Antibodies to Platelet Factor 4–Heparin Complex and Outcome in Hemodialysis Patients with Diabetes

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Background and objectives: Hemodialysis patients with type 2 diabetes exhibit an excessive cardiovascular risk and regularly receive heparin. We tested whether antibodies to the platelet factor 4–heparin complex (PF4-H-AB) contribute to outcome.

Design, setting, participants, & measurements: In 1255 hemodialysis patients with type 2 diabetes, the German Diabetes Dialysis Study evaluated the effect of atorvastatin (20 mg/d) *versus* placebo. In a *post hoc* analysis, the association among PF4-H-ABs, biochemistry, and prespecified, centrally adjudicated end points (combined cardiovascular end point [CVE], all-cause mortality, sudden death, myocardial infarction, stroke) was investigated.

Results: During 4 years, 460 patients reached the CVE; 605 died, 159 of sudden death. Myocardial infarction and stroke occurred in 199 and 97 patients, respectively. Positive PF4-H-AB status was found in 231 (18.7%) of 1236 tested patients and was associated with lower albumin, higher C-reactive protein, and arrhythmia. In a multivariate model adjusted for demographics, comorbidities, and biochemistry, PF4-H-ABs were associated with sudden death. No significant association between PF4-H-ABs and all-cause mortality, myocardial infarction, stroke, or the CVE was observed. Detecting an interaction between acetylsalicylic acid and PF4-H-ABs regarding sudden death and mortality, we found that the association between PF4-H-ABs and outcomes was restricted to patients with acetylsalicylic acid use, most likely because of indication bias.

Conclusions: In hemodialysis patients who have type 2 diabetes and are treated with acetylsalicylic acid, PF4-H-ABs are associated with sudden and all-cause death. Further studies are needed to elucidate this association.

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Annual mortality rates in hemodialysis patients with type 2 diabetes are extremely high (250 to 300 per 1000 patient-years) (1). Treatment of established cardiovascular risk factors shows only limited benefit; therefore, the existence of other nontraditional risk factors has been assumed (2,3).

One of these emerging risk factors might be antibodies to the platelet factor 4–heparin complex (PF4-H-ABs), which can lead to platelet activation and loss of antithrombogenic endothelial surface properties (4). According to the "Iceberg Model" (5), only a minority of patients with increased PF4-H-ABs at the apex of the "iceberg" will develop the full-blown picture of heparin-induced thrombocytopenia type 2 with arterial and venous thromboses. Investigations have demonstrated that patients who are at the broad base of this iceberg and have no signs of thrombosis and have normal platelet counts might also have an increased cardiovascular risk (6).

Hemodialysis patients are repeatedly exposed to heparin and, therefore, may have an increased risk for formation of PF4-H-ABs. Indeed, some investigators found PF4-H-ABs to be increased and associated with cardiovascular events (7–10).

We performed a *post hoc* analysis of the German Diabetes Dialysis Study (Die Deutsche Diabetes Dialyse Studie [4D Study]) to evaluate the efficacy and safety of atorvastatin in 1255 hemodialysis patients with type 2 diabetes (11). The main question was whether the extreme cardiovascular morbidity and mortality in these patients could be attributed to circulating PF4-H-ABs.

Materials and Methods

Study Design and Participants

Design and methods of the 4D Study have previously been reported (11,12). The 4D Study was a randomized, multicenter trial of 1255 patients who had type 2 diabetes, were 18 to 80 years of age, and had a previous duration of hemodialysis of <2 years. Between March 1998 and October 2002, patients were recruited in 178 dialysis units throughout Germany. After a run-in period of 4 weeks, patients were randomly assigned to receive double-blind treatment with either 20 mg of atorvastatin once daily ($n = 619$) or placebo ($n = 636$). Data were recorded

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at 4 weeks and every 6 months. At each follow-up, a blood sample was taken and information was recorded about any suspected study end point or other serious adverse experience. Further details related to study end points were sought from family doctors, emergency physicians, hospitals, and local health authorities.

