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Heparin-induced thrombocytopenia (HIT II) in liver transplant recipients: a retrospective multivariate analysis of prognostic factors

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No conflict of interest.

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Summary

We investigated the prevalence of HIT II in liver transplant recipients and analysed associated factors. In recipients with clinically suspected HIT II in the 4Ts pretest clinical scoring system HIPA-assay was performed. Next, 37 clinical variables were analysed retrospectively for their association with HIT II. Factors significantly correlated to our findings in univariate analysis were included in a multivariate model and binary logistic regression analysis. Among 46 recipients 21 patients were suspicious in the 4Ts pretest and 14 of them (30.4%) were diagnosed HIT-antibody positive. Patient's age ($P = 0.001$), postoperative dialysis ($P = 0.028$), and postoperative hospital stay ($P = 0.035$) were significantly associated with development of HIT-antibodies in univariate analysis. Postoperative dialysis and postoperative hospital stay turned out as epiphenomena of patient's age, the only independent predictor ($P = 0.021$). Using multiple χ^2-testing, a cut-off could be calculated, assigning patients younger than 59 years to a low risk group and patients of 59 years and older to a high risk group. High incidence of peri-operative HIT II seroconversion in liver transplant recipients is not associated with factors known to induce thrombocyte activation, like blood products or cell-saver. Only patients' age was identified as independent predictor.

Introduction

Heparin-induced thrombocytopenia (HIT) is a prothrombotic, iatrogenic complication of heparin therapy, which may lead to disseminated thromboembolic events and cause significant peri-operative morbidity and mortality. Hereby, any dosage and way of heparin application entail the potential risk of thrombosis [1]. Pathophysiological investigations allow the differentiation between 2 types of HIT: the nonimmune-mediated type I and the perilous immune-mediated type II. Whereas the type I HIT is a transient, asymptomatic phenomenon within the first days of heparin application because of direct agglutinating effects between platelets in the presence of heparin, which even disappears under heparin therapy, type II HIT typically appears between day 5 and 14 after beginning of heparin therapy, a typical time period in which the postoperative platelet count normally increases [2]. In HIT II, heparin-dependent antibodies induce platelet aggregation by binding to complexes of platelet factor 4 (PF4) and heparin [3]. Next, immune-complexes crosslink platelets via FcγRIIa-receptors which leads to their activation. In a cascade, more PF4 will be released from platelets, released proteins neutralise heparin and therefore its antithrombotic effect, and platelets start clotting [4]. Comparing unfractionated heparin (UFH) and low molecular weight heparin (LMWH), the latter has less potential to trigger the production of antibodies and is therefore assumed less likely to cause HIT II [5].

In literature, the definition of HIT II is considered to be a clinicopathological syndrome because the diagnosis is based on both clinical and serological grounds. Thus,

HIT antibody seroconversion without thrombocytopenia or other clinical sequelae is not considered HIT II, whereas a diagnosis of HIT II is made when HIT antibody formation is accompanied by an otherwise unexplained platelet count fall, thromboembolic event, or by skin lesions at heparin injection sites or acute systemic reactions (e.g. chills, cardiorespiratory distress) after intravenous heparin bolus administration [2].

In literature, up to 20% of patients with distinct clinical findings are tested negative for antibodies [6].

Laboratory investigations for HIT-antibody detection are based on either a direct enzyme-linked immunosorbent assay (ELISA) test for the identification of anti-PF4/heparin antibodies or a rapid, specific and sensitive platelet activation assay [heparin-induced platelet activation test (HIPA test), a functional test to detect HIT-antibodies using plasma from the citrated blood of healthy donors and a patient's serum sample that is incubated with various concentrations of heparin]. Although, a higher sensitivity, the ELISA implicates a decreased specificity because antibodies are detected that do not induce HIT [7,8].

The risk of induction of HIT-antibodies and therefore the risk of HIT II differs between operative disciplines. Along these subgroups, HIT may affect 1–2% of the patients undergoing cardiac surgery [9] or coronary interventions for acute coronary syndromes [10], and rises up to as far as 5% in orthopaedic surgery patients [5,11]. Nevertheless, the ACCP guidelines discourage routine HIT antibody testing in the absence of clinical indications, but recommend careful platelet count observation to discover patients with HIT [2].

However, the assessment of HIT in the postoperative period is generally complicated by postoperative ''reactive'' thrombocytosis, which unfortunately happens between day 2 and 14. Therefore, HIT suspicion must be eliminated in all postoperative patients with a decrease in platelets to less than 50% of the highest postoperative summit [1]. Even suspicion of HIT II requires immediate stop of heparin application and change to alternative therapeutically dosed anticoagulants such as heparinoids, hirudin, argatroban or fondaparinux.

