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Platelet Factor 4–Positive Thrombi Adhering to the Ventricles of a Ventricular Assist Device in Patients with Heparin-Induced Thrombocytopenia Type II

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ABSTRACT

Background. Thromboembolism is a major complication in patients with ventricular assist devices (VADs). Drug anticoagulation and the use of biocompatible surfaces, such as coating with heparin, aim to reduce thromboembolism in these patients. Administration of heparin can lead to heparin-induced thrombocytopenia (HIT) type II, mainly through heparin/platelet factor 4 (PF4) antibodies. We assessed the presence of PF4 antibodies in VAD thrombi of patients with heparin-coated VADs and HIT II.

Methods. Thrombi (n = 6) were obtained from the replaced Excor ventricles of patients with HIT II after biventricular VAD implantation (Excor Adult; Berlin Heart, Germany). Excor ventricles were changed after clinical examination and suspicion of thrombi in the polyurethane valves. Expression of PF4- antibodies was assessed with the use of a polyclonal rabbit antibody (anti-PF4 antibody; Abcam, USA). Expression was assessed by 2 independent observers.

Results. Biopsies of all thrombi showed an extreme positive immunoreaction for PF4. No differences between the different thrombi and localization (left/right Excor ventricle) were observed. The thrombi were organized, without lamination of fibrin and cellular layers.

Conclusions. Platelet surface expression of PF4 in the thrombi reflects HIT antigen presentation. The physical relationship between the PF4-positive thrombi and the heparin-coated surface suggests that onset of HIT II could be influenced by the immobilized heparin coating.

Thromboembolism is a major complication in patients with ventricular assist devices (VADs).[1] Standard long-term anticoagulation protocols are usually based on a combination of coumarine and antiplatelet agents to reduce thromboembolic events. However, during the perioperative period the systemic continuous administration of unfractionated heparin represents the standard anticoagulation therapy, for reasons of low cost, short half-life, and well established monitoring by activated clotting time or activated partial thromboplastin time.[2] Administration of heparin can lead to 2 types of heparin-induced thrombocytopenia (HIT). Type I HIT is characterized by a moderate reduction in platelet counts early in heparin therapy and usually occurs within the first 1–3 days. Type II HIT is immunologically mediated, mainly through heparin/platelet factor 4 (PF4) antibodies,[3] being a frequent complication (up to 7.8 %) in this group of patients.[4] Clinical characteristics of HIT II are potentially life-threatening arterial and venous thromboembolic complications and bleeding.[5] However, the use of biocompatible surfaces, such as tube-coating with heparin, is recommended to reduce thromboembolism in patients with mechanical assistance. The relationship between the heparin coating of these devices and the development of HIT II is poorly understood. The

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aim of the present study was to investigate the local expression of PF4 antibodies in thrombi of patients with heparin-coated VAD and HIT II.

MATERIALS AND METHODS

Biventricular support (Excor; Berlin Heart, Berlin, Germany) was indicated after acute decompensation of 2 patients presenting with dilative cardiomyopathy. Surgery and postoperative course were uneventful. Postoperatively (5 and 7 days, respectively) a drop in platelet count of >50%, as well as the presence of thrombi in the Excor ventricle after visual inspection suggested the possibility of HIT II. Laboratory tests for HIT II, including functional tests and antigen assays, were performed. Heparin administration was immediately terminated and an alternative anticoagulation strategy with argatroban initiated. However, despite adequate anticoagulant therapy, both patients developed new thrombi in the right ventricle, which were detected by regular visual inspection. Indication for an exchange was given mainly after clinical examination and suspicion of thrombi in the polyurethane valves (Fig 1). Thrombi (n = 6) were obtained from replaced left (n = 2) and right (n = 4) Excor ventricles. The thrombi were stored in 4% formalin for 48–72 hours and embedded in paraffin and cut into 5-μm serial sections for immunohistochemical and histologic studies. Hematoxylin-eosin staining was performed to localize and to differentiate cell nuclei, fibrin, and collagen. Expression of PF4 antibodies was assessed with the use of a polyclonal rabbit antibody (anti-PF4 antibody; Abcam, Cambridge, Massachusetts). Briefly, the sections were consecutively incubated in: 1) polyclonal anti-PF4 antibody at a dilution of 1:100 for 1 hour; 2) 3% hydrogen peroxide (Merck, Darmstadt, Germany) to block endogenous peroxidase for 10 minutes; 3) visualization reagent (dextran polymer conjugated with horseradish peroxidase and affinity-isolated goat anti–rabbit immunoglobulins; (Envision; Dako, Glostrup, Denmark) for 30 minutes; and 5) 3,3′-diaminobenzidine tetrahydrochloride solution (Dako). Control for specificity of immunohistochemistry was performed by omitting any essential step of the immunoreaction. PF4 expression was assessed by 2 independent observers.

