CLINICAL SCIENCE

PRESENCE OF ANTI-TF4/HEPARIN ANTIBODIES IN PATIENTS PREOPERATIVELY TREATED WITH ENOXAPARINE AFTER ORTHOPEDIC SURGERY

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Abstract

Heparin-induced thrombocytopenia (HIT) is a condition caused by antibodies against the platelet factor 4 (PF4)/heparin complex. This significantly increases the risk of bleeding and thrombosis in patients, which is essential in the postoperative period. In this study we examined the rate of seroconversion of anti-PF4/heparin antibodies in patients with rheumatoid arthritis (RA) and osteoarthritis (OA) after total knee or hip arthroplasty. The aims of the study were to assess the risk of HIT by evaluation of induction of anti-PF4/heparin antibodies in patients with RA and OA after total knee or hip arthroplasty, treated prophylactically with enoxaparine. Material and methods: We followed 36 patients aged 18 to 80 years, after total knee or hip arthroplasty, treated prophylactically with enoxaparine. Patients were divided in two groups: patients with RA and patients with OA. They were examined for occurrence of HIT. Blood was sampled twice, from a peripheral vein, for immunologic tests. The first time it was done before enoxaparine application and the second time postoperatively 10 days after surgery. We noted demographic data, anti-PF4/heparin antibodies, erythrocyte sedimentation rate (ESR), CRP, RF, antiCCP and anti-nuclear antibodies Hep2 (ANA). Results: There was no significant difference in the values of anti-PF4/heparin antibodies in patients with RA and OA preoperatively. The presence of anti-PF4/heparin antibodies was significantly lower in RA patients compared to OA (7.14% versus 27.27%, p=0.034). There was no significant association between levels of anti-PF4/heparin antibodies and ESR, CRP, RF, CCP, ANA. Conclusion: The results obtained showed a lower level of anti-PF4/heparin antibodies in patients with RA than in patients with OA. This shows that there may be a difference in the generation of this antibody in patients with RA compared to patients with OA, prophylactically treated with enoxaparine after total knee or hip arthroplasty.

КЛИНИЧКИ ИСТРАЖУВАЊА

ПРИСУСТВО НА АНТИ-ТФ4/ХЕПАРИН АНТИТЕЛА КАЈ ПАЦИЕНТИ ПРОФИЛАКТИЧКИ ТРЕТИРАНИ СО ЕНОКСАПАРИН ПО ОРТОПЕДСКИ ОПЕРАТИВЕН ЗАФАТ

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Клучни зборови: хепарин-индуцирана тромбоцитопенија, анти-ПФ4/хепарин антитела, имплантација на протеза на колк или колено, еноксапарин, постоперативен ризик

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Извадок

Хепарин-индуцираната тромбоцитопенија (ХИТ) е предизвикана од антитела кон тромбоцитниот фактор 4 (ТФ4)/хепарин комплексот. Таа значително го зголемува ризикот од крвавење и тромбоза кај пациентите, што е особено есенцијално во постоперативниот период. Во оваа студија ја проценуваме стапката на сероконверзија на анти-ТФ4/хепарин антителата кај пациенти со ревматоиден артритис и остеоартритис по имплантација на протеза на колк или колено лекувани профилактички со еноксапарин. Целта на истражувањето беш да се процени ризикот од хепарин-индуцирана тромбоцитопенија преку евалуација на индукцијата на анти-ТФ4/хепарин антителата кај пациенти со ревматоиден артритис и остеоартритис по имплантација на протеза на колена или колк, лекувани профилактички со еноксапарин. Материјал и методи: Беа испитани 36 пациенти, на возраст од 18 до 80 години, по имплантација на протеза на колк или колено, лекувани профилактички со еноксапарин. Пациентите беа поделени во две еднакви групи, односно пациенти со ревматоиден артритис (РА) и пациенти со остеоартритис (ОА). Пациентите беа следени за време на хоспитализацијата на Клиниката за ортопедски болести за појава на ХИТ. Во два наврата беше земена венска крв, од периферна вена, за имунолошки иследувања. Прв пат тоа беше направено пред почеток на лекување со еноксапарин, а втор пат постоперативно, 10 дена по оперативниот зафат. Беа нотирани демографски податоци, анти-ТФ4/хепарин антитела, седиментација на еритроцити (ESR), CRP, RF, CCP, ANA, појава на ХИТ. Резултати: Немаше сигнификантна разлика во вредностите на анти-ТФ4/хепарин кај пациентите со ОА и РА предоперативно. Стапката на анти-ТФ4/хепарин антитела кај пациентите со РА беше сигнификантно пониска од онаа кај пациентите со ОА (7,14% наспроти 27,27%, p=0,034). Немаше сигнификантна поврзаност на вредностите на анти-ТФ/4 антителото со вредностите на ESR, CRP, RF, CCP или ANA. Заклучок: Резултатите укажаа на намалена инциденција на анти-ТФ4/хепарин антитела кај пациентите со РА во споредба со оние со ОА. Ова укажува дека постои разлика во анти-ТФ4/хепарин имуниот одговор кај пациенти со РА наспроти оние со ОА, профилактички лекувани со еноксапарин, по ортопедски оперативен зафат за имплантација на протеза на колк или колено.