Liver transplantation is the only curative therapy for irreversible liver cirrhosis of any underlying origin such as ethyl-toxic, hepatitis, primary biliary hepatitis, primary sclerosing cholangitis, hemochromatosis, Wilson's disease, or alpha-1-antitrypsin deficiency. Besides chronic hepatic failure, acute liver failure (ALF) – most frequently because of toxic metabolites – requires liver transplantation too. Patients with advanced hepatic failure show severe clinical symptoms of impaired haemostasis such as cutaneous bleeding on the one hand and laboratory conspicuousness in haemostasis on the other hand. These changes in partial thromboplastin time (PTT) and especially in prothrombin time (PT) serve as objective laboratory parameters in different scores (Child-Pugh classification, MELD score) that facilitate an equitable allocation of liver transplants, dependent on the patients' stage of liver disease.

In a retrospective analysis of 46 consecutive patients who underwent liver transplantation at our institution between June 2008 and September 2010, HIT II antibodies could be found in 30.4%. This incidence revealed to be much higher than previously reported (5.6%) by Kaneko *et al.* in a series of 52 liver transplant recipients in 2008 [12]. We therefore investigated potential causing clinical factors that could be attributed to influence HIT development.

Patients and methods

Our transplant centre is an interdisciplinary part of the University Hospital of the Technische Universität München and we perform between 25 and 40 deceased-donor liver transplantations per year. Liver transplantation is performed using a modified piggyback technique as described previously by Belghiti [13]. Postoperative regimen on our ICU includes triple immunosuppression (Calcineurin-inhibitor, Mycophenolic acid and steroids), and cardiocirculatory, respiratory, metabolic or antibiotic therapy as required. Anticoagulant therapy starts with porcine intestinal mucosal unfractionated heparin sodium (UFH) on the day of transplantation and is then switched to low-molecular weight heparin (LMWH, as commonly used in anticoagulant protocols to prevent hepatic or portal vein thrombosis after liver transplantation) on postoperative day (POD) 3–4. Liver function is monitored by daily laboratory investigations (ALAT, ASAT, bilirubin, cholinesterase, PTT, PT, serum-proteins), colour Doppler and duplex sonography and in case of doubtful findings, percutaneous fine needle biopsy is taken for histopathological investigations and specific therapy modifications.

In the period between June 2008 and September 2010 we performed 46 orthotopic liver transplantations (OLT) and 6 near-term retransplantations. However, all recipients were included into this retrospective study. The most frequent underlying hepatic diseases were ethyl-toxic liver cirrhosis ($n = 17$; 36.9%), hepatitis B- and hepatitis C-related cirrhosis ($n = 7$; 15.2%), hepatocellular carcinoma (HCC) because of alcoholic liver cirrhosis ($n = 6$; 13%), HCC because of hepatitis B- and C-related cirrhosis ($n = 5$; 10.9%), fulminant/acute hepatic failure ($n = 4$; 8.6%), HCC ($n = 3$; 6.6%), cholangiocellular carcinoma (CCC) ($n = 1$; 2.2%) and others ($n = 3$; 6.6%).

Liver grafts were allocated from deceased donors via Eurotransplant Foundation in Leiden, The Netherlands. Among the recipients there were 34 men (74%) and 12 women (26%) and the mean age was 57.7 years (range 29

– 72 years, SD 8.6). Median MELD score at point in time of OLT was 28.9 (minimum 10; maximum 40; SD 8.3) and in 12 cases liver transplantation was performed after high-urgency request. Altogether, six patients were retransplanted between the 5th and the 14th POD (mean 8.2 days) because of primary nonfunction. Baseline patients' data are shown in Table 1. Platelet counts were analysed daily for at least two weeks. Follow-up in our outpatients' clinic was for at least 6 months. HIT occurrence in the 46 consecutive liver transplant recipients of our single centre was analysed retrospectively. However, laboratory HIT diagnostics had been performed during the patients' hospital stay in case of HIT suspicion. For decision guidance for or against laboratory HIT investigation, the 4Ts pretest clinical scoring system as a predictor of HIT probability had been applied. In this scoring system with a negative predictive value of 91% [14], which could recently be revalidated prospectively in our centre in an independent in-house series of HIT-antibody negative liver transplant recipients, clinical features of HIT – extent of thrombocytopenia, timing of platelet count fall, thrombosis or other sequelae, and identification of alternative causes for thrombocytopenia – are graded and a score ranging 0–3 (= low risk), 4–5 (= intermediate risk), and 6–8 (= high risk) is calculated [1,14,15]. Patients with suspected HIT II (≥4 points in the 4Ts score) had been investigated immediately for detection of HIT-antibodies using standardised, validated HIPA test as described elsewhere [16,17] and anticoagulation was continued with argatroban or danaparoid.

