RESEARCH ARTICLE

Anti-PF4/ Heparin Antibodies Early Seroconversion in Hip Fracture Patients Receiving Low Molecular Weight Heparin Prophylaxis: a Pilot Study of 100 Consecutive Patients

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Abstract

Objectives: Heparin-induced thrombocytopenia (HIT) represents a serious adverse reaction triggered by antibodies (anti-PF4/H) in heparin regimens. It is not clear if different low-molecular weight heparins (LMWHs) prompt distinct immunogenic responses in anti-PF4/H production and if these antibodies correlate with thrombocytopenia, thrombotic events, and early postoperative mortality. This pilot prospective study investigates the early output of anti-PF4/ H in elderly patients undergoing proximal femoral nailing for an intertrochanteric hip fracture surgery.

Methods: A total of 100 consecutive patients (72 females) with surgically treated intertrochanteric hip fractures were prospectively included. Ninety-four patients were available for the final follow-up. Twenty-seven patients received bemiparin, 42 enoxaparin and 25 tinzaparin. The levels of anti-PF4/H using the semi-quantitative latex-enhanced immunoassay; HemosIL® HIT-Ab(PF4-H) and platelets (PLT) levels were measured on the admission day and on day 5 following LMWH administration. Patients were followed up for at least 3 months for major thrombotic events and all-cause mortality.

Results: No patient developed clinically evident HIT, while 6 (6.4%) experienced thrombotic complications, and 22 (23.4%) passed away within 3 months after surgery. None of the patients with thrombotic complications tested positive for anti-PF4/H. Upon evaluating patients' seroconversion by day 5, six out of 94 (6.4%) patients tested positive for anti-PF4/H. Among them, three patients received bemiparin, two tinzaparin, and one enoxaparin. No statistically significant variance was observed in anti-PF4/H seroconversion between different types of LMWHs (p-value = 0.545) or in PLT count deviations (p-value = 0.990).

Conclusion: This pilot prospective study investigated anti-PF4/H production in older patients with hip fractures receiving different LMWHs. Preliminary results suggest that all tested anticoagulants have similar immunogenicity profiles in terms of PF4/H sensitization. These findings highlight the overall safety of LMWHs in elderly hip fracture patients. Moreover, the presence of anti-PF4/H appears unrelated to PLT fluctuations, subsequent VTE events and early postoperative mortality.

Level of evidence: II

Keywords: Anti/ PF4, Femoral nail, Heparin, Hip fracture, HIT, LMWH

Introduction

A rare but potentially life-threatening complication of low molecular weight heparins (LWMHs) administration is heparin-induced thrombocytopenia (HIT), an immunological complication caused by antibodies against platelet factor 4-heparin complex (PF4/H) that bind and activate platelets (PLTs).[1,2] HIT presents primarily in two types: Type I is a non-immunologic response to heparin treatment, characterized by mild transient thrombocytopenia without thrombotic consequences, while type II represents a severe

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immunologic drug reaction. In type II, antibodies develop against the antigenic complex PF4/H, leading to platelet activation and subsequently activation of the coagulation cascade.[3,4] For the diagnosis of HIT, the 4T scoring system considers the magnitude and timing of the PLTs decrease, the association with venous thromboembolism (VTE) and the presence or absence of alternative causes of thrombocytopenia.[5] HIT is reported to occur in approximately 0.1–5% of patients exposed to heparin, with variability depending on clinical settings and heparin type. In elderly patients undergoing hip fracture surgery, the incidence of HIT is estimated to be lower, around 0.5–1%, due to reduced immunogenic response in this population.[3,4] However, when HIT occurs, it is associated with significant morbidity, including thrombotic events in up to 50% of cases, and mortality rates ranging from 10–30% depending on the severity of complications and the timeliness of intervention. Although clinically evident HIT rarely occurs in patients treated with LMWHs, anti-PF4/H develops in up to 25% of them. The immunogenicity of LMWHs differs between different regimens, and even batches of products can be affected by impurities.[6-8] There is lack of evidence regarding the clinical significance of these antibodies in patients suffering hip fractures with or without clinically evident HIT.

