Platelet-activating protamine-heparin-antibodies lead to higher protamine demand in patients undergoing cardiac surgery

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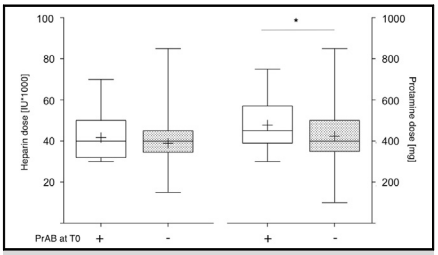
ABSTRACT

Objectives: Platelet-activating antibodies against protamine-heparin-complexes were described in patients undergoing cardiac surgery, but their clinical consequences remain unclear. This prospective single-center observational study aimed to describe the prevalence and clinical consequences of protamine-heparin-complex antibodies in patients undergoing cardiac surgery with cardiopulmonary bypass.

Methods: A total of 200 patients undergoing cardiac surgery with cardiopulmonary bypass were included. Blood samples were collected preoperatively and 1 hour, 24 hours, and 7 days after weaning from cardiopulmonary bypass. All sera were tested for the presence of protamine-heparin-complex antibodies using a modified heparin-induced platelet-activation assay. Specific Fcγ receptor IIa–dependent platelet activation was confirmed by repeated testing in the presence of the Fcγ receptor IIa–blocking antibody IV.3.

Results: Samples from 185 patients were obtained, of whom 24 patients (13%) were positive for protamine-heparin-complex antibodies preoperatively. In all positive samples, functional reactivity was reversible in the presence of IV.3. Although patients with a preoperative presence of protamine-heparin-complex antibodies were significantly older compared with patients negative for protamine-heparin-complex antibodies (73 ± 9.8 years vs 68 ± 10 years, $P = .037$), no other potential risk factors were identified at 1 day before operation. Patients with protamine-heparin-complex antibodies required significantly more protamine to neutralize heparin (47.66 mg vs 41.67 mg, $P = .027$). Protamine-heparin-complex antibodies have no significant influence on perioperative platelet numbers, bleeding complications, transfusion requirement, thromboembolic events, major cardiovascular and cerebrovascular events, inflammation parameters, or kidney function.

Conclusions: Protamine-heparin-complex antibodies occur frequently in patients undergoing cardiac surgery on cardiopulmonary bypass, resulting in specific platelet activation in vitro. Protamine-heparin-complex antibodies are associated with increased protamine requirement after cardiopulmonary bypass and possibly slower recovery of platelet numbers. (J Thorac Cardiovasc Surg 2015;150:967-73)



Although heparin doses are similar, PrAB-positive patients require higher doses of protamine.

Central Message

PrAB in patients undergoing cardiac surgery have effects resulting in higher protamine demand to neutralize heparin-mediated anticoagulation.

Perspective

Platelet-activating PrAB affect the perioperative protamine management and might influence the perioperative outcome of patients undergoing cardiac surgery. Further characterization of the effects in larger patient cohorts will lead to a better understanding of the clinical relevance of these antibodies. This could imply preoperative testing for the presence of these antibodies in the future.

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Abbreviations and Acronyms

ACT = activated clotting time
CI = confidence interval
CPB = cardiopulmonary bypass
FcγRIIa = Fcγ receptor IIa
HIT = heparin-induced thrombocytopenia
MACCE = major cardiovascular and cerebrovascular events
NPH = neutral protamine Hagedorn
OR = odds ratio
PrAB = protamine-heparin-complex antibody
T0 = 1 day before operation
T1 = 1 hour after weaning from cardiopulmonary bypass
T2 = 24 hours after operation
T3 = 7 days after operation

Supplemental material is available online.

