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Thrombotic Events in Patients with Anti-Platelet Factor 4/Heparin-Antibodies

Short title: Heparin-induced antibodies and thrombosis

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ABSTRACT

Background. Antibodies to the heparin/platelet factor 4 (PF4) complex are linked to the pathogenesis of heparin-induced thrombocytopenia type II, and to the thrombotic complications associated with this syndrome. We investigated the long-term relationship between antibody concentration and thrombosis.

Methods. Two-hundred and fifty patients who had been treated with unfractionated heparin as part of cardiac surgery management were included in the study. Immunoassay ELISA test was used to detect the presence and the plasma concentration of heparin-PF4 antibodies (as optical density value, O.D.). Follow-up lasted one year and new thrombotic events (myocardial infarction, stroke, pulmonary embolism), and death from any cause, were evaluated.

Results. Seventy-nine of 250 patients (31.6 %) developed anti-PF4/heparin antibodies after cardiac surgery. Nadir platelet count was significantly lower in patients who developed antibody positivity (82,000±31,000/mm^3 versus 105,000±52,000/mm^3, P<0.001). At follow up, patients with anti-PF4/heparin antibodies were more likely to die or develop myocardial infarction (25.3% versus 10.5%, P<0.001), pulmonary embolism (20.2% versus 5.8%, P<0.001), or stroke (12.6% versus 5.8%, P<0.001), than patients who were antibody-negative. Patients were categorized in quintiles of antibody concentration according to the O.D. The risk of developing thrombotic events markedly increased with increasing quintile of O.D., with the highest group showing an OR of 7.68 (4.04-9.20) (P<0.001).

Conclusions. Patients who develop antibodies to the PF4-heparin complex have a significantly higher rate of thrombotic events during a one year follow-up than those who lack these antibodies; within this group the risk of developing thrombosis increases with increasing plasma concentration of antibodies.

Key words: heparin, antibodies, thrombosis, platelets

Heparin is one of the most commonly used drugs in patients requiring therapeutic interventions for cardiovascular disease. However, use of heparin may have major side effects, one of the most dramatic being heparin-induced thrombocytopenia (HIT) (1, 2). HIT is a drug-induced IgG-mediated thrombocytopenic disorder. Because patients with HIT may develop multiple and recurrent arterial and venous thromboembolism, it is often stated that heparin may paradoxically cause the same complications that it is intended to prevent.

Anticoagulation-associated thrombocytopenia can be life-threatening, and difficult to diagnose without criteria for suspicion of the HIT syndrome (3). Estimates of the frequency of HIT vary, but it has been found that in 27% to 50% of post-cardiac surgery patients anti-PF4/heparin antibodies are detected by enzyme immunoassay (4, 5, 6). In previous retrospective studies, occurrence of HIT after heart surgery was associated with high post-operative mortality and increased incidence of arterial and venous thromboembolic events at short term follow-up (7,8). Similarly, patients who develop antibodies following heparin treatment during acute coronary syndrome also show a high risk of myocardial infarction at 30 days follow-up (9). In a previous study that involved patients with acute coronary syndromes we found a higher risk of thrombotic events (death, MI, recurrent angina, urgent revascularization, stroke) in patients that developed anti-PF4 antibodies (10). However, although the early prognostic significance of anti-PF4/heparin antibodies is well established, whether this also portends a worse prognosis on the long term, and whether this is related to antibody titer, is largely unknown.

The aim of the present study was to investigate thrombotic events during long-term follow up in patients who develop anti-PF4/heparin antibodies in response to heparin administered because of cardiac surgery and to ascertain whether there is a relationship between antibody concentration and events.

METHODS

Study design: Patient population included 320 consecutive patients undergoing elective cardiac surgery; of this group we selected 250 patients that matched inclusion criteria (see below). Detailed clinical data were recorded for each patient, including age, gender, degree of coronary stenosis, cardiovascular risk factors, as well as drug treatment and smoking status within the preceding year. Patients with anti-PF4/heparin antibodies, known history of HIT, history of prior major bleeding (defined according to Fitzmaurice) (11), history of hypercoagulation (12), chronic coumadin treatment, and those at high risk for bleeding according to Serebruany (13) were excluded. Other exclusion criteria were: any stroke within the previous 3 months; history of active peptic ulcer in the previous 3 months; major surgery in the previous week; thrombocytopenia (<100,000/mm^3); anemia (men Hb <12.5 g/L, women Hb <11.0 g/L); renal insufficiency (creatinine clearance <30 mL/min); liver insufficiency; suspected endocarditis, pericarditis or myocarditis; malignant hypertension; known heparin sensitivity; pregnancy.