Data regarding anti-PF4/H seroconversion in geriatric orthopedic patients and in particular hip fracture patients receiving thromboprophylaxis and its clinical significance is scarce. Warkentin et al investigated several clinical factors affecting the risk of anti-PF4/H formation in different clinical settings and reported that patients undergoing hip fracture surgery displayed higher seroconversion rates compared to those undergoing elective hip operation; indicating that antibody levels are likely to be elevated secondary to trauma.[9] However, no correlation with clinical outcome or postoperative mortality has been established. Also, it remains unclear whether different LMWH molecules have distinct effects on the immune response against PF4/H complexes. To date, no study has established differences in the production of anti-PF4/H among various LMWHs and its impact on VTE complications and mortality in geriatric hip fracture patients.

This pilot prospective study aims to evaluate the early production of anti-PF4/H in elderly patients undergoing hip fracture surgery. Three different LMWH prophylaxis regimens were compared in terms of anti-PF4/H production. We also assessed whether the production of anti-PF4/H is related to thrombocytopenia, symptomatic VTE and early postoperative mortality. Specifically, our objectives were: 1) to determine the impact of overall LMWHs treatment on the production of anti-PF4/H and further seroconversion, 2) to compare the frequency of anti-PF4/H seroconversion among three LMWHs regimens (bemiparin, tinzaparin and enoxaparin), 3) to investigate whether the incidence of anti-PF4/H seroconversion is linked to fluctuations in PLT levels, and finally and 4) to investigate whether different LMWHs are associated with fluctuations in PLT levels, major thrombotic events, and early (up to 3 months) postoperative mortality.

Materials and Methods

Population sample

One hundred consecutive patients, older than 65 years undergoing operative treatment for intertrochanteric hip fracture caused by a low-energy fall and receiving prophylactic subcutaneous administration of LMWH, were prospectively included in this study. Written informed consent was obtained from each patient and the study protocol was approved by the Ethics Committee of the hospital (Ref. No 61/5-10/22-06-2020).

Patients with exposure to unfractionated heparins (UFHs) or LMWHs during the last three months and patients with pre-existing thrombocytopenia, active neoplastic disease, active COVID-19 infection or high-energy trauma were excluded from this study. In total, six patients were excluded: two patients because of thrombocytopenia at admission, one because of positive anti-PF4/H (6.7 U/ml) at admission, and three were lost during the follow-up. Overall, ninety-four patients were available for the final assessment.

Patients' characteristics are shown in Table 1 [Table 1]. Twenty-seven patients (28.7%) received bemiparin 3500 international units (IU) anti–Xa, 42 patients (44.6%) enoxaparin 4000 IU anti–Xa and 25 (26.5%) tinzaparin 4500 IU anti–Xa. Drug selection was random, depending on the preference of the attending surgeon. Age, gender, differences between days 1 and 5 in PF4/H antibodies and PLT number, the presence or absence of thrombotic events, and mortality within three months after surgery were recorded for each group. The 4T scoring system was used for the diagnosis of HIT. In particular, the resultant clinical probability was divided into high (6–8 points), intermediate (4–5 points), and low risk (≤ 3 points) groups.[5]

Table 1. Patients' characteristics

LMWH	Gender	Mean age (range, 95% C.I.)
Enoxaparin	30 F 12 M	82,74 (65 – 90, 80,58 – 84,89)
Bemiparin	22 F 5 M	86,26 (73 – 92, 84,09 – 88,43)
Tinzaparin	18 F	84,52 (66 – 92, 81,75 – 87,29)

M (males), F (females)

All fractures were treated with close reduction and fracture fixation with the proximal femoral nail (G-nail) (Stryker, MI, USA).