Next, we further analysed potentially HIT-related factors. Statistics were calculated with SPSS 18.0 software (SPSS Inc. Chicago, IL, USA). Overall, 37 variables were tested for their association with development of HIT II after OLT, using Mann–Whitney *U*-test, Student's *t*-test or χ^2-test where appropriate. Factors that were significantly correlated to the development of HIT II in univariate analysis were included in a multivariate model and binary logistic regression analysis was performed. A cut-off was calculated using multiple χ^2-testing for the factor that was found to be an independent predictor in the multivariate model.

All patients gave their informed consent for general data ascertainment before OLT and database analysis was performed, according to ethical standards as laid down in the Declaration of Helsinki 2000 and the Declaration of Istanbul 2008.

Table 1. Baseline demographic and clinical data of liver graft recipients (n = 46); HIT II antibody positive versus no HIT II / not tested (nt) because of low risk (≤3 points) in the 4Ts score.

	HIT II-antibodies	no HIT II / nt
Number (men / women)	14 (11 / 3)	32 (23 / 9)
Age of recipient (year; median; range)	62 (53–72)	55 (29–71)
Transplantations	16 (30.8%)	36 (69.2%)
Retransplantation	2	4
Thrombotic complications	1	2
Urgent transplantation	5	7
MELD (median ± SD) score	31.5 ± 8.2	28 ± 7.7
Operation time (median ± SD)	355 ± 93.7 min	330 ± 88.9 min

Results

For our study we performed a retrospective database analysis to evaluate the occurrence of HIT-antibodies, HIT II, and HIT-causing factors after OLT.

Independently from the study, we first revalidated the results of the recently performed assessment of the 4Ts clinical scoring system as a predictor of HIT by Strutt *et al.* [14]. Therefore, we evaluated the occurrence of HIT-antibodies in an independent collective of consecutive liver transplant recipients of our centre without suspected HIT in the 4Ts score (≤3 points). In this prospective series, we could corroborate its validity as no case of HIT or any hints for HIT-suspicion could be identified in this control group (n = 10, results of 4Ts pretests: range 2–3, mean 2.8, SD 0.42, HIPA test POD10; Fig. 1). Hence, the pretest can be assumed to qualify as a reliable decision criterion for or against HIT-diagnostics in our patient population.

Next, we analysed 46 liver transplant recipients for the retrospective study and compared the score of all patients without suspicious results in the 4Ts pretest (≤3 points) and no HIPA investigation (n = 25, results of 4Ts

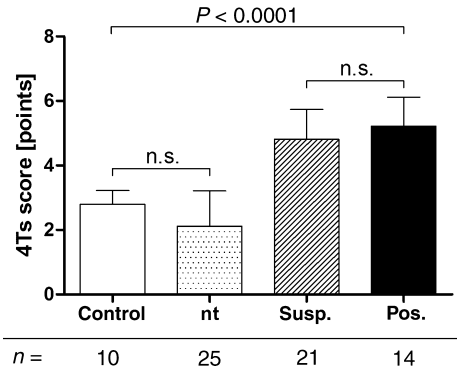

Figure 1 Results of 4Ts clinical scoring system as a predictor of HIT in (a) an independent prospective control group (clear), and (b) in relevant subpopulations of the study: i, HIPA not tested (nt) because of low risk (≤3 points) in the 4Ts score, (dotted); ii, suspicious 4Ts test (>4 points), (striped); iii, confirmed positive HIPA test, (black).

pretests: range 0–3, mean 2.12, SD 1.10), to the independent control group ($P = 0.10$). However, in 21 patients (45.7%), an intermediate to high risk in the 4Ts score had been observed and HIT was suspected (results of 4Ts pretests: range 4–6, mean 4.81, SD 0.93). These patients had been investigated immediately for detection of HIT-antibodies and in 14 patients (14/46, 30.4%) antibody-positivity could be detected by HIPA assay (results of 4Ts pretests: range 4–6, mean 5.21, SD 0.89; comparison of means of antibody-positive patients versus suspicious study patients: $P = 0.21$, and versus the independent prospective controls: $P < 0.0001$; Fig. 1). HIPA-assays were usually conducted between the second and ninth POD.

No HIT patient suffered from postoperative thrombosis of portal vein or hepatic vein and artery, but one patient developed lethal fulminant pulmonary embolism on the 18th POD. Two patients developed a deep venous thrombosis on POD 8 and 14, respectively, but both patients were not diagnosed positive for HIT. Basic characteristics of evaluated patients are summarised in Table 1.