Blood samples were taken at the time of hospitalization and 6 days after surgery for analysis of anti-PF4/heparin antibodies. Platelet count was monitored daily until discharge. Heparin was given as a 5,000 IU i.v. bolus followed by a continuous infusion, with monitoring of aPTT. The choice of background therapy was left to the discretion of the treating physician. After 40 days all patients were re-evaluated, and blood samples were collected to measure antibody concentration (see below) and platelet count. Written informed consent was obtained from all patients. The protocol was approved by the Ethics Committee of our University.

Definition of thrombocytopenia and heparin-induced thrombocytopenia. Thrombocytopenia was defined as at least two consecutive platelet counts below 150,000 /mm^3, or a drop below 50% of the initial value (4, 10). “Early” thrombocytopenia was defined as thrombocytopenia occurring between the 1st and 5th day of heparin treatment. “Late” thrombocytopenia was defined as thrombocytopenia occurring after at least 6 days of heparin treatment. Heparin-induced thrombocytopenia was defined when a patient with possible HIT also had positive results of laboratory antigen assay for HIT-IgG.

Assay for Heparin-induced Antibodies: Three blood samples were checked, the first sample at baseline before the beginning of heparin infusion, the second on day 6 and the third sample on day 40 from the beginning of therapy. All laboratory testing was performed by experienced personnel, unaware of clinical variables. Serum samples were tested by a commercial immunoassay (Asserachrom® HPIA, Diagnostica STAGO (France), distributed by Roche Diagnostics (Monza, Italy). The heparin-PF4 ELISA is reported with an optical density (O.D.) value that is directly proportional to the antibody concentration (14,15). A positive result was defined as an O.D. at 492 nm of >0.50 units, a cut-off value recommended by the manufacturer based on studies of PF4/heparin ELISA in patients with established HIT and various control populations (10, 16). The heparin-PF4 ELISA test has high sensitivity (97%) and moderate specificity (54.8%) for the diagnosis of HIT (16,17).

Clinical outcome: Patients came back for clinical evaluation every three months for one year. The following events were evaluated: death from any cause, new myocardial infarction, recurrent angina, stroke and pulmonary embolism.

Statistical Analysis. Continuous variables are presented as mean±SD. Groups were compared using a Student T-test. Data were analyzed using SPSS V.11. The cumulative proportion of patients who had an event over time was estimated by the Kaplan-Meier method, and comparisons were made using the log-rank test. Comparison of proportion was performed using the McNemar test. Cox proportional hazards regression was used to relate antibodies to the primary outcomes of all-cause mortality and secondary outcomes of cardiovascular and thrombotic events. We also evaluated the association between continuously modeled O.D. and thrombosis, and the *P* for trend was calculated from regression with continuous variables. Pearson correlations were calculated to examine univariate relation of antibodies to continue variables. Differences in thrombotic events among antibodies quintiles were compared by analysis of variance (ANOVA). Multiple logistic regression analysis was performed to identify independent predictors of thrombotic events. Candidate variables included: age, gender, body mass index, positivity to PF4/heparin on day 6 postoperatively, previous MI, previous CABG and/or PCI, hypertension, smoking habits, admission platelet count 150.000 to 200.000, 200.000 to 250.000, or >250.000. A *P* value <0.05 was considered significant.

RESULTS

At day 6, heparin-PF4 antibodies were found in 79 patients (31.6 %). The clinical characteristics of the two groups of patients are reported in Table 1.

