The severity of trauma determines the immune response to PF4/heparin and the frequency of heparin-induced thrombocytopenia

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Heparin can induce heparin-induced thrombocytopenia (HIT). The combined effect of type of surgery (major vs minor) and heparin on this prothrombotic immune reaction to platelet factor 4 (PF4)/heparin was analyzed. In a randomized, double-blind study, trauma patients receiving low-molecular-weight (LMWH) or unfractionated heparin (UFH) for thrombosis prophylaxis were assessed for PF4/heparin-antibody seroconversion, HIT, and thrombosis according to type of surgery. The risk for seroconversion was higher than major versus minor surgery odds ratio, 7.98 [95% confidence interval, 2.06-31.00], *P* = .003, controlled for potential confounders, as was the risk for HIT (2.2% [95% confidence interval, 0.3%-4.1%] vs 0.0%, *P* = .010). During LMWH compared with UFH thromboprophylaxis, HIT (1 of 298 vs 4 of 316; *P* = .370) and PF4/heparin seroconversion (1.7% vs 6.6%; *P* = .002) were less frequent, driven by differences in patients undergoing major surgery (incidence of HIT: LMWH 0.8% vs UFH 4.0%; *P* = .180; seroconversion rates: 4.0% vs 17.0%; *P* = .001). After minor surgery, no case of HIT occurred. The severity of trauma and the need for major surgery strongly influence the risk of an anti-PF4/heparin immune response, which is then increased by UFH. In major trauma certoparin may be safer than UFH because it induces HIT-antibody seroconversion, and the corresponding risk of HIT, less frequently. (Blood. 2010;115: 1797-1803)

Introduction

Low-molecular-weight heparin and unfractionated heparin, the most widely used anticoagulants for thrombosis prophylaxis after surgery,[1] can induce a serious adverse drug reaction, heparin-induced thrombocytopenia (HIT).[2] HIT is an immune-mediated prothrombotic syndrome in which patients develop platelet-activating antibodies against platelet factor 4 (PF4)/heparin complexes,[3] resulting in a decrease of platelet counts, enhanced thrombin generation,[4-6] and the paradox of an increased risk for thromboembolic complications despite heparin application.

The risk estimates for HIT in surgical patients are made on the basis of studies in patients undergoing elective major joint-replacement surgery,[7-10] in which the investigators demonstrated the risk for HIT being only approximately one-tenth as great when low-molecular-weight heparin is used for thrombosis prophylaxis compared with unfractionated heparin.[11,12] Whether the findings of these studies can be applied to other surgical patient populations is unresolved. It is especially unclear whether platelet count monitoring during the second week of heparin, for recognizing HIT,[12] should be recommended for the large group of patients with minor trauma, such as ankle fracture. This question is of major clinical importance because regular platelet count monitoring in this outpatient group is a burden for the patient and the health-care system. We assessed the incidence of the immune response against PF4/heparin, and of clinical HIT, in a general trauma patient population with respect to type of heparin (unfractionated vs low-molecular-weight heparin).

Furthermore, heparin is a classic biotherapeutic. Understanding the immune response to PF4/heparin might allow a better understanding of the mechanism of immune reactions toward other biotherapeutics. In this regard, the most important and surprising finding of our study is that the risk for HIT is largely influenced by a nondrug factor, namely the severity of trauma and the requisite need for either major or minor surgery.

Methods

This study was a double-blind randomized controlled trial in which we used a 2-arm parallel treatment design to demonstrate a lower incidence of HIT antibody seroconversion (superiority) in patients treated with low-molecular-weight heparin compared with unfractionated heparin (ClinicalTrials.gov ID NCT00196417). The study was approved by the Ernst-Moritz-Arndt-Universität Institutional Research Board. All patients gave informed consent in accordance with the Declaration of Helsinki. Data analysis was performed by the authors, all of whom had access to the primary clinical trial data.

Patients

From January 2003 to June 2005, consecutive patients admitted to the trauma surgery department at the University Hospital Greifswald, Germany,

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An Inside *Blood* analysis of this article appears at the front of this issue.

were eligible for the study if they met all of the following criteria: age 18 years or older, expected in-patient period of more than 4 days, and anticipated need for thrombosis prophylaxis of more than 5 days. Exclusion criteria were pregnancy, known drug or alcohol abuse, need for therapeutic dose anticoagulation, intensive care treatment, and participation in another clinical study within the last 30 days. Patients who provided informed consent were immediately randomly allocated to 1 of 2 treatment strategies by the use of sealed envelopes. The persons assessing eligibility and assigning treatment allocation to the patient were not aware of the allocation schedule. No heparin was administered before randomization.