The levels of anti-PF4/H, as well as the number of PLTs in the plasma, were measured for each patient on the admission day (before treatment initiation) and on day 5 following the initiation of LMWH administration. The variation (Δ parameter) for the two aforementioned variables (anti- PF4/ H and PLT difference) between days 1 and 5, as well as the development of seroconversion and or thrombocytopenia, were calculated. Additionally, the 4T score was applied in all patients with seroconversion, thrombocytopenia or thrombosis on day 5. The patients were followed up for three months, during which major thrombotic events and all-cause mortality were recorded.

Blood samples analysis

Citrated plasma samples were collected at baseline before treatment initiation (day 1) and at day 5 and stored at -70°C.

Antibodies against PF4/ H complex were measured using a commercially available semi-quantitative latex-enhanced immunoassay, HemosIL® HIT-Ab (PF4-H) analyzed by the ACL TOP 750 analyzer (Instrumentation Laboratory, Bedford, MA, USA).[10]

Statistical Analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 28 program (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp). The patients were randomized into three groups based on the specific LMWH regimen (bemiparin, tinzaparin and enoxaparin). Age, gender, differences between days 1 and 5 in PF4/H antibodies and PLT number, the presence or absence of thrombotic events, and mortality within three months after surgery were recorded for each group. Homogeneity of the three groups of patients in terms of age and gender was tested using the one-way analysis of variance (ANOVA) and Pearson's chi-square test respectively. The assessment of normality of the parametric values was performed via the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Levene's test was applied to assess homogeneity in anti-PF4/H and PLT differences among the groups receiving the three medications. One sample Wilcoxon signed-rank test was used to test the impact of LMWH on the PF4/H antibodies seroconversion. Given the non-Gaussian distribution of differences in PF4/H antibodies and PLT numbers, the Kruskal-Wallis H test for nonparametric independent samples was employed to identify whether any of the included medications had more pronounced impact on the changes in anti-PF4/H and PLT. Nonlinear regression was used to evaluate the correlation between the differences in anti-PF4/H and PLTs. An ordinal regression model was applied to investigate whether there is any correlation between major thrombotic events, as well as mortality within three months and the received medication.

Additionally, statistical analysis was conducted with the assumption that the anti-PF4/H is a nominal variable (with the cut-off value set at 1 U/ml). Pearson's chi-square test was used to identify if there is any difference regarding the anti-PF4/H seroconversion among the three LMWHs. The Student's t–test was used to identify whether the anti-PF4/H seroconversion was associated with the presence of thrombocytopenia. The correlation of a major thrombotic event (DVT or PE) to the age, type of LMWH and anti-PF4/H Δ parameter was investigated using a logistic regression model. Logistic regression was also performed to study the correlation of the less than three months mortality to the LMWH product, age, anti-PF4/H Δ parameter, gender, PLTs difference and a major thrombotic event.

Results

Overall, 6 (6.4%) patients tested positive for anti-PF4/H on the 5th day following LMWHs thromboprophylaxis administration. Three patients were commenced on bemiparin (2.6-5.6 U/ml), two on tinzaparin (1.1-1.4 U/ml), and one with a very high antibody titer (14.1 U/ml), on enoxaparin. Figures 1 and 2 present box plots illustrating the differences in anti-PF4/H and PLT numbers for the three LMWHs, respectively [Figure 1, Figure 2]. No statistically significant difference was found among the three regimens of LMWHs in terms of age and gender (p-value: 0.186 and 0.612 respectively). The Kolmogorov-Smirnov and the Shapiro-Wilk tests indicated that the differences in anti-PF4/H and PLT numbers were not normally distributed across all groups (p-value< 0.001). Levene's test showed homogeneity in the anti-PF4/ H and PLT differences among the three medications (p-value: 0.477 and 0.112 respectively). No patient developed clinical HIT based on the calculated 4T score (low-risk probability <3). However, six (6.4%) patients experienced clinical thrombotic complications (2 patients DVT, 3 PE and 1 both DVT & PE), and 22 (23.4%) passed away within three months after surgery. None of the patients who developed thrombotic complications tested positive for anti-PF4/H.

