Heparin-induced antibodies and cardiovascular risk in patients on dialysis

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Summary

The clinical relevance of heparin-induced antibodies (HIA) in the absence of thrombocytopenia remains to be defined. The aims of this study were (i) to determine the prevalence of HIA in patients treated by dialysis, (ii) to determine the prevalence of thrombocytopenia and heparin-induced thrombocytopenia (HIT), and (iii) to test whether HIA are associated with adverse outcomes. Sera from 740 patients treated by hemodialysis (HD, n=596) and peritoneal dialysis (PD, n=144) were tested for HIA (IgG, IgA or IgM) by masked investigators at approximately six months after enrolment in the Choices for Healthy Outcomes in Caring for End-Stage Renal Disease (CHOICE) study. We assessed, with time-to-event Cox proportional hazards models, whether the presence of HIA predicted any of four clinical outcomes: arterial cardiovascular events, venous thromboembolism, vascular access occlusion and mortality. HIA prevalence was 10.3% overall. HIA positivity did not predict development of thrombocytopenia or any of the four clinical outcomes over a mean follow-up of 3.6 years, with hazard ratios for arterial cardiovascular events of 0.98 (95% confidence interval 0.70–1.37), venous thromboembolism 1.39 (0.17–11.5), vascular access occlusion 0.82 (0.40–1.71), and mortality 1.18 (0.85–1.64). Chronic intermittent heparin exposure was associated with a high seroprevalence of HIA. In dialysis patients these antibodies were not an independent risk factor for cardiovascular events and mortality. Our data do not suggest that dialysis patients should be monitored for HIA antibodies in the absence of thrombocytopenia.

Keywords

Cardiovascular risk, dialysis, heparin-induced antibodies, survival analysis, thrombocytopenia

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Introduction

Heparin-induced thrombocytopenia (HIT) is an acquired thrombocytopenia mediated by anti-heparin/platelet factor 4 (PF4) antibodies in the setting of heparin therapy. Affected patients are at substantial risk for arterial and venous thrombotic complications (1–4). While HIT is defined as thrombocytopenia in the presence of heparin-induced antibodies (HIA), many patients exposed to heparin acquire HIA in the absence of thrombocytopenia. Up to 50% of cardiac surgery patients will develop HIA, although only 2% of these patients will also have thrombocytopenia (5). Some reports suggest that HIA even in the absence

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of thrombocytopenia are an independent predictor of myocardial infarction in patients with acute coronary ischemic syndromes (6), although others have not confirmed this (7).

Patients with chronic kidney disease (CKD) are at high risk for cardiovascular events. Five-year survival of patients on hemodialysis (HD) is less than 50% (8). More than half of this mortality is related to myocardial infarction alone, but other arterial events including stroke and peripheral artery disease are also highly prevalent. Venous thromboembolic events also occur in HD patients, but data regarding incidence are scarce (9, 10). Additionally, vascular access occlusion occurs in 33–56% of HD patients and leads to annual costs exceeding $1 billion in the United States (11, 12).

Known cardiovascular risk factors do not entirely account for the high cardiovascular disease-related morbidity and mortality in these patients (13). Several reports, most of them based on small patient cohorts, have analysed the frequency of HIA in patients chronically exposed to heparin in the setting of HD. However, these studies were not designed to assess the clinical relevance of these antibodies. Hence, we sought to test the hypothesis that HIA are a "non-traditional" cardiovascular risk factor in this population using time to event analysis. The aims of this study were (i) to determine the prevalence of HIA in patients treated by dialysis, (ii) to determine the prevalence of thrombocytopenia and HIT in this group of patients, and (iii) to test whether HIA are associated with adverse outcomes including arterial cardiovascular events, venous thromboembolic events, events of vascular access occlusion, and mortality.