Table 1. Clinical characteristics of patients

Clinical characteristics	Antibody positive	Antibody negative	*P*
N. patients	79	171	
Female, nr of pts (%)	18 (22.7)	37 (21.6)	n.s.
Mean Age, y	69 $\pm$ 10	68 $\pm$ 7	ns
Weight, Kg	76 $\pm$ 20	78 $\pm$ 18	ns
BMI	26.3 $\pm$ 2.5	28.1 $\pm$ 3.0	ns
Previous MI; nr of pts (%)	44 (55.6)	91 (53.2)	0.05
Previous CABG or PCI; nr of pts (%)	28 (35.4)	52 (30.4)	0.001
Hypercholesterolemia nr of pts (%)	37 (46.8)	83 (48.5)	n.s.
Hypertension; nr of pts (%)	32 (40.5)	71 (41.5)	n.s.
Smoking habit; nr of pts (%)	29 (36.7)	60 (35)	n.s.
4 T low probability; nr of pts (%)	4 (5.0)	122 (71.3)	0.001
4 T intermediate probability; nr of pts (%)	26 (32.9)	43 (25.1)	0.001
4 T high probability; nr of pts (%)	49 (62.0)	6 (3.5)	0.001
CABG only, nr of pts (%)	54 (68.3)	120 (70.1)	n.s.
Concomitant CABG and valve, nr of pts (%)	10 (12.6)	38 (22.2)	0.001
Valve only, nr of pts (%)	15 (15.1)	13 (7.6)	0.001

Mean baseline platelets count was similar in patients who eventually developed antibodies compared with those who did not (200,000±53,000/mm^3 vs 210,000±51,000/mm^3; *P*=n.s.). However, mean nadir value of platelet count was significantly lower in patients who developed antibodies (82,000±31.000/mm^3 vs 105,000±52,000/mm^3, *P*<0.001).

Embolic events were evaluated during a follow-up lasting 1 year. A total of 84 thrombotic events were documented; patients with HIT developed 46 thrombotic events in 40 patients (6 patients with HIT later had multiple events); the vascular location for all HIT related thrombosis was evenly distributed between arterial (20 new myocardial infarction and death, 10 stroke, and 3 graft occlusion) and venous systems (16 pulmonary embolism).

The global analysis of incidence of events in patients with and without antibodies, determined by the log-rank test, showed a significant difference between the two groups in the composite of death, new myocardial infarction, stroke and embolic events (Figure 1). We found an increased risk of cardiac and embolic events in patients positive to antibodies: the O.R. for composite end-point was. 4.1 (95%C.I. 1.9 to 9.5; *P*<0.001).

With regard to specific end-points, patients with anti-PF4/heparin antibodies were more likely to have 1-year new myocardial infarction (25.3% vs 10.5%, *P*<0.001), pulmonary embolism (20.2% vs 5.8%, *P*<0.001), or stroke (12.6% vs 5.8%, *P*<0.001), than patients who were antibody-negative.

After multiple logistic regression analysis, anti-PF4/heparin positivity was an independent predictor of thrombotic events (OR 2.31; 95% CI, 1.96-4.25; *P*<0.001). Antibody positivity predicted thrombotic events independently of baseline platelet count.

In patients with positive test, optical density at discharge averaged 0.824±0.547 O.D. units, compared to 0.131±0.145 O.D. units in antibody-negative patients. Patients were then categorized in quintiles according to their O.D. value. Median O.D. was 0.206 units, and the 25th and 75th percentiles were 0.001 O.D. units and 1.219 O.D. units, respectively. By quintile analysis, the median nadir platelet value decreased significantly with increasing quintiles of O.D (Figure2)

Mean O.D. was 0.913±0.670 units in individuals with thrombosis vs. 0.212±0.225 units (P<0.001) in patients who remained free of thrombotic complications at one year. By quintile analysis, patients with higher O.D. values were at significantly increased risk for thrombosis; using quintiles of optical density, the ORs (95% CI) for risk of thrombosis were: 1.04 (0.26-2.54) in the second quintile; 2.25 (1.90-3.75) in the third quintile; 5.10 (2.40-7.16) in the fourth quintile, and 7.68 (4.04-9.20) in the fifth quintile (P<0.001 for trend). Patients in the highest quintile experienced 36 thrombotic events as compared with patients in the lowest quintile who developed only 2 thrombotic events (P<0.001) (Figure 3).

