.com).

RESULTS

Aim 1: The effect of lower-leg trauma on the coagulation system

Study population

A total of 1288 patients with lower-leg injury were included, who were compared with 1018 control subjects (**Fig 1**). Both populations had comparable distributions of general patient characteristics, i.e., a median age of 46 years for patients and 50 years for controls, and 50% of patients and 57% of controls were male (**Table 1**). Most patients had a fracture of the foot (54%) or ankle (34%) (**Table S1**).

Main analyses

Patients with lower-leg injury had the following mean levels: FVIII 149.2%, FIX 135.9%, FXI 117.5%, VWF 166.8%, fibrinogen 339.2 mg/dL, D-dimer 723.6 ng/mL (**Table 2**). These means were all higher compared with controls, especially for FVIII (adjusted mean difference 26.6%, 95%CI 23.6 to 29.6), VWF (adjusted mean difference 29.4%, 95%CI 25.7 to 33.1) and fibrinogen 34.0 mg/dL (95%CI 27.4 to 40.7). Mean D-dimer levels were threefold higher (95%CI 3.2 to 3.7) in patients compared with controls. Mean TF in patients (i.e. 37.4 pg/mL) was lower compared with controls: adjusted mean difference -3.4 pg/mL (95%CI -6.2 to -0.6). An adjusted left-censored mean difference of 163.1 pg/mL (95%CI -335.9 to 662.2) was found for detectable IL-6 levels.

Subgroup analyses

1) Severity of injury

An association between injury severity and levels of coagulation factors was demonstrated, mainly for FVIII and VWF (**Table S2**, **Fig 2**). More specifically, patients with phalanx fractures (labelled as the least severe injury type) had the lowest levels of FVIII (136.0%) and VWF (152.8%), while patients with fractures in need for surgery (considered as the most severe injury type) had the highest levels of FVIII (167.0%) and VWF (182.8%). Achilles' tendon rupture, considered as minimally severe, was associated with relatively high levels of FVIII (154.8%) and VWF (169.3%). At the same time, D-dimer levels were rather low (499.0 ng/mL) in patients with Achilles' tendon rupture. Individuals with an ankle distortion or contusion had relatively high FVIII and VWF levels as opposed to phalanx fractures.

2) Time between trauma and blood sampling

Of all patients, 62.1% presented within 24 hours after trauma occurrence. As shown in **Fig S3**, time between trauma and blood sampling did not affect coagulation factor levels.

Table 1 – General characteristics of study populations.

		AIM 1		AIM 2
		Patients with lower-leg injury (N=1288)*	**Controls (N=1018)†**	**Knee arthroscopy patients (N=729)‡**
Sex	Male, *n (%)*	646 (50.2)	581 (57.1)	416 (57.1)
Age	*Median in years*	46.2	50.0	49.4
	(5th-95th percentile)	(20.6 – 73.0)	(24.0 – 67.0)	(24.0 – 67.0)
BMI	< 18.5 kg m^{-2}, *n (%)*	12 (1.0)	1 (0.1)	1 (0.1)
	18.5 - 25.0 kg m^{-2}, *n (%)*	569 (47.3)	351 (35.0)	257 (35.8)
	25.0 - 30.0 kg m^{-2}, *n (%)*	439 (36.5)	432 (43.2)	308 (43.0)
	> 30.0 kg m^{-2}, *n (%)*	182 (15.2)	217 (21.7)	151 (21.1)
Comorbidity	Yes, *n (%)*	138 (11.3)	83 (8.3)	58 (8.1)
Infections in last 2 months	Yes, *n (%)*	175 (14.6)	139 (13.9)	106 (14.8)
Smoking	Yes: currently, *n (%)*	320 (26.8)	190 (19.1)	139 (19.4)
	Yes: formerly, *n (%)*	329 (27.5)	333 (33.4)	232 (32.4)
Current use of oral contraceptives§	Yes, *n (%) of women*	110 (18.2)	85 (19.9)	54 (17.6)
Malignancy in last 5 years	Yes, *n (%)*	34 (2.8)	17 (1.7)	12 (1.7)
ABO-blood type	Homozygote O, *n (%)*	521 (43.3)	403 (44.4)	300 (46.5)
	Heterozygote O, *n (%)*	549 (45.6)	411 (45.2)	279 (43.3)
	Homozygote non-O, *n (%)*	133 (11.1)	94 (10.4)	66 (10.2)
Factor II 20210A mutation	Heterozygote, n (%)	26 (2.1)	22 (2.2)	15 (2.1)
Factor V Leiden	Heterozygote, *n (%)*	49 (4.0)	34 (3.7)	25 (3.8)
	Homozygote, *n (%)*	1 (0.1)	0 (0.0)	0 (0.0)
Prophylactic LMWH (after blood sampling)	Yes, *n (%)*	639 (49.6)	513 (50.4)	371 (50.9)

Percentages indicate proportions in non-missing data only.

* Numbers of missing data for lower-leg injury patients: BMI (N=86), comorbidity (N=70), infection (N=86), smoking (N=93), oral contraceptives (N=37), malignancy (N=74), ABO blood type (N=85), factor II mutation (N=73), factor V Leiden mutation (N=77).

† Control samples involved preoperative samples of knee arthroscopy patients. Numbers of missing data for controls: BMI (N=17), comorbidity (N=18), infection (N=18), smoking (N=21), oral contraceptives (N=9), malignancy (N=22), ABO blood type (N=110), factor II mutation (N=37), factor V Leiden mutation (N=88).

‡ Numbers of missing data for knee arthroscopy patients: BMI (N=12), comorbidity (N=12), infection (N=12), smoking (N=12), oral contraceptives (N=7), malignancy (N=16), ABO blood type (N=84), factor II mutation (N=28), factor V Leiden mutation (N=70).

§ Oral contraceptives included any type of hormonal therapy, including oral contraceptives and intrauterine devices.

Table 2 – Aim 1: Mean differences in coagulation and inflammatory factor levels in patients with lower-leg injury compared with controls.

	Reference range	Mean (SD)*	Mean difference/ratio (95%CI)*	Adj. mean difference/ ratio (95%CI)*†
FVIII activity (%)	50-150%			
Controls (N=1018)		125.6 (29.4)	Reference	Reference
Patients (N=1288)		149.2 (40.3)	23.6 (20.6 to 26.5)	26.6 (23.6 to 29.6)‡
FIX activity (%)	65-135%			
Controls (N=1018)		125.1 (22.4)	Reference	Reference
Patients (N=1288)		135.9 (24.8)	10.9 (8.9 to 12.8)	13.9 (12.1 to 15.7)‡
FXI activity (%)	65-140%			
Controls (N=1018)		114.3 (21.3)	Reference	Reference
Patients (N=1288)		117.5 (23.8)	3.3 (1.4 to 5.1)	5.0 (3.2 to 6.9)‡
VWF antigen (%)	50-150%			
Controls (N=1018)		140.5 (39.5)	Reference	Reference
Patients (N=1288)		166.8 (47.7)	26.2 (22.6 to 29.9)	29.4 (25.7 to 33.1)‡
Fibrinogen (mg/dL)	200-393 mg/ dL			
Controls (N=1018)		312.1 (73.4)	Reference	Reference
Patients (N=1288)		339.2 (93.7)	27.1 (20.1 to 34.1)	34.0 (27.4 to 40.7)‡
D-dimer (ng/mL)*	<500 ng/mL			
Controls (N=1018)		*216.5 (205.4 to 228.3)**	Reference	Reference
Patients (N=1288)		*723.6 (685.0 to 764.4)**	*3.3 (3.1 to 3.6)**	*3.4 (3.2 to 3.7)*‡*
TF (pg/mL)	<62.2 pg/mL			
Controls (N=83)		40.5 (10.0)	Reference	Reference
Patients (N=88)		37.4 (9.3)	-3.1 (-6.0 to -0.2)	-3.4 (-6.2 to -0.6)
cfDNA (ug/mL)	<1.14 ug/mL			
Controls (N=28)		0.9 (0.1)	Reference	Reference
Patients (N=67)		1.0 (0.2)	0.0 (0.0 to 0.1)	0.0 (-0.1 to 0.1)
MPO-DNA (ng/mL)*	<0.64 ng/mL			
Controls (N=28)		*0.1 (0.1 to 0.1)**	Reference	Reference
Patients (N=67)		*0.1 (0.1 to 0.1)**	*0.9 (0.6 to 1.2)**	*0.9 (0.7 to 1.3)**
IL-6 (pg/mL)	<10 pg/mL			
Controls (N=80)		NA	Reference	Reference
Patients (N=75)		NA	193.6 (-291.8 to 679.0)§	163.1 (-335.9 to 662.2)§

Control samples involved the preoperative samples of knee arthroscopy patients.
* This symbol and italics indicate geometric means (with 95%CIs) and mean ratios of ln-retransformed data.
† Adjusted for sex, age, BMI, comorbidity, infections, malignancy.
‡ Adjusted for sex, age, BMI, comorbidity, infections, malignancy, use of oral contraceptives.
§ Left-censored results, i.e., only detectable levels (>8.0 pg/mL) were analysed; means could not be obtained.