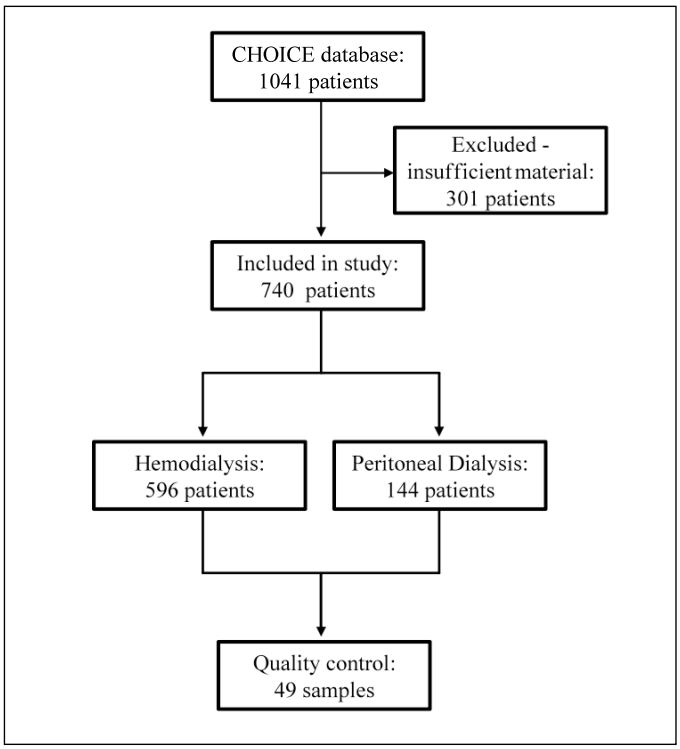

Figure 1: Consolidated standards of reporting trials (CONSORT) diagram of patient enrolment. The diagram shows the numbers of patients who met the criteria of inclusion in or exclusion from the study. Quality control samples included hemodialysis (n=40) and peritoneal dialysis patients (n=9).

Methods

Patient population and blood sampling

The study subjects were a subpopulation of patients participating in the Choices for Healthy Outcomes in Caring for End-Stage Renal Disease (CHOICE) Cohort Study (14,15). CHOICE is a national, prospective cohort study of incident HD and peritoneal dialysis (PD) patients initiated in 1995 to investigate treatment choices of modality and dose and outcomes of dialysis care. From October 1995 to June 1998, 1,041 (767 HD and 274 PD) patients were enrolled from 81 dialysis clinics associated with Dialysis Clinic, Incorporated (DCI, Nashville, TN, USA), New Haven CAPD (New Haven, CT, USA), and the Hospital of Saint Raphael (New Haven, CT, USA) (Fig. 1). All study participants were incident kidney failure patients starting outpatient dialysis, were over 17 years of age, and spoke English or Spanish. Patients were enrolled a median of 45 days from initiation of chronic dialysis (98% within 4 months). The mean duration of follow-up was 3.6 years (5^{th} and 95^{th} percentiles, 5.8 months and 9.1 years, respectively). Follow-up was complete (100%) in regard to cardiovascular mortality. The prospectively designed study was approved by the Johns Hopkins University School of Medicine Institutional Review Board prior to the initiation of HIA testing.

Routine blood draw sera, collected as close as possible to six months after the first dialysis (mean time to testing 5.7 months, 5^{th} and 95^{th} percentile: 71 days and 1.1 years), were available from 740 dialysis (HD and PD) patients. HD patients were chronically exposed to heparin during dialysis, whereas PD patients putatively were not. The 301 patients that could not be included in the study due to lack of available serum samples had similar baseline characteristics compared to patients that were included in the study. The excluded patients did not differ by sex, hypertension, diabetes, smoking status, albumin, cholesterol, triglyceride levels and body mass index (BMI). They did tend to be younger, were less likely to be white, were less likely to have an elevated comorbidity score (ICED =3; see "baseline data collection" for definition) and had higher creatinine, higher low-density lipoproteins (LDL) cholesterol and lower C-reactive protein (CRP).