DISCUSSION

The major finding of the present study is that patients who develop antibodies against PF4-heparin complex have a significantly higher rate of thrombotic events during follow-up than those who lack these antibodies; the risk of thrombosis increase with increasing antibody titer.

Thrombocytopenia has long been known to represent a clinically relevant complication in patients with cardiac disease. Patients with heparin-induced thrombocytopenia have an increase rate of bleeding complications, ischemic events, and early mortality (18,19) Vanderschueren and coworkers evaluated the prognostic significance of thrombocytopenia in patients admitted to an intensive care unit, and found that it is a powerful predictor of adverse events (20). A similar finding was reported by Overgaard: in patients referred for PCI, baseline thrombocytopenia was an independent predictor of in-hospital mortality (19).

Antibodies to heparin-PF4 were detected in 31.6% of our patients treated with unfractionated heparin. Our results are in accordance with those previously reported in CABG population (21, 22). Cardiopulmonary bypass is known to induce strong platelet activation and the release of large amount of PF4 into the plasma. This is one possible reason for the high frequency of HIT-IgG formation that occurs after cardiac surgery; Bauer found that 50% of patients undergoing cardiopulmonary bypass had antibodies detected by antigen assay post-operatively (6). Furthermore, the typical onset of HIT (beginning 5 to 10 days after cardiac surgery) coincides with the time period in which the platelet count typically is falling to thrombocytotic levels following perioperative hemodilution. In fact, recent guidelines suggests that in patients who have undergone cardiac surgery, clinicians should consider a fall in the platelet count >50% occurring between postoperative days 5 and 14 to represent potential HIT, and should prompt laboratory investigations for HIT antibodies (23).

Previous exposure to heparin could also influence the development of antibodies (24). To avoid this confounding factor, we excluded from the study patients with above-normal antibody titer before surgery. In the present study, antibodies to the PF4-heparin complexes were detected by using an antigen assay. Both the functional platelet activation assays and the antigen assays are very sensitive for detecting HIT antibodies. Antigen assays detect all antibodies classes (IgG, IgA, IgM) to the PF4-heparin complexes; however they are less specific than functional assay in detecting clinically relevant antibodies (25). Platelets activation assays, such as the serotonin-release assay, exclusively detect platelet-activating antibodies of IgG class. Therefore they have much higher specificity for detecting clinically relevant antibodies. However, performance of these functional assays required experienced staff and is restricted to a few references laboratories (26). Previous studies showed that IgM and IgA class of antibodies did not activate platelets, only IgG antibodies can activate platelets via their FcγIIα or IgG-receptors (25). The test we used detects antibodies of all three Ig classes, mainly of IgG class (27, 28). The diagnostic interpretation of the test was performed in the context of platelet counts. Prospective data indicate that an increased risk of thrombosis occurs in the group of patients whose platelet count has fallen in relation to HIT antibody formation. (23, 29, 30)

The specificity of this assay for clinical HIT increases in relation to the magnitude of a positive test results (expressed in O.D. units, especially >1.0) (14)

We found a relation between optical density value of antibodies and thrombosis. In vitro study have demonstrated that platelet aggregation in HIT patients is dependent on the relative quantity of heparin-PF4 antibodies, which is consistent with our observation that patients with higher O.D. had a significant higher number of thrombotic events (31) .

To our knowledge, only few retrospective studies, performed in non-cardiac patients, evaluated the relationship between antibody concentration and risk of thrombosis (14, 32). Pena de la Vega and coworkers found that higher concentrations of antiheparin/ PF4 antibodies were associated with increased cardiovascular and all-cause mortality in chronic hemodialysis patients (32). Higher antibody levels were also associated with elevated circulating markers of thrombin generation in patients with suspected HIT (33). Zuicker and coworker, in a retrospective study, found that patients with HIT antibodies suffering thrombosis had higher ELISA O.D. values. They identified a threshold of O.D., and found a near 6-fold increase in the risk of thrombosis in those with OD values >1.0 compared with lower O.D. (14).