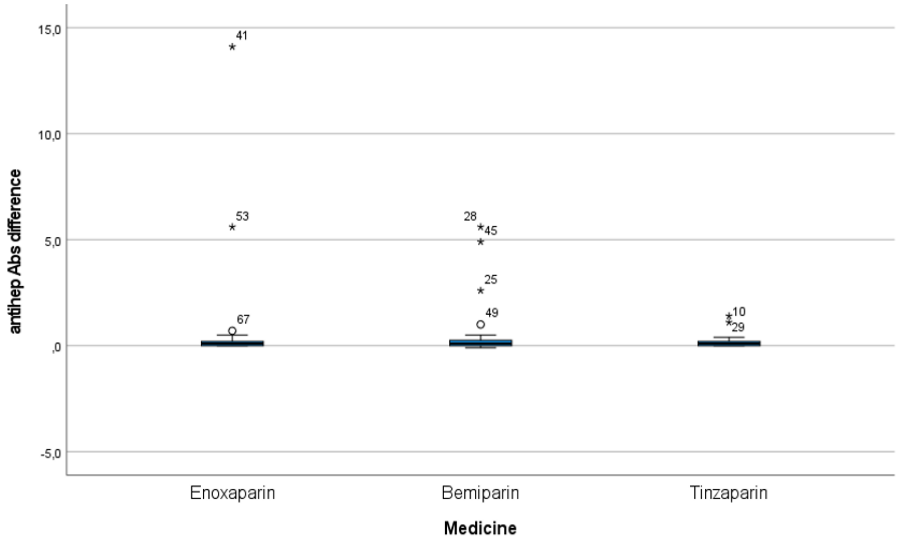

Figure 1. Box plots of the PF4-H antibody Δ-parameter between day 1 and 5 for each one of the LMWH studied

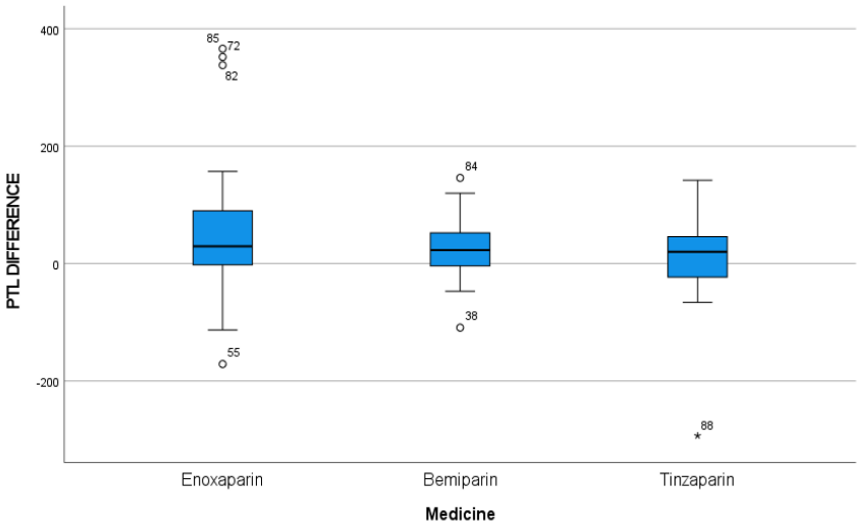

Figure 2. Box plots of PLT Δ-parameter between day 1 and 5 for each one of the LMWH studied