Outcomes

Our outcomes of interest were thrombocytopenia and four clinical outcomes. Platelet counts were obtained from routine monthly blood draw data provided by the clinics. Thrombocytopenia was defined as a platelet count below 150 G/l (150,000/µl) or a decrease of 50% or more from a previous platelet count. Mortality was ascertained from dialysis centre reports, medical records, and data from the CMS and the National Death Index (NDI). Arterial cardiovascular events included any of the following events during the follow-up period: myocardial infarction, cerebrovascular accident, coronary artery bypass graft, percutaneous coronary angioplasty, peripheral artery bypass, amputation, abdominal aortic aneurysm repair, carotid endarterectomy, and sudden coronary death. These events were identified in hospitalisation records, reviewed and adjudicated

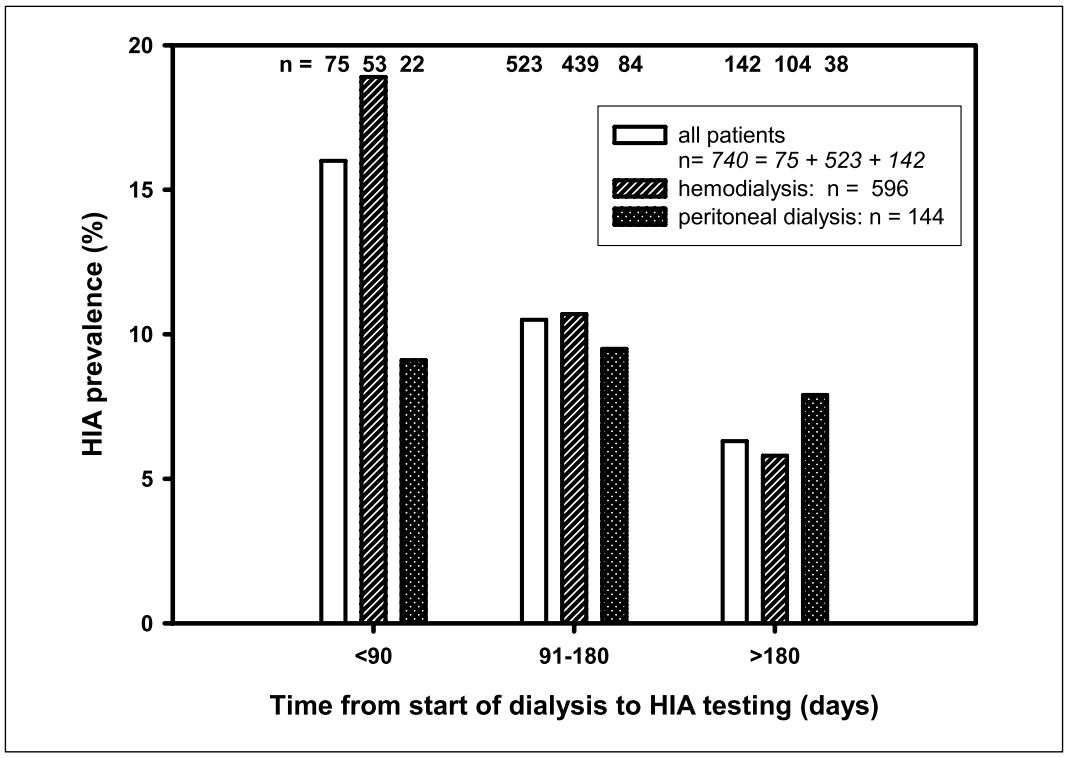

Figure 2: Temporal aspects of HIA prevalence. For the 740 patients in whom analysable samples were available, some did not have samples available at six months. In these cases the temporally closest available samples were chosen. Depicted is the prevalence of HIA according to sample collection time relative to start of dialysis: <90 days (n=75), 91–180 days (n=523) and >180 days (n=142). Corrected ELISA results were used to determine prevalence.

by two members of the study's outcomes committee using uniformly applied criteria modified from the Cardiovascular Health Study (16), HEMO study (17), or, in the absence of an adjudicated record, from CMS hospitalisation data. Deep vein thrombosis and pulmonary embolism events were identified in CMS hospitalisation data as defined by ICD-9-CM codes (451.1x, 451.2, 451.81, 451.83, 451.89, 452.x, 453.x and 415.1x). Finally, vascular access information was obtained through review of discharge summaries, dialysis flow sheets, and dialysis clinic progress notes, as described elsewhere (18). All vascular access occlusion events were reviewed, and only those coded as thrombosis-related were used as outcomes in this study.