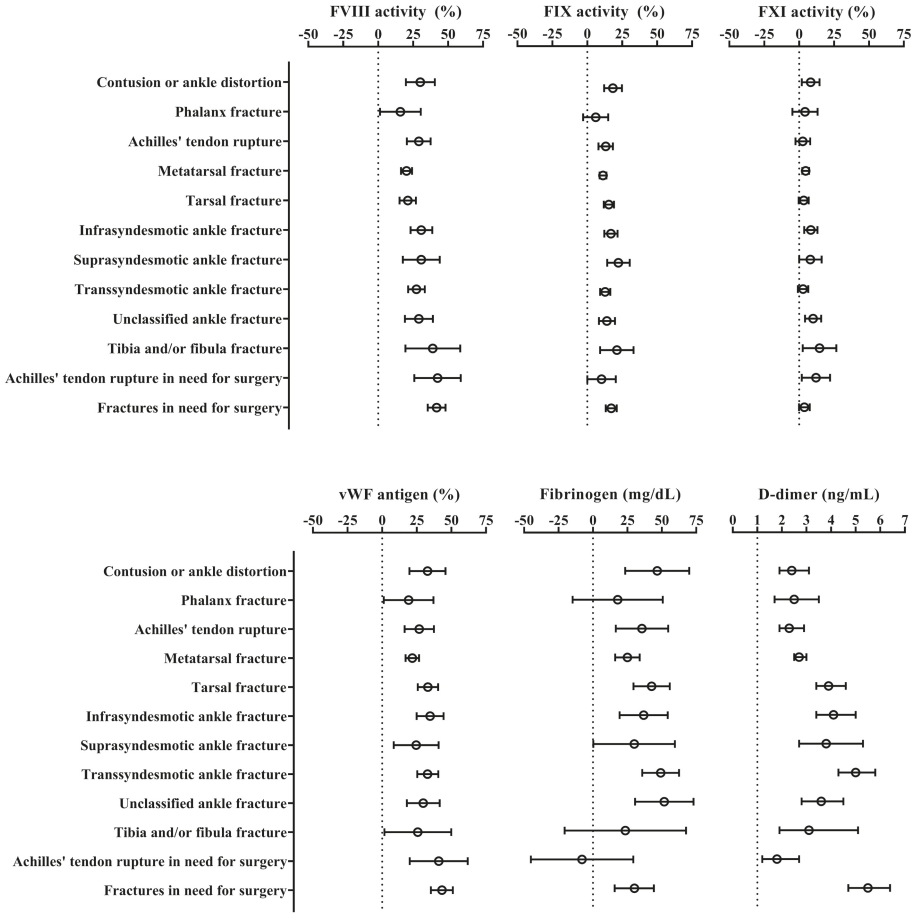

Fig 2 – Aim 1: Error bars reflecting adjusted mean differences with 95%CIs for FVIII, FIX, FXI, VWF, fibrinogen and D-dimer per injury type (ordered by severity) relative to controls.

Furthermore, the sensitivity analysis restricted to patients who presented within 24 hours provided similar results, which are not shown.

3) Synergistic effects

As shown in **Table 3**, the combination of lower-leg trauma and a known risk factor for VTE did not affect FVIII, FIX, FXI, VWF, fibrinogen or D-dimer levels in a synergistic way. For higher age and for obesity some combinations led to supra-additive effects.

Validation in population controls

The main results were verified by comparing 1204 patients with lower-leg injury with 2921 population controls, of which general characteristics are shown in **Table S3**. Similar

Table 3 – Aim 1: Combined effects of lower-leg injury and male, sex, age >55 years, BMI>30, comorbidity, oral contraceptives, Factor V Leiden mutation, Factor II mutation and non-O blood group on the patterns of coagulation factor levels.

Risk factor	Lower-leg injury		Number of persons (N=2306)	FVIII (%)	FIX (%)	FXI (%)	VWF (%)	Fibrinogen (mg/dL)	D-dimer (ng/mL)
				Adj. mean difference (95%CI)*	Adj. mean difference (95%CI)*	Adj. mean difference (95%CI)*	Adj. mean difference (95%CI)*	Adj. mean difference (95%CI)*	Adj. mean ratio (95%CI)*
Male sex	Absent	Absent	437	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*1.0 (Ref)*
	Present	Absent	581	-1.4 (-5.9 to 3.1)	0.5 (-2.3 to 3.2)	-9.7 (-12.4 to -6.9)	3.7 (-1.9 to 9.3)	-20.2 (-30.3 to -10.1)	0.8 (0.7 to 0.9)
	Absent	Present	642	24.1 (19.8 to 28.9)	13.2 (10.5 to 15.8)	3.9 (1.3 to 6.6)	26.5 (21.2 to 31.9)	31.3 (21.6 to 41.0)	3.0 (2.7 to 3.4)
	Present	Present	646	27.3 (22.7 to 31.8)	14.9 (12.1 to 17.7)	-3.8 (-6.6 to -1.0)	35.6 (29.9 to 41.2)	15.9 (5.6 to 26.1)	3.1 (2.7 to 3.4)
Age > 55 years	Absent	Absent	703	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*1.0 (Ref)*
	Present	Absent	315	11.4 (6.7 to 16.2)	6.9 (4.0 to 9.8)	2.4 (-0.5 to 5.2)	17.5 (11.7 to 23.4)	15.7 (5.0 to 26.4)	1.5 (1.3 to 1.7)
	Absent	Present	870	24.3 (20.7 to 27.9)	11.4 (9.2 to 13.6)	2.4 (0.2 to 4.6)	26.5 (22.0 to 31.0)	20.5 (12.3 to 28.6)	3.2 (2.9 to 3.5)
	Present	Present	418	38.4 (33.9 to 43.0)	23.0 (20.2 to 25.7)	10.8 (8.0 to 13.6)	46.9 (41.3 to 52.5)	66.1 (55.9 to 76.3)	5.4 (4.8 to 6.0)
Obesity†	Absent	Absent	352	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*1.0 (Ref)*
	Present	Absent	649	3.0 (-2.2 to 8.3)	12.9 (9.6 to 16.2)	5.3 (2.1 to 8.6)	1.6 (-4.8 to 8.1)	32.8 (21.0 to 44.7)	1.2 (1.0 to 1.3)
	Absent	Present	586	25.3 (22.0 to 28.5)	12.4 (10.3 to 14.4)	3.6 (1.6 to 5.6)	28.2 (24.1 to 32.2)	30.2 (22.8 to 37.7)	3.5 (3.3 to 3.8)
	Present	Present	616	32.8 (27.0 to 38.5)	28.4 (24.8 to 31.9)	13.1 (9.6 to 16.7)	34.7 (27.6 to 41.8)	71.0 (58.1 to 83.8)	3.5 (3.0 to 4.0)
Comorbidity	Absent	Absent	917	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*1.0 (Ref)*
	Present	Absent	83	-1.9 (-9.8 to 6.0)	5.9 (1.2 to 10.7)	1.8 (-3.1 to 6.6)	9.1 (-0.5 to 18.8)	12.4 (-5.3 to 30.1)	1.2 (1.0 to 1.4)
	Absent	Present	1080	26.3 (23.2 to 29.4)	14.4 (12.5 to 16.4)	5.4 (3.5 to 7.3)	29.2 (25.4 to 33.1)	34.3 (27.3 to 41.3)	3.5 (3.2 to 3.8)
	Present	Present	138	27.0 (20.6 to 33.4)	13.9 (10.0 to 17.8)	2.0 (-1.9 to 5.9)	39.5 (-10.5 to -2.6)	42.2 (27.9 to 56.5)	3.3 (2.8 to 3.9)
Oral contraceptives use in women	Absent	Absent	343	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*1.0 (Ref)*
	Present	Absent	85	14.4 (5.9 to 23.0)	26.2 (20.5 to 31.8)	13.6 (8.0 to 19.2)	14.6 (3.8 to 25.3)	28.8 (10.0 to 47.7)	1.4 (1.2 to 1.8)
	Absent	Present	495	26.4 (21.6 to 31.2)	15.0 (11.8 to 18.2)	6.4 (3.2 to 9.6)	28.2 (22.2 to 34.3)	35.8 (25.2 to 46.4)	3.1 (2.8 to 3.5)
	Present	Present	110	30.9 (23.0 to 38.9)	32.8 (27.5 to 38.0)	6.5 (1.3 to 11.7)	39.3 (29.4 to 49.3)	49.5 (32.0 to 67.0)	4.0 (3.3 to 4.8)

Table 3 – Aim 1: Combined effects of lower-leg injury and male, sex, age >55 years, BMI>30, comorbidity, oral contraceptives, Factor V Leiden mutation, Factor II mutation and non-O blood group on the patterns of coagulation factor levels. (continued)