For bemiparin, tinzaparin and enoxaparin, the mean anti-PF4/H difference was 0.611 (95% C.I.: 0.042 –1.180), 0,188 (95% C.I.: 0.048 –0.328) and 0.598 (95% C.I.: -0.12 –1.315), while the mean PLT difference 28.41 (95% C.I.: 7.37–49.45), 7.88 (95% C.I.: -27.03–42.79) and 47.88 (95% C.I.: 14.39–81.37), respectively. A statistically significant increase in anti-PF4/H production on day 5 irrespective of the given regimen was found, as determined by the one sample Wilcoxon test (p-value< 0.001). The Kruskal-Wallis H test revealed no statistically significant difference in anti-PF4/H production among the tested drugs (x2= 0.190, p-value= 0.910), with a mean rank anti-PF4/H difference of 49.31 for bemiparin, 46.42 for tinzaparin and 46.98 for enoxaparin. Similarly, there was no statistically significant difference regarding the PLT numbers between the different regimens (x2=1.923, p=0.382), with mean rank PLT difference of 48.37 for bemiparin, 41.22 for tinzaparin and 50.68 for enoxaparin.

Nonlinear regression analysis was conducted to assess the correlation between anti-PF4/H differences and PLT differences. The scatter plot in Figure 3 displays the best-fitted line of the nonlinear model, with R^2 of 0.007 (p-value: 0.786), suggesting no significant correlation between anti-PF4/H differences and PLT differences [Figure 3]. The ordinal regression model showed that neither the type of LMWHs regimen, the age nor the difference in anti-PF4/H had significant influence on the presence of major thrombotic events (OR - p-value: 0,427 - 0.690, 0,412 - 0.268 and 0,504 - 0.566 for bemiparin, tinzaparin and enoxaparin, respectively). Similarly, the same model showed that early postoperative mortality was not significantly affected by the type of LMWH (OR - p-value: 0,273 - 0.211), the age of the patient (p-value: 0.06), the gender (OR - p-value: 0,767 - 0.831), the Δ in anti-PF4/H (p-value: 0.275), the Δ in PLTs (p-value: 0.398), or any major thrombotic event (OR - p-value: 0,01 - 0.999).

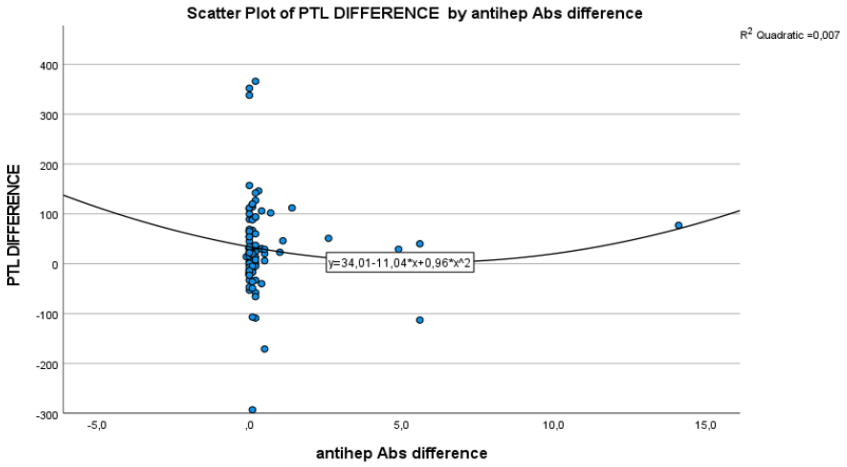

Figure 3. Nonlinear regression model for the correlation of the anti-PF4/H difference to the PLT difference

Upon evaluating patients' seroconversion by day 5, no statistically significant difference was observed in anti-PF4/H seroconversion between different types of LMWH (p-value= 0.545) or in platelet count deviations (p-value= 0.990). The logistic regression analysis indicated that the presence of a major thrombotic event was not significantly influenced by seroconversion on day 5, the type of LMWH used, or the patients' age (R2= 0.099). Finally, the LMWH given, the age, the seroconversion, the gender and a major thrombotic event were not found to have a significant impact to the early (up to 3 months) mortality (R2= 0.180).

