Incidence and clinical outcomes of heparin-induced thrombocytopenia: 11 year experience in a tertiary care university hospital

INCIDENCE AND CLINICAL OUTCOMES OF HEPARIN INDUCED THROMBOCYTOPENIA: 11 YEAR EXPERIENCE IN A TERTIARY CARE UNIVERSITY HOSPITAL.

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ABSTRACT

Background: heparin induced thrombocytopenia (HIT) is a rare immuno-mediated adverse reaction with high thrombotic and mortality risk.

Aims: to evaluate incidence and outcomes of HIT cases diagnosed at a tertiary care hospital from 2007 to 2018.

Materials and Methods: a retrospective study was conducted. Patients with suspected HIT underwent 4Ts score and anti-heparin PF4 IgG antibodies ELISA screening test. If the latter was positive, heparin induced platelet aggregation test (PAT) was performed. If the latter was positive, any form of heparin was stopped and alternative anticoagulants were started, and then overlapped with warfarin. HIT incidence was calculated by dividing HIT cases by the mean yearly number of admitted patients over 11 years. Follow-up was 90 days.

Results: Among 2125 screening tests, 90 (4.2%) were positive with confirmatory PAT in 82 (3.8%). Median age was 75, 39 patients were surgical and 51 medical. The median 4Ts score was 5. Unfractionated heparin was employed in 40 (44%). HIT incidence was 0.16/1000/patient/years (95% CI: 0.13-0.19) in surgical and 0.15/1000/patient/years (95%: 0.12-0.20) in medical patients. HIT with thrombosis (HIT-T) was observed in 31 patients (0.05/1000/patient/years 95% CI: 0.04-0.08), with venous thromboses in 25 (80%). HIT without thrombosis was observed in 59 patients (0.1/1000 patient/years; 95% CI: 0.08-0.13, 2 fold vs HIT-T). All cause mortality was 25.5% (95% CI: 17.6-35.4), major bleeding 5.5% (95% CI:1.8-12.5) and thromboembolic complications 3.3% (95% CI:1.1-9.3) **Conclusions**: HIT is a rare event with high mortality, in spite of the use of non heparin anticoagulants.

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- Heparin induced thrombocytopenia (HIT) is a rare immuno mediated adverse reaction
- HIT incidence varies according to the type of heparin and patient settings
- HIT is still associated with high mortality and thrombotic risk in spite of the use of alternative non heparin anticoagulants.

- Further studies are needed to improve HIT diagnosis and treatment.

Key words: heparin, low molecular weight heparin, heparin induced thrombocytopenia, argatroban, danaparoid.

INTRODUCTION

Heparin induced thrombocytopenia (HIT) is a rare immuno-mediated adverse reaction which can be associated with thrombotic events with a high mortality risk [1]. HIT is mediated by the formation of IgG antibodies against platelet factor 4 (PF4) complexes with polyanions such as heparin. Immunocomplexes of heparin/PF4 and IgG bind to the platelet receptor FcRγIIa with receptor cross-linking which can lead to an intense procoagulant response with the generation of platelet microparticles, monocyte and endothelial cell procoagulant activity [2]. Such process is associated with thrombocytopenia, due to intravascular consumption, and paradoxically with potential life-threatening thrombotic complications, but usually not with bleeding [3-6].

Clinical HIT develops in only a minority of patients with HIT-IgG, depending upon the patient population and heparin type, with unfractionated heparin (UFH) being the most immunogenic, and low molecular weight heparin (LMWH) less immunogenic [4].

Thromboembolic complications can affect any vascular bed, (HIT with thrombosis - HIT-T) although not all patients with HIT develop thrombosis [1]. Venous thromboses are the majority in medical and orthopaedic patients, whereas arterial and venous thromboses occur at a similar frequency in patients who have undergone cardiovascular surgery [1].