The relative course of the platelet counts in the seroconverted patients is displayed in Fig. 2. The mean time point of the platelet counts' nadirs was at 5.14 ± 2.28 days and all 14 recipients showed a >50% relative decrease in platelets after transplantation and a mostly rapid recovery after heparin discontinuation and switch to argatroban or danaparoid.

Of 37 clinical variables, patients' age ($P = 0.001$), postoperative dialysis ($P = 0.028$), and postoperative hospital stay ($P = 0.035$) were the only parameters associated with the development of HIT-antibodies in univariate analysis (Table 2). These factors were subsequently analysed for their independent association in a multivariate model using binary logistic regression analysis. Surprisingly, factors known to induce thrombocyte activation, e.g. blood products or intra-operative use of a cell saver, did not turn out to have a significant impact on the development of HIT II.

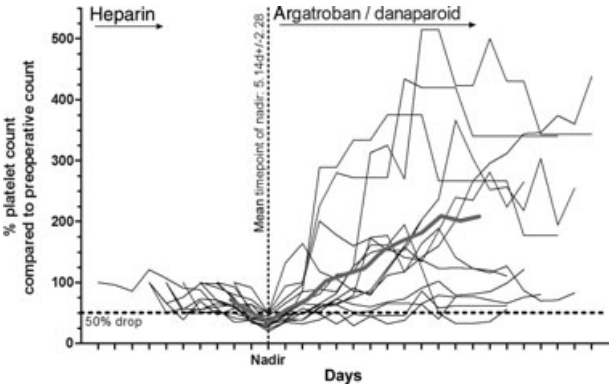

Figure 2 Relative platelet counts of HIT-antibody positive liver transplant recipients ($n = 14$) and mean platelet course (thick grey line) after liver transplantation, discontinuation of heparin and switch to argatroban or danaparoid. Curves of platelet counts are centred on the respective nadirs as overlay graphic.

Furthermore, postoperative haemodialysis and postoperative hospital stay could be identified to be epiphenomena of patients' age, which was found to be the only independent predictor ($P = 0.021$) of perioperative HIT seroconversion in patients undergoing OLT (Table 2).

By multiple χ^2-testing a cut-off could be generated, dividing patients into a low-risk group (patients younger than 59 years) and a high-risk group (patients of 59 years and older) concerning the individual risk for developing HIT-antibodies and HIT (Fig. 3).

Discussion

In contrast to the benign, nonimmune-mediated HIT type I, HIT II leads to limb- or life-threatening thromboembolic events. HIT II is a prothrombotic, immune-mediated complication of heparin therapy, caused by the development of antibodies recognising complexes of platelet factor 4 (PF4) and heparin, [18] and consecutive further formation of HIT-IgG/PF4/heparin complexes on the platelets' surface. Specific concentrations of heparin and PF4 are required in the circulation [19] to induce complex forming tendency, which explains that HIT II occurs less frequently in a setting of low-dose heparin prophylaxis compared to high dose administration, e.g. after cardiac surgery [9] or in orthopaedic surgery patients [20]. However, few data are available for patients undergoing general surgery [1]. In these patients, the transient postoperative thrombocytosis interferes with the detection of thrombocytopenia. In the postoperative setting, HIT II typically occurs with a 50% platelet count drop. As a result of its intense predilection for thrombosis, HIT II must be suspected whenever thrombosis occurs despite heparin prophylaxis, especially 5–14 days after the start of heparin therapy.

After cardiac surgery, Warkentin *et al.* pointed out that only 2.4% of the patients developed HIT II, even though 25–50% of these patients could be revealed as HIT-antibody positive after 5–10 days [9]. A similar distribution was observed in a study with orthopaedic surgery patients. Whereas 15% were positive for HIT-antibodies after the operation, merely 3% developed HIT II [20]. A recent prospective study by Kaneko and co-workers demonstrated that the outcome of HIT II after OLT was inconsistent. The percentage of HIT-antibody positive patients was 0.5% preoperatively and rose to 5.6% on POD 7 and 14, respectively. Nevertheless, none of the patients developed UFH-related HIT [12]. An explanation for the high frequency of HIT-antibody positivity among

Table 2. Univariate and multivariate analyses with tested HIT II as dependent variable. (nt, not tested because of low risk (≤3 points) in the 4Ts score).

