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

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running head: HIT in trauma surgery

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Abstract
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 PF4/heparin was analyzed. In a randomized, double-blind, single center study, trauma patients receiving low-molecular-weight (LMWH) or unfractionated heparin (UFH) for thrombosis prophylaxis were assessed for PF4/heparin-antibody seroconversion, clinical HIT, and thrombosis according to type of surgery. The risk for seroconversion increased with the magnitude of surgical trauma: major vs minor surgery OR 7.98 [95%CI 2.06-31.00; p=0.003, controlled for potential confounders] as did the risk for HIT (2.2% [95%CI 0.3-4.1%] vs 0.0%, p=0.010). During LMWH compared to UFH thromboprophylaxis, HIT (1/298 vs 4/316; p=0.370) and PF4/heparin seroconversion (1.7% vs.6.6%; p=0.002) were less frequent, driven by differences in patients undergoing major surgery: incidence of HIT (LMWH 0.8% vs UFH 4.0%; p=0.180); seroconversion rates (4.0% vs 17.0%; p=0.001). After minor surgery no case of HIT occurred. The severity of trauma and the need for major surgery strongly influence the risk of forming an anti-PF4/heparin immune response, which is then increased by UFH. In major trauma certoparin may be safer than UFH as it induces HIT-antibody seroconversion and the corresponding risk of HIT - less frequently. This study is registered at http://clinicaltrials.gov as NCT00196417.

Key words
HIT, heparin, platelets, trauma, orthopedic surgery
Introduction

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

The risk estimates for HIT in surgical patients are based on studies in patients undergoing elective major joint replacement surgery, which showed the risk for HIT being only approximately one-tenth as great when low-molecular-weight heparin is used for thrombosis prophylaxis as compared to unfractionated heparin. 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, should be recommended in the large group of patients with minor trauma, such as ankle fracture. This is of major clinical importance, as 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 towards 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
non-drug factor, namely the severity of trauma and the requisite need for either major or minor surgery.
Patients and methods

This study was a double blind randomized controlled trial using a two arm parallel treatment design to demonstrate a lower incidence of HIT antibody seroconversion (superiority) in patients treated with low-molecular-weight heparin as compared to unfractionated heparin (ClinicalTrials.gov ID NCT00196417).

The study was approved by the Ernst-Moritz-Arndt-Universität IRB. Data analysis was performed by the authors, all of which had access to the primary clinical trial data.

Patients

From 01/2003–06/2005 consecutive patients admitted to the trauma surgery department, University Hospital Greifswald, Germany, were eligible for the study if they met all of the following criteria: age ≥18 years, expected in-patient period >4 days, and anticipated need for thrombosis prophylaxis >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 gave informed consent were immediately randomly allocated to one of two treatment strategies using sealed envelopes. The individuals assessing eligibility and assigning treatment allocation to the patient were not aware of the allocation schedule. No heparin was given before randomization.

Study medication

Patients received either unfractionated heparin 5,000 IU (B. Braun, Melsungen, Germany) t.i.d. s.c. or the low-molecular-weight heparin certoparin (Monoembolex, Novartis, Nürnberg, Germany) 3,000 anti-factor XaU o.d. s.c. and two 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 two 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 two independent investigators and tested
positive for anti-PF4/heparin IgG antibodies and showed platelet activating antibodies
in the HIPA test.\textsuperscript{13} Patients were observed until a study endpoint (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 thrombembolic 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\textsuperscript{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 heparin-induced platelet activation (HIPA) test,\textsuperscript{15} and
by an in-house PF4/heparin enzyme-immunoassay (EIA) for IgG, IgM, and IgA, as
described (cutoff, 0.5 optical density [OD] units).\textsuperscript{16} HIT antibody seroconversion was
defined as negative HIPA test and PF4/heparin-immunoassay on admission and a
positive test ≥day 5. Daily platelet counts were measured in capillary blood (SE 9000, Sysmex, Kobe, Japan).

All patients showing at least one positive result in the immunoassay or the HIPA test were assessed independently by two investigators using a validated clinical probability score for HIT, the 4Ts.\textsuperscript{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 $P=0.05$, two sided); 270 patients per study arm were required.

Statistical analysis
Data were evaluated using SAS (version 8.0, SAS Inc., Cary, NC, USA). Patient characteristics, including age, gender, duration of treatment, field of underlying disease, were compared by Chi-square and by Wilcoxon test. Additionally the differences between study medication and type of procedure were described by proportion and 95\% confidence intervals [CI] and compared by Chi-square or Fisher’s 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, gender, type of heparin, length of heparin application). The increase of platelet counts was compared by the chi-square test. The differences of thrombosis rates were compared by 2-sample t-test. All tests were two-sided and considered to be statistically significant below 0.05.