Risk factor	Lower-leg injury		Number of persons (N=2306)	FVIII (%)	FIX (%)	FXI (%)	VWF (%)	Fibrinogen (mg/dL)	D-dimer (ng/mL)
				Adj. mean difference (95%CI)*	Adj. mean difference (95%CI)*	Adj. mean difference (95%CI)*	Adj. mean difference (95%CI)*	Adj. mean difference (95%CI)*	Adj. mean ratio (95%CI)*
Factor V Leiden mutation‡	Absent	Absent	896	*0.0 (Ref)*	*0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*1.0 (Ref)*
	Present	Absent	34	5.7 (-6.3 to 17.8)	0.4 (-6.9 to 7.6)	-4.3 (-11.6 to 3.0)	-0.4 (-15.0 to 14.2)	-5.4 (-32.2 to 21.4)	1.2 (0.9 to 1.6)
	Absent	Present	1161	26.8 (23.6 to 30.0)	14.3 (12.3 to 16.2)	4.9 (3.0 to 6.9)	29.4 (25.5 to 33.3)	35.9 (28.8 to 43.1)	3.5 (3.2 to 3.8)
	Present	Present	50	30.9 (20.7 to 41.1)	11.6 (5.5 to 17.8)	3.9 (-2.3 to 10.1)	39.5 (26.8 to 52.1)	20.8 (-2.0 to 43.5)	4.0 (3.1 to 5.1)
Factor II 20210A mutation‡	Absent	Absent	959	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref)*	*1.0 (Ref)*
	Present	Absent	22	-3.5 (-18.3 to 11.3)	-1.4 (-10.4 to 7.5)	-4.0 (-13.0 to 5.0)	-5.6 (-23.5 to 12.4)	0.6 (-32.3 to 33.5)	1.2 (0.8 to 1.7)
	Absent	Present	1189	26.3 (23.2 to 29.4)	13.7 (11.8 to 15.6)	4.7 (2.8 to 6.6)	29.1 (25.3 to 32.9)	35.1 (28.2 to 42.0)	3.5 (3.2 to 3.8)
	Present	Present	26	27.2 (12.9 to 41.4)	10.8 (2.2 to 19.3)	-1.0 (-9.6 to 7.7)	28.9 (11.3 to 46.5)	25.3 (-6.2 to 56.9)	4.1 (2.9 to 5.9)
Non-O blood group‡	Absent	Absent	403	*0.0 (Ref)*	*0.0 (Ref)*	*0.0 (Ref*	*0.0 (Ref)*	*0.0 (Ref)*	*1.0 (Ref)*
	Present	Absent	505	21.2 (16.8 to 25.7)	6.4 (3.6 to 9.2)	6.6 (3.8 to 9.5)	36.6 (31.4 to 41.8)	-2.2 (-12.6 to 8.1)	1.1 (0.9 to 1.2)
	Absent	Present	521	25.7 (21.2 to 30.2)	14.5 (11.7 to 17.3)	5.8 (3.0 to 8.7)	31.5 (26.2 to 36.8)	40.4 (29.9 to 50.9)	3.6 (3.1 to 4.0)
	Present	Present	682	47.8 (43.6 to 52.1)	19.6 (16.9 to 22.3)	11.0 (8.3 to 13.7)	64.1 (59.1 to 69.1)	28.4 (18.5 to 38.4)	3.6 (3.3 to 4.1)

* Adjusted for sex, age, BMI, comorbidity, presence of malignancy, use of oral contraceptives.

† Obesity defined as BMI>30.

‡ Heterozygote and homozygote variants of factor V Leiden mutation, factor II mutation and non-O blood group were combined.

results were observed, except for fibrinogen of which the adjusted mean difference was smaller (i.e. 10.8 mg/dL, 95%CI 6.0 to 15.7) (**Table S4**).

Aim 2: The effect of knee arthroscopy on the coagulation system

Study population

In total, 729 knee arthroscopy patients were included (**Fig 1**). Their median age was 49 years and 57% of the patients were male (**Table 1**). Meniscectomy was performed most (63%), and procedures were performed predominantly under general anaesthesia (65%) and with the use of a thigh-tourniquet (99%) (**Table S5**).

Main analyses

All coagulation factors decreased after arthroscopy, the largest decreases were found for D-dimer, mean change -51.3 ng/mL (95%CI -86.3 to -16.2), and fibrinogen, mean change -32.0 mg/dL (95%CI -34.6 to -29.5). FVIII and VWF showed the smallest decrease of 9% (95%CI -11.1 to -7.0) and 7.2% (95%CI -8.6 to -5.8), respectively. TF was also reduced after knee arthroscopy which corresponded to a mean change of -3.3 pg/mL (95%CI -4.1 to

Table 4 – Aim 2: Coagulation and inflammatory factor levels compared before and after knee arthroscopy.

	Reference range	Mean (SD)*		Mean change (95%CI)
		Preoperative	Postoperative	
All patients (N=729)				
FVIII activity (%)	50-150%	123.4 (28.0)	114.3 (35.8)	-9.0 (-11.1 to -7.0)
FIX activity (%)	65-135%	123.2 (21.9)	110.3 (21.7)	-12.9 (-13.9 to -11.9)
FXI activity (%)	65-140%	112.5 (20.7)	101.1 (21.0)	-11.4 (-12.6 to -10.3)
VWF antigen (%)	50-150%	140.7 (39.2)	133.3 (40.7)	-7.2 (-8.6 to -5.8)
Fibrinogen (mg/dL)	200-393 mg/dL	309.4 (72.3)	277.4 (67.4)	-32.0 (-34.6 to -29.5)
D-dimer (ng/mL)*	<500 ng/mL	*218.5 (204.4 to 233.6)**	*207.7 (196.0 to 220.1)**	-51.3 (-86.3 to -16.2)
Random subset (N=80)				
IL-6 (pg/mL)†	<10 pg/mL			
≤8 pg/mL		N=52	N=52	NA
>8 pg/mL		N=28	N=28	-34.5 (-63.9 to -5.1)
Random subset (N=70)				
TF (pg/mL)	<62.2 pg/mL	42.2 (9.3)	39.0 (8.5)	-3.3 (-4.1 to -2.4)
Random subset (N=28)				
cfDNA (ug/mL)	<1.14 ug/mL	0.9 (0.1)	0.9 (0.1)	-0.1 (-0.1 to 0.0)
MPO-DNA (ng/mL)*	<0.64 ng/mL	*0.1 (0.1 to 0.1)**	*0.1 (0.1 to 0.1)**	0.0 (0.0 to 0.1)

* This symbol and italics indicate geometric means (with 95%CIs) and mean ratios of ln-retransformed data.
† Number of patients per category of IL-6 is given instead of means. Mean change could only be calculated for detectable levels (>8 pg/mL).

-2.4). Plasma levels of cfDNA and MPO did not change after surgery. Preoperative IL-6 levels did not change from detectable (>8.0 pg/mL) to undetectable (≤8.0 pg/mL) or vice versa in any patient. Detectable IL-6 levels decreased with a mean change of -34.5 pg/mL (95%CI -63.9 to -5.1 pg/mL) after arthroscopy. Subgroup analyses showed that arthroscopic surgery >30 minutes was associated with increased postoperative levels of FVIII, VWF and D-dimer relatively to preoperative levels. For the other subgroups no differences were identified (**Table S6**).

DISCUSSION

In this study, we observed an association between lower-leg injury and increased plasma levels of FVIII, FIX, FXI, VWF, fibrinogen and D-dimer. The increase of D-dimer levels was most pronounced with a threefold increase, followed by FVIII and VWF with a 1.2-fold increase for both factors. Furthermore, IL-6 levels were elevated after lower-leg injury, whereas soluble TF, cfDNA and MPO-DNA were unaffected. Knee arthroscopy did not affect any of the measured factors. In contrast, plasma levels of FVIII, FIX, FXI, VWF, fibrinogen and D-dimer decreased postoperatively.

The extensive increase in D-dimer levels following lower-leg injury indicates *in vivo* activation of coagulation. This is most probably the result of the elevated plasma levels of FVIII, FIX and FXI. The elevations in FVIII levels were the strongest, which is probably explained by FVIII being an acute phase reactant, i.e., homeostatic disturbances such as traumatic injury lead to increased FVIII levels.[18-24] Although fibrinogen is also an acute phase protein,[20] only a moderate increase in fibrinogen levels was observed which was not verified in the comparison with population controls. The increased levels of VWF indicate endothelial activation, which suggests an inflammatory response due to lower-leg trauma.[24-26] The enhanced posttraumatic IL-6 levels support this theory. This is also in line with studies that found that trauma induces short-term hyperinflammation.[27] During this acute phase after trauma, remnants of tissue damage are removed by platelets and immune cells, by which the tissue is prepared for repair.[27] It seems contradictory that neutrophil extracellular traps (NETs) were not detected, based on the unaffected levels of MPO-DNA, despite a hyperinflammatory state. However, it is known that minor trauma leads to hyperinflammation without NETs formation and lower-leg trauma can be considered relatively minor.[27] In order to obtain an indication of the extent of cell damage, cfDNA and soluble TF levels were measured. Both parameters were not enhanced in patients with lower-leg injury, although this might be explained by dilution of (local) effects as plasma levels were measured only systemically. Of note, soluble TF does not give an indication of the level of TF-dependent activation of coagulation.