Protamine, an arginine-rich, positively charged protein physiologically involved in sperm head stabilization during spermatogenesis,[1] is widely used in medicine as a stabilizing adjunct to insulin and a neutralizer of heparin-mediated anticoagulation, particularly after cardiac surgical procedures using cardiopulmonary bypass (CPB).[2,3] In addition to various side effects including systemic hypotension, anaphylactic reaction, and pulmonary vasoconstriction with secondary right heart failure,[4-6] thrombocytopenia after protamine administration following CPB has been described.[7,8] Furthermore, protamine and protamine-heparin-complexes are immunogenic.[9-11] Lee and colleagues[12] retrospectively screened patients undergoing CPB for the presence of protamine-heparin-complex antibodies (PrAB). The prevalence of PrAB in patients undergoing cardiac surgery ranged from 1% to 9.6% preoperatively increasing to 26.6% to 31% 10 and 30 days postoperatively, respectively.[10,12] Different risk factors for the preoperative presence of PrAB have been suggested, including obesity, arterial hypertension, previous myocardial infarction, previous cardiac surgery with CPB, nicotine abuse, and diabetes mellitus; however, no significant independent predictor has been characterized so far.[10,12,13] A subgroup of PrAB shows similar characteristics as antiplatelet factor 4/heparin antibodies in heparin-induced thrombocytopenia (HIT). These antibodies were shown to be capable of heparin-dependent platelet activation via Fcγ receptor IIa (FcγRIIa).[10,12,14] Immunogenic platelet-activating features of protamine or protamine-heparin-complexes and the perioperative presence of PrAB raise the question for a PrAB-mediated pathomechanism leading to perioperative platelet activation. Therefore, we prospectively analyzed the perioperative prevalence of platelet-activating PrAB in patients undergoing cardiac surgery on CPB and their impact on clinical outcome parameters.

MATERIAL AND METHODS

Study Design

The present study was a prospective single-center observational study. It aimed to describe the prevalence and time course of the occurrence of PrAB in patients undergoing cardiac surgical procedures with the use of CPB. The study was approved by the ethical committee of the Faculty of Medicine at Justus Liebig University Giessen, Germany. The trial was designed and conducted in accordance to the Declaration of Helsinki.

Patients and Samples

Patients were eligible for participation if they were aged more than 18 years, were undergoing any cardiac surgical procedure with the use of CPB, and gave informed consent for study participation. Exclusion criteria were thrombocytopenia (<100 G/nL) at the time of admission, any known chronic thrombocytopenia or thrombopathy, a history of HIT, or an allergic reaction to heparin or protamine. All patients being referred to the study center during the inclusion period were screened on the day before surgery and, if eligible, invited to participate in the trial. The participants were interviewed, and their clinical data were reviewed for a history of arterial hypertension, obesity, myocardial infarction, thromboembolic events (ischemic cerebral infarction, transitory ischemic attacks, thrombosis), cardiac surgery with CPB, nicotine abuse, or atopy and diabetes mellitus with or without insulin treatment. A sample of 10 mL native blood was obtained from the participants 1 day before operation (T0), 1 hour after weaning from CPB (T1), 24 hours after operation (T2), and 7 days after operation (T3). Clinical follow-up was continued until discharge from the hospital. We prespecified to exclude patients from analysis if follow-up until postoperative day 7 was not available. Sample tubes were immediately centrifuged, and sera were stored at −80°C until further processing.

Serologic Studies

All serologic analyses were conducted after completion of the inclusion period. Therefore, PrAB testing results were not known to the clinicians during the patients' treatment period. Laboratory personnel were blinded to the patients' clinical data. All sera were tested for the presence of antibodies reactive to protamine-heparin-complexes. For this purpose, an established heparin-induced platelet activation assay protocol[15] was modified. In brief, each sample was tested with washed platelets from 4 different donors in the presence of protamine (2 $\mu g \times mL^{-1}$) and protamine and heparin in low (0.2 IU $\times$ mL^{-1}) or high (100 IU $\times$ mL^{-1}) concentration. Reactions were placed in microtiter wells containing spherical stir bars and stirred at approximately 500 rpm. Wells were examined optically at 5-minute intervals for loss of turbidity. A serum was interpreted as reactive (positive) if a shift from turbidity to transparency (assessed optically) occurred within 30 minutes in at least 2 of 4 platelet suspensions in the presence of protamine or protamine and 0.2 IU $\times$ mL^{-1} heparin. Specificity of positive reactions was confirmed by repeated testing in the presence of the FcγRIIa-blocking monoclonal antibody IV.3 (10 $\mu g \times mL^{-1}$).

To identify patients positive for heparin-dependent HIT antibodies, all sera also were tested for the presence of HIT-antibodies using a heparin-induced platelet activation assay protocol as previously reported.[15] In brief, each sample was tested with washed platelets from 4 different platelet donors in the absence (buffer alone) or presence of heparin (0.2 U $\times$ mL^{-1} and 100 U $\times$ mL^{-1}). Similar to the PrAB testing, a serum was interpreted as reactive if a shift from turbidity to transparency occurred within 30 minutes in at least 2 of 4 platelet suspensions in the presence of 0.2 U $\times$ mL^{-1}, but not 100

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