Variables	HIT II-antibodies	no HIT II / nt	univariate *P* value	multivariate *P* value
Underlying disease	*n* (%)	*n* (%)		
Alcoholic liver cirrhosis	10 (20)	15 (30)	0.059§	
Other causes	4 (8)	21 (42)		
– fulminant hepatic failure	1 (2)	3 (6)		
– hepatitis related cirrhosis	1 (2)	6 (12)		
– HCC because of hepatitis B-/C-cirrhosis	0	6 (12)		
– PBC	0	2 (4)		
– Budd Chiari Sydrome	1 (2)	0		
– Vanishing bile duct	0	1 (2)		
HCC	1 (2)	2 (4)		
CCC	0	1 (2)		
Age	Median ± SD	Median ± SD		
	61.5 ± 4.89	58.00 ± 9.32	<0.001*	0.021
Gender	*n* (%)	*n* (%)		
Male	14 (28)	25 (50)	0.729‡	
Female	3 (6)	11 (22)		
CMV constellation (don./rec.)	*n* (%)	*n* (%)		
Neg./neg.	10 (20)	29 (58)	0.586‡	
Pos./neg.	2 (4)	5 (10)		
Neg./pos.	0	0		
Pos./pos.	2 (4)	2 (4)		
Blood group	n (%)	n (%)		
0	3 (6)	14 (28)	0.302‡	
A	9 (18)	20 (40)		
B	1 (2)	2 (4)		
AB	1 (2)	0		
Urgency	n (%)	n (%)		
T	10 (20)	28 (56)	0.718‡	
HU	4 (8)	8 (16)		
MELD-score	Median ± SD	Median ± SD		
	31.50 ± 8.23	28.00 ± 7.61	0.255*	
In-hospital mortality *n* (%)	3 (6)	9 (18)	0.552‡	
Time setting	Median ± SD	Median ± SD		
Operation time (min)	355.00 ± 93.68	330.00 ± 99.65	0.721*	
Cold ischemia (h)	8.50 ± 1.70	7.50 ± 2.10	0.416*	
Warm ischemia (min)	125.00 ± 38.60	115.00 ± 60.82	0.803*	
Hospitalisation (d)	Median ± SD	Median ± SD		
Postop. hospitalisation	53.00 ± 27.52	23.50 ± 30.76	0.035*	0.155
Preop. hospitalisation	0.00 ± 14.19	0.50 ± 15.08	0.991†	
dialysis	n (%)	n (%)		
Preop. haemodialysis	3 (6)	6 (12)	0.697‡	
Postop. haemodialysis	11 (22)	25 (30)	0.028‡	0.051

Table 2. continued

Variables	HIT II-antibodies	no HIT II / nt	univariate *P* value	multivariate *P* value
Operative setting	*n* (%)	*n* (%)		
Application of heparin preop.	7 (14)	19 (38)	0.860‡	
Cell saver	9 (18)	15 (30)	0.151‡	
G5% in situ perfusion	9 (18)	16 (32)	0.208‡	
Retransplantation	1 (2)	7 (14)	0.542‡	
Application of blood-products	Median ± SD	Median ± SD		
Fresh frozen plasma preop.	0.00 ± 40.20	0.00 ± 28.71	0.306†	
Units of packed red blood cells preop.	0.00 ± 14.43	0.00 ± 9.11	0.715†	
Thrombocyte transfusion preop.	0.00 ± 2.57	0.00 ± 4.30	0.813†	
Fresh frozen plasma intraop.	29.50 ± 36.61	26.50 ± 28.39	0.880†	
Units of packed red blood cells intraop.	6.5 ± 15.68	10.00 ± 9.40	0.848*	
Thrombocyte transfusion intraop.	2.00 ± 1.70	1.00 ± 1.56	0.359†	
Fresh frozen plasma postop.	13.00 ± 35.52	12.5 ± 46.56	1.000#	
Units of packed red blood cells postop.	10.00 ± 13.21	7.50 ± 16.51	0.335†	
Thrombocyte transfusion postop.	3.00 ± 2.96	2.00 ± 7.64	0.790†	
Fresh frozen plasma overall	51.00 ± 61.22	61.00 ± 65.34	0.845†	
Units of packed red blood cells overall	20.00 ± 23.50	19.00 ± 24.22	0.604†	
Thrombocyte transfusion overall	5.50 ± 4.18	5.50 ± 9.10	0.939†	

*Student's *t*-test.
†Mann–Whitney *U*-test.
‡Chi-square-test according to Pearson or Fisher's Exact-test, where appropriate.