The prevalence of HIT depends both on the type and dose of heparin, and of patients admitted to different hospital settings (either medical or surgical). It is estimated that approximately one in 40 patients receiving UFH and one in 500 patients LMWH develops HIT [7-10] with reported prevalence varying from 1-5% and 0.1-1% in post-operative patients receiving UFH or LMWH, respectively; 1-3% in cardiac surgery patients , 1% in cancer patients, 0.1-1% in medical patients receiving UFH and 0.6% in patients receiving LMWH [11]. Such figures derive from studies evaluating patients enrolled in randomized clinical trials or prospective or retrospective studies conducted in 90' and first decade of 2000 with different criteria to diagnose HIT. More recently larger studies have been conducted based on national administrative databases in different settings in which however HIT diagnosis was based on ICD-9 CM codes. In these studies, HIT was

reported in 0.3%-0.4% of cardiac surgery procedures [12, 13], 0.49% of transcathether aortic valve replacements [14], 0.3% of vascular surgery procedures [15], 0.02% of total hip and 0.01% of total knee replacements [16] and 0.08% of solid malignancy hospitalized patients [17]. These data indicate that HIT prevalence and incidence in hospitalized patients vary according to the type of heparin preparation, patient setting, dose and duration of heparin, and criteria for HIT diagnosis. HIT diagnosis requires the assessment of pre-test clinical probability in combination with the measurement of pathogenic platelet activating IgG antibodies against PF4-heparin complexes with immunological and functional assays [18].

When HIT is diagnosed, all guidelines recommend stopping any form of heparin and starting a non heparin alternative anticoagulant [18]. Argatroban and danaparoid are currently the only drugs licensed for HIT, with different country availability, while lepirudin has been withdrawn in EU by the European Commission at the request of the manufacturer in 2012. Bivalirudin is an option in cardiac surgery and procedures in HIT patients. Fondaparinux and direct oral anticoagulants (DOACS) are also alternative non heparin anticoagulants, although they are not formally licensed for HIT treatment.

In spite of such treatments, the mortality rate is still reported high (8 to 20%) [1], with high bleeding and recurrent thrombosis risk. In a recent meta-analysis of 4698 patients, effectiveness and safety outcomes were similar among various anticoagulants. The pooled rates of thromboembolic events ranged from 1% (fondaparinux) to 7% (danaparoid), major bleeding from 1% (DOAC) to 14% (bivalirudin), and death from 7% (fondaparinux) to 19% (bivalirudin). Results were not influenced by patient population, diagnostic test used, study design, or type of article [19].

In addition, many clinicians may not be familiar with non heparin alternative anticoagulants such argatroban or bivalirudin as their use is a “niche”, they require strict laboratory monitoring and do not have reversal agents in case of bleeding and may pose risk of litigation issues. Fondaparinux and DOAC are more manageable as clinicians are more familiar with them, but their use may be limited to less severe HIT cases such those without extensive thrombosis.

The aims of our study were to evaluate HIT incidence, clinical characteristics and outcomes of a cohort of HIT cases among hospitalized patients in a large tertiary teaching care hospital.

MATERIALS AND METHODS.

We conducted a retrospective study in patients in whom HIT was diagnosed during hospital admission from Jan 1st 2007 to March 31st 2018 at S.Orsola-Malpighi University Hospital Research Institute IRCCS, University of Bologna, Bologna, Italy, a 1420 bed tertiary care teaching hospital. The study was approved by the Centralized Ethics Committee.

In case of suspected HIT, a standardized form was filled by the attending physician for the calculation of the pre-test probability with the 4Ts score [20] . Items to be filled were:1- patients demographic characteristics; 2- current/previous ward of admission; 3-date of platelet count before beginning of heparin; 3- any known heparin exposure in the preceding 3 months; 4- date of heparin initiation with type (UFH or LMWH) and dosage ; 5- indication for heparin – thromboprophylaxis or treatment or cardiac or vascular surgery or haemodialysis; 6- date of first platelet count decrease; 7- platelet count on the day of 30-50% decrease and on the day of blood sampling; 8-any overt thromboembolism; 9-any comorbidity; 10- possible alternative cause of thrombocytopenia; 11- date of blood sampling for HIT screening test. Such standardized form was sent along with the blood sample for HIT screening tests and the 4Ts score was calculated by the attending consultant of the Division of Angiology and Blood Coagulation. HIT screening tests were conducted within 24 hours in business days and in case they were positive, confirmatory tests were performed on the same day.