Baseline data collection

Demographic characteristics, primary cause of kidney failure, and date of first chronic dialysis were ascertained from the Centres for Medicare & Medicaid Services (CMS) Medical Evidence Form (Form 2728), which was completed at initiation of chronic dialysis. Race was categorised as black, white, or other (including Native American and Asian). Comorbidity assessment was performed at enrolment using the Index of Coexistent Disease (ICED), a composite scoring system based on 19 medical and 11 physical impairment categories (19). The scores are compiled into a summary score representing mild (0 or 1), moderate (2), or severe comorbidity (3).

Assay for HIA

We utilised a standard enzyme-linked immunosorbent assay (ELISA) for HIA, the GTI-PF4 enhanced assay (Genetic Testing Institute, Waukesha, WI), which detects IgG, IgM and IgA directed against platelet factor 4 (PF4) bound to polyvinylsulfonate (6,20). All assays were performed in duplicate (45 samples, a positive control, a negative control and a blank per each 96-well plate) by blinded investigators and according to the manufacturer's instructions. Absorbance for positive controls had to be ≥1.8 units, negative controls ≤0.3 units; if the mean of the sample absorbance ≥0.4 units, the test was considered positive. To detect false-positive results, such as when antibodies are directed against heparin alone or when antibodies are non-specific, the heparin neutralisation procedure (HNP) was performed according to the manufacturer's instructions. An inhibition of a positive result by more than 50% in the presence of heparin is considered confirmatory for HIA.

Quality control, correction procedure, and heparin neutralisation

ELISA results were validated on a per-plate and per-assay basis. Plates were considered valid if no more than four sample results exceeded the limits for the coefficient of variability (CV>15%) and if the positive/negative control criteria were met. For individual assays, the manufacturer tolerates 20% variability of the mean of the two samples; we applied a more stringent CV criterion of 15%. Any individual sample with a coefficient of variability exceeding 15% was considered invalid if the mean absorbance was ≥0.150. Tests with a mean absorbance value <0.15 were considered to be valid, regardless of the CV, as they were clearly negative. Forty-nine blinded quality control samples were analysed. Due to the quality control criteria, 0.6% of samples (5/789) but no (0/18) whole plates had to be retested. Agreement for 49 pairs of blinded quality control samples was 90% with a kappa value of 0.4.

In agreement with the manufacturer's specifications intra-plate variability of results was minimal (mean coefficient of

variability (CV) for positive controls was 1.86 ± 1.32%, range 0.06–4.83%). We observed an interplate variability range for positive controls of 2.3–3.6. According to the manufacturer's specifications any result ≥1.8 is acceptable for positive controls, resulting in a theoretical range of 1.8–3.6. This illustrates that the same sample measured on different plates can give results that vary by 64% (as observed) or 100% (in theory). Our results suggest that, due to the assay characteristics, results from different plates can only be evaluated on a categorical scale (positive/negative). To permit evaluation of optical density (OD) results on a continuous scale we designed a correction procedure that allowed us to compare OD values stemming from different plates. For this procedure mean OD values were calculated for all plates after exclusion of extreme OD values (<0.1 and >1.0). The mean OD values were normalised to the mean OD of an index plate defined to have a correction coefficient of 1. All results on an individual plate (including the extreme values) were then multiplied by the plate's correction coefficient. When this per-plate correction procedure was performed, the quality control parameters improved to 93% agreement and a kappa value of 0.6, respectively.

Table 1: Patient characteristics by HIA status at baseline.