Outcome Measures

End points were evaluated by a specialized committee blinded to study treatment, according to prespecified criteria (11–13). The primary study outcome was a composite of death from cardiac causes, myocardial infarction (MI), and stroke, whichever occurred first (composite cardiovascular end point [CVE]). Sudden death, stroke, MI, and death from any cause were defined as secondary outcomes. These five end points were the outcome measures in this *post hoc* analysis.

Laboratory Procedures

All laboratory measurements of the initial clinical trial and the *post hoc* analyses of PF4-H-ABs and high-sensitivity C-reactive protein (CRP) were performed centrally at the Department of Clinical Chemistry, University of Freiburg (Freiburg, Germany). Total PF4-H-ABs (IgG, IgA, and IgM) were measured by a solid-phase ELISA in duplicate (PF4 ENHANCED; GTI, Waukesha, WI). Results were reported as OD. An OD >0.4 was considered positive for statistical analyses. Interassay coefficients of variance for PF4-H-ABs were $<7\%$. Blood samples were taken before start of dialysis and administration of heparin or additional drugs.

Statistical Analysis

Patient data are presented according to baseline PF4-H-AB status (negative or positive). *P* values for comparison between groups were derived from a general linear model for continuous and a logistic regression analysis for categorical variables adjusted for age and gender, as appropriate.

Kaplan-Meier estimates for cumulative incidences were calculated for all outcomes. The Cox proportional hazards regression model was used to estimate relative risks and corresponding 95% confidence intervals (CIs). The association of baseline PF4-H-ABs with outcome was analyzed as a continuous variable (logarithmically transformed, because values were not normally distributed) and as categorical variables: (*1*) Negative or positive (patients with negative PF4-H-AB status served as reference) and (*2*) quartiles of PF4-H-Abs (the first quartile served as the reference). The interaction of PF4-H-AB status and study treatment regarding the five end points was tested within the Cox regression model.

The following explanatory variables were considered for inclusion in the various Cox proportional hazards regression models: Treatment assignment, gender, age, phosphate, LDL, hemoglobin, glycated hemoglobin, CRP, smoking, systolic and diastolic BP, body mass index, ultrafiltration volume, duration of dialysis, hemodialysis shunt, history of stroke/transient ischemic attack, coronary artery disease (MI, coronary artery bypass grafting, percutaneous coronary intervention, and angiographically documented coronary artery disease), arrhythmia, peripheral vascular disease, and congestive heart failure (predominantly New York Heart Association class II). A stepwise selection procedure was used to determine the variables that were included in the final model (treatment assignment was always included), separately for each end point. This procedure starts examining the variable with the largest adjusted χ^2 statistic and adds variables to the model if $P \leq 0.25$ and retains them if $P \leq 0.15$ in the following steps. In the final analysis $P \leq 0.05$ was considered to be significant (in an exploratory sense). All *p* values are reported two-sided. Analyses were done using SAS 9.1.3 (SAS Institute, Chicago, IL).

Results

Of 1255 patients who took part in the 4D Study, 1236 (626 on placebo and 610 on atorvastatin) had a baseline PF4-H-AB measurement. The mean follow-up-period of the 4D Study was 3.96 years (median 4.00 years) for patients on atorvastatin and 3.91 years (median 4.08 years) for those on placebo. During follow-up, 605 patients died, 159 of sudden death. Furthermore, 460 patients reached the CVE (cardiac death, nonfatal MI, and stroke), with MI and stroke occurring in 199 and 97 patients, respectively. Increased PF4-H-ABs were found in 231 (18.7%) of 1236 patients analyzed.

Patient Characteristics

Patients with positive PF4-H-AB status showed lower albumin and higher CRP concentrations compared with those with negative PF4-H-AB status. Furthermore, they more often experienced arrhythmias, were less likely to present with a hemodialysis shunt, and were on maintenance hemodialysis for a shorter period (Table 1).

Baseline PF4-H-ABs and Outcome

Analyses with the PF4-H-ABs as continuous variable showed a significant increase in the risk for sudden death per unit increase in log PF4-H-ABs (hazard ratio [HR] 1.41; 95% CI 1.03 to 1.92; $P = 0.03$). Accordingly, patients with a positive PF4-H-AB status at baseline tended to be at higher risk for sudden death (HR 1.39; 95% CI 0.95 to 2.03; $P = 0.09$) compared with those with negative PF4-H-AB status at baseline. No statistically significant association between the PF4-H-ABs (continuous variable and categorization into positive or negative) and any other outcome measure was observed (Table 2).