Subgroup analyses stratifying for the severity of injury demonstrated a strong association with increased FVIII and VWF plasma levels. This is in line with the results of a study performed in mice that showed that FVIII levels correlate with the extent of vascular injury.[28] The highest levels of these factors were observed in patients with more extensive lower extremity fractures, i.e., ankle fractures and tibia fractures and with injuries in need for surgical treatment. Ankle fractures were subdivided into infra-, trans- and suprasyndesmotic fractures.[29, 30] As these fractures are assumed to impact the stability of the ankle joint differently and to affect distinct parts of the bone (i.e., infrasyndesmotic fractures affect the epiphysis, transsyndesmotic fractures affect the metaphysis and suprasyndesmotic fractures affect the diaphysis), considerable differences in the effect on coagulation factor levels were expected between these fracture types.[31] Therefore, we chose to subdivide ankle fractures into these categories for analyses. However, no strong differences were observed. Somewhat surprisingly, patients with a contusion or ankle distortion also had relatively high FVIII and VWF levels. This may be explained because only severe contusions or ankle distortions are treated with cast immobilisation and were hence included in the POTCAST trial. Achilles' tendon ruptures were also associated with high levels of FVIII and VWF, which corresponds with the high rates of VTE following Achilles tendon ruptures.[32-34] However, since tendons are not well vascularized and the extent of injury is thought to be low, it is unclear why these subjects showed such high factor levels. Moreover, it is contradictory that patients with this type of injury had relatively low D-dimer levels.

In patients who underwent knee arthroscopy, we observed an opposite effect on the coagulation system, i.e., levels of coagulation factors decreased postoperatively. A possible explanation could be consumption of coagulation factors due to the trauma. However, this seems unlikely since arthroscopy is considered as a minimally invasive procedure. Furthermore, the lack of a postoperative increase in D-dimer levels (in fact a decrease was observed), indicates there was no (additional) coagulation *in vivo* by which coagulation factors were consumed. Alternatively, postoperative levels could be relatively low compared to preoperative levels as a result of physiological variation over the day (circadian rhythm). In line with this theory, we observed that both pre- and postoperative factor levels were slightly higher in patients operated in the morning compared with those operated in the afternoon. In support of this, several studies have reported FVIII, VWF, and fibrinogen levels to be highest in the morning.[35-40] Third, preoperative stress could cause preoperative elevation of factor levels, which has only been described for FVIII.[41] Compared to the population controls (used in validation for aim 1), preoperative levels of FVIII, FIX and VWF were higher (10% to 29%), indicating that perioperative stress could have been involved. However, this does not explain the consistent decrease for all factors measured. Fourth, we considered haemodilution due to perioperative administration of intravenous saline. As patients received 0.5L saline at most during

surgery, this would lead to a maximum 14% reduction in postoperative factor levels assuming an average plasma volume of 3L,[42] which is probably lower when excretion of the exogenous fluid by the kidneys is taken into account. Finally, the postoperative blood draw may have been too early to detect an effect. However, since FVIII, VWF, fibrinogen and D-dimer are acute phase reactants, an immediate effect is to be expected.

Important strengths of our study are that we had access to a large, unselected number of individuals. Moreover, blood was drawn shortly after trauma and before and after knee arthroscopy. Of note, prophylactic LMWH could not have affected our results, because LMWH was administered after (postoperative) blood sampling. Furthermore, for the comparison with lower-leg injury patients, two control groups were used from which comparable results were obtained. In order to assess possible misclassification caused by differences in laboratory measurements between these groups, a sensitivity analysis was performed which showed that misclassification was negligible. The arthroscopy patients were compared with themselves which allows the most optimal comparison. There are also limitations which should be mentioned. First, blood was drawn systemically and not locally (in the injured or operated leg). Therefore, we may have missed more subtle, local effects. Second, since multiple comparisons were made, type I errors might have occurred. However, since we focused on global changes in marker levels, rather than on significancy, correction for multiple testing would not change our conclusions. Third, IL-6 could not be quantified if <8.0 pg/mL, while IL-6 levels associated with a procoagulant state in patients with deep vein thrombosis have previously been observed to be in the ~1 pg/mL range.[43] However, our analysis likely allowed for the detection of postoperative IL-6 levels, which are known to range from 19-484 pg/mL >12-72 hours for knee arthroplasty.[44-46] Fourth, regarding aim 2, some postoperative samples were missing, mostly due to logistic reasons and were therefore completely at random, which should therefore not have affected our results. That the general characteristics were similar for the 1018 knee arthroscopy patients with preoperative blood samples and the 729 knee arthroscopy patients with a pre- and postoperative blood sample confirms this. Finally, plasma samples of arthroscopy patients were collected postoperatively within 4 hours. An extra measurement at 12-24 hours postoperatively might have added more information to understand the pathophysiology of thrombus formation. Also for lower-leg injury patients multiple measurements in time would be useful to evaluate the dynamics of coagulation activation, for which further research is necessary. Our study should be considered as a first step in this direction.

The information about the effect of lower-leg trauma and knee arthroscopy on plasma levels of relevant coagulation factors, i.e., which are related to VTE risk in general, sets a first step in understanding the mechanism of VTE in both conditions. It shows that specific alterations in blood composition (defined as a pillar of Virchow's triad) are induced, at least by lower-leg trauma. Eventually, this knowledge can help to improve thromboprophylaxis

treatment by providing new targets for prevention following lower-leg trauma, such as FXI(a) or FVIII inhibitors. FXI(a) inhibitors are already studied in humans with promising results,[47] whereas FVIII inhibitors as yet only have been studied in animal models or *in vitro*.[48, 49] For arthroscopy, such an approach seems less appropriate given our findings. Furthermore, the strong differences that were found between lower leg injury and knee arthroscopy, suggest that several mechanistic pathways lead to the development of VTE. More specifically, whereas lower-leg trauma initiates an inflammatory state in which coagulation factors are increased, knee arthroscopy apparently does not. This opens the question as to which mechanistic pathways are at the basis of VTE in knee arthroscopy. Possibly, the use of tourniquet during the surgery is associated with VTE, which needs to be established in further research. For lower-leg injury, the persistence of the alterations in coagulation factor levels needs to be evaluated, including the exact effect of (duration of) lower-leg cast application on coagulation.

In conclusion, lower-leg trauma was associated with strongly increased levels of procoagulant factors, especially D-dimer and FVIII and VWF. In patients who underwent knee arthroscopy, on the other hand, procoagulant factor levels did in fact decrease. It seems that elevations in procoagulant factor levels are the result of an inflammatory reaction directly resulting from the trauma, indicated by increased plasma levels of IL-6. This mechanism differs from that seen in knee arthroscopy patients. Additional research is needed to further explore the mechanism of VTE in both patient groups, especially in knee arthroscopy patients.

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SUPPLEMENTARY MATERIAL

Validation in population controls derived from the MEGA study (aim 1)

The MEGA (Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis) study is a population-based case-control study in which patients diagnosed with a first VTE (i.e. cases) and persons with no current or history of VTE (i.e. controls) were included from March 1999 to August 2004. All participants were aged between 18 years and 70 years. Controls were either partners of cases or participants approached by random digit dialling (RDD). Shortly after VTE was diagnosed in the cases, the partner controls filled in a questionnaire on possible thrombotic risk factors, which is similar to the questionnaire used in the POT-(K)CAST trials. Their blood was sampled at least three months after discontinuation of anticoagulant treatment of the cases. RDD control subjects completed the questionnaire shortly after the moment they were approached to participate in the study. After they returned the questionnaire, they were asked to draw blood. In order to verify our main results of the first aim, these population controls were included as a second control group.

Of the 5150 controls, only 2942 controls provided a blood sample, which were included for validation analysis. In order to obtain a comparable group for patients with lower-leg injury, 21 pregnant controls were excluded (since this was also an exclusion criterion in the POT-CAST trial). This resulted in 2921 included population controls. Likewise, 84 lower-leg injury patients older than 70 years were excluded for these analyses since controls in the MEGA study were aged up to 70 years, resulting in 1204 lower-leg injury patients included for validation analysis.

Laboratory measurements

Blood samples provided by MEGA controls were drawn into S-monovette® citrate tubes (0.106 M, 3.2%) using the aspiration technique.[1] After centrifuging these tubes during 10 minutes at 2500G at 18°C, plasma was obtained. Aliquots of the plasma were stored at -80°C, within 4 hours after blood draw. Measuring device STA-R coagulation analyser was used to quantify factor VIII activity, factor XI activity and fibrinogen activity. The latter was also measured according to the Clauss method.[2] Immunoturbidimetric method, which uses STA Liatest kit, was applied in order to measure von Willebrand Factor (VWF) antigen levels. D-dimer was quantified in the same way as in the POT-(K)CAST trial, i.e. by using the HemosIL D-dimer HS on the ACL-TOP 700 analyser. Instructions of the manufacturer were followed when using these measurement devices. Factor IX activity was not quantified in the MEGA study.