RESULTS

Macroscopic examination revealed in all cases well organized thrombi adhering to the sinuses of the polyurethane valves inside the Excor ventricle (Fig 1). No floating macroscopic thrombi were observed in the device. Histologically, VAD thrombi were organized (Fig 2A), without histologic lamination of fibrin and cellular layers (Fig 2A and B). Thrombocytes were localized in the external side of the thrombus, surrounding a collagenous core in the center. Biopsies of all thrombi showed an extreme positive immunoreaction for PF4 (Fig 2C and D). No differences in PF4 expression and histologic appearance between the different thrombi independently from their localization (left/right Excor ventricle) were observed.

DISCUSSION

Optimal anticoagulation therapy in patients with biventricular VADs is essential to long-term outcome, because thromboembolism and bleeding events are the most common complications of these patients.[6] HIT II is a life-threatening disease that is particularly difficult to diagnose and to treat in VAD patients. The pathophysiologic mechanism of HIT II has been previously described. Briefly, heparin binds to the platelet surface and leads to release of PF 4. The heparin-PF4 (HPF4) complex undergoes a conformational change, leading to formation of antibodies. The HPF4 complex–bound antibody then binds to platelets, further initiating the coagulation cascade causing thrombin production and platelet activation. The antibody complex can also activate endothelial cells, leading to local thrombus formation and endothelial permeability.[3]

Diagnosis of HIT II is based on clinical and laboratory symptoms, and the presumption of diagnosis demands an immediate change of the anticoagulation protocol. However, diagnosis of HIT II in patients treated with extracorporeal devices remains a challenge, because thrombocytopenia is a common finding in patients on extracorporeal membrane oxygenation (ECMO) and HIT II might be present despite negative testing.[7] On the other hand, positive testing for HPF4 antibodies is found in 88% of patients with VADs, and HIT II was diagnosed in 3 of 9 acute respiratory distress syndrome patients on ECMO in another series.[1,7]

Expression of PF4 in the thrombi reflects the HPF4 complex binding to platelets. The physical relationship

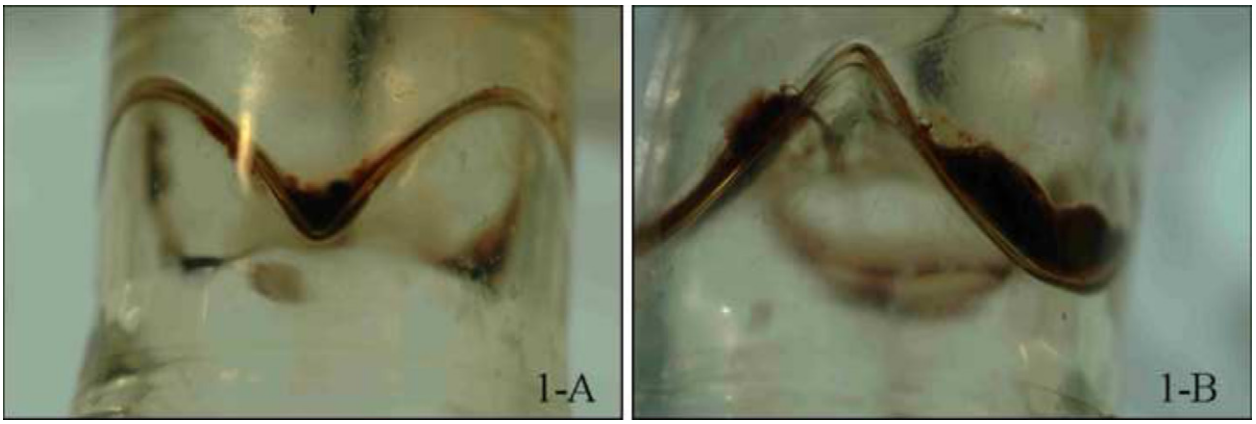

Fig 1. Thrombi in the polyurethane valves after ventricle change.