Study medication

Patients received either unfractionated heparin 5000 IU (B. Braun) subcutaneously 3 times daily or the low-molecular-weight heparin certoparin (Monoembolex, Novartis) 3000 anti-factor XaU once daily subcutaneously and 2 placebo injections to ensure blinding of patients and study personnel. The first injection (given immediately after admission of the patient) always contained active study drug. During subsequent days, the morning injection always contained low-molecular-weight heparin in patients allocated to that study arm (secured by special coding of the morning injection in both groups).

Outcome measures

The primary outcome of this study was the difference in frequency of HIT-antibody seroconversion (defined subsequently) between the 2 treatment arms. Secondary outcomes were the occurrence of clinical HIT and occurrence of all-cause thrombosis (both non-HIT and HIT associated). HIT was considered if the patient had a 4T score of 4 or more points as agreed by 2 independent investigators and tested positive for anti-PF4/heparin immunoglobulin (Ig) G antibodies and showed platelet-activating antibodies in the heparin-induced platelet activation (HIPA) test.[13] Patients were observed until a study end point (HIT or new thrombosis) was reached. In case of an uneventful course, patients were followed from admission to discharge. After day 10, all patients received low-molecular-weight heparin. A follow-up for thromboembolic events was performed 3 months after discharge.

In a second, prespecified analysis, patients were analyzed for seroconversion, clinical HIT, and thromboses depending on severity of trauma and type of surgery. Before study unblinding, patients were classified by the Abbreviated Injury Score (AIS; 2005 revision[14]) and also grouped into "major surgery" (fractured humerus, hip, femur, tibia, pelvis, or extended tissue trauma), "minor" (all other surgical interventions), or "no surgical procedure."

Laboratory testing, adjudication of HIT, sonography, follow-up

HIT antibody testing was performed on admission, at discharge (if before day 10) and between day 10 and 14 by the HIPA test[15] and by an in-house PF4/heparin enzyme-immunoassay for IgG, IgM, and IgA, as described (cutoff, 0.5 optical density units).[16] HIT antibody seroconversion was defined as negative HIPA test and PF4/heparin-immunoassay on admission and a positive test from on day 5 of heparin. Daily platelet counts were measured in capillary blood (SE 9000; Sysmex).

All patients demonstrating at least 1 positive result in the immunoassay or the HIPA test were assessed independently by 2 investigators by the use of a validated clinical probability score for HIT, the 4Ts.[13] Disagreement was resolved by consensus.

Compression ultrasonography was performed as screening at discharge, or in case of clinical suspicion of deep-vein thrombosis (DVT). All findings that were reported not to be within normal limits were adjudicated by an independent investigator blinded to treatment and outcome.

All patients received a questionnaire about clinical symptoms of thrombosis to be completed 3 months after discharge. In case this questionnaire was not returned, a telephone interview was conducted.

Sample size

Sample size was calculated from estimated probabilities of seroconversion for low-molecular-weight heparin of 7.5% and of 14.1% in the control group of unfractionated heparin–treated patients. With a type I error of 0.05 and a type II error of 0.2 (estimated $P = .05$, 2-sided); 270 patients per study arm were required.

Statistical analysis

Data were evaluated by the use of SAS (version 8.0; SAS Inc). Patient characteristics, including age, sex, duration of treatment, and field of underlying disease, were compared by χ^2 and by the Wilcoxon signed rank test. In addition, the differences between study medication and type of procedure were described by proportion and 95% confidence intervals (CIs) and compared by χ^2 or the Fisher exact test. The risk of immune response was calculated by logistic regression analysis with major and minor surgery as predictor and adjusted for potential confounders (age, sex, type of heparin, length of heparin application). The increase of platelet counts was compared by the χ^2 test. The differences of thrombosis rates were compared by a 2-sample *t* test. All tests were 2-sided and considered to be statistically significant at less than 0.05.