Discussion

This prospective pilot study aimed to investigate the immunogenicity of various LMWHs and the incidence of anti-PF4/H seroconversion in older patients surgically treated due to intertrochanteric hip fracture and receiving three distinct regimens of LMWHs (bemiparin, tinzaparin and enoxaparin) for VTE prophylaxis. Additionally, the study sought to determine whether any of the tested LMWHs exhibited any differences in terms of safety and effectiveness. Hence, the anti-PF4/H production was correlated, in each of these 3 patients' groups, with alterations in PLT levels, symptomatic VTE events and early (up to 3 months) postoperative mortality. The elderly population is generally expected to exhibit lower antibody production, while the development of non-pathogenic PF4/H antibodies following exposure to LMWH has not been extensively investigated within this orthopedic population sample.[10,11]

The present study findings indicate that all tested LMWHs similarly induce the production of anti-PF4/H antibodies, which is statistically significant compared to pre-treatment levels. However, the development of anti-PF4/H antibodies did not lead to any clinical consequences, such as thrombocytopenia, symptomatic VTE, or early postoperative mortality.

Currently, routine screening for anti-PF4/H antibodies in patients without clinical signs of HIT is not recommended; however, recent data suggests that under certain circumstances or trigger events, these antibodies may become pathogenic or cause immune dysregulation.[12-14] The widespread adoption of immunoassays for detecting anti-PF4/H has highlighted that asymptomatic immune responses to PF4/H may occur significantly more frequently than clinical complications of HIT, such as thrombocytopenia and/or thrombosis.[15] Moreover, healthy individuals are also susceptible to anti-PF4/H production when exposed to UFHs and LMWHs with no subsequent thrombotic sequelae.[16,17] Our results align with studies that support the absence of a relationship between nonpathogenic anti-PF4/H production and adverse thrombotic effects in patients without any clinical evidence of HIT.[18,19] Nevertheless, there are other studies supporting the development of anti-PF4/H after UFH exposure, even in patients without clinical HIT syndrome, which may be related to a higher risk of symptomatic VTE events.[20,21] In this study, anti-PF4/H antibodies were detected using a semi-quantitative latex-enhanced immunoassay, which is widely used for its simplicity and accessibility. However, alternative immunoassays, such as enzyme-linked immunosorbent assays (ELISAs) or functional platelet activation assays (e.g., serotonin release assay), offer different sensitivities and specificities in detecting anti-PF4/H antibodies. [15,17,20]

It remains unclear whether the findings from these studies can be generalized to trauma patient populations. In a randomized, double-blind study, 614 trauma patients receiving LMWH or UFH for thromboprophylaxis were assessed for anti-PF4/H seroconversion, HIT, and VTE thrombosis according to the type of surgery (major versus minor).[22] The risk for seroconversion was higher in major surgery patients, as was the risk for HIT. LMWH treatment resulted in less frequent anti-PF4/H seroconversion and HIT events compared to UFH thromboprophylaxis. After minor surgery, no case of HIT occurred. The authors concluded that the severity of trauma and the need for major surgery strongly influence the risk of anti-PF4/H production, which is further increased by UFH.[22]

Studies in orthopedic patients are scarce and have been conducted almost entirely in patients undergoing total hip or total knee arthroplasty. Motokawa et al. reported the incidence of symptomatic VTE in 374 Japanese patients undergoing total hip or total knee reconstruction surgery under different anticoagulant prophylaxis therapeutics, who tested positive for IgG-class PF4/H antibodies.[23] The seroconversion incidence of anti-PF4/H was higher in patients receiving UFH (32.7%) when compared to those receiving LMWHs (9.5%) or fondaparinux (14.8%). Seroconversion of the IgG-class PF4/H antibodies was identified as an independent risk factor for symptomatic VTE.[23] Similar research had previously reported that the seroconversion of anti-PF4/H triggered by UFH is associated with a high risk of VTE in patients undergoing elective joint replacement surgery.[23] In a larger cohort of patients involving 2,726 patients undergoing total knee and hip arthroplasty, Warkentin et al, found that the frequency of forming anti-PF4/H was the same for patients receiving fondaparinux or enoxaparin suggesting that both drugs share similar immunogenicity patterns.[24] Griffin et al found that anti-PF4/H production is higher in hip fracture patients when treated with UFH compared to enoxaparin; however, the authors did not examine any correlation between their findings with VTE complications.[25] There appears to be a notable gap in the literature regarding direct comparisons of different LMWH products and their impact on anti-PF4/H antibody production. Even data from pharmacovigilance databases do not provide sufficient evidence to safely extract direct information regarding differences in the incidence of HIT between the various LMWH formulations.[26]