Patient venous blood samples were collected in 10 mL spray coated silica tubes with an a polymer gel for serum separation (BD Vacutaneir, Fisher Scientific, Rodano, Milan, Italy) and sent for the determination of anti-heparin PF4 IgG antibodies to the Specialized Coagulation Laboratory. After collection, whole blood was allowed to clot at room temperature and serum was prepared by centrifugation 20 min at 2.800 x g at controlled temperature (20°C). Serum was then collected and

transferred in a clean polypropylene tube and maintained at 2-8° C if handled and analyzed immediately, while 0.5 ml aliquots were stored at -80 ° C for further studies.

Enzyme-linked immunosorbent assay (ELISA) screening test was performed on the DSX ELISA processor (Technogenetics, Milan, Italy) using the PF4 IgG assay test kit (Immucor GTI Diagnostics Inc, Milan, Italy), according to the manufacturer's instructions [21].

A test was considered positive in case of Optical Density (OD) > 0.400, negative if OD < 0.400; dubious if OD = 0.400 (10).

In case of a positive ELISA test, the confirmatory heparin induced platelet aggregation test (PAT) was performed, according to Chong et al. [22]. Blood of 4–5 normal donors who had not taken aspirin or other antiplatelet drugs for at least 10 days was collected from the antecubital vein into 0.109 mM trisodium citrate; platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared by centrifugation for 20 min at controlled temperature at 150 and 2.500 x g, respectively. PAT was performed using the patient serum heated at 56°C for 30 min to inactivate complement and traces of thrombin; any precipitates formed were removed by centrifugation for 5 min at 5.000 x g at controlled temperature.

PAT was performed using a four channel Chrono-Log platelet aggregometer (model 540, Chrono-Log Corp. Havertown, PA, USA); the platelet count in PRP was standardized to 300 $x10^9$/L and PRP was stored at room temperature in capped tubes until use. After blanking the aggregometer with PPP, 290 µL of pre-heated serum was added to 160 µL of pre- warmed PRP in a stir bar containing cuvette. After 5 min to rule out spontaneous aggregation, 10 µL of UFH (1 and 100 IU/ml final concentration) was added to the cuvette and the aggregation response was monitored for 20 minutes. The presence of HIT was confirmed if the aggregation was > 20% with 1 IU/ml UFH and completely inhibited or < 20% with 100 IU/ml UFH. The presence of HIT was excluded if the platelet aggregation was < 20% with 1 IU/ml UFH or was not inhibited in the presence of 100 IU/ml UFH.

After HIT confirmation, in patients without signs or symptoms of overt thrombosis, lower and upper limb ultrasound was performed to exclude deep vein trombosis (DVT). Afterwards, the Angiology and Blood Coagulation attending consultant recommended stopping any form of heparin and starting non heparin alternative anticoagulants (either lepirudin from Jan 2007 to Dec 2011 when lepirudin was withdrawn from market, and then in the remaining patients argatroban or fondaparinux) which were then overlapped with warfarin when platelet count recovered (above 100-150.000 mm3), for at least 3 months.

All cause mortality was reported at 90 days with cause of death as assessed by the attending physician on the basis of clinical chart review performed by LB. Major bleeding was assessed according the ISTH criteria [23] by LB, as well as objectively diagnosed thromboembolic complications at 90 days on the basis of clinical charts review.

STATISTICAL ANALYSIS

Continuous variables were expressed as median and range. Categorical variables were expressed as percentage with 95% confidence intervals (95% CI according to Wilson score interval). The significance level was set at <=0.05.

The incidence of HIT was calculated by the formula: total HIT cases/ (mean yearly number of hospital admissions x years of observation). The mean yearly number of hospital admissions were based on the official figures provided by the hospital for the different wards. Day hospital and day surgery admissions were excluded.

Analysis was carried out using the SPSS software package (version 19.0; SPSS Inc. Chicago, Illinois, USA).

RESULTS

A total of 2125 ELISA screening tests (mean: 190/year) were performed from January 2007 to March 2018, among which 90 had OD values > 0.400 (4.2%). A positive confirmatory PAT was

observed in 82 (3.8%) while 8 had a negative confirmatory test (0.37%). Six patients in whom HIT was diagnosed were excluded from the analysis for missing data.

Table 1 shows patients characteristics.