Results

Patients

Of 696 patients enrolled, 614 were evaluable per protocol (low-molecular-weight heparin, 298 [48.5%]; unfractionated heparin, 316 [51.5%]). A total of 53 patients received study medication for less than 5 days, and 29 withdrew consent during the study, primarily because they objected to 3 injections per day and additional daily blood sampling. Baseline characteristics were similar in both groups (Table 1; Figure 1).

Type of surgery and risk for the anti-PF4/heparin immune response and clinical HIT

The types of injury/procedure are given in Table 2. Patients were grouped into major, minor, or no surgical procedures (Table 2). The corresponding AIS were as follows: AIS 1 (n = 76; 62 minor; 14 no surgical procedure); AIS 2 (n = 374; 120 major; 222 minor; 32 no surgical procedures); AIS 3 (n = 135; 99 major; 32 minor; 4 no surgical procedures); and AIS 4 (no patient); AIS 5 (n = 2; 1 minor; 1 no surgical procedures).

The study drug was given for a median of 10 days (range, 5-20 days) in patients undergoing major surgical procedures (unfractionated heparin: median, 10 days; range, 5-19 days; low-molecular-weight heparin: median, 10 days; range, 5-20 days; $P = .19$) and for a median of 7 days (range, 5-19 days) in patients undergoing minor surgical procedures (unfractionated heparin: median, 7 days; range, 5-19; low-molecular-weight heparin: median, 7 days, range, 5-19; $P = .46$).

The 3 most common major surgical procedures in women were fractured neck of femur, n = 51 (38.9%; men: n = 15, 16.1%); fractured humerus, n = 36 (27.5%; men: n = 24, 25.8%); and fractured tibia, n = 15 (11.5%; men: n = 27, 29.0%). Patients requiring major surgery were older (66 vs 43 years, $P < .001$) and more often female (131 [58.5%] of 224 vs 108 [32.0%] of 337, $P < .001$) compared with patients with minor surgery. Blood samples for HIT antibody testing were obtained at day 11.0 (± 3.1 days) in patients with major and at day 10.6 (± 3.3 days) in patients with minor surgical procedures ($P = .18$).

Five patients developed clinical HIT, all of them after major surgical procedures (Table 2). The difference in risk of HIT between patients undergoing major and minor surgery was significant (absolute risk 2.2% [95% confidence interval [CI], 0.3%-4.1%] vs 0.0%, $P = .01$). Seroconversion rates also strongly differed between patients undergoing major or minor surgical procedures (total seroconversion rates: 9.8% [95% CI, 5.9%-13.7%] vs 0.9% [95% CI, 0.0%-1.9%, $P < .001$]; immunoassay

Table 1. Characteristics of study patients

	UFH, n = 316 or 51.5%	LMWH, n = 298 or 48.5%	*P*
Female sex	144 (45.6%)	121 (40.6%)	.21
Median age, y (min-max)	49.0 (18-98)	50.0 (18-94)	.99
Median treatment duration, d (min-max)	8 (5-19)	8 (5-20)	.27
Location of injury			.54
Upper arm	43	41	
Forearm/hand	69	56	
Hip/thigh	61	61	
Lower leg/foot	74	76	
Other*	69	64	
Major surgery	n = 100	n = 124	
Female sex	66 (66.0%)	65 (52.4%)	.04
Median age, y (min-max)	68.5 (18-96)	61.5 (18-94)	.07
Median treatment duration, d (min-max)	10 (5-19)	10 (5-20)	.19
Minor surgery	n = 189	n = 148	
Female sex	64 (33.9%)	44 (29.7%)	.42
Median age, y (min-max)	42.0 (18-92)	45.0 (18-81)	.71
Median treatment duration, d (min-max)	7 (5-19)	7 (5-19)	.46

*For example, head injury, thorax injury, burns.

IgG and HIPA test seroconversion 4.9% [95% CI, 2.1%-7.7%] vs 0.3% [95% CI, 0.0%-0.9%, $P < .001$]; Figure 2) and also correlated to the AIS (AIS group 1 [0%], AIS group 2 [3.7%; 95% CI, 1.8%-5.6%]; AIS group 3 [7.4%; 95% CI, 3.0%-11.8%], $P = .03$).