Introduction

Heparin-induced thrombocytopenia (HIT) is an immunologic condition which can develop in patients treated with heparin ([1]), through the generation of antibodies which recognize the complex between platelet factor-4 (PF4) and heparin[2]. PF4 is released rapidly after platelet activation and binds to heparin, forming PF4-heparin complexes[3]. These molecules elicit an immune response, thus the creation of anti-PF4/heparin antibodies[4]. Recent data suggests that these antibodies bind to the PF4/heparin complexes and activate platelets thus accelerating the process of coagulation[5]. There is data showing the presence of anti-PF4/heparin antibodies in patients who have not received heparin[6]. PF4 could also be an antigen target for autoimmune diseases[7]. Anti-PF4/heparin antibodies could be induced in patients after major surgery without exposure to heparin[8]. HIT is even discovered after the postoperative prophylactic use of fondaparinux, an inhibitor of factor Xa[9]. Studies have reported the existence of "spontaneous" HIT, potentially caused by inflammation or infection[10]. Crauel et al. showed an association between bacterial infections and occurrence of anti-PF4/heparin antibodies[11]. These were present in about 20% of patients after total knee arthroplasty[12]. Besides the routine prophylactic use of heparin products in these patients, the prothesis itself is a major challenge for the immune system because of the mechanical damage to bone and connective tissue during the surgery[13].

Compared to different surgical procedures, arthroplasty results in a high postoperative incidence of anti-PF4/heparin antibodies. The procedure itself can induce their creation in patients with RA (which is an autoimmune disease by itself) or in patients with OA. The presence of anti-PF4/heparin antibodies and its risks in patients with RA, treated prophylactically with enoxaparine after total knee or hip arthroplasty has not been well examined.

We investigated the induction of anti-PF4/heparin antibodies in patients with RA and OA treated prophylactically with enoxaparine after total knee or hip arthroplasty and its potential association with erythrocyte sedimentation rate in the first hour (ESR), C-reactive protein (CRP), rheumatoid factor (RF), anti-CCP (anti-cyclic citrullinated peptide), antinuclear antibodies by Hep2 (ANA).

The aims of this study were to examine the induction of anti-PF4/heparin antibodies in patients with RA and OA after total knee or hip arthroplasty, prophylactically treated with enoxaparine. We also examined whether there was any association in the induction of these antibodies and ESR, CRP, RF, anti-CCP, ANA. This was done with the goal to recommend an optimal model for postoperative management of these patients.

Material and methods

We investigated the induction of anti-PF4/heparin antibodies in patients with RA and OA, prophylactically treated with enoxaparine after total hip or knee arthroplasty and its association with ESR, CRP, RF, anti-CCP, ANA. The study was conducted at PHI University Clinic for TOARILUC where recruitment and follow-up of

patients was done. Laboratory tests were conducted at the immunology laboratory of the University Clinic for Rheumatology.