There were more females than males (52%), median age was 75 years (range: 15-102). More patients were medical than surgical (51- 57%- vs 39 patients), 9 patients (10%) were from nephrology wards, 12 were cancer patients (13%). The 4TS score median value was 5 (intermediate probability) (range: 1-8). UFH was employed in 40 patients (44%, mostly in cardiovascular surgery or interventional cardiology or nephrology wards), while LWMH was employed in the remaining, mostly in orthopedic or general surgery or medical wards at prophylactic doses. ELISA median OD was 1.898 (range: 1.029-4.180), median platelet aggregation with was 63% (range: 20 -100%) with heparin 1 IU/mL and median of 5% (range:0-19%) with heparin 100 IU/mL. Median duration of hospitalization was 28 days (range 3-131).

All cause death at 90 days was recorded in 23 patients (25.5 %; 95% CI: 17.6-35.4; 18 ; 14 deaths occurring during hospitalization) of whom 8 died for metastatic cancer, 4 for heart failure, 2 for kidney failure, 2 for acute myocardial infarction, 2 for pneumonia with respiratory failure, 1 for ischemic stroke and 4 for bleeding. Among

Major bleeding events at 90 days were observed in 5 patients (5.5%; 95% CI:2.4-12.3, in 4 patients considered contributing to death), while recurrent thrombosis in 3 (3.33%; 95% CI:1.1-9.3) all during fondaparinux treatment.

Table 2 shows HIT incidence in different patient settings. It was 0.16/1000/ patient/years (95% CI:0.12-0.23) in all surgical patients, with the highest incidence among cardiac or vascular surgery or interventional cardiology patients (0.44/1000 patients/years; 95% CI:0.23-0.83) and in orthopedic surgery (0.85/1000/patient/years 95 CI%: 0.54-1.3, including those post-surgical patients transferred from the orthopedic/orthogeriatric ward to rehabilitation wards and intensive care), while it was lowest in general surgery (0.08/1000/ year 95% CI: 0.04-0.14).

The incidence of HIT in medical patients was 0.15/1000 patient/years (95% CI:0.12-2.2) while it was 0.8/1000/patient/years (95 CI%:0.4-1.5) in nephrology wards were all patients received UFH. Medical cancer patients had an observed incidence of HIT of 1.2/1000/patient/years (95% CI: 0.8-1.8).

Table 3 shows the characteristics of patients with HIT-T, which was observed in 31 subjects with a similar incidence among surgical and medical patients (Table 2). Among the HIT-T cases, venous thrombosis was observed in 25 while arterial thrombosis was observed in 6. Mortality at 90 days was 25.8% (95% CI:13.7-43); .major bleeding was 9.6% (95% CI:3.3-24) and recurrent thrombosis was 3.2%; (95% CI:0.05-16%).

Table 4 shows the characteristics of patients with HIT without thrombosis which was observed in 59 patients with a similar incidence in surgical and medical patients. All cause mortality at 90 days was 25.4 % (95% CI: 16.6-38); major bleeding was observed in 3.4% (95 CI: 0.93-12); 2 thromboembolic events were observed at 30 days (3.4 %; 95% CI: 0.9-12).

All cause mortality was similar from 2007 to 2011 (11 patients all treated with lepirudin) and from 2012 to 2018 (12 patients).

DISCUSSION

Our study confirms that HIT is a rare drug related adverse event , with high risk of mortality, and bleeding and thrombosis, in spite of the use of recommended non heparin alternative anticoagulants.

Our data are consistent with recent data reported in literature both regarding incidence and clinical characteristics and outcomes [11-17]. We observed a slight prevalence of women and, as expected, a higher incidence of HIT in subjects receiving UFH compared to LMWH. In particular cardiovascular surgery or interventional cardiology patients had a higher risk of HIT. A high risk was also observed in orthopedic surgery (most hip fractures) and in cancer patients in spite of the use of LMWH. General surgery patients had a lower risk of HIT.

All cause mortality at 90 days was attributed mostly to concomitant comorbid conditions, such as metastatic cancer, and in one case to HIT, while most major bleeding events were considered contributing to death.

Recurrent thrombosis at 90 days were observed in 3 patients treated with fondaparinux, 1 in a post-surgical cancer patients with acute limb ischemia undergoing limb amputation.