The definition of a positive antibody status (OD >0.4) is based on correlations with the syndrome of heparin-induced thrombocytopenia. The appropriate threshold in a population without thrombocytopenia is not known; therefore, we further analyzed the association between quartiles of PF4-H-ABs and outcome (Table 3), and we looked for the optimal cutoff value to select a high-risk group regarding sudden death, which was an OD of 0.341 according to the minimal *P* value in the multivariate Cox model (HR 1.54; 95% CI 1.11 to 2.14; $P = 0.01$) (Figure 1).

Furthermore, we performed subgroup analyses of patients with high (more than the median) and low (less than or equal to the median) platelets, which showed that the HR of PF4-H-AB positive *versus* PF4-H-AB negative for sudden death tended to be higher in patients with low platelets (patients with platelets $<249\ 500/\mu l$: HR 1.42 [95% CI 0.82 to 2.46; $P = 0.21$]; patients with platelets $>249\ 500/\mu l$: HR 1.16 [95% CI 0.68 to 1.98; $P = 0.58$]) compared with those with high platelets.

Regarding MI, we found a significant interaction between PF4-H-AB status (negative or positive) and treatment ($P = 0.04$); therefore, the association between PF4-H-AB status and MI was evaluated in each treatment group separately: No significant association between positive PF4-H-AB test and the

Table 1. Patient characteristics according to baseline PF4-H-AB status

Variable	Group 1: Negative PF4-H-AB Test (n = 1005)	Group 2: Positive PF4-H-AB Test (n = 231)	P[a]
Age (years; mean [SD])	65.5 ± 8.3	66.3 ± 8.2	0.299
Gender (male/female)	548/457	118/113	0.495
Ever smoked (% [n])	42 (418)	36 (83)	0.213
BMI (kg/m^2; mean [SD])	27.4 ± 4.8	28.0 ± 4.7	0.067
SBP (mmHg; mean [SD])	146 ± 22	145 ± 22	0.460
DBP (mmHg; mean [SD])	76 ± 11	76 ± 11	0.817
Ultrafiltration volume (kg; median [25th to 75th percentiles])	2.0 (1.5 to 3.0)	2.0 (1.0 to 3.0)	0.443
Hemodialysis shunt (% [n])	94 (944)	90 (208)	0.045
Central venous dialysis catheter (% [n])	6 (60)	10 (23)	0.045
Time on dialysis (months; mean [SD])	8.6 ± 7.1	6.7 ± 5.8	<0.001
History of[b]			
arrhythmia (% [n])	17 (174)	25 (57)	0.017
MI, CABG, PCI, or CHD[c] (% [n])	29 (293)	30 (69)	0.811
CHF (% [n])[d]	34 (345)	39 (91)	0.210
stroke or TIA	18 (158)	16 (37)	0.383
peripheral vascular disease (% [n])	45 (453)	42 (98)	0.512
Hemoglobin (g/dl; mean [SD])	10.9 ± 1.3	10.8 ± 1.4	0.394
Platelets (1000/μl; mean [SD])	257 ± 80	259 ± 84	0.608
HbA_{1c} (%; mean [SD])	6.74 ± 1.27	6.63 ± 1.22	0.225
Phosphate (mg/L; mean [SD])	6.02 ± 1.59	6.08 ± 1.66	0.453
Albumin (g/dl; mean [SD])	3.83 ± 0.31	3.77 ± 0.28	0.016
CRP (mg/L; median [25th to 75th percentiles])	4.9 (2.2 to 12.0)	5.6 (2.6 to 14.2)	0.042
LDL cholesterol (mg/dl; mean [SD])	125 ± 29	128 ± 32	0.211

To convert hemoglobin values to millimoles per liter, multiply by 0.62. To convert platelet values to platelets per liter, multiply by 10^6. To convert values for phosphate to millimoles per liter, multiply by 0.32. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.03. BMI, body mass index; CABG, coronary artery bypass grafting surgery; CHD, coronary heart disease; CHF, congestive heart failure; DBP, diastolic BP; HbA_{1c}, glycated hemoglobin; PCI, percutaneous coronary intervention; SBP, systolic BP; TIA, transient ischemic attack.