On the basis of these earlier observations, we hypothesized that antibodies titer could predict which patients undergoing cardiac surgery were at high risk of thrombosis; consequently, we evaluate the risk according to increasing value of O.D. A sensitive and specific assay to detect heparin-dependent IgG antibodies was used. Our results, obtained in a prospectively enrolled cohort, followed up for one year, provide direct support to earlier retrospective findings about the fact that a prothrombotic status can persist long after initial exposure to heparin; furthermore, for the first time we were able to document a significant relationship between initial antibody titer and long-term outcome. Of note, the risk of thrombosis increased with increasing quintile of optical density, with patients in the highest quintile having a nearly 7-fold increased risk of thrombosis than those in lowest quintile.

In conclusion, exposure to heparin is associated with a substantial risk of developing antiheparin/ PF4 antibodies. This phenomenon, besides its acute negative effects on platelet count and pro-thrombotic status, portends a significantly worse prognosis also in the long term. Detection of antiheparin/ PF4 antibodies in the post-operative phase after cardiopulmonary bypass surgery, may suggest that re-exposure to heparin should be avoided in such patients, and that they should be managed as a high-risk group for thrombotic events.

Figure legend

Figure 1. Event free survival curves, comparison between patients who developed antibodies (dashed line) and patients who did not developed antibodies after surgery.

Figure 2. Distribution of platelet count in patients categorized according to quintiles of optical density. The nadir platelet counts decreased with increasing optical density value.

Figure 3. Risk of thrombosis in patients categorized according to quintiles of optical density. The risk of developing thrombosis increased with increasing optical density of antibodies.