Our study has several limitations such as the retrospective single center design, the use of PAT for the confirmatory functional test and not the gold standard test, that is C-14 serotonin release assay. This approach may have potentially missed or overestimated HIT diagnosis in some of the patients included in this study. However PAT is an accepted confirmatory method with a rapid turnaround time [24]. In addition the median 4Ts score was intermediate and it was associated with an ELISA mean OD of 1.898, even in those patients with a negative platelet aggregation test , which were all patients without thrombosis and who were treated with non heparin alternative anticoagulants, on clinical grounds.

No inter-observer agreement for the 4Ts score was calculated between the ward attending physician and Division of Angiology and Blood Coagulation consultant.

Data regarding time to platelet recovery were not collected but vitamin K antagonists were overlapped with alternative anticoagulants when platelet count was at least 100.000 mm3. No post-mortem was available.

No data regarding thrombotic complications or death were collected in patients beyond 90 days.

Incidence was calculated on the basis of yearly admissions in different surgical and medical patients, as the overall number of patients exposed to heparin per year during hospital stay was not captured. As a result, HIT incidence may be underestimated and may fluctuate on the basis of varying type of hospital admissions. However, in our hospital all patients admitted to both surgical and medical wards are assessed for thromboprophylaxis and the majority of them receive LMWH during hospital stay.

Several strengths can be considered. Our study was conducted in a tertiary teaching hospital with many diverse surgical and medical wards and the referral center for cardiovascular surgery, where UFH is routinely used. The Specialized Coagulation Laboratory was the referral for HIT screening test. Mortality, bleeding complications and thrombotic complications were in line with those reported in the literature, regardless of treatment.

Our study confirms the rarity of drug adverse event, which may go unrecognized in settings where heparin is less used as well as the challenge of treatment, with high mortality and bleeding risk in spite of treatment with non alternative anticoagulants.

Stewardship programs have been advocated to raise awareness and ameliorate HIT clinical management [25]. Further studies are needed to improve HIT diagnosis as well as treatment.

Statements and declarations

The authors declare no conflict of interest related to the paper

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Table 1 Characteristics of all HIT patients

Total	N= 90
M/F (M%)	43/47 (48%)
Age: ; median, (range)	75; (15-102)
Surgical pts cardiac or vascular surgery or interventional cardiology orthopedic surgery general surgery	39 (43%); 9 18 12
Medical pts Nephrology/hemodialysis	51 (48%) 9 (10%)
Cancer patients surgical; medical	19 (13.3%) 8;11
4T' score: ;median (range) all pts 4T' score: median; (range) in 8 pts with neg confirmatory test	5 (1-8) 5 (3-8)
% platelet fall over baseline: median, (range)	67%; (11-95%)
Type of heparin UFH (prophylactic dose calcium sc;therapeutic dose ev or calcium sc) LMHW (prophylactic dose; therapeutic doses) Cases shifted from LMWH to UFH Heparin in the previous 3 months Interval between heparin initiation and blood sampling for suspected HIT days, median (range) Interval between heparin initiation and platelet decrease days median (range)	 40 (41%) (18; 22) 50 (35 ; 15) 3 34 11.5 (2-105) 9; (1-64)
ELISA screening test OD : , median (range) OD , median (range) in 8 pts with neg confirmatory test	 1.898 (1.029-4.180) 1.289 (1.144-2.300)
Positive aggregation test aggregation with heparin 1U /mL; median, (range) % aggregation with heparin 100 U/mL; median, (range)	82 (91%) 63% (20-100) 5% (0-19)
All cause mortality at 90 days Major bleeding at 90 days (1 in septic shock due to cholecistis, 1 with 2 gr HB loss, 2 haematuria 1 with rectus muscle haematoma, CID in uterine cancer, all contributing to death, 3 with lepirudin) Recurrent thrombosis at 30 days (all with fondaparinux, 1 with acute limb ischemia undergoing limb amputation)	23 (25.5 %; 95% CI: 17.6-35.4) 5 (5.5%; 95% CI:1.8-12.5) 3 (3.33%; 95% CI:1.1-9.3)