[a]P values for comparison between groups of patients with positive and negative PF4-H-AB test at baseline were derived from a general linear model for continuous variables or logistic regression for categorical variables both adjusted for age and gender, as appropriate.

[b]Types of disease and intervention are not mutually exclusive.

[c]Documented by coronary angiography.

[d]Predominantly New York Heart Association class II.

risk for MI was found (placebo-treated patients only: HR 1.50 [95% CI 0.94 to 2.38; P = 0.09]; atorvastatin-treated patients only: HR 0.64 [95% CI 0.35 to 1.18; P = 0.15]). There was no significant interaction between treatment and PF4-H-AB status with respect to the other outcome measures.

No significant influence of low molecular weight heparin, acetylsalicylic acid or anticoagulants on sudden death was observed; however, we found an interaction among acetylsalicylic acid use at baseline, PF4-H-ABs, and outcome. The P values for the interaction were as follows: $P < 0.01$ for all-cause death, $P = 0.12$ for CVE, $P = 0.01$ for sudden death, $P = 0.47$ for stroke, and $P = 0.57$ for MI. Consequently, the associations between PF4-H-ABs, sudden death, and all-cause mortality were analyzed separately for patients with and without acetylsalicylic acid use at baseline. Patients who were on acetylsalicylic acid and showed a positive PF4-H-AB status were at higher risk for dying compared with those who had negative PF4-H-AB status and were on acetylsalicylic acid (all-cause death: HR 1.72 [95% CI 1.33 to 2.24; $P < 0.01$]; sudden death: HR 2.20 [95% CI 1.36 to 3.56; $P < 0.01$]). In patients who were not treated with acetylsalicylic acid, the risk for dying (all-cause mortality and sudden death) did not differ significantly between those with positive and those with negative PF4-H-AB status (all cause death: HR 0.77 [95% CI 0.57 to 1.04; P = 0.08]; sudden death: HR 0.75 [95% CI 0.39 to 1.44; P = 0.38]).

Discussion

We tested the hypothesis that PF4-H-ABs were linked to the excessive cardiovascular risk in patients who had type 2 diabetes and were on long-term hemodialysis in a large prospec-

Table 2. Risk for all-cause death, CVE, sudden death, stroke, and MI by baseline PF4-H-ABs

Parameter	Patients with	
	Negative PF4-H-AB Status (n = 1005)	Positive PF4-H-AB Status (n = 231)
All-cause death		
no. of events during study	486	119
Kaplan-Meier estimate (95% CI)[a]	0.49 (0.45 to 0.52)	0.54 (0.47 to 0.62)
adjusted HR (95% CI; P)[b]	[c]	1.15 (0.94 to 1.41; 0.18)
adjusted HR (95% CI; P)[b] with PF4-H-ABs as continuous variable		1.12 (0.95 to 1.32; 0.19)
CVE (MI, cardiac death, and stroke)		
no. of events during study	372	88
Kaplan-Meier estimate (95% CI)[a]	0.43 (0.39 to 0.47)	0.44 (0.36 to 0.52)
adjusted HR (95% CI; P)[b]	[c]	1.09 (0.86 to 1.38; 0.48)
adjusted HR (95% CI; P)[b] with PF4-H-ABs as continuous variable		1.08 (0.89 to 1.30; 0.44)
Sudden death		
no. of events during study	124	35
Kaplan-Meier estimate (95% CI)[a]	0.16 (0.13 to 0.19)	0.19 (0.12 to 0.25)
adjusted HR (95% CI; P)[b]	[c]	1.39 (0.95 to 2.03; 0.09)
adjusted HR (95% CI; P)[b] with PF4-H-ABs as continuous variable		1.41 (1.03 to 1.92; 0.03)
Stroke		
no. of events during study	82	15
Kaplan-Meier estimate (95% CI)[a]	0.10 (0.08 to 0.13)	0.08 (0.04 to 0.13)
adjusted HR (95% CI; P)[b]	[c]	0.79 (0.46 to 1.38; 0.41)
adjusted HR (95% CI; P)[b] with PF4-H-ABs as continuous variable		0.85 (0.54 to 1.32; 0.46)
MI		
no. of events during study	163	36
Kaplan-Meier estimate (95% CI)[a]	0.22 (0.19 to 0.25)	0.23 (0.15 to 0.31)
adjusted HR (95% CI; P)[b]	[c]	1.03 (0.71 to 1.48; 0.89)
adjusted HR (95% CI; P)[b] with PF4-H-ABs as continuous variable		0.97 (0.72 to 1.30; 0.81)