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figure 1

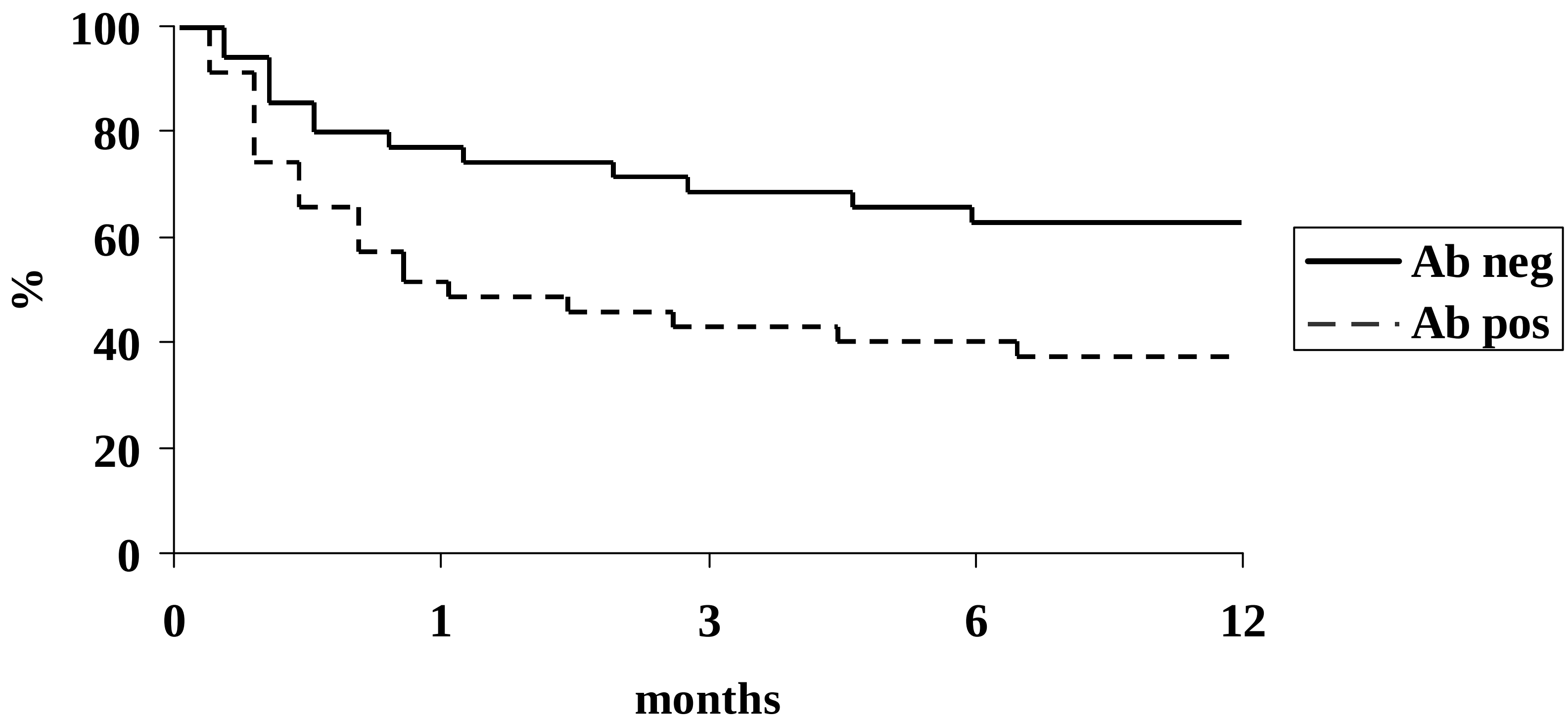

figure 2

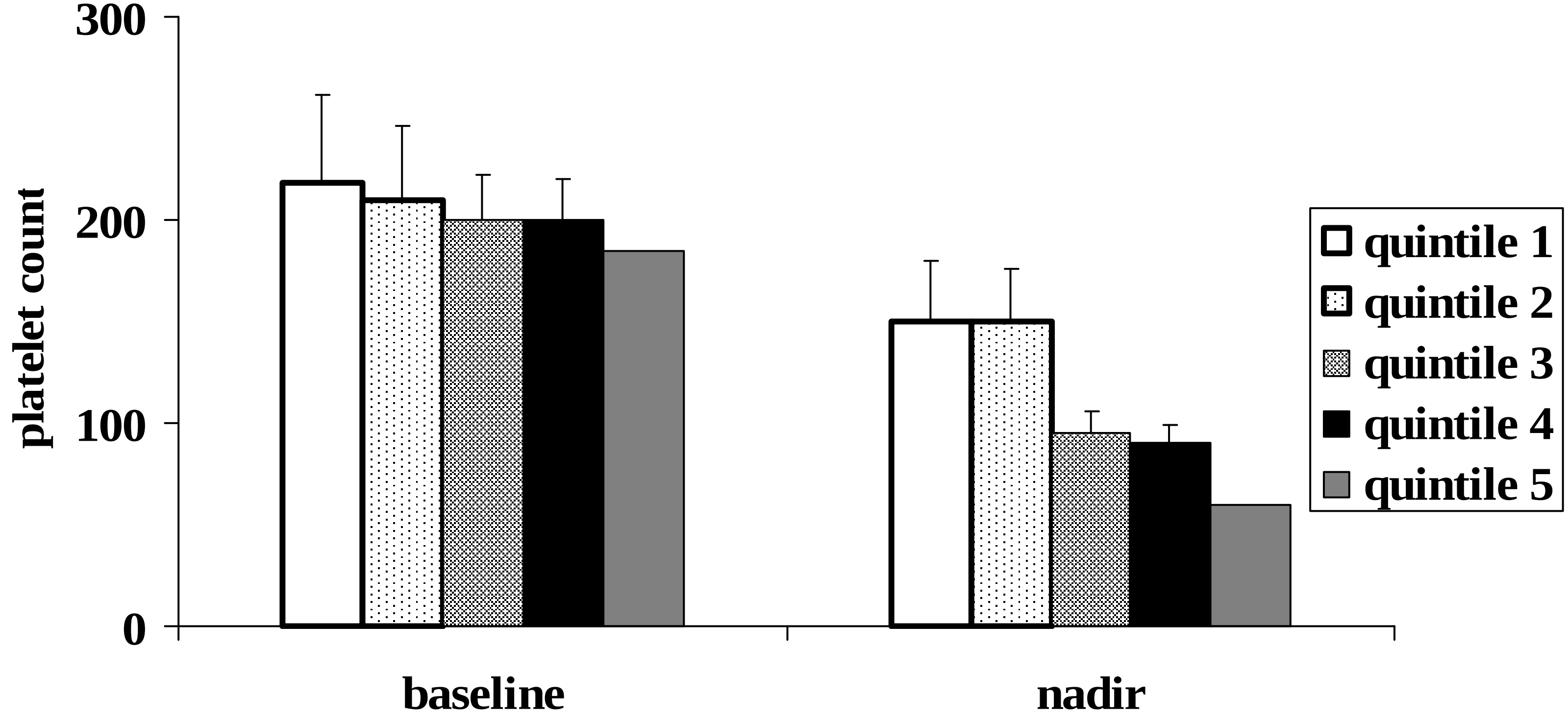