The study included patients aged 18 to 80 years, previously diagnosed with RA or OA, hospitalized at TOARILUC Department of Orthopedic Surgery for total knee or hip arthroplasty, prophylactically treated with enoxaparine. All patients were informed about the goals and procedures involved in the study and signed an informed consent form before being included.

A total of 36 patients were divided into two groups, patients with OA and RA. The groups were comparable in regards to size, age and sex distribution.

Patients with an infection, thrombosis on admission to hospital, those treated with heparin in the last month before admission or patients with other autoimmune diseases such as systemic lupus, systemic sclerosis, sarcoidosis, Lyme borreliosis, etc. were not included in the study.

After a detailed anesthesiologic examination, detailed demographic data were collected. Blood was collected preoperatively from a peripheral vein for anti-PF4/heparin antibodies, ESR, CRP, RF, anti-CCP, ANA. Postoperatively blood was collected on the tenth day of enoxaparine prophylaxis for anti-PF4/heparin antibodies, ESR, CRP, RF, anti-CCP, ANA.

The blood was analyzed at the immunology laboratory of the University Clinic for Rheumatology in Skopje. RF and CRP test were done with a BioSystem A15 biochemical analyzer. The levels of anti-CCP antibodies was done with Elisis Duo (Human) ELISA analyzer, while for the anti-PF4/heparin antibodies a Mindray MR-96A ELISA analyzer was used. Antinuclear antibodies were tested by immunofluorescence by the same certified physician on an Olympus CX31 immunofluorescence microscope.

During the hospitalization all patients received standard follow-up regarding blood tests and physical examination. Patients were assessed for the emergence of HIT, which was done using the 4T test.

Data were entered into an electronic database and analyzed by SPSS, v19.1 (SPSS, Chicago, IL, USA). Comparison was done with the Student's t-and Chi-square tests. We used a multivariant logistic regression to identify independent risk factors for the induction of anti-PF4/heparin antibodies. Correlation was assessed using the Pearson's analysis of correlation. P values <0.05 were considered statistically significant.

Results

Patient's average age was 70.1 +/- 9.23 in the RA group and 72.7 +/- 7.5 years in the OA group. In the RA group 2 (14.28%) were men and 12 (85.72%) women, while in the OA group 7 (31.82%) were men and 15 (68.18%) women. All patients with RA were previously diagnosed according to the EULAR 2010 criteria. Of these patients, 10 (71.43%) were anti-CCP positive, 9 (64.29%) were positive for RF IgG and 2 (14.29%) were ANA Hep2 positive. Twelve patients (85.71%) with RA were treated with disease-modifying antirheumatic drugs (DMARDs) according to the definition of EULAR (biologic, methotrexate, leflunomide, sulfasalazine or antimalarial).

Table 1. Postoperative occurrence of anti-PF4/heparin antibodies. Demographic, clinical, serologic and immunologic characteristics

	Rheumatoid arthritis Postoperative conversion			Osteoarthritis Postoperative conversion		
	Positive n=1	Negative n=13	P value	Positive n=6	Negative n=16	P value
Sex (male/female)	0/1	2/11	0.584	2/4	5/11	0.291
Age +/- SD	66.7 +/- 9.2	70.4 +/- 7.99	0.630	72.9 +/- 7.6	72.6 +/- 7.5	0.601
BMI (kg/m2) +/- SD	24.8 +/- 1.1	25.2 +/- 3.8	0.901	28.1 +/- 3.8	27.6 +/- 3.9	0.599
RF IgG (IU/ml)	18.8 +/- 25.9	60.1 +/- 70.9	0.137	5.6 +/- 11.2	7.8 +/- 13.4	0.145
CRP (mg/dl)	1.62 +/- 1.13	1.32 +/- 1.45	0.316	0.15 +/- 1.01	0.21 +/- 0.96	0.532
ESR (mm/1 час)	54.4 +/- 23.4	58.1 +/- 30.1	0.334	61.2 +/- 21.1	59.4 +/- 16.9	0.312
Anti-CCP (mg/dl)	0.6 +/- 0.1	250 +/- 543.2	0.022	0.0 +/- 55.6	0.0 +/- 0.0	0.667
ANA (Hep2)	0/1 (0%)	2/13 (15.38%)	0.879	1/6 (16.67%)	2/16 (12.5%)	0.918
Use of DMARD	1/1 (100%)	11/13 (84.61%)	0.856	0/6 (0%)	0/16 (0%)	NA
HIT	0/2 (0%)	0/13 (0%)	NA	0/6 (0%)	0/16 (0%)	NA