[a]Unadjusted Kaplan-Meier estimates at the end of year 4.

[b]Explanatory variables were selected by a stepwise process with adjustment for treatment assignment (was always included in the final model), gender, age, phosphate, LDL, hemoglobin, glycated hemoglobin, CRP, ever smoked, systolic and diastolic BP, body mass index, ultrafiltration volume, duration of dialysis, hemodialysis shunt, history of stroke/transient ischemic attack, coronary artery disease (MI, coronary artery bypass grafting, percutaneous coronary intervention, and angiographically documented coronary artery disease), arrhythmia, peripheral vascular disease, and congestive heart failure (predominantly New York Heart Association class II).

[c]Patients with negative PF4-H-AB status (OD <0.4) at baseline served as the reference.

tive patient cohort with a high incidence of prespecified and centrally adjudicated end points during 4 years of follow-up. For these patients, PF4-H-ABs at baseline were found to be predictive of sudden death. No statistically significant association between PF4-H-AB status and combined cardiovascular events, MI, all-cause death, and stroke was detected. PF4-H-ABs were a common finding and associated with signs of inflammation.

Sudden death was the major component of mortality in the 4D Study. Similar information has been available from the 2007 data set of the US Renal Data System for the general hemodialysis population showing that only 9% of deaths have been caused by acute MI or atherosclerotic heart disease, whereas 27% have been classified as cardiac arrest or arrhythmia (1). Corresponding data from the 4D Study were 11% for coronary heart disease deaths and 26% for sudden deaths (13).

In this analysis, no significant association between PF4-H-ABs and MI or stroke was identified; therefore, it was unlikely that macrovascular disease (*e.g.*, MI) has been responsible for the increase in the risk for sudden death. Consequently, alternative causes came into focus. Indeed, microvessel disease and cardiac fibrosis were found to be a typical abnormality of cardiac structure in uremia (14–16). Wall thickening of intramyocardial arteries and inadequate capillary density were

Table 3. Risk for all-cause death, CVE, sudden death, stroke, and MI by quartiles of baseline PF4-H-ABs

Parameter	Quartile 1 ≤0.208 PF4-H-ABs (n = 310)	Quartile 2 >0.208 to ≤0.268 PF4-H-ABs (n = 309)	Quartile 3 >0.268 to ≤0.362 PF4-H-ABs (n = 309)	Quartile 4 >0.362 PF4-H-ABs (n = 308)
All-cause death				
no. of events during study	152	152	142	159
adjusted HR (95% CI)[a]	[b]	1.27 (1.01 to 1.59)	1.12 (0.88 to 1.41)	1.27 (1.02 to 1.60)
P		0.05	0.36	0.04
CVE (MI, cardiac death, and stroke)				
no. of events during study	120	108	107	125
adjusted HR (95% CI)[a]	[b]	1.12 (0.86 to 1.46)	1.07 (0.82 to 1.39)	1.28 (1.00 to 1.66)
P		0.41	0.62	0.06
MI				
no. of events during study	55	45	44	55
adjusted HR (95% CI)[a]	[b]	0.97 (0.65 to 1.45)	0.96 (0.64 to 1.44)	1.23 (0.84 to 1.79)
P		0.89	0.85	0.29
Sudden death				
no. of events during study	39	31	40	49
adjusted HR (95% CI)[a]	[b]	0.98 (0.61 to 1.57)	1.12 (0.72 to 1.75)	1.50 (0.98 to 2.30)
P		0.92	0.62	0.06
Stroke				
no. of events during study	29	26	18	24
adjusted HR (95% CI)[a]	[b]	1.11 (0.65 to 1.90)	0.75 (0.41 to 1.36)	0.94 (0.55 to 1.63)
P		0.70	0.34	0.84