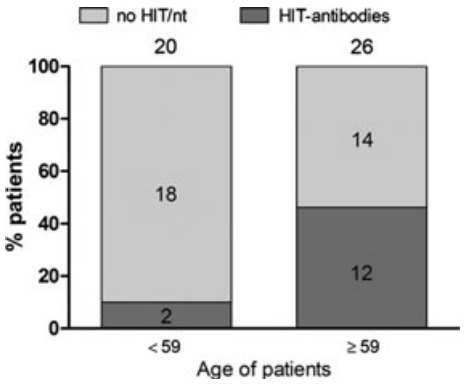

Figure 3 Constellation of HIT in a low- (<59 years) and high-risk (≥59 years) population of 46 liver transplant recipients; not tested (nt).

patients undergoing cardiac or orthopaedic surgery could be the use of high doses of heparin during the operation and in the peri-operative setting, with subsequent release of PF4 from platelets [5]. This implies that a higher incidence of HIT-antibodies must be anticipated in liver transplant recipients receiving high doses of heparin perioperatively. We therefore analysed 46 consecutive patients who underwent OLT at our institution. Retrospectively, this could be confirmed in 14 patients (30.4%) by a heparin-induced platelet activation test (HIPA) [16,17].

Several factors were reported to modify the risk of HIT II. In case of LMWH application, less multimolecular complexes are formed [21], which leads to a decreased induction of immune response (compared to UFH) because of a reduced affinity to PF4, platelets, and endothelial cells [19]. Besides the type of heparin used, the duration of heparin treatment is associated with HIT II development [1]. Heparin therapy for 4–14 days and previous heparin application within the last 100 days, correlated with the highest risk [1]. Also the type of patients' treatment (surgical versus medical) and the patients' gender – with up to two times higher relative risk in women compared to men – have an impact on HIT II development [22,23]. However, the American College of Chest Physicians (ACCP) guidelines discourage routine HIT-antibody testing in the absence of clinical indication. Platelet count monitoring is considered more useful in the identification of patients at risk instead [2,24].

All our patients were treated with UFH during their initial ICU stay with consecutive LMWH-therapy in the follow-up. One patient with proven HIT II developed lethal fulminant pulmonary embolism on the 18th POD despite therapy with heparinoid danaparoid, (Orgaran®;

Essex Pharma, Munich, Germany) which was begun immediately after suspicion of HIT II.

The observed high incidence of HIT II antibodies was not associated with currently known triggers of thrombocyte activation. Particularly, blood transfusion and intraoperative usage of cell saver did not correlate with the occurrence in our patients. In contrast, postoperative haemodialysis correlated with the occurrence in univariate analysis. Underlying alcoholic liver cirrhosis missed significance level with a *P*-value of 0.059. In multivariate analysis, where postoperative haemodialysis and postoperative stay (significant in univariate testing) turned out as epiphenomena of patients' age, only patients' age could be identified as independent predictor in these patients. Hereby, patients younger than 59 years could be assigned to the low risk group, whereas patients of 59 years and older had a high risk of HIT II seroconversion. These results enable us to characterise our patients in more detail than any of the previously published studies on increased incidence of HIT II before. So far, no additional analyses have been performed within no study on cardiac surgery patients [9], coronary interventions for acute coronary syndromes [10], orthopaedic patients [5,11], nor in the study by Kaneko on liver transplant recipients [12]. Comparing the results from the last study to ours, we found a more than fivefold higher incidence of HIT-antibodies in our liver transplant recipients. However, this apparent discrepancy might be explained by the setting (living donor liver transplantation versus deceased donor liver transplantation and elective versus unplanned surgery including ALF and high-urgency transplantation of far advanced cirrhosis with high MELD scores), the operative technique (partial liver graft versus whole liver graft), different ethnic groups (Asiatic versus European), different laboratory tests, and potentially also the younger age of the patients in the Japanese collective (median 53 years versus 59 years) as the calculated cut-off for the high-risk group is 59 years.

Literature reveals that not all patients who develop HIT-antibodies automatically show clinically manifest HIT II [1,4]; however, anti-PF4/heparin antibodies are known to be associated with an increased morbidity and mortality e.g. in cardiac surgery (increased hospital mortality) or with an elevated incidence of thrombotic events in vascular reconstruction and orthopaedic surgery patients [1,25–27].

To this day, the reason for different clinical manifestations of sequelae because of HIT-antibodies remains indeterminate. Greinacher postulates different HIT-antibody-subclasses that cannot be distinguished or different HIT-antibody titres in affected patients to result in – to current pathophysiological understanding – inhomogeneous outcome concerning HIT II manifestation. Furthermore, disparity in sensitivity of platelets' FcγRIIa-receptors towards immune-complexes might influence the clinical manifestation of HIT II in case of identical antibody features [4,28,29]. There are hints, that distinct phenotypes of FcγRIIa-receptors have impaired mechanisms of PF4/heparin-antibody-complex elimination and therefore are associated with a prolonged activation of platelets and endothelium [4,30,31].