Role of the funding source

This study was supported by Novartis, Nürnberg, Germany. The sponsor had no role in study design, collection, analysis and interpretation of data, or in the writing of the manuscript.
Results

Patients

Of 696 patients enrolled, 614 were evaluable per protocol (low-molecular-weight heparin 298 [48.5%]; unfractionated heparin 316 [51.5%]). 53 patients received study medication <5d, and 29 withdrew consent during the study, primarily as they objected to three injections/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: 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); 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) in patients undergoing major surgical procedures (unfractionated heparin median 10 days, range 5-19; low-molecular-weight heparin median 10 days, range 5-20; p=0.19) and for a median of 7 days (range 5-19) 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=0.46).

The three 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<0.001) and more often female (131/224 [58.5%] vs. 108/337 [32.0%], p<0.001) compared to 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=0.18).

Five patients developed clinical HIT, all of them following major surgical procedures (table 2). The difference in risk of HIT between patients undergoing major and minor surgery was significant (2.2% [95%CI 0.3-4.1%] vs 0.0%, p=0.01). 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<0.001]; immunoassay IgG and HIPA test seroconversion 4.9% [95%CI 2.1-7.7%] vs 0.3% [95%CI 0.0-0.9%, p<0.001] (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=0.03

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=0.002; both tests positive: 11 [3.5%; 95%CI 1.5-5.5%] vs 2 [0.7%; 95%CI 0.0-1.6%], p=0.02; IgG immunoassay positive: 13 [4.1%;95%CI 1.9-6.3%] vs 2 [0.7%;95%CI 0.0-1.6%], p=0.007) (table 2). Of the five patients that were adjudicated as having clinical HIT (table 3), four received unfractionated heparin, and one received low-molecular-weight heparin (p=0.37). All had a positive HIPA and PF4/heparin IgG immunoassay, and associated DVT (4 proximal, 1 distal).
Logistic regression analysis of the immune response to PF4/heparin

The odds ratio (OR) for seroconversion (functional test and/or immunoassay) after major surgery vs minor surgery was 11.57 (95%CI 3.41-39.29; p<0.001); controlled for confounders (age, gender, type of heparin, length of heparin application) by logistic regression analysis, it was 7.98 (95%CI 2.06-31.00; p=0.003). The OR for a positive HIPA was 9.50 (95%CI 2.11-42.99; p=0.003); controlled for the above mentioned confounders, it was 7.30 (95%CI 1.37-38.94; p=0.020).

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%), two patients had pulmonary embolism (0.3%; 95% CI 0.0-0.7%). In addition seven proximal DVT (1.1%; 95%CI 0.3-1.9%) and 18 distal DVT (2.9%; 95%CI 1.5-4.3%) were found by screening at discharge. In proximal DVT/PE following major surgical procedures (n=19) there was no difference in seroconversion rates depending on type of heparin (UFH vs. LMWH 3 [37.5% 95%CI: 4-71%] vs. 1 [9.1% 95%CI: 0-26.1%] p=0.134) but this 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 following 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 low-molecular-weight heparin prophylaxis following a fractured humerus. There was no platelet count decrease, and HIT antibody tests at day 10 were negative.
**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<0.001]; distal DVT 6.7% vs 2.1%, OR 3.4 [95% CI, 2.5-9.1, p=0.007]). 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=0.002]; proximal DVT OR 2.8 [95%CI, 1.0-9.3; p=0.09]; distal DVT OR 5.6 [95% CI, 1.7-18.3; p=0.002]). This correlation was only significant for IgG-antibodies (p=0.02), but not for IgM-antibodies (p=0.21).

In patients undergoing minor surgical procedures there was a trend for an increased risk for DVT with PF4/heparin antibody seroconversion (HIPA and/or ELISA vs no antibodies, p=0.07).

**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=0.56); the risk for developing an asymptomatic distal DVT (p=0.88); or the risk for any thrombotic event (p=0.91).

**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 vs minor surgery: nadir (mean ± SD), day 3 (193,000 ± 65,100/mm³ vs. 210,500 + 56,100/mm³; p<0.001) with a higher reactive platelet count increase compared with the postoperative platelet count nadir (p<0.001): >50% (48.3% vs 15.6%); >30-49% (18.4% vs 14.3%); <30% (33.3% vs 72.6%) (Fig 3a).