[a]Explanatory variables were selected by a stepwise process (atorvastatin treatment was always included in the final model) with adjustment for treatment assignment (was always included in the final model), gender, age, phosphate, LDL, hemoglobin, glycated hemoglobin (HbA1c), CRP, ever smoked, systolic and diastolic BP, body mass index, ultrafiltration volume, duration of dialysis, hemodialysis shunt, history of stroke/transient ischemic attack, coronary artery disease (MI, coronary artery bypass grafting, percutaneous coronary intervention, and angiographically documented coronary artery disease), arrhythmia, peripheral vascular disease, and congestive heart failure (predominantly New York Heart Association class II).

[b]The first quartile was used as the reference for each of the other three.

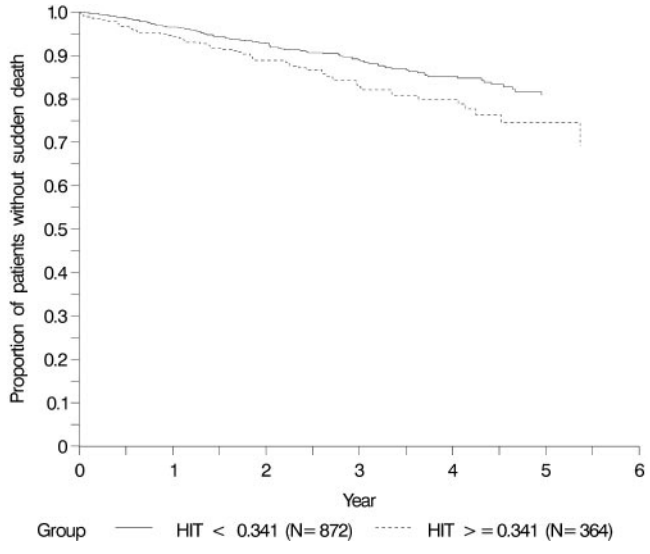

Figure 1. Kaplan-Meier estimate for time to sudden death according to baseline PF4-H-AB status (OD >0.341 was chosen to define positive PF4-H-AB testing).

described to restrict compensatory vasodilation and the ability of the heart to cope with increased oxygen demand (17). This might have been of special importance in the presence of PF4-H-Abs, which are known to induce platelet activation (6) and to act *via* the F(ab)2 fragment of IgG PF4-H-ABs binding to and activating microvascular endothelial cells (18), thereby potentially aggravating the already impaired cardiac perfusion and promoting cardiac fibrosis. Cardiac fibrosis had both mechanical and electrical sequelae affecting cardiovascular prognosis (14). Fibrosis was found to reduce the ventricular compliance and to promote arrhythmia. Fibrous tissue with high electrical resistance, interposed between cardiomyocytes, has been described to cause local delay in the spread of the action potential favoring the development of reentry types of arrhythmias (19,20).

Data with respect to macrovascular disease and all-cause death were not unequivocal. Previous studies found that patients who had acute coronary syndrome and tested positive for PF4-H-ABs were more likely to experience an MI (21) and a combined end point of death, revascularization, MI, and stroke (21,22); however, for patients who underwent percutaneous coronary intervention and for patients who were on dialysis, no

significant increase in thrombotic events in antibody-positive patients was found (23,24). With respect to all-cause mortality, two studies showed PF4-H-ABs to be predictive of death in hemodialysis patients (7,10). The Choices for Healthy Outcomes in Caring for End-Stage Renal Disease study (24) did not confirm these findings. In this analysis, a significant association between PF4-H-ABs and all-cause death was found when PF4-H-ABs were categorized into quartiles that identified patients within the first quartile being at lowest risk for all-cause death. No significant association was detected when PF4-H-ABs were analyzed as a continuous or categorical (positive or negative PF4-H-AB status) variable; however, we detected an interaction between acetylsalicylic acid use and PF4-H-ABs regarding sudden and all-cause death most likely as a result of indication bias. Patients who are treated with acetylsalicylic acid are supposed to be at higher risk. In these patients, adding another risk factor might result in a significant increase in risk.