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Assessment of misclassification risk

Since we used different methods and techniques to measure coagulation factor levels in POT-CAST trial and MEGA study, direct comparison between lower-leg injury patients and population controls could be hampered. In order to estimate the degree of potential misclassification, 73 MEGA samples were re-measured with ACL-TOP 700 analyser (i.e. POT-CAST device). We used reverse sample size calculation to calculate the smallest mean difference which could be detected with this sample size of 73 controls when comparing original measurement (MEGA device) with current measurement (POT-CAST device). **Figure S1** shows that FVIII activity measured in MEGA samples using ACL-TOP analyser correlates well with the original measurement. Same applies to FXI, fibrinogen and vWF. Originally, mean of 125.8% (SD 37.8) for FVIII was found in MEGA, compared with a mean of 117.0% (SD 28.9) when measured with ACL-TOP. This corresponded with a paired mean change of -8.8 (95%CI -11.0 to -6.4), calculated with paired t-test. Therefore, we concluded that a good correlation was observed between both measurements, in which results obtained with the ACL-TOP were slightly lower compared to the results measured with STA-R, i.e. comparisons of patients with lower-leg injury to population controls will be underestimated.

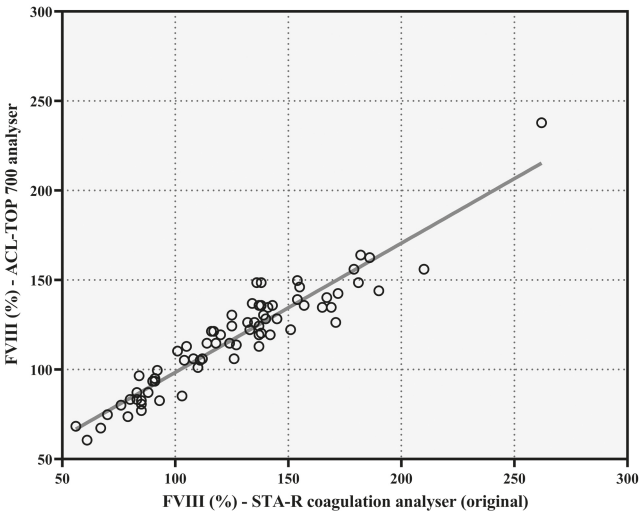

Fig S1 – Aim 1: Correlation between measurements of selected MEGA samples with STA-R coagulation analyser (i.e., original analysis) and with ACL-TOP 700 analyser (i.e., sensitivity analysis).

Statistical analyses – Data transformation using natural logarithms

In case of not normally distributed data, transformation were performed using natural logarithms (*ln*). In order to obtain geometric means, the *ln* of all values of the variable concerned (in this example referred to as '*X*') were computed, after which the mean of *ln-X* was calculated and retransformed by calculating *e^(mean ln-X)*, resulting in the geometric mean. In linear regression models, *ln-X* was included as the dependent variable. Since the outcome estimate of linear regression is mean difference, for ln-transformed variables, this involves *ln(b) – ln(a)*, which equals *ln(b/a)*. Therefore, by retransforming the outcome of linear regression, i.e. *e^(ln(b/a))*, a mean ratio (i.e. *b/a*) is obtained. The geometric means and mean ratios were obtained with their 95% confidence intervals (95%CIs).

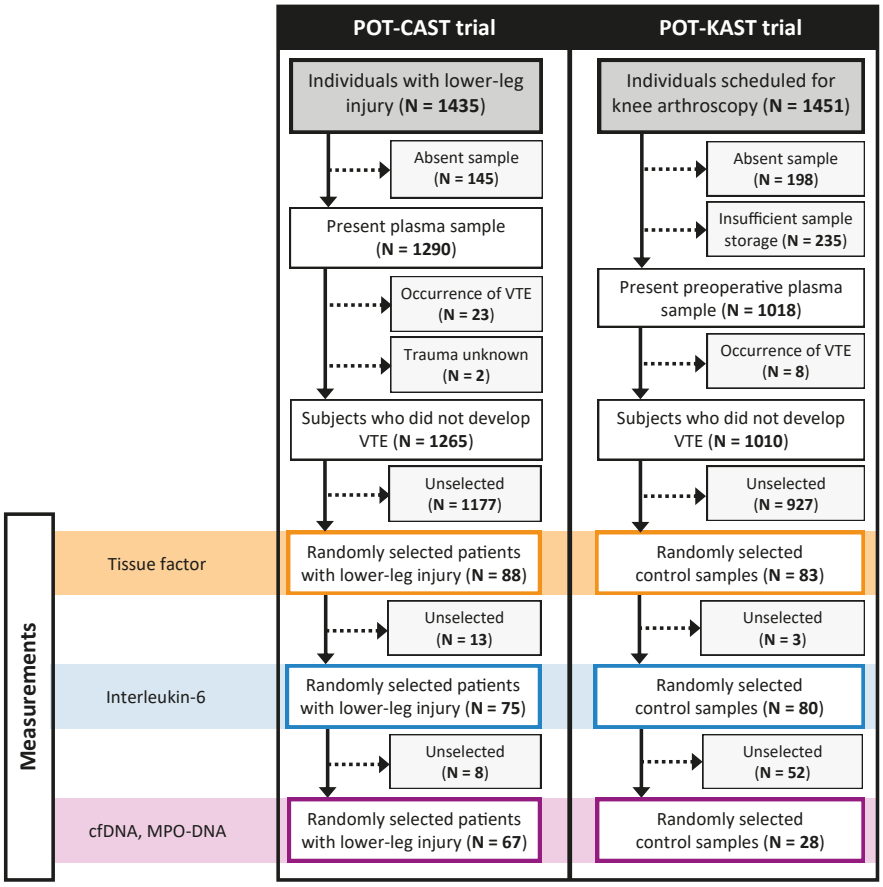

Fig S2A – Aim 1: Flowchart for random selections from POT-CAST and POT-KAST populations for measurements of TF, IL-6, cfDNA and MPO-DNA.

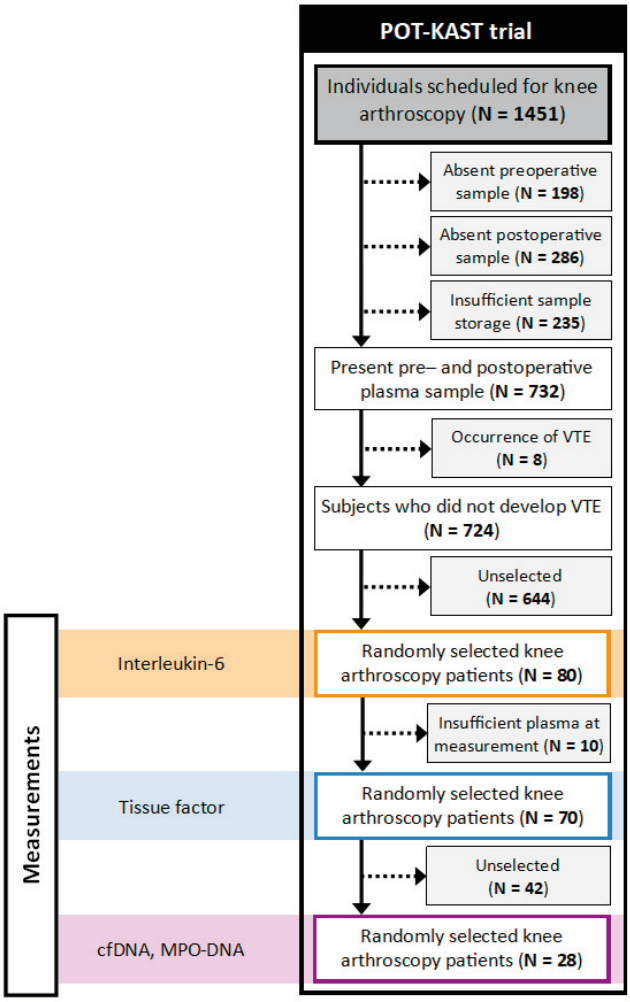

Fig S2B – Aim 2: Flowchart for random selections from POT-KAST population for measurements of TF, IL-6, cfDNA and MPO-DNA.

Table S1 – Aim 1: Lower-leg injury specific characteristics.

		Patients with lower-leg injury (N = 1288)*
Indication for lower-leg cast	*Foot fractures*	
	Phalanx, *n (%)*	23 (1.8)
	Metatarsal, *n (%)*	480 (37.3)
	Tarsal, *n (%)*	192 (14.9)
	Ankle and leg fractures	
	Infrasyndesmotic	90 (7.0)
	Transdesmotic	223 (17.3)
	Suprasyndesmotic	53 (4.1)
	Unclassified ankle, *n (%)*	71 (5.5)
	Tibia and/or fibula shaft, *n (%)*	14 (1.1)
	Other injuries	
	Ankle distortion, *n (%)*	34 (2.6)
	Contusion, *n (%)*	19 (1.5)
	Achilles' tendon rupture, *n (%)*	89 (6.9)
Indication for surgical treatment	Yes, *n (%)*	150 (11.6)
	No, *n (%)*	1138 (88.4)
Time between trauma and blood sampling	Within 24 hours, *n (%)*	786 (62.1)
	Within 7 days, *n (%)*	419 (33.1)
	After 7 days, *n (%)*	60 (4.7)

Percentages indicate proportions in non-missing data only.
* Numbers of missing data: timing blood sampling (N=23).

Table S2 – Aim 1: Correlation between procoagulant factor levels and injury severity.