The 5 HIT patients developed their symptoms between day 5-10 (Fig 3b). The platelet count decrease in these patients was moderate only. This might have been the effect of close monitoring of patients within this prospective study.
3 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, one pulmonary embolism suspected clinically), stroke (n=2), were not related to low-molecular-weight heparin/unfractionated heparin (p=0.73) or HIT antibody status at discharge (1/26 vs. 7/573, p=0.30).
Discussion

The most interesting finding of our study was that patients after major surgical procedures have a much higher risk for developing the immune response to PF4/heparin than patients undergoing minor surgical procedures irrespective of heparin type received. Thus a non-drug 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, e.g. age and gender. We therefore controlled for potential confounders (age, gender, type of heparin, length of heparin application) by logistic regression analysis and still those patients undergoing major surgery had a much higher risk to develop the immune response against PF4/heparin (OR 7.98; 95%CI 2.06-31.00; p=0.003). 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 immunogenic complexes 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 would be consistent with the observation that HIT is less frequent in medical patients and in pregnant women as compared to surgical patients. 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. The somewhat longer exposure to
heparin after major surgical procedures may also 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). As most immune responses to PF4/heparin start within the first week\textsuperscript{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 FVIII in patients with hemophilia receiving FVIII concentrates during episodes of inflammation and surgery.\textsuperscript{23,24} This 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 non-drug patient-related factors, e.g. 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 as compared to unfractionated heparin. It was not powered to detect a statistically significant difference in clinical HIT between the two heparin groups. However, in the view of earlier studies, \textsuperscript{7-11} it provides further evidence that using 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. As these previous studies utilized the LMWH enoxaparin, the fact that risk reduction was seen in our study with another LMWH (certoparin) is worth noting as possible evidence of a class effect.

Our study corroborates the previously noted gender imbalance in HIT\textsuperscript{18}, as well as the beneficial effect of LMWH in reducing the risk for HIT predominantly in females.\textsuperscript{18} four of the five patients with HIT were female, all of whom received UFH i.e. 4/66 (6.1%) with major trauma; in comparison none of the 65 females receiving LMWH after major trauma developed HIT (p=0.12). The only man to suffer from HIT
received LMWH. This 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 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 would reduce a major burden of care during prolonged thrombosis prophylaxis in out-patients with minor trauma.

Interestingly, in this study unfractionated heparin and low-molecular-weight heparin showed the same efficacy for preventing DVTs. This is different to the study of Geerts et al (1996) which found a significant risk reduction of all DVTs of 30% (95% CI 4-50%, p=0.014) by low-molecular-weight heparin compared to unfractionated heparin. However, these authors enrolled only patients with major trauma and compared a higher dose of low-molecular-weight heparin (enoxaparin 30 mg b.i.d.) with a lower dose of unfractionated heparin (5,000 IU b.i.d). We tested 3,000 anti-FXaU certoparin once per day and unfractionated heparin (5,000 IU t.i.d.).

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 towards biotherapeutics in humans.
Acknowledgements

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Authorship Contributions and Disclosure of Conflicts of Interest

AG and NL designed the study concept, NL, PH, AE and AG interpreted the data and wrote the draft, TL performed statistical analyses, SS adjudicated clinical events. ST, MV, AL, MJ, MN, KW, GE did the data collection. All authors helped to write the draft. The corresponding author (AG) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

The authors declare no conflict of interests.
References


### Patient characteristics

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<td>17.0%[9.6-24.4]</td>
<td>4.0%[0.6-7.4]</td>
<td>4.0%[0.2-7.8]</td>
<td>0.8%[0.0-2.4]</td>
<td>8.0%[2.7-13.3]</td>
</tr>
</tbody>
</table>

| minor surgical procedures         |                    | p=0.001 | p=0.18 | p=0.82 | p=0.87 |
| lower arm/hand                    | 123                | -       | -      | -      | -      |
| Shoulder                          | 21                 | -       | -      | -      | -      |
| knee (e.g. cruciate ligament      | 23                 | -       | -      | -      | -      | 1    | -    |
| rupture)                          | fracture ankle joint | 57     | 2      | -      | -      | -    | 2    | 1    |
| Foot                              | 19                 | 1       | -      | -      | 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%) |
|                                   |                    | 1.6%[0.0-3.9] | 0.0%[0.0-0.3] | 0.0%[0.0-0.7] | 0.0%[0.0-0.7] | 0.53%[0.0-1.0] | 0.0%[0.0-0.3] | 1.6%[0.0-3.9] | 2.7%[0.0-5.4] |
| 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%) |
|                                   |                    | 3.7%[0.0-10.6] | 0.0%[0.0-0.3] | 0.0%[0.0-0.7] | 0.0%[0.0-0.7] | 0.0%[0.0-0.7] | 0.0%[0.0-0.7] | 7.4%[2.0-14.9] | 0.0%[0.0-0.7] |
| total                             | 614                | 21/316 (6.6%) | 5/298 (1.7%) | 4/316 (1.3%) | 1/298 (0.3%) | 9/316 (2.8%) | 11/298 (3.7%) | 12/316 (4.0%) | 12/298 (4.0%) |
|                                   |                    | 6.6%[3.9-9.3] | 1.7%[0.2-3.2] | 1.3%[0.1-2.5] | 0.3%[0.0-0.9] | 2.8%[1.0-4.6] | 3.7%[1.6-5.8] | 3.8%[1.7-5.9] | 4.0%[1.8-6.2] |