Type of heparin and risk for the immune response to PF4/heparin and HIT

Seroconversion occurred more often in unfractionated heparin–than in low-molecular-weight heparin–treated patients (immunoassay and/or HIPA positive: 21 [6.6%; 95% CI, 3.9%-9.3%] vs 5 [1.7%; 95% CI, 0.2%-3.2%], $P = .002$; both tests positive: 11 [3.5%; 95% CI, 1.5%-5.5%] vs 2 [0.7%; 95% CI, 0.0%-1.6%], $P = .02$; IgG immunoassay positive: 13 [4.1%; 95% CI, 1.9%-6.3%] vs 2 [0.7%; 95% CI, 0.0%-1.6%], $P = .007$; Table 2). Of the 5 patients who were adjudicated as having clinical HIT (Table 3), 4 received unfractionated heparin, and 1 received low-molecular-weight heparin ($P = .37$). All had a positive HIPA and PF4/heparin IgG immunoassay and associated DVT (4 proximal, 1 distal).

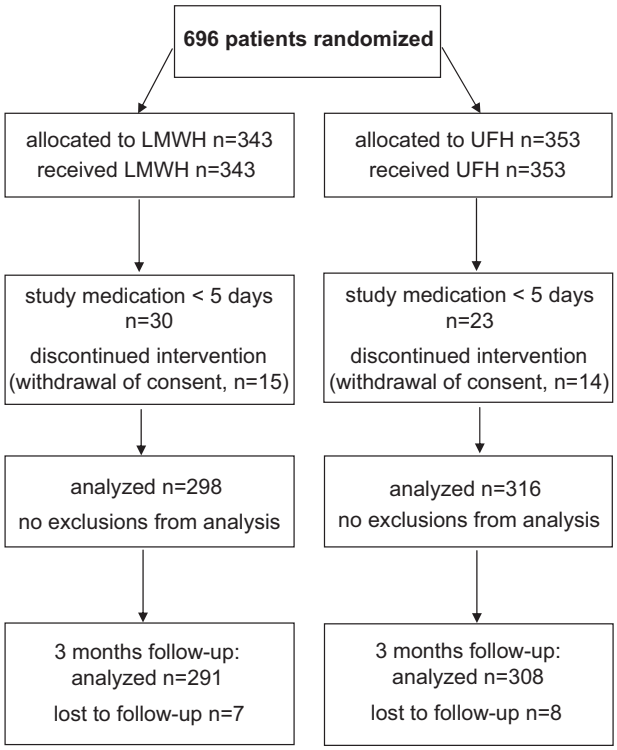

Figure 1. Overview of patients included in the study. Consent was withdrawn because of the inconvenience of daily platelet count monitoring and the need for 3 daily injections. The patients lost to the 3 months' follow-up could be tracked neither via the general practitioner nor by the registry office.

Logistic regression analysis of the immune response to PF4/heparin

The odds ratio (OR) for seroconversion (functional test and/or immunoassay) after major surgery versus minor surgery was 11.57 (95% CI, 3.41-39.29; $P < .001$); controlled for confounders (age, sex, type of heparin, length of heparin application) by logistic regression analysis, it was 7.98 (95% CI, 2.06-31.00; $P = .003$). The OR for a positive HIPA was 9.50 (95% CI, 2.11-42.99; $P = .003$); controlled for the aforementioned confounders, it was 7.30 (95% CI, 1.37-38.94; $P = .020$).

Risk for thrombosis related to type of surgery, type of heparin, and immune response to PF4/heparin

All thromboses. In the entire study population 17 patients had symptomatic DVT (11 proximal [1.8%; 95% CI, 2.4%-4.6%] and 6 distal [0.98%; 95% CI, 0.2%-1.8%]), and 2 patients had pulmonary embolism (0.3%; 95% CI, 0.0%-0.7%). In addition, 7 cases of proximal DVT (1.1%; 95% CI, 0.3%-1.9%) and 18 cases of distal DVT (2.9%; 95% CI, 1.5%-4.3%) were found by screening at discharge. In proximal DVT/PE after major surgical procedures (n = 19) there was no difference in seroconversion rates depending on type of heparin (unfractionated heparin vs low-molecular-weight heparin: 3 [37.5%; 95% CI, 4%-71%] vs 1 [9.1%; 95% CI, 0%-26.1%], $P = .134$), but this finding might also be an effect of small numbers.