	Ranking severity injury	Lower-leg injuries (N = 1288)	FVIII (%)		FIX (%)		FXI (%)	
			Mean	Adj. mean diff. (95%CI)*	Mean	Adj. mean diff. (95%CI)*	Mean	Adj. mean diff. (95%CI)*
POT-KAST controls (N = 1018)†	NA	NA	125.6	*Ref*	125.1	*Ref*	114.3	*Ref*
Injuries without need for surgery								
Contusion or ankle distortion	1	53 (4.1%)	147.2	30.2 (19.8 to 40.6)	135.3	18.4 (12.1 to 24.8)	116.3	8.3 (1.9 to 14.6)
Phalanx fracture	1	23 (1.8%)	136.0	15.9 (1.3 to 30.5)	127.9	6.1 (-2.9 to 15.0)	114.2	4.2 (-4.8 to 13.2)
Achilles' tendon rupture	2A	71 (5.5%)	154.8	29.1 (20.6 to 37.6)	138.3	13.2 (8.0 to 18.4)	116.3	2.6 (-2.6 to 7.9)
Metatarsal fracture	2B	460 (35.7%)	143.6	20.3 (16.4 to 24.3)	133.2	11.3 (8.8 to 13.7)	117.9	4.7 (2.2 to 7.1)
Tarsal fracture	2C	183 (14.2%)	141.6	21.2 (15.3 to 27.0)	135.4	15.5 (11.9 to 19.1)	114.0	3.3 (-0.3 to 6.9)
Ankle fracture								
Infrasyndesmotic	2C	87 (6.8%)	154.2	30.9 (23.2 to 38.7)	140.1	17.0 (12.2 to 21.7)	121.6	8.4 (3.6 to 13.1)
Suprasyndesmotic	2D	30 (2.3%)	161.3	30.8 (17.6 to 44.0)	147.8	22.3 (14.3 to 30.4)	124.6	8.1 (0.0 to 16.2)
Transsyndesmotic	2D	154 (12.0%)	151.1	27.4 (21.5 to 33.4)	136.1	12.8 (9.2 to 16.5)	115.6	2.8 (-0.9 to 6.5)
Unclassified	2D	64 (5.0%)	152.4	29.1 (19.1 to 39.2)	133.4	14.1 (8.4 to 19.9)	121.1	10.0 (4.2 to 15.8)
Tibia and/or fibula fracture	2E	13 (1.0%)	159.0	39.1 (19.5 to 58.7)	141.5	21.2 (9.2 to 33.2)	123.4	14.6 (2.6 to 26.7)
Injuries in need for surgery								
Achilles' tendon rupture	2F	18 (1.4%)	163.1	42.5 (25.9 to 59.1)	130.0	10.3 (0.1 to 20.4)	120.1	12.1 (1.9 to 22.2)
Fractures	2F	132 (10.2%)	167.0	41.8 (35.5 to 48.2)	142.4	17.2 (13.3 to 21.1)	118.2	3.8 (-0.1 to 7.6)
Adjusted overall coefficients*	NA	NA	3.6 (3.2 to 4.0)		1.6 (1.4 to 1.9)		0.6 (0.3 to 0.8)	

Table S2 – Aim 1: Correlation between procoagulant factor levels and injury severity. (continued)

	Ranking severity injury	Lower-leg injuries (N = 1288)	VWF (%)		Fibrinogen (mg/dL)		D-dimer (ng/mL)	
			Mean	Adj. mean diff. (95%CI)*	Mean	Adj. mean diff. (95%CI)*	Mean	Adj. mean diff. (95%CI)*
POT-KAST controls (N = 1018)†	NA	NA	140.5	*Ref*	312.1	*Ref*	216.5	*Ref*
Injuries without need for surgery								
Contusion or ankle distortion	1	53 (4.1%)	159.4	32.8 (19.7 to 45.8)	330.4	46.7 (23.4 to 70.0)	402.4	2.4 (1.9 to 3.1)
Phalanx fracture	1	23 (1.8%)	152.8	19.1 (1.2 to 37.1)	310.2	18.0 (-14.8 to 50.8)	452.3	2.5 (1.7 to 3.5)
Achilles' tendon rupture	2A	71 (5.5%)	169.3	26.8 (16.3 to 37.4)	346.2	35.6 (16.6 to 54.7)	499.0	2.3 (1.9 to 2.9)
Metatarsal fracture	2B	460 (35.7%)	159.4	21.8 (16.9 to 26.7)	330.8	25.0 (16.1 to 34.0)	583.0	2.7 (2.5 to 3.0)
Tarsal fracture	2C	183 (14.2%)	167.2	33.1 (25.8 to 40.4)	340.0	42.7 (29.5 to 55.9)	824.1	3.9 (3.4 to 4.6)
Ankle fracture								
Infrasyndesmotic	2C	87 (6.8%)	171.6	34.7 (24.9 to 44.4)	349.1	36.9 (19.4 to 54.3)	894.7	4.1 (3.4 to 5.0)
Suprasyndesmotic	2D	30 (2.3%)	173.2	24.6 (8.4 to 40.8)	344.4	30.0 (0.3 to 59.6)	1011.1	3.8 (2.7 to 5.3)
Transsyndesmotic	2D	154 (12.0%)	170.8	32.8 (25.4 to 40.6)	359.1	49.2 (35.8 to 62.6)	1051.9	5.0 (4.3 to 5.8)
Unclassified	2D	64 (5.0%)	171.3	29.7 (17.9 to 41.6)	352.4	51.8 (30.6 to 73.1)	823.8	3.6 (2.8 to 4.5)
Tibia and/or fibula fracture	2E	13 (1.0%)	167.5	25.8 (1.7 to 49.9)	323.8	23.6 (-20.5 to 67.7)	657.4	3.1 (1.9 to 5.1)
Injuries in need for surgery								
Achilles' tendon rupture	2F	18 (1.4%)	177.8	40.9 (20.0 to 61.9)	287.0	-7.9 (-45.1 to 29.3)	339.1	1.8 (1.2 to 2.7)
Fractures	2F	132 (10.2%)	182.8	43.2 (35.3 to 51.1)	343.7	30.1 (15.8 to 44.3)	1177.6	5.5 (4.7 to 6.4)
Adjusted overall coefficients*	NA	NA	3.8 (3.3 to 4.3)		3.8 (2.9 to 4.7)		*1.17 (1.16 to 1.19) §*	

* Adjusted for sex, age, BMI, comorbidity, malignancy, use of oral contraceptives.
† Preoperative samples of controls obtained from POT-KAST.
‡ D-dimer was ln-retransformed due to skewedness, resulting in geometric means.
§ D-dimer was ln-retransformed due to skewedness; overall coefficient should be interpreted as follows: for every one-unit increase of injury severity, D-dimer increases 17%.

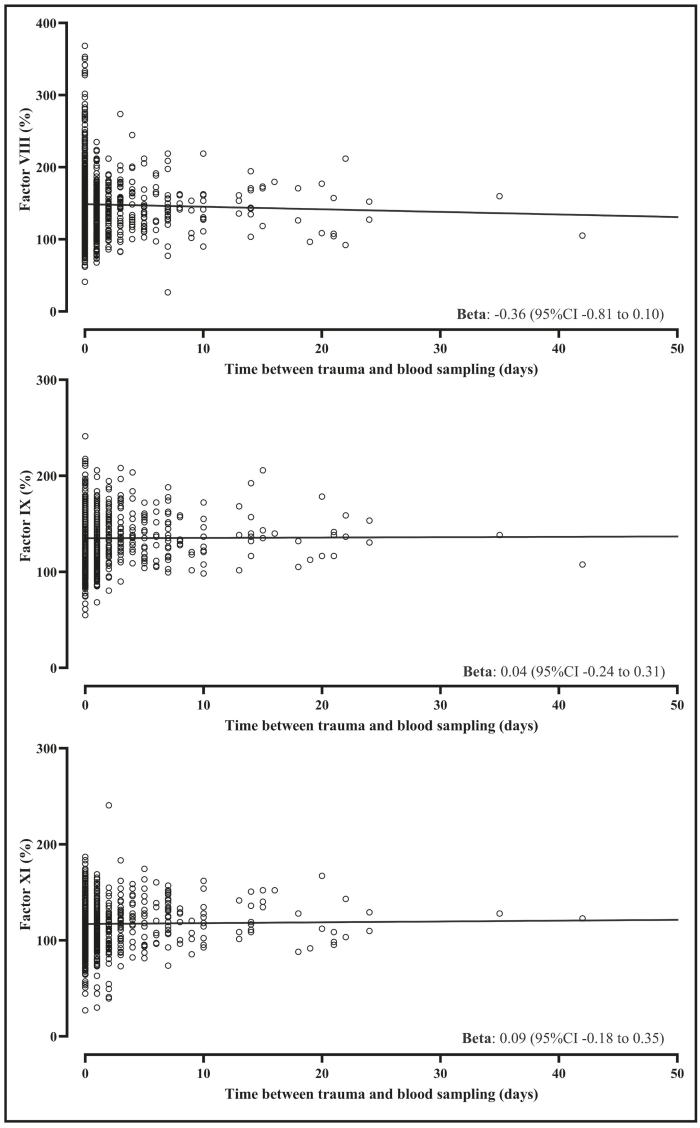

Fig S3A – Aim 1: Procoagulant factors plotted over time between trauma and blood sampling.