In line with previous work (7,25), we identified increased PF4-H-ABs in a high percentage (18.7%) of patients. We underline that the study took place before the problems with chondroitin sulfate, but the actual purity of heparin that was used from 1998 through 2002 remains unclear. It is important, because oversulfated chondroitin sulfate is associated with an increased prevalence of PF4-H-ABs in hemodialysis patients (26).

Patients with positive PF4-H-AB status presented with lower albumin and higher CRP concentrations compared with those with negative PF4-H-AB status. PF4-H-ABs may have elicited an inflammatory response (4,27). Alternatively, they were markers of a general inflammatory process. Evidence was found to support this: Higher levels of CRP, myeloperoxidase, and soluble adhesion molecules have been demonstrated in patients with heparin antibodies (28,29). In this context, the higher prevalence of dialysis catheters in patients with positive PF4-H-AB status might as well have played a role, because catheter use was associated with lower albumin (mean albumin 3.71 *versus* 3.83 g/dl; $P < 0.01$) and higher CRP (mean logCRP 1.94 *versus* 1.64 mg/L; $P = 0.03$). It remains unclear whether this results from selection bias or from catheter use; however, increased markers of microinflammation have generally been associated with a higher risk for mortality, cardiovascular events, and also sudden death (30–32). Our multivariate analysis was adjusted for this.

In patients with positive PF4-H-AB status, we found a lower prevalence of hemodialysis shunts. Whether this was due to a higher rate of preceding fistula thromboses (9,33) or a result of the permanent exposition to heparin to prevent thrombus formation within the catheter is unknown.

Our finding of more arrhythmias in patients with positive PF4-H-AB status could have pointed to a myocardial microcirculation already compromised by platelet clots (34).

Reasons for the negative correlation between PF4-H-AB status and the time that patients had been on hemodialysis (a similar association has been described [9]) need to be elucidated further; however, this was in line with our finding that increasing PF4-H-ABs were associated with a higher risk for sudden death. This was the case at each time point. Consequently, patients within the antibody-positive group might have died earlier and stayed shorter on dialysis. Another reason might have been that with repeat exposure, tolerance to heparin developed.

Our study had several limitations. We performed a *post hoc* analysis within a selected cohort of German patients who had type 2 diabetes and were on hemodialysis. Results may not necessarily be generalizable to other populations, and cause cannot be inferred from these associations. Although our analyses have been adjusted carefully for multiple variables, residual confounding cannot be excluded. Furthermore, we detected an interaction between acetylsalicylic acid and PF4-H-ABs regarding sudden death. The association between PF4-H-ABs and sudden death was restricted to patients with acetylsalicylic acid use, most likely because of indication bias. Patients who are treated with acetylsalicylic acid are supposed to be at higher risk; adding another risk factor might result in a significant increase in risk. An effect of acetylsalicylic acid cannot be excluded; however, there is no plausible pathophysiologic explanation. Our assay analyzed the PF4-H-ABs without detecting the IgG-specific PF4-H-ABs. Some investigators recommended the combination of a functional test of platelet activation in conjunction with measurements of PF4-H-ABs to increase specificity (35). Because of the *post hoc* study design, we did not have functional data on platelet activation because fresh platelet preparations were not available; however, these limitations should have been balanced by the large study population, the high incidence of prespecified and centrally adjudicated end points, and the long follow-up period.

Conclusions

This study found PF4-H-ABs to be associated with sudden and all-cause death in patients who had type 2 diabetes, were on hemodialysis, and were treated with acetylsalicylic acid, whereas it did not support an association with MI and stroke. These results further endorse a possible link between increased PF4-H-ABs and microinflammation. PF4-H-ABs are a common finding in hemodialysis patients with type 2 diabetes. Whether treating these patients with nonheparin anticoagulants would decrease the risk for sudden death needs to be tested in an intervention trial.

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Disclosures

None.

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