Table 2-HIT incidence , according to the type of patients setting

	Mean yearly admissions	HIT incidence n/ 1000/pts-year; (95% CI)
Type of patient setting (n HIT cases)	52.171	---
Surgical (39)	21.535	0.16 (0.12-0.23)
cardiac or vascular surgery or interventional cardiology (9)	1875	0.44 (0.23-0.83)
orthopedic surgery (18)	1935	0.85 (0.54-1.3)
general surgery (12)	13954	0.08 (0.04-0.14)
Medical (51)	30.636	0.15 (0.12-0.20)
Nephrology/hemodialysis (9)	1059	0.8 (0.4-1.5)
Cancer (19)	1579	1.2 (0.8-1.8)
HIT -T	---	
surgical- (15)		0.06 (0.04-0.1)
medical- (16)		0.05 (0.03-0.08)
HIT without thrombosis	---	
surgical- (25)		0.11 (0.07-0.17)
medical- (26)		0.08 (0.06-0.11)
nephrology/haemodialysis- (8)		0.07 (0.03-1.3)

Table 3 Characteristics of patients with HIT and thrombosis

Total N	n= 31
M/F	14/17 (M:45%)
Age: median (range)	70 (31-84)
Surgical pts cancer pts	15 (52%) 6
Medical pts cancer pts	16 (41%) 2
Nephrology/hemodialysis pts	2 (6%)
4T' score; median (range) all pt with a positive confirmatory test	6 (4-8)
platelet fall over baseline: median (range)	70% (11-97%)
Type of heparin UFH (prophylactic dose calcium sc; therapeutic dose sodium ev, calcium sc) LMHW (Prophylactic dose;therapeutic doses) Heparin in the previous 3 months Days between heparin initiation and blood sampling for HIT; median (range) Days between heparin initiation and platelet decrease median (range)	 11 (36%) (3; 8) 20 (64%) (11;9) 10 (30%) 14 (3-65) 11 (2-64)
ELISA screening test OD : median (range)	 1.806 (1.029-4.180)
Positive aggregation test % aggregation with heparin 1U /mL median, (range) % aggregation with heparin 100 U/mL median, (range)	 70; (31-100) 5 (0-19)
All cause mortality at 90 days Major bleeding at 90 days (2 pts with loss < 2 gr% HB, CID in uterine cancer, contributing to death) Recurrent thrombosis at 90 days (fondaparinux, 1 with acute limb ischemia undergoing limb amputation)	8 (25.8%; 95% CI:13.7-43) 3 (9.6%; 95% CI:3.3-24) 1 (3.2%; 95% CI:0.05-16)
Venous thrombosis	25 (12 lower limb proximal DVT; 3 upper limb due to CVC, 2 lower limb DVT + PE, 2 PE, 4 distal DVT, 1 cerebral vein thrombosis, 1 upper vena cava)
Arterial thrombosis	6 (of which 2 post-surgical) and 1 in multiple sites

Table 4 Characteristics of patients with HIT without thrombosis

Total N	n= 59
M/F (M%)	31/28 (48%)
Age: median, (range)	76 (15-101)
Surgical pts cancer pts	25 (42%) 2
Medical pts cancer pts	26 (44%) 9
Nephrology/hemodialysis	8 (14%)
4T' score; median (range) all pts 4T' score: median (range) in 8 pts with neg confirmatory test	4 (2-8) 5 (3-8)
% platelet fall over baseline: median, (range)	66%; (12-96%)
Type of heparin UFH (prophylactic dose calcium sc;therapeutic dose sodium ev, calcium sc) LMHW (prophylactic dose; therapeutic doses) Cases shifted from LMWH to UFH Heparin in the previous 3 months Interval between heparin initiation and blood sampling for suspected HIT days, mean, median (range) Interval between heparin initiation and platelet decrease days mean, median (range)	 22 (37%) (10,12) 37 (63%) (23,14) 1 9 12 ; 11 (2-63) 11; 9 (1-62)
ELISA screening test OD : median (range)	 1.914 (1.056-3.372)
Positive aggregation test % aggregation with heparin 1U /mL ;median, (range) % aggregation with heparin 100 U/mL; median, (range)	 59 (20-100) 5 (0-19)
All cause mortality at 90 days (4 heart failure, 4 respiratory failure, 4 metastatic cancer, 2 acute myocardial infarction, 1 major bleeding) Major bleeding at 90 days (hematuria, rectus muscle haematoma) Thromboembolic events at 90 days (2 DVT)	15 (25.4 % ; 95% CI: 16.6-38) 2 (3.4%; 95 CI: 0.93-12) 2 (3.4 %; 95% CI: 0.9-12)

Statements and declarations

The authors declare no conflict of interest related to the paper