	HIA-positive	HIA-negative
N (%)	76 (10.3)	664 (89.7)
Age (years)	59	56
Sex (f : m)	0.51 : 0.49	0.47 : 0.53
Race (%):		
– African American	40.8	31.2
– Caucasian	53.9	63.1
– Other	5.3	5.7
ICED: 1/2/3 (%)	24/45/31	34/38/28
Albumin (g/dl)	3.60 (3.51–3.69)	3.63 (3.60–3.66)
Creatinine (mg/dl)	7.22 (6.66–7.77)	7.49 (7.29–7.68)
Smoking status (% ES)	67.1	58.3
Hypertension (%)	97.4	95.6
Diabetes (%)	57.9	54.8
Cholesterol total (mg/dl)	186.7 (174.1–199.3)	188.2 (184.5–192.0)
Cholesterol LDL (mg/dl)	82 (73–91)	87 (84–90)
Triglycerides (mg/dl)	209.9 (177.9–241.9)	195.4 (185.6–205.2)
Body mass index	27.8 (25.8–29.7)	26.9 (26.4–27.4)
C-reactive protein (mg/ml)	3.65	3.91

Baseline time of HIT antibody testing was at a mean of 5.7 months with 5th and 95th percentiles at 71 days and 1.1 years, respectively. HIT: heparin-induced thrombocytopenia; f: female, m: male; ICED: index of coexistent disease, comorbidity score; ES: ever-smoker includes current and former smokers; LDL low-density lipoprotein. Where applicable 5th and 95th percentile values are given in parentheses.

Data analysis

We compared patient characteristics by HIA status using Pearson's Chi^2 tests for categorical variables and analysis of variance for continuous variables. We used logistic regression to examine the association between HIA status and incidence of thrombocytopenia. Cox proportional hazards models were used to assess the strength and independence of an association between HIA status and arterial cardiovascular events, deep vein thrombosis/pulmonary embolism, vascular access occlusion, and mortality. Relative hazards for these events were calculated by HIA status using time from first dialysis to death or censoring (at transplant, loss to follow-up, or closeout) as the survival time variable. Variables were chosen for adjustment (e.g. race, ICED, age at enrolment, and baseline albumin level) in the regression models based on either their demonstration to be confounders or prior evidence of their association with the outcome in question. All analyses were performed using Stata version 8.1 (StataCorp, College Station, TX, USA).

Patients at the same clinic cannot be considered independent observations (21). We accounted for this by obtaining robust variance-covariance matrix estimates in all logistic regression and Cox proportional hazards models (stata option cluster) (22).

Table 2: Mean platelet count and mean absorbance by HIA status and treatment modality.

	HD	PD	Overall
N			
– HIA-positive (%)	63 (10.6)	13 (9.3)	76 (10.3)
– HIA positive by HNP	53	13	66
– HIA-negative	533	131	664
Mean platelet count			
– HIA-positive	230 ± 62	265 ± 77	226 ± 67
– HIA-negative	242 ± 77	298 ± 65	242 ± 75
Mean (median) absorbance			
– HIA-positive	0.70 ± 0.38 (0.57)	0.71 ± 0.43 (0.55)	0.70 ± 0.39 (0.57)
– HIA-negative	0.17 ± 0.09 (0.16)	0.15 ± 0.08 (0.14)	0.17 ± 0.09 (0.15)

N: number of patients; HNP: number of patients with results confirmed by heparin neutralisation procedure; HD: haemodialysis; PD: peritoneal dialysis.

Table 3: Adjusted risk of adverse events by the presence of HIA at baseline.

	Hazard ratio	95% CI	No. of events
Arterial cardiovascular events*	0.98	0.70 – 1.37	372
Venous thromboembolism	1.39	0.17 – 11.5	7
Vascular access occlusion	0.82	0.40 – 1.71	86
Mortality†	1.18	0.85 – 1.64	448

CI: confidence interval; *adjusted for age, sex, race, dialysis modality, and smoking status; †adjusted for age, race, albumin and comorbidity score (ICED).