+ HIT-patient, § patient with pulmonary embolism (PE) 95% confidence intervals given in square brackets.
<table>
<thead>
<tr>
<th>Gender</th>
<th>type of heparin</th>
<th>underlying disease</th>
<th>platelet count decrease</th>
<th>thromboembolic complication (all asymptomatic)</th>
<th>4T score</th>
<th>day of onset of platelet count fall</th>
<th>day of diagnosis of thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>UFH</td>
<td>fracture femur</td>
<td>44.9 %</td>
<td>pelvic thrombosis, calf vein thrombosis</td>
<td>7</td>
<td>7</td>
<td>10</td>
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<tr>
<td>Female</td>
<td>UFH</td>
<td>fracture head of tibia</td>
<td>34.8 %</td>
<td>thromboses popliteal vein, tibiofibular truncus, tibial post. vein, peroneal veins</td>
<td>6</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>UFH</td>
<td>fracture neck of femur</td>
<td>61.3 %</td>
<td>thromboses calf vein, tibiofibular truncus, peroneal veins, tibial post. Vein</td>
<td>7</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Female</td>
<td>UFH</td>
<td>gonarthrosis</td>
<td>19.6 %</td>
<td>thromboses calf vein, peroneal vein</td>
<td>5</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Male</td>
<td>LMWH</td>
<td>fracture femur</td>
<td>27.4 %</td>
<td>thromboses popliteal vein, fibular veins, fibular post. veins, gastrocnemic veins</td>
<td>5</td>
<td>9</td>
<td>15</td>
</tr>
</tbody>
</table>
Legends

table 1  Characteristics of study patients.
table 2  Type of injury/surgical procedure of the study patients, type of heparin and outcome with regard to thrombosis, seroconversion and HIT.
table 3  Clinical and laboratory details of the 5 study patients in whom clinical HIT occurred.
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 three daily injections. The patients lost to the 3 months follow-up could be tracked neither via the general practitioner nor by the registry office.
figure 2  Clinical and laboratory findings in patients with minor (upper part) and major (lower part) surgery. Black bars depict patients receiving UFH, white bars patients receiving LMWH.
figure 3  a) Platelet count profiles in patients after major vs minor procedures. Solid line: major surgery, platelet count mean ± standard deviation (hatched area). Dotted lines: minor surgery, platelet count mean (bold line) ± standard deviation. Platelet count nadir (p<0.001) as well as profiles from on day 5 (p<0.001) differ significantly between the two groups. Platelet count decrease was greater after major surgery vs minor surgery (nadir day 3; 193,000/mm³, mean + 65.1 vs. 210,500/mm³ mean + 56.1; p<0.001) with a higher reactive platelet count increase (p<0.001): >50 % (48.3% vs 15.6%); >30-49% (18.4% vs 14.3%); <30% (33.3% vs 72.6%).
b) Platelet count range of all patients with major procedures (platelet count mean (bold line) ± standard deviation (hatched area)) in relation to the platelet count profile of the 5 HIT-patients (black, grey and dotted lines). Asterisks depict time of diagnosis of thrombosis.
figure 1  Overview of patients included in the study

696 patients randomized

allocated to LMWH n=343
received LMWH n=343

study medication < 5 days
n=30
discontinued intervention (withdrawal of consent) n=15

analyzed n=298
no exclusions from analysis

3 months follow-up:
analyzed n=291
lost to follow-up n=7

allocated to UFH n=353
received UFH n=353

study medication < 5 days
n=23
discontinued intervention (withdrawal of consent) n=14

analyzed n=316
no exclusions from analysis

3 months follow-up:
analyzed n=308
lost to follow-up n=8
figure 2 Clinical and laboratory findings in patients with minor and major surgery
figure 3a  Platelet count profiles major vs minor procedures.
**figure 3b** Platelet count range of all patients with major surgical procedures in relation to the platelet count profile of the 5 HIT-patients.
The severity of trauma determines the immune response to PF4/heparin and the frequency of heparin-induced thrombocytopenia

Norbert Lubenow, Peter Hinz, Simone Thomaschewski, Theresia Lietz, Michael Vogler, Andrea Ladwig, Michael Junger, Matthias Nauck, Sebastian Schellong, Kathrin Wander, Georg Engel, Axel Ekkernkamp and Andreas Greinacher