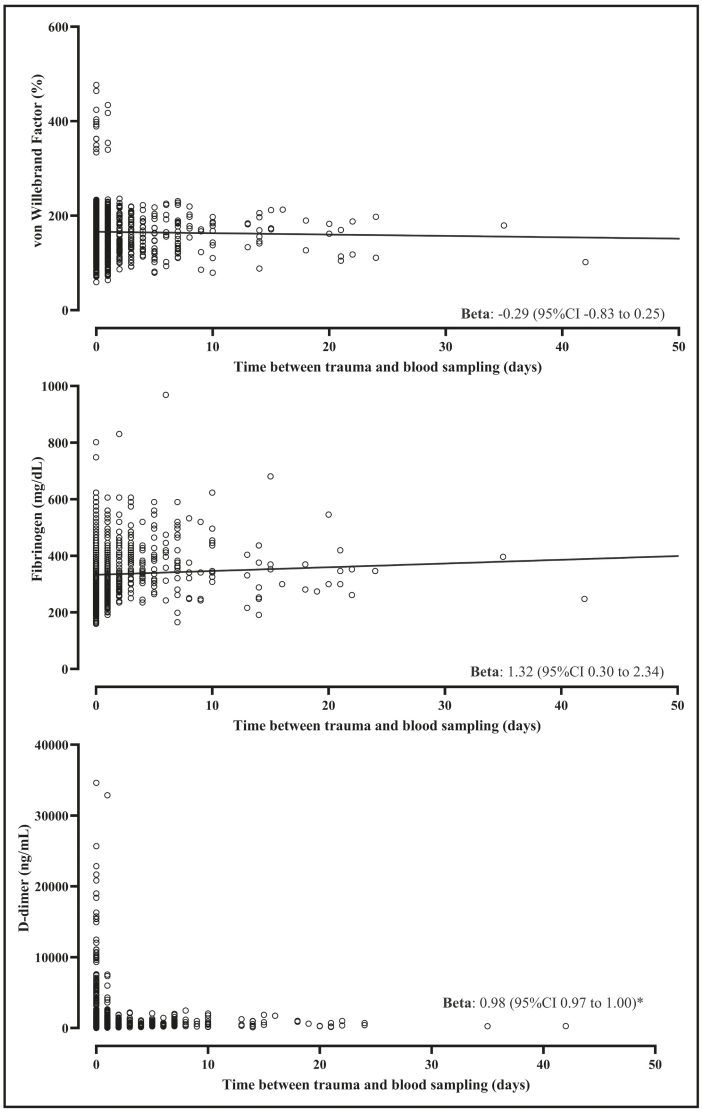

Fig S3B – Aim 1: Procoagulant factors plotted over time between trauma and blood sampling.
* Beta of D-dimer was ln-retransformed; the regression line is not presented while the original (not transformed) data is shown in the scatterplot.

Table S3 – Aim 1: General characteristics of study populations (including population controls), available for validation analyses.

		Patients with lower-leg injury (N = 1204)*	Population controls (N = 2921)†
Sex	Male, *n (%)*	621 (51.6)	1395 (47.8)
Age	*Median in years (5th-95th percentile)*	44.7 (20.4 – 66.8)	49.9 (27.1 – 67.0)
BMI	< 18.5 kg m-2, *n (%)*	11 (1.0)	25 (0.9)
	18.5 - 25.0 kg m-2, *n (%)*	538 (48.0)	1344 (47.3)
	25.0 - 30.0 kg m-2, *n (%)*	403 (36.0)	1105 (38.9)
	> 30.0 kg m-2, *n (%)*	168 (15.0)	365 (12.9)
Comorbidity	Yes, *n (%)*	119 (10.5)	301 (10.4)
Smoking	Yes: currently, *n (%)*	315 (28.2)	874 (30.4)
	Yes: formerly, *n (%)*	294 (26.3)	868 (30.1)
Current use of oral contraceptives‡	Yes, *n (%) of women*	103 (18.6)	434 (29.2)
Malignancy in last 5 years	Yes, *n (%)*	25 (2.2)	57 (2.0)
ABO-blood type	Homozygote O, *n (%)*	489 (43.4)	1364 (46.8)
	Heterozygote O, *n (%)*	511 (45.4)	1254 (43.0)
	Homozygote non-O, *n (%)*	126 (11.2)	297 (10.2)
Factor II 20210A mutation	Heterozygote, n (%)	24 (2.1)	51 (1.7)
Factor V Leiden	Heterozygote, *n (%)*	47 (4.1)	137 (4.7)
	Homozygote, *n (%)*	1 (0.1)	6 (0.2)
Prophylactic LMWH (after blood sampling)	Yes, *n (%)*	593 (49.3)	NA

Percentages indicate proportions in non-missing data only.
* Numbers of missing data for lower-leg injury patients: BMI (N=84), comorbidity (N=69), smoking (N=87), oral contraceptives (N=29), malignancy (N=72), ABO blood type (N=78), factor II mutation (N=66), factor V Leiden mutation (N=70).
† Control samples involved population controls (derived from the MEGA study). Numbers of missing data for controls: BMI (N=82), comorbidity (N=17), smoking (N=42), oral contraceptives (N=40), malignancy (N=5).
‡ Oral contraceptives included any type of hormonal therapy, including oral contraceptives and intrauterine devices.

Table S4 – Aim 1: Mean differences in coagulation factor levels in patients with lower-leg injury compared with population controls (validation).

	Mean (SD)*	Adj. mean difference/ratio (95%CI)*†
FVIII activity (%)		
Population controls (N=2921)	112.8 (44.4)	*Reference*
Patients (N=1204)	148.2 (40.7)	39.1 (36.1 to 42.1)
FXI activity (%)		
Population controls (N=2921)	100.3 (19.2)	*Reference*
Patients (N=1204)	117.1 (23.7)	18.4 (17.0 to 19.8)
vWF antigen (%)		
Population controls (N=2921)	111.6 (46.5)	*Reference*
Patients (N=1204)	165.4 (47.7)	58.1 (54.9 to 61.3)
Fibrinogen (mg/dL)		
Population controls (N=2921)	330.7 (66.7)	*Reference*
Patients (N=1204)	334.9 (91.6)	10.8 (6.0 to 15.7)
D-dimer (ng/mL) *		
Population controls (N=2921)	*254.0 (248.2 to 260.0)**	*Reference*
Patients (N=1204)	*677.8 (641.8 to 715.8)**	*2.9 (2.7 to 3.0)**

Population controls involved MEGA controls.
* This symbol and italics indicate geometric means (with 95%CIs) and mean ratios of ln-retransformed data.
† Adjusted for sex, age, BMI, comorbidity, presence of malignancy, use of oral contraceptives.

Table S5 – Aim 2: Knee arthroscopy specific characteristics.

		Knee arthroscopy patients (N = 729)*
Type of procedure	Meniscectomy, *n (%)*	460 (63.1)
	Diagnostic arthroscopy, *n (%)*	41 (5.6)
	Removal of loose bodies, *n (%)*	13 (1.8)
	Other, *n (%)*	92 (12.6)
	Multiple procedures, *n (%)*	123 (16.9)
ASA classification	ASA I, *n (%)*	453 (64.1)
	ASA II, *n (%)*	250 (35.4)
	ASA III, *n (%)*	4 (0.6)
Type of anaesthesia	General, *n (%)*	468 (65.2)
	Spinal, *n (%)*	249 (34.7)
	Epidural, *n (%)*	1 (0.1)
Use of tourniquet	Yes, *n (%)*	702 (98.7)
	No, *n (%)*	9 (1.3)
Total duration of knee arthroscopy†	*Median in minutes (5th-95th percentile)*	21.0 (11.0 – 48.0)
Duration of surgery‡	*Median in minutes (5th-95th percentile)*	13.0 (6.0 – 31.0)

Percentages indicate proportions in non-missing data only; ASA: American Society of Anaesthesiologists classification.
* Numbers of missing data: ASA classification (N=22), anaesthesia (N=11), tourniquet (N=18).
† From moment of receiving anaesthesia to moment of leaving operating room.
‡ From moment of incision to moment of wound closure.

Table S6 – Aim 2: Stratified analyses for characteristics of knee arthroscopy patients.