Results

HIA prevalence

Antibodies directed against heparin/PF4 were detected in nearly 20% of patients early after treatment initiation (Fig. 2) and at six months following initiation of dialysis seventy-six of 740 (10.3%) patients had a positive HIA assay (Table 1). In samples taken at later time points after HD initiation, the prevalence was lower. For patients receiving PD, the prevalence was stable over time at 9%. Patient characteristics by antibody positivity at study inclusion are depicted in Table 2. There were no significant differences between the HIA-positive and -negative groups by demographics, comorbidity score, cardiovascular risk factors, or relevant laboratory data with the exception of a smoking history at study inclusion.

HIA and thrombocytopenia

Mean platelet counts were lower in HIA-positive patients but did not differ significantly from those in HIA-negative patients at the time of antibody testing (Table 2). The presence of HIA did not predict subsequent thrombocytopenia at three (p=0.89), six (p=0.56), or nine months (p=0.76) after measurement.

HIA and clinical outcome

Survival analyses showed no association between positive HIA status and any of the clinical outcomes (Table 3). A total of 372 arterial events were recorded in 678 individuals. The corresponding numbers were 7 and 737 for venous thromboembolism, 86 and 601 for venous access occlusion, and 448 and 732 for mortality. The discrepancy between the number of patients in the study (740) and the number of individuals cited above is due to events taking place prior to HIA testing and missing covariates. The highest hazard ratio (HR) was 1.4 for the association between HIA and venous thromboembolism but the confidence interval (CI) was wide and included 1.0. Unadjusted and adjusted results yielded similar results.

Survival analysis performed on corrected (see per-plate correction procedure under methods; shown) and uncorrected data (not shown) yielded the same results. The results of the survival analyses were also the same when we analysed all positive samples (n=76) or only the ones confirmed by HNP (n=66).

HIT and HIT-related thrombosis

Nine patients had results compatible with HIT, defined as a positive HIA assay and thrombocytopenia in the ensuing three months (9/740, 1.2%). The mean minimal platelet count of the potential HIT patients during their thrombocytopenic episode was 124 (range 106–147). One of the nine patients had thrombocytopenia as defined by a relative drop of platelet count by more than 50%. All nine patients with potential HIT were receiving HD. One patient with potential HIT had a venous thromboembolic event (1/740, 0.13%) resulting in a HR of 13.7 (95% CI 1.63–115.5) for HIT and thrombosis. Six out of nine potential HIT patients' (including the patient with thrombosis) ELISA results were confirmed by the HNP.

Discussion

We have demonstrated that approximately 10% of CKD patients treated by dialysis develop HIA at six months. Most previous studies suggested a lower prevalence of HIA in HD patients ranging from 0 to 8.8% (23–31) with one exception at 17.9% (32). In these studies, screening was often limited to patients with thrombocytopenia or thrombotic complications. Furthermore, the assay used to detect HIA and timing of the test varied across studies. While most of these studies have not shown a correlation of HIA with adverse outcomes (29, 32), others have (30, 31). Limitations of these studies included small patient cohorts (n<100) (23, 24, 27, 29–31), fewer than 10 HIA positive patients (23–27, 30, 31), or study design that not did allow prospective recording and assessment of multiple clinical outcomes. None of these studies reported quality control and/or performance of HNP.

In the absence of thrombocytopenia, we found that HIA positivity was not associated with adverse outcomes including arterial cardiovascular events, venous thromboembolic events, vascular access occlusion, and mortality. Our data, as well as data from prospective studies in populations without CKD (5, 7, 20, 33) and with CKD (24, 29), support the hypothesis that adverse cardiovascular events associated with HIA require the activation of platelets by HIA, which manifests as thrombocytopenia. Our findings are not consistent with the model in which HIA-mediated platelet activation is bypassed and in which there is direct HIA-mediated activation of target cells such as endothelial cells or monocytes (1, 2, 34).