There were no anti-CCP positive patients in the OA group, while 2 (9.09%) were RF IgG positive and 3 (13.64%) were ANA Hep2 positive.

We compared the presence of anti-PF4/heparin antibodies in both groups. The rate of postoperative conversion was significantly lower in patients with RA compared to patients in OA group (7.14% versus 27.27%, p=0.034). There was no statistical significance in the association of anti-PF4/heparin antibody incidence and ESR, CRP, RF, anti-CCP or ANA.

There were no patients diagnosed with heparin-induced thrombocytopenia in the study period.

Discussion

In this study we examined the occurrence of anti-PF4/heparin antibodies in patients with RA and OA after surgery, prophylactically treated with enoxaparine. Previous studies have shown the presence of anti-PF4/heparin antibodies in patients with systemic erythematous lupus (SLE) and antiphospholipid syndrome[5,14]. To date there are very few studies examining the association of anti-PF4/heparin antibodies with other diseases and risk factors such as RA, OA and knee or hip arthroplasty. Izumi et al. presented data that the generation of anti-PF4/heparin antibodies was reduced in patients with RA, compared to patients with OA after total knee arthroplasty, prophylactically treated with edoxaban[15]. According to this study, 25.5% of patients with OA after total knee arthroplasty were positive for anti-PF4/heparin antibodies. This correlates well with our data, since we observed a lower postoperative seroconversion in the RA compared to the OA group (7.14% versus 27.27%, p=0.034).

Heparin-induced thrombocytopenia is caused by antibodies against the complex of platelet factor-4 and heparin 2. Heparin has high affinity to-

wards PF4 and after binding together they become a center of a powerful antigen stimulation, with the creation of anti-PF4/heparin antibodies (16). The presence of these antibodies is shown in patients after arthroplasty who have not been treated with heparin products[17]. It is considered to be an effect of the postoperative inflammatory process[18].

There are several theories regarding the lower incidence of anti-PF4/heparin antibodies in patients with RA. According to Ohayama et al.[19] the serum of patients with RA has many immune complexes containing PF4, so this molecule is much less available for the formation of anti-PF4/heparin antibodies. Other studies 15 present the opinion that treatment with DMARDs causes immunomodulation and immunosuppression which may be the reason for the lower generation of anti-PF4/heparin antibodies. Brauweiler et al. demonstrated the presence of B-cell anergy in patients with autoimmune inflammatory diseases because of which the generation of anti-PF4/heparin antibodies was inhibited[20].

Our data did not show an association between the use of DMARDs and the induction of anti-PF4/heparin antibodies. Previous studies have presented data that 52% of patients with RA who were anti-CCP positive had immune complexes containing PF4[21,22]. It is possible that patients, especially those with high levels of anti-CCP and RF are preimmunized towards PF4 which inhibits the production of anti-PF4/heparin antibodies[22].

This study t showed no statistical significance between the presence of anti-PF4/heparin antibodies and inflammatory markers such as ESR and CRP, immunologic factors such as RF, anti-CCP, ANA or clinical elements BMI, or the use of DMARDs.

Conclusion

The rate of postoperative seroconversion is significantly higher in patients with OA compared to the RA group. This suggests that OA patients require more attention from clinicians especially regarding potential HIT, after total hip or knee arthroplasty, prophylactically treated with heparin products such as enoxaparine.

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