Risk factors	Number of patients (n=729), n (%)	Factor VIII activity (%)			Factor IX activity (%)			Factor XI activity (%)		
		Preop. mean	Postop. mean	Mean change (95% CI)	Preop. mean	Postop. mean	Mean change (95% CI)	Preop. mean*	Postop. mean*	Mean change (95% CI)
Sex										
Male	416 (57.1)	121.9	114.7	-7.3 (-9.9 to -4.6)	121.6	109.1	-12.6 (-13.9 to -11.2)	108.0	98.1	-6.2 (-8.2 to -4.2)
Female	313 (42.9)	125.3	113.9	-11.4 (-14.5 to -8.3)	125.2	111.9	-13.3 (-14.8 to -11.8)	118.5	105.0	-13.5 (-15.3 to -11.7)
Age										
≤ 55 years	481 (66.0)	119.7	110.3	-9.4 (-11.6 to -7.0)	121.1	108.5	-12.7 (-13.9 to -11.5)	111.2	99.7	-11.5 (-13.0 to -10.1)
> 55 years	248 (34.0)	130.5	122.1	-8.4 (-12.2 to -4.6)	127.1	113.8	-13.2 (-15.0 to -11.4)	115.0	103.7	-11.3 (-13.0 to -9.6)
BMI										
≤ 25 kg m^{-2}	258 (36.0)	122.6	112.2	-10.4 (-13.9 to -6.9)	115.1	102.9	-12.2 (-13.7 to -10.7)	110.0	98.5	-11.5 (-13.4 to -9.5)
> 25 kg m^{-2}	459 (64.0)	123.8	115.7	-8.2 (-10.8 to -5.6)	127.7	114.6	-13.1 (-14.5 to -11.8)	114.3	102.9	-11.5 (-12.9 to -10.0)
Comorbidity										
No	659 (91.9)	123.1	113.8	-9.3 (-11.4 to -7.2)	122.4	109.6	-12.7 (-13.7 to -11.7)	112.4	100.8	-11.6 (-12.8 to -10.5)
Yes	58 (8.1)	126.6	122.1	-4.5 (-12.1 to 3.1)	131.8	118.5	-13.3 (-18.6 to -7.9)	116.4	107.6	-8.8 (-13.5 to -4.1)
Type of procedure										
Meniscectomy	460 (63.1)	123.9	113.5	-10.4 (-12.6 to 8.1)	123.6	110.5	-13.1 (-14.4 to -11.9)	112.0	100.9	-11.1 (-12.5 to -9.7)
Diagnostic arthroscopy	41 (5.6)	122.1	118.4	-3.7 (-13.7 to 6.3)	129.3	118.3	-11.0 (-15.9 to -6.1)	110.0	98.9	-11.1 (-16.8 to -5.3)
Removal of loose bodies	13 (1.8)	129.6	113.3	-16.3 (-28.6 to -4.0)	119.5	106.0	-13.5 (-21.6 to -5.4)	114.0	103.3	-10.7 (-20.8 to -0.6)
Other	92 (12.6)	119.5	111.4	-8.1 (-13.8 to -2.4)	119.1	108.8	-10.3 (-13.0 to -7.7)	114.8	101.9	-12.9 (-16.4 to -9.5)
Multiple procedures	123 (16.9)	124.2	118.3	-5.8 (-12.3 to 0.7)	122.9	108.6	-14.3 (-17.1 to -11.5)	113.4	101.6	-11.8 (-14.5 to -9.0)
Type of anaesthesia										
General	468 (65.2)	123.6	110.9	-12.7 (-14.8 to -10.5)	122.7	108.5	-14.2 (-15.4 to -13.0)	112.8	99.2	-13.6 (-15.0 to -12.2)
Spinal/epidural	250 (34.8)	123.2	121.7	-1.6 (-5.6 to 2.5)	123.9	113.9	-10.1 (-11.9 to -8.2)	111.9	104.8	-7.2 (-9.0 to -5.3)
Time of surgery										
Morning (before 12 PM)	476 (72.1)	123.6	114.2	-9.4 (-11.7 to -7.1)	124.7	111.7	-13.0 (-14.1 to -11.8)	113.4	102.1	-11.2 (-12.6 to -9.9)
Afternoon (after 12 PM)	184 (27.9)	121.1	111.7	-9.4 (-13.9 to -4.8)	117.9	104.7	-13.2 (-15.7 to -10.7)	108.9	96.1	-12.7 (-15.3 to -10.2)
Surgery ≤30 min										
Yes	658 (90.3)	123.4	114.0	-9.4 (-11.5 to -7.4)	123.2	110.1	-13.1 (-14.1 to -12.1)	112.5	100.7	-11.8 (-13.0 to -10.6)
No	35 (4.8)	118.5	123.6	5.1 (-6.1 to 16.2)	119.8	116.2	-3.6 (-7.7 to -0.6)	110.5	108.2	-2.3 (-6.8 to 2.1)

Table S6 – Aim 2: Stratified analyses for characteristics of knee arthroscopy patients. (continued)

Risk factors	Number of patients (n=729), n (%)	Von Willebrand Factor (%)			Fibrinogen (mg/dL)			D-dimer (ng/mL)		
		Preop. mean	Postop. mean	Mean change (95% CI)	Preop. mean	Postop. mean	Mean change (95% CI)	Preop. mean*	Postop. mean*	Mean change (95% CI)
Sex										
Male	416 (57.1)	140.5	134.3	-6.2 (-8.2 to -4.2)	295.4	264.1	-31.3 (-34.5 to -28.1)	188.4	184.1	-38.7 (-81.4 to 4.1)
Female	313 (42.9)	140.9	132.3	-8.6 (-10.5 to -6.6)	327.9	294.8	-33.1 (-37.2 to -28.9)	266.0	243.7	-67.9 (-126.8 to -9.0)
Age										
≤ 55 years	481 (66.0)	134.9	127.3	-7.5 (-9.3 to -5.8)	302.5	271.7	-30.8 (-34.0 to -27.5)	187.6	185.2	-28.3 (-61.1 to 4.6)
> 55 years	248 (34.0)	151.8	145.2	-6.6 (-9.1 to -4.2)	322.8	288.3	-34.5 (-38.7 to -30.3)	293.1	259.1	-95.5 (-176.3 to -14.6)
BMI										
≤ 25 kg m^{-2}	258 (36.0)	139.5	131.6	-8.0 (-10.3 to -5.6)	292.7	263.1	-29.6 (-34.1 to -25.1)	194.8	188.4	-78.5 (-150.4 to -6.7)
> 25 kg m^{-2}	459 (64.0)	141.2	134.5	-6.7 (-8.5 to -4.9)	318.6	285.4	-33.3 (-36.5 to -30.1)	232.3	219.3	-36.6 (-75.5 to 2.3)
Comorbidity										
No	659 (91.9)	139.1	131.9	-7.2 (-8.7 to -5.7)	306.8	274.8	-32.0 (-34.7 to -29.4)	212.6	203.3	-40.4 (-73.1 to -7.6)
Yes	58 (8.1)	157.2	150.6	-6.6 (-11.3 to -1.8)	337.7	307.3	-30.4 (-42.2 to -18.7)	290.8	263.8	-175.1 (-413.7 to 63.5)
Type of procedure										
Meniscectomy	460 (63.1)	143.3	134.9	-8.5 (-10.0 to -7.0)	310.2	279.8	-30.4 (-33.7 to -27.0)	228.6	212.9	-64.0 (-118.4 to -9.6)
Diagnostic arthroscopy	41 (5.6)	132.5	129.2	-3.3 (-10.1 to 3.4)	311.4	280.4	-31.0 (-40.2 to -21.8)	198.0	197.9	-26.2 (-75.1 to 22.7)
Removal of loose bodies	13 (1.8)	143.5	131.3	-12.2 (-17.1 to -7.4)	291.7	260.7	-31.0 (-51.3 to -10.7)	216.2	252.4	19.8 (-51.3 to -10.7)
Other	92 (12.6)	131.0	127.3	-3.6 (-7.7 to 0.5)	299.9	270.3	-29.5 (-35.4 to -23.7)	169.4	181.7	-13.1 (-35.4 to -23.7)
Multiple procedures	123 (16.9)	140.2	134.2	-6.0 (-10.9 to -1.1)	314.6	273.9	-40.7 (-47.5 to -33.9)	231.3	208.2	-48.3 (-86.7 to -9.9)
Type of anaesthesia										
General	468 (65.2)	140.2	130.2	-10.0 (-11.5 to -8.4)	310.8	276.3	-34.5 (-37.8 to -31.2)	208.2	196.4	-41.1 (-76.3 to -5.9)
Spinal/epidural	250 (34.8)	142.4	140.6	-1.7 (-4.5 to 1.0)	306.9	279.4	-27.5 (-31.5 to -23.5)	236.2	228.1	-71.5 (-149.8 to 6.8)
Time of surgery										
Morning (before 12 PM)	476 (72.1)	140.1	133.7	-6.4 (-7.8 to -5.0)	312.0	282.1	-30.0 (-33.0 to -26.9)	222.8	208.8	-42.8 (-74.5 to -11.0)
Afternoon (after 12 PM)	184 (27.9)	137.4	127.6	-9.8 (-13.1 to -6.5)	298.3	262.1	-36.2 (-42.1 to -30.4)	199.5	203.0	-47.4 (-127.5 to 32.8)
Surgery ≤30 min										
Yes	658 (90.3)	141.0	133.5	-7.5 (-9.0 to -6.1)	311.4	278.0	-33.4 (-36.1 to -30.7)	221.3	207.9	-54.4 (-91.5 to -17.4)
No	35 (4.8)	137.7	139.6	2.0 (-4.5 to 8.5)	287.2	272.2	-15.0 (-26.1 to -3.8)	244.7	284.7	11.9 (-205.8 to 229.6)

* Geometric means due to ln-retransformation.