Our study at hand is the first clinical series demonstrating an extremely high incidence of HIT II antibodies in liver transplant recipients. Based on a retrospective database analysis and consistent with numerous reports on elevated antibody titres in other surgical subgroups, our data underline the thesis that detection of HIT-antibodies does not mandatorily lead to clinical HIT II manifestation with skin lesions or thromboembolic events [4,28]. This finding is important to liver transplant surgeons, physicians on ICUs, and hepatologists to be aware of its high frequency in almost one third of the patients after OLT. However, in case of an otherwise unexplained thrombocytopenia, especially between POD 5 –14, after the onset of heparin treatment and intermediate or high risk in the 4Ts score, HIT II is an important differential diagnosis that has to be taken into consideration and laboratory investigations have to be performed immediately to verify the suspected diagnosis and avoid thromboembolic complications. Once identified, a HIT II patient can receive complex surgery like OLT, provided that an adapted heparin-free anticoagulation management is applied [32]. Attentive observation of platelet counts displays the most effective approach to early detection and prevention of HIT II-antibody positivity and HIT II manifestation [4].

Put together, both the observations on seemingly inconsistent development of HIT II – most likely because of molecular characteristics of antibodies and receptors [29–31] – on the one hand and our findings that exclude platelet-activating factors and reveal patient's age as independent factor, on the other hand, underline the hypothesis that first, presence of HIT-antibodies or even HIT II diagnosis does not mandatorily cause HIT II associated complications and second, that HIT II is a multifactorially influenced affection.

On the basis of the results of this study, additional prospective trials are necessary to confirm our data and detect – or refute – additional possible risk factors for HIT II and the antibodies' impact on development of redoubtable HIT II syndrome. Comprehensive trials are essential to ensure maximum safety for liver transplant recipients and to find out the fundamental principles of circulating anti-PF4/heparin antibodies, because the resulting risk for HIT II manifestation in terms of severe venous and arterial thrombosis is still unexplained.

Authorship

NH and VA: wrote manuscript, collected data, designed analysis, idea. DR, AN and ZC: analysed data. ST, AK and HF: designed analysis. ST, HF, PB and EM: performed liver transplantations. EM: wrote manuscript.