Two patients died during the in-hospital period: an 86-year-old female patient died of pulmonary embolism (diagnosed clinically, no autopsy) at day 8 of unfractionated heparin prophylaxis after a fractured femur. There was no platelet count decrease suggestive of HIT. No blood sample for antibody testing was available. A 55-year-old female patient died of pulmonary embolism (proven at autopsy) at day 14 after 7 days of low-molecular-weight heparin prophylaxis after a fractured humerus. There was no platelet count decrease, and HIT antibody tests at day 10 were negative.

Table 2. Type of injury/surgical procedure related to outcome

		Seroconversion		HIT cases		Thromboses			
						Proximal or PE		Distal	
Surgical procedures	**Number of patients**	**UFH**	**LMWH**	**UFH**	**LMWH**	**UFH**	**LMWH**	**UFH**	**LMWH**
Major surgical procedures									
Fracture humerus	63	5	1	—	—	—	1*	1	1
Fracture hip/pelvis	54	3	2	—	—	1	3	3	2
Fracture femur	59	5	2	2	1	5*,†,†	7†	1	4
Knee endoprothesis	3	1	—	1	—	—	—	1†	—
Fracture head of tibia	15	2	—	1	—	2†	—	1	—
Fracture tibia	30	1	—	—	—	—	—	—	1
Total major surgical procedures (95% CI)	224	17/100, 17.0% (9.6-24.4)	5/124, 4.0% (0.6-7.4)	4/100, 4.0% (0.2-7.8)	1/124, 0.8% (0.0-2.4)	8/100, 8.0% (2.7-13.3)	11/124, 8.9% (6.0-16.0)	7/100, 7.0% (2.0-12.0)	8/124, 6.5% (2.2-10.8)
		$P = .001$		$P = .18$		$P = .82$		$P = .87$	
Minor surgical procedures									
Lower arm/hand	123	1	—	—	—	—	—	—	—
Shoulder	21	—	—	—	—	—	—	—	—
Knee (eg, cruciate ligament rupture)	23	—	—	—	—	—	—	1	—
Fracture ankle joint	57	2	—	—	—	—	—	2	1
Foot	19	—	—	—	—	1	—	—	—
Removal of metal	35	—	—	—	—	—	—	—	—
Fracture spine	14	—	—	—	—	—	—	—	1
Tendon injury	26	—	—	—	—	—	—	—	2
Others	19	—	—	—	—	—	—	—	—
Total minor surgical procedures	337	3/189 (1.6%)	0/148 (0.0%)	0/189 (0.0%)	0/148 (0.0%)	1/189 (0.53%)	0/148 (0.0%)	3/189 (1.6%)	4/148 (2.7%)
Total no surgical procedures	53	1/27 (3.7%)	0/26 (0.0%)	0/27 (0.0%)	0/26 (0.0%)	0/27 (0.0%)	0/26 (0.0%)	2/27 (7.4%)	0/26 (0.0%)
Total (95% CI)	614	21/316, 6.6% (3.9-9.3)	5/298, 1.7% (0.2-3.2)	4/316, 1.3% (0.1-2.5)	1/298, 0.3% (0.0-0.9)	9/316, 2.8% (1.0-4.6)	11/298, 3.7% (1.6-5.8)	12/316, 3.8% (1.7-5.9)	12/298, 4.0% (1.8-6.2)
		$P = .002$		$P = .37$		$P = .56$		$P = .88$	

Type of injury/surgical procedure of the study patients, type of heparin and outcome with regard to thrombosis, seroconversion, and HIT.
— indicates no patient in this category.
*Patient with pulmonary embolism (PE).
†HIT patient.