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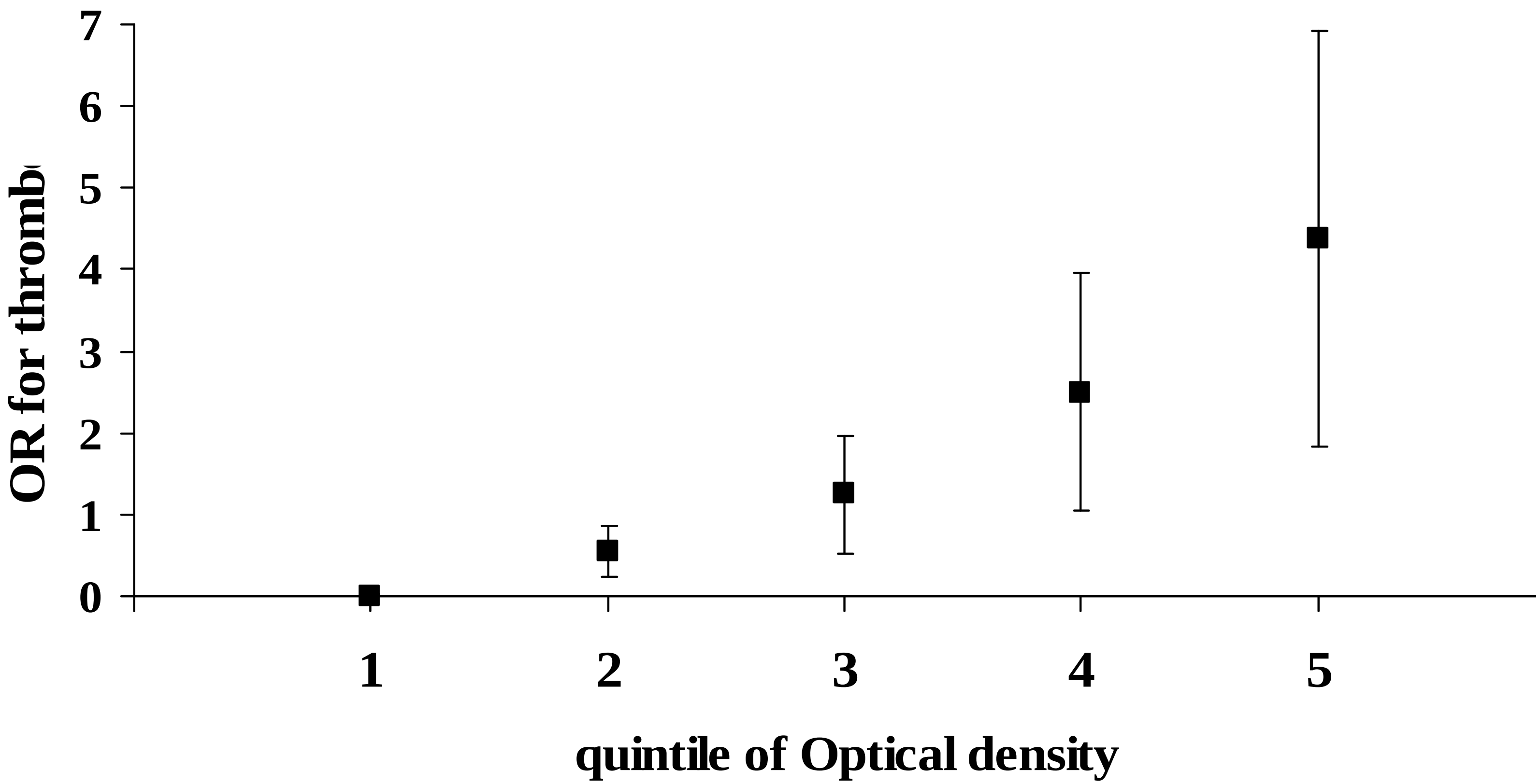
figure 3



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