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References

1. Battistelli S, Genovese A, Gori T. Heparin-induced thrombocytopenia in surgical patients. *Am J Surg* 2010; **199**: 43.
2. Warkentin TE, Greinacher A. Heparin-induced thrombocytopenia: recognition, treatment, and prevention: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004; **126**(3 Suppl): 311S.
3. Warkentin TE. Heparin-induced thrombocytopenia. *Hematol Oncol Clin North Am* 2007; **21**: 589.
4. Greinacher A. Heparin-induced thrombocytopenia – pathogenesis and treatment. *Thromb Haemost* 1999; **82**(Suppl. 1): 148.
5. Warkentin TE, Sheppard JA, Horsewood P, Simpson PJ, Moore JC, Kelton JG. Impact of the patient population on the risk for heparin-induced thrombocytopenia. *Blood* 2000; **96**: 1703.
6. Fabris F, Ahmad S, Cella G, Jeske WP, Walenga JM, Fareed J. Pathophysiology of heparin-induced thrombocytopenia. Clinical and diagnostic implications – a review. *Arch Pathol Lab Med* 2000; **124**: 1657.
7. Walenga JM, Jeske WP, Fasanella AR, Wood JJ, Bakhos M. Laboratory tests for the diagnosis of heparin-induced thrombocytopenia. *Semin Thromb Hemost* 1999; **25**(Suppl. 1): 43.
8. Greinacher A, Janssens U, Berg G, *et al.* Lepirudin (recombinant hirudin) for parenteral anticoagulation in patients with heparin-induced thrombocytopenia. Heparin-Associated Thrombocytopenia Study (HAT) investigators. *Circulation* 1999; **100**: 587.
9. Warkentin TE, Greinacher A. Heparin-induced thrombocytopenia and cardiac surgery. *Ann Thorac Surg* 2003; **76**: 2121.
10. Matsuo T, Tomaru T, Kario K, Hirokawa T. Incidence of heparin-PF4 complex antibody formation and heparin-induced thrombocytopenia in acute coronary syndrome. *Thromb Res* 2005; **115**: 475.
11. Warkentin TE, Roberts RS, Hirsh J, Kelton JG. An improved definition of immune heparin-induced thrombocytopenia in postoperative orthopaedic patients. *Arch Intern Med* 2003; **163**: 2518.
12. Kaneko J, Sugawara Y, Tamura S, Togashi J, Matsui Y, Makuuchi M. Heparin-induced thrombocytopenia after liver transplantation. *Transplant Proc* 2008; **40**: 1518.
13. Belghiti J, Panis Y, Sauvanet A, Gayet B, Fékété F. A new technique of side to side caval anastomosis during orthotopic hepatic transplantation without inferior vena caval occlusion. *Surg Gynecol Obstet* 1992; **175**: 270.
14. Strutt JK, Mackey JE, Johnson SM, Sylvia LM. Assessment of the 4Ts pretest clinical scoring system as a predictor of heparin-induced thrombocytopenia. *Pharmacotherapy* 2011; **31**: 138.
15. Warkentin TE. Heparin-induced thrombocytopenia: diagnosis and management. *Circulation* 2004; **110**: e454.
16. Eichler P, Budde U, Haas S, *et al.* First workshop for detection of heparin-induced antibodies: validation of the heparin-induced platelet activation test (HIPA) in comparison with a PF4/heparin ELISA. *Thromb Haemost* 1999; **81**: 625.
17. Zimmermann I, Pachmann U, Seybold D, Moessmer G, Neumeier D. Antibodies and HIT II- therapeutically relevant diagnostics. *Haemostaseologie* 2004; **24**: A59.
18. Rauova L, Poncz M, McKenzie SE, *et al.* Ultralarge complexes of PF4 and heparin are central to the pathogenesis of heparin-induced thrombocytopenia. *Blood* 2005; **105**: 131.
19. Horne 3rd MK, Alkins BR. Platelet binding of IgG from patients with heparin-induced thrombocytopenia. *J Lab Clin Med* 1996; **127**: 435.
20. Greinacher A, Eichler P, Lietz T, Warkentin TE. Replacement of unfractionated heparin by low-molecular-weight heparin for postorthopedic surgery antithrombotic prophylaxis lowers the overall risk of symptomatic thrombosis because of a lower frequency of heparin-induced thrombocytopenia. *Blood* 2005; **106**: 2921.
21. Greinacher A, Alban S, Dummel V, Franz G, Mueller-Eckhardt C. Characterization of the structural requirements for a carbohydrate based anticoagulant with a reduced risk of inducing the immunological type of heparin-associated thrombocytopenia. *Thromb Haemost* 1995; **74**: 886.
22. Greinacher A, Warkentin TE. Recognition, treatment, and prevention of heparin-induced thrombocytopenia: review and update. *Thromb Res* 2006; **118**: 165.
23. Martel N, Lee J, Wells PS. Risk for heparin-induced thrombocytopenia with unfractionated and low-molecular-weight heparin thromboprophylaxis: a meta-analysis. *Blood* 2005; **106**: 2710.
24. Warkentin TE, Greinacher A, Koster A, Lincoff AM, American College of Chest Physicians. Treatment and prevention of heparin-induced thrombocytopenia: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008; **6**(Suppl.): 340S.
25. Bennett-Guerrero E, Slaughter TF, White WD, *et al.* Preoperative anti-PF4/heparin antibody level predicts adverse

outcome after cardiac surgery. *J Thorac Cardiovasc Surg* 2005; **130**: 1567.
26. Greinacher A, Eichler P, Lietz T, *et al.* Replacement of unfractionated heparin by low-molecular-weight heparin for postorthopedic surgery antithrombotic prophylaxis lowers the overall risk of symptomatic thrombosis because of a lower frequency of heparin-induced thrombocytopenia. *Blood* 2005; **106(8)**: 2921.
27. Hirsh J, Heddle N, Kelton JG. Treatment of heparin-induced thrombocytopenia: a critical review. *Arch Intern Med* 2004; **164**: 361.
28. Greinacher A. Heparin-induced thrombocytopenia: frequency and pathogenesis. *Pathophysiol Haemost Thromb* 2006; **35**: 37.
29. Suh JS, Mohammad IM, Aster RH, Visentin GP. Characterization of the humoral immune response in heparin-induced thrombocytopenia. *Am J Hematol* 1997; **54**: 196.
30. Bachelot-Loza C, Saffroy R, Lasne D, Chatellier G, Aiach M, Rendu F. Importance of the FcgammaRIIa-Arg/His-131 polymorphism in heparin-induced thrombocytopenia diagnosis. *Thromb Haemost* 1998; **79**: 523.
31. Denomme GA, Warkentin TE, Horsewood P, Sheppard JA, Warner MN, Kelton JG. Activation of platelets by sera containing IgG1 heparin-dependent antibodies: an explanation for the predominance of the Fc gammaRIIa ''low responder'' (his131) gene in patients with heparin-induced thrombocytopenia. *J Lab Clin Med* 1997; **130**: 278.
32. Fretschner R, Dietrich K, Unertl K, Greinacher A. Management of liver transplantation in a patient with a history of heparin-induced thrombocytopenia. *Transpl Int* 2005; **18**: 664.