The mortality rate in our study is comparable to the literature, however, VTE clinical complications were much higher (6.4%) than previously reported data (1.5–2.5%).[27-29] Our findings align more with a recent cohort of 5,184 patients who received postoperative thromboprophylaxis for hip fracture. In this cohort, LMWH was administered for 35 days in 87% of cases and the risk of VTE was reported to be 4.7±0.5% at 3 months.[30] The authors suggested that the difference in their results was explained by the use of larger

databases in the previous studies, which was probably more prone to miss VTE events, and that many of the previous studies did not take into consideration mortality in their statistical analysis. We believe that higher VTE rates observed in our study can be attributed to the closer follow-up of the patients.

Our study is subject to several limitations that should be taken into consideration. Firstly, the relatively small size of the cohort (n=94) and the even fewer anti-PF4/H positive patients (three patients who were receiving bemiparin, two tinzaparin, and one patient enoxaparin) limit our capability to extract solid conclusions. Power analysis typically used for statistical validity could not be applied due to the absence of similar studies. Secondly, anti-PF4/H seroconversion was assessed relatively early (on the 5th day following LMWHs initiation). Ideally, a more comprehensive understanding of antibody kinetics could be achieved by extending the follow-up to one month. Furthermore, the presence of antibodies was detected using only one immunological assay. A comparative analysis with other immunological assays and more importantly, confirmation with functional assays could offer a more comprehensive approach. Fourthly, the effect of the surgical intervention was not taken into consideration. Finally, data regarding previous COVID- 19 infection or vaccination, medications, comorbidities, and the history of previous exposure to heparin were not obtained for this study. Nevertheless, this represents a pilot study, offering some valuable insights into the immunogenicity of various LMWHs and the incidence of anti-PF4/H seroconversion in older patients with intertrochanteric hip fractures. Future studies should also take into account the impact of age, particularly comparing patients older and younger than 65 years, as well as the differences between low-energy and high-energy trauma. Comparative cohort or multi-center studies could help clarify these factors' roles in seroconversion and clinical outcomes. Additionally, larger studies are needed to validate these findings and to explore other biomarkers or assays that could provide deeper insights into HIT in this patient population. Moreover, parameters such as comorbidities and concurrent medications may influence seroconversion and should be considered in the design of future research.

Conclusion

The present pilot study has demonstrated comparable anti-PF4/H production in older patients with hip fractures receiving different LMWH prophylaxis (bemiparin, tinzaparin and enoxaparin) and suggests that all tested anticoagulants have similar immunogenicity profiles in terms of PF4/H sensitization, with tinzaparin showing the best immunogenicity profile without reaching statistical significance though. Additionally, the presence of anti-PF4/H seems to be unrelated to PLT fluctuations, subsequent VTE events and early postoperative mortality. Considering that HIT diagnosis is mainly clinical, seroconversion was not correlated with the 4T score. This implies that the observed antibodies may not exert clinical impact; thereby, underscoring the overall safety and equivalence of the tested LMWHs. A more extensive investigation involving larger number of patients and employing diverse laboratory methodologies is essential to comprehensively assess the significance if any of the presence of these non-pathogenic antibodies.

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