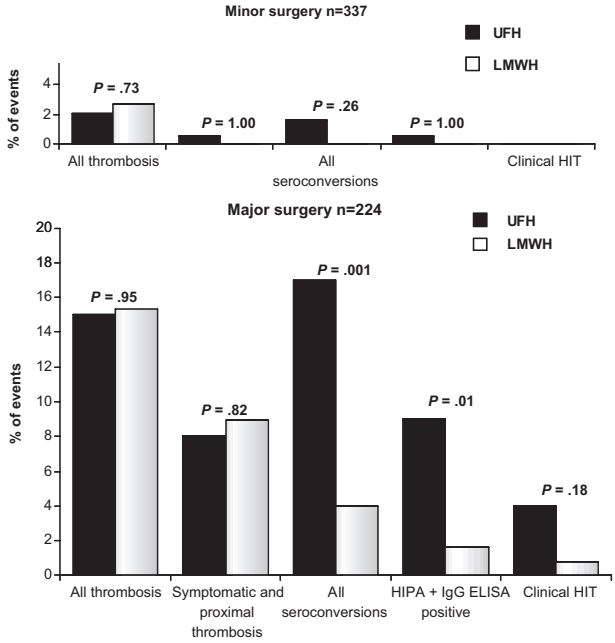

Figure 2. Clinical and laboratory findings in patients with minor and major surgery. Clinical and laboratory findings in patients with minor (top) and major (bottom) surgery. ■ depicts patients receiving unfractionated heparin (UFH); and □, patients receiving low-molecular-weight heparin (LMWH).

Type of surgery. Patients undergoing major surgical procedures had more thromboses than those undergoing minor surgical procedures (proximal DVT: 8.5% vs 0.3%; OR 31.1 [95% CI, 4.1-234.0, $P < .001$]; distal DVT: 6.7% vs 2.1%, OR 3.4 [95% CI, 2.5-9.1, $P = .007$]). Their risk for DVT was also correlated to anti-PF4/heparin seroconversion (HIPA and/or immunoassay; all DVT: OR 4.9 [95% CI, 1.9-12.8; $P = .002$]; proximal DVT: OR 2.8 [95% CI, 1.0-9.3; $P = .09$]; and distal DVT: OR 5.6 [95% CI, 1.7-18.3; $P = .002$]). This correlation was only significant for IgG antibodies ($P = .02$) but not for IgM antibodies ($P = .21$). In patients undergoing minor surgical procedures, there was a trend for an increased risk for DVT with PF4/heparin antibody seroconversion (HIPA and/or enzyme-linked immunoassay vs no antibodies, $P = .07$).

Type of heparin. The type of heparin (low-molecular-weight heparin/unfractionated heparin) did not correlate with the combined risk for proximal DVT and pulmonary embolism ($P = .56$), the risk for developing an asymptomatic distal DVT ($P = .88$), or the risk for any thrombotic event ($P = .91$).

Platelet count profiles after major and minor surgical procedures

Platelet count profiles are shown in Figure 3. The platelet count decrease was greater after major surgery versus minor surgery: nadir (mean ± SD, day 3: 193 000 ± 65 100/mm^3 vs 210 500 ± 56 100/mm^3; $P < .001$) with a greater reactive platelet count increase compared with the postoperative platelet count nadir ($P < .001$): greater than 50% (48.3% vs 15.6%); greater than 30% to 49% (18.4% vs 14.3%); less than 30% (33.3% vs 72.6%; Figure 3A). The 5 HIT patients developed their symptoms between days 5 and 10 (Figure 3B). The platelet count decrease in these patients was moderate only. This finding might have been the effect of close monitoring of patients within this prospective study.

Three months' follow-up

In 599 patients (97.6%) follow-up information was obtained after 3 months. Of those, 26 (4.3%) had tested positive for PF4/heparin antibodies at discharge. The events after discharge, DVT (n = 3), death (n = 3, 1 pulmonary embolism suspected clinically), and stroke (n = 2), were not related to low-molecular-weight heparin/unfractionated heparin ($P = .73$) or HIT antibody status at discharge (1 of 26 vs 7 of 573, $P = .30$).

Discussion

The most interesting finding of our study was that patients after major surgical procedures have a much greater risk for developing the immune response to PF4/heparin than patients undergoing minor surgical procedures, irrespective of the type of heparin received. Thus, a nondrug factor, namely the severity of trauma determining the need for major or minor surgery, is a marker for the immune response leading to the adverse drug reaction, HIT. The patients undergoing major and minor surgery differed in some of their characteristics, for example, age and sex. We therefore controlled for potential confounders (age, sex, type of heparin, length of heparin application) by logistic regression analysis, and still those patients undergoing major surgery had a much greater risk to develop the immune response against PF4/heparin (OR 7.98; 95% CI, 2.06-31.00; $P = .003$). However, we cannot differentiate whether this is a direct effect of trauma and inflammation or a more indirectly related association.