There is a recently published study by Carrier et al. which reports that IgG specific HIA found in nine of 419 patients (univariate HR 2.40, 95% CI 0.98 –5.89; multivariate HR 2.68, 95%CI 1.08–6.63) are associated with increased mortality in HD patients. The authors also looked at non IgG specific HIA – using the same test that we used – in their patient population. No association between non-specific HIA and mortality was found (35). Our study confirms the results of Carrier et al. in that 12.9% were HIA positive (using the non IgG-specific assay) while we found 10.3% seropositive patients. The study by Carrier et al., however, does not report on platelet count. One can thus not evaluate and differentiate the outcome of HIA-positive thrombocytopenic patients, i.e. those with true HIT from that of HIA positive non-thrombocytopenic patients. We looked at HIA status and platelet count and found no increased mortality of

other adverse outcome in the HIA positive non-thrombocytopenic group. There is literature showing that IgG-specific HIA testing offers enhanced test characteristics as compared to non IgG-specific HIA tests (36). However, there are also data showing that non IgG-mediated forms of HIT occur (37). This together with the aspect that at the time of study design there were only two marketed, clinically validated and Food and Drug Administration (FDA) approved tests available (both were non IgG-specific, one of which we chose for this study) motivated our choice of HIA assay.

In this study we examined 740 patients, including nearly 600 HD patients. HIA testing was performed by blinded investigators using a standardised protocol with rigorous quality control, and HNP was performed to rule out false-positive results. Clinical outcomes were collected from multiple sources, and all events were validated. Limitations of our study include a potential underestimation of the frequency of HIT-related events. As we tested HIA status at six months after HD initiation, any cardiovascular events in patients whose seroconversion occurred after the initial testing would be missed. However, late seroconversion is less likely than seroconversion close to the time of heparin initiation. Finally, we did not assess the persistence of HIA until the time of the event.

Previously, one study described temporal aspects of HIA prevalence in patients acutely exposed to heparin (20). Only limited data are available for chronically exposed patients. Mean time between starting of HD and development of HIT in a UK survey was 61 days (range 5–390) (38). We did not serially follow up our dialysis patients, our study nonetheless gives insight into the temporal aspects of HIA seroconversion in this type of heparin exposure (Fig. 2). The above mentioned findings are compatible with a model in which a subset of heparin-exposed patients have a propensity to develop HIA and in which a "time window" exists during which HIA formation is most likely to occur. According to this hypothesis the large majority of patients will develop HIT when both conditions are met. The "time window" may vary depending on duration and type of heparin exposure as well as the clinical setting. As the UK survey and our data suggest chronic intermittent exposure in dialysis patients may lead to a time window of several weeks to months, whereas for acute heparin exposure the time of maximum risk appears to be from day 4 to day 14 (4).

The high prevalence of HIA in PD patients remains unexplained. All HIA in PD patients were shown to be heparin-specific through the HNP, which detects false-positive results (see Table 2). PD patients are not routinely exposed to therapeutic doses of heparin. They may, however, be exposed to heparin in intravenous flushes, heparin additives to dialysis fluids, heparin prophylaxis at time of immobilisation and other situations with increased venous thromboembolic risk or also during angiographic procedures.

We conclude that although there is a high prevalence of HIA of the IgG, IgA or IgM class, these antibodies directed against heparin/PF4 (as detected in a non IgG-specific assay) in the absence of thrombocytopenia are not associated with cardiovascular events or increased mortality in patients on dialysis. Our results do not support the hypothesis that HIA lead to adverse clinical outcomes in the absence of platelet activation. In addition to giving insight into the pathophysiology of HIT, our data have implications for monitoring and therapeutic guidelines. Firstly, our data suggest that following the initiation of dialysis monitoring of HIA antibodies is not warranted in patients in the absence of thrombocytopenia or clinical evidence suggestive of HIT. Secondly, our data provide no evidence that would justify switching from heparin to an alternative potentially more expensive anticoagulant in dialysis patients who are HIA positive and non-thrombocytopenic. Finally, the data provide reassurance that the induction of HIA by chronic intermittent exposure to heparin is not associated with a high risk of adverse outcomes in patients with CKD treated by dialysis.

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