If one considers a more direct correlation between major surgery and the immune response against PF4/heparin possible, an increased release of PF4 during major surgery resulting in more

Table 3. Patients with clinical HIT

Sex, age	Type of heparin	Underlying disease	Platelet count decrease, %	Thromboembolic complication, all asymptomatic	4T score	Day of	
						Onset of platelet count fall	Diagnosis of thrombosis
Female, 77 y	UFH	Fracture femur	44.9	Pelvic thrombosis, calf vein thrombosis	7	7	10
Female, 67 y	UFH	Fracture head of tibia	34.8	Thromboses popliteal vein, tibiofibular truncus, tibial post. Vein, peroneal veins	6	10	11
Female, 90 y	UFH	Fracture neck of femur	61.3	Thromboses calf vein, tibiofibular truncus, peroneal veins, tibial post. Vein	7	6	8
Female, 64 y	UFH	Gonarthrosis	19.6	Thromboses calf vein, peroneal vein	5	4	7
Male, 81 y	LMWH	Fracture femur	27.4	Thromboses popliteal vein, fibular veins, fibular post. Veins, gastrocnemic veins	5	9	15

Clinical and laboratory details of the 5 study patients in whom clinical HIT occurred.

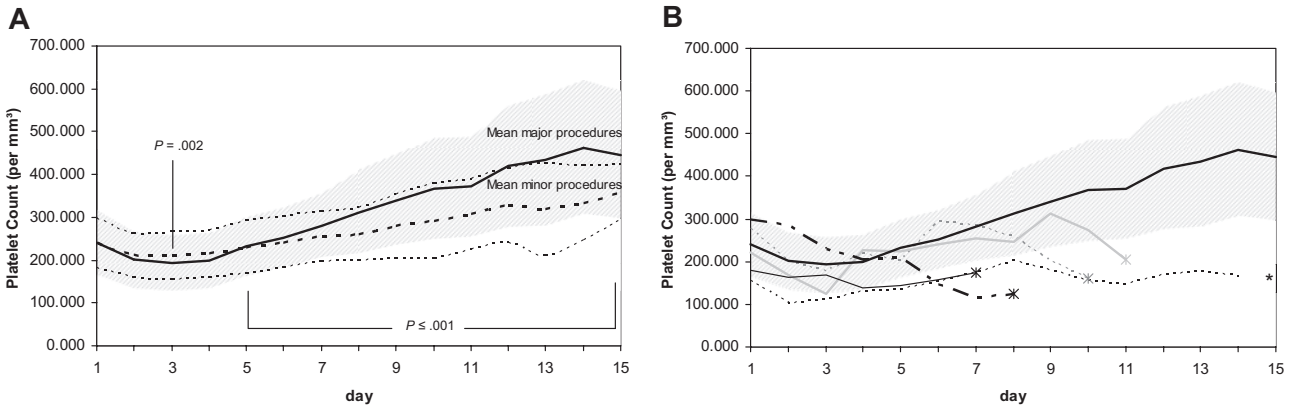

Figure 3. Platelet count profiles major versus minor procedures and platelet count range of all patients with major surgical procedures in relation to the platelet count profile of the 5 HIT patients. (A) Platelet count profiles in patients after major versus minor procedures. Solid line indicates major surgery, platelet count mean ± SD (hatched area). Dotted lines indicate minor surgery, platelet count mean (bold line) ± SD. Platelet count nadir ($P < .001$) as well as profiles from on day 5 ($P < .001$) differ significantly between the 2 groups. Platelet count decrease was greater after major surgery versus minor surgery (nadir day 3; 193 000/mm^3, mean ± 65.1 vs 210 500/mm^3 mean ± 56.1; $P < .001$) with a greater reactive platelet count increase ($P < .001$): > 50% (48.3% vs 15.6%); > 30% to 49% (18.4% vs 14.3%); and < 30% (33.3% vs 72.6%). (B) Platelet count range of all patients with major procedures (platelet count mean [bold line] ± SD [hatched area]) in relation to the platelet count profile of the 5 HIT patients (black, gray, and dotted lines). *Time of diagnosis of thrombosis.

immunogenic complexes[17] is one potential explanation for the enhanced immune response after major trauma. An alternative explanation might be that the surgical trauma by itself, or the inflammation associated with major surgery, modifies the immune system in a way to trigger B cells specific for PF4 complexes. This explanation would be consistent with the observation that HIT is less frequent in medical patients and in pregnant women compared with surgical patients.[18] However, it is difficult to control for the effect of inflammation in a clinical study in trauma patients and to differentiate between the effect of inflammation and potential other confounders. This issue might be better addressed in one of the mouse models of the HIT immune response.[19,20] The somewhat-longer exposure to heparin after major surgical procedures also may have contributed to the difference in immunization rates. However, both groups were tested for PF4/heparin antibodies at the same time (day 10.6 and day 11.0, respectively). Because most immune responses to PF4/heparin start within the first week[21,22] the immune response should have been detected by our study in both groups with a similar likelihood.

An interesting parallel to the present study is the enhanced risk for the induction of antibodies against factor VIII in patients with hemophilia receiving factor VIII concentrates during episodes of inflammation and surgery.[23,24] This finding indicates the intriguing possibility that not only the immune response of HIT but also the immune responses to other biotherapeutics might be driven considerably by transient nondrug patient-related factors, for example, induced by tissue trauma.

The study further corroborates that the immune response to PF4/heparin is less frequent in trauma patients receiving low-molecular-weight heparin compared with unfractionated heparin. It was not powered to detect a statistically significant difference in clinical HIT between the 2 heparin groups. However, in the view of earlier studies,[7-11] it provides further evidence that the use of low-molecular-weight heparin instead of unfractionated heparin for thrombosis prophylaxis is most likely an efficient measure to reduce the risk for HIT in a general trauma patient population. Because these previous studies[7-11] used the low-molecular-weight heparin enoxaparin, the fact that risk reduction was observed in our study with another low-molecular-weight heparin (certoparin) is worth noting as possible evidence of a class effect.

Our study corroborates the previously noted sex imbalance in HIT,[18] as well as the beneficial effect of low-molecular-weight heparin in reducing the risk for HIT predominantly in women[18]: 4 of the 5 patients with HIT were women, all of whom received UFH, that is, 4 (6.1%) of 66 women with major trauma; in comparison, none of the 65 women receiving low-molecular-weight heparin after major trauma developed HIT ($P = .12$). The only man to suffer from HIT received low-molecular-weight heparin. This result also underscores the importance of adjustment for potential confounders such as sex in clinical studies on HIT.

Our study also indicates that in trauma patients the recommendation of guidelines[12,25] and package inserts (Germany, France) to monitor platelet counts in all patients who receive heparin could be restricted to patients with major trauma and potentially limited to patients receiving unfractionated heparin. This step would reduce a major burden of care during prolonged thrombosis prophylaxis in outpatients with minor trauma.

Interestingly, in this study unfractionated heparin and low-molecular-weight heparin demonstrated the same efficacy for preventing DVTs. This finding is different from the study of Geerts et al,[26] in which they found a significant risk reduction of all DVTs of 30% (95% CI, 4%-50%, $P = .014$) by low-molecular-weight heparin compared with unfractionated heparin. However, these authors enrolled only patients with major trauma and compared a greater dose of low-molecular-weight heparin (enoxaparin 30 mg, 3 times daily) with a lower dose of unfractionated heparin (5000 IU twice daily). We tested 3000 anti-FXaU certoparin once per day and unfractionated heparin (5000 IU, 3 times daily).

In conclusion, our study has shown for the first time that the magnitude of trauma, and resulting need for major versus minor surgery, is a major risk factor influencing the immune response of HIT. It further extends the finding that low-molecular-weight heparin may be safer than unfractionated heparin with regard to triggering HIT in patients receiving thrombosis prophylaxis after major trauma as it induces HIT-antibody seroconversion—and the corresponding risk of HIT—less frequently. Given the wide use of heparin, the anti-PF4/heparin immune response is a potential model to better understand important confounders of immune reactions toward biotherapeutics in humans.

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Authorship

Contribution: A.G. and N.L. designed the study concept; N.L., P.H., A.E., and A.G. interpreted the data; T.L. performed statistical analyses; S.S. adjudicated clinical events; S.T., M.V., A.L., M.J., M.N., K.W., and G.E. did the data collection; and all authors helped to write the manuscript. A.G had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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