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Pathomorphological aspects of heparin-induced thrombocytopenia II (HIT-II syndrome)

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Abstract The therapeutic use of heparin results in thrombocytopenia in 5–30% of patients. In 0.1–1% of patients treated with heparin, the platelet count decreases to between 100 × 10^9/l and 50 × 10^9/l and leads to severe synchronous central arterial and venous thrombosis with a mortality of 18–36%. This is known as "white-clot syndrome" or heparin-induced thrombocytopenia II (HIT-II syndrome). Whilst the clinical aspects and the central type of thrombosis in HIT-II syndrome are well documented, the histomorphology and differential diagnosis of thrombosis are not. We report three cases of HIT-II syndrome with thrombosis of the central arteries and veins. The HIT-II thrombi could be differentiated from thrombi of other origins, particularly from mural thrombi. Heparin-induced thrombi were seen on microscopical examination to be like onion skin in structure, and immunohistochemistry showed that they had a markedly reduced content of fibrin and clearly enhanced amounts of IgG and IgM. The layered structure thus implied appositional growth. The thrombi in HIT-II syndrome do not seem to be induced by activation of the coagulation cascade, but by platelet aggregation mediated by anti-platelet antibodies.

Key words Heparin · Thrombocytopenia · Thrombosis · Pathomorphology · Immunohistochemistry

Introduction

Heparin is an endogenously synthesized mucopolysaccharide, which binds to and activates antithrombin III, thus augmenting the inhibitory effects of coagulation proteins. As a drug for prevention and therapy of thromboembolic events it is widely used in all medical disciplines. For therapeutic use, synthetic heparin or natural heparin derived from bovine lung or porcine intestinal mucosa is available. Depending on the manufacturing process, heparin has a molecular weight between 6,000 and 20,000 Da.

In general, heparin is well tolerated, but side effects such as bleeding, osteoporosis, and alopecia and hypersensitivity reactions such as arthralgia, urticaria, itching, dyspnoea, bronchospasm, hypotension, nausea, vomiting, fever, headaches and anaphylactoid reactions may occur. In 5–30% of patients thrombocytopenia also develops [1, 2]. Two clinical types of heparin-induced thrombocytopenia (HIT) are known. In HIT-I syndrome thrombocytopenia is transitory, without thrombus formation [3]. It usually occurs 1–2 days after the administration of heparin and involves a mild reduction of platelet counts to between 150 × 10^9/l and 100 × 10^9/l. Even when heparin therapy is continued the platelet count returns to normal values [4, 5]; thrombocytopenia is caused by platelet aggregation as a direct effect of heparin [6, 7]. In HIT-II syndrome platelet numbers decrease more dramatically, to between 100 × 10^9/l and 50 × 10^9/l [2], but a normal platelet count does not exclude the diagnosis [8, 9]. The thrombocytopenia usually develops within 6–14 days after the first administration of heparin [10, 11]. In patients who have had previous contact with heparin thrombocytopenia may appear earlier [12]. A characteristic feature is the occurrence of thrombi in great central vessels, and often in both arteries and veins [13]. HIT-II syndrome is found in 0.1–2% of heparin-treated patients [1, 14] and is accompanied by a mortality of 18–36% [2, 11, 15, 16]. The form of heparin administration (subcutaneous, venous, or minimal quantities for catheter flushing) and the dosage have no significant influence on the appearance of HIT-II syndrome and there is no male or female predominance [17]. All this is in contrast to the significance of the origin of the heparin: bovine heparin seems to have a significantly greater capacity to induce HIT-II syndrome than does porcine

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heparin [18]. However, the likelihood of HIT-II syndrome is significantly higher if common heparin is used rather than low-molecular-weight heparin [19].

HIT-II syndrome was first described in 1958 [20]. We now report three cases and add new findings relating to pathomorphology [21] and histomorphological differentiation; its pathophysiology is almost completely understood [22, 23].

Clinical histories

Case 1

A 65-year-old woman with a history of coronary artery disease was admitted following an anterior wall myocardial infarction. Systemic thrombolytic therapy with 100 mg tissue plasminogen activator (100 mg/2 h, Actilyse, Thomae, Germany) was started. Because of continued chest pain percutaneous transluminal coronary angioplasty of the left marginal artery was performed, while heparin (unfractioned porcine heparin, 1200 IU/h) was administered for systemic anticoagulation. On day 10 she developed thrombosis of the common iliac vein and right femoral vein; on day 17 her platelet count decreased to 85×10^9/l and the high-molecular-weight heparin was replaced by to low-molecular-weight heparin (Fragmin, Pharmacia, Germany). With this treatment, the platelet count increased to 200×10^9/l. Nevertheless, on day 23 a complete thrombosis of the left common iliac and femoral vein occurred. Two days later transoesophageal echocardiography (TEE) and computerized tomography (CT) revealed a large thrombus in the aortic arch, which was partly -attached to the wall and partly free-floating, effecting a partial occlusion of the descending aorta. Thrombi were also found in the left pulmonary artery, the inferior vena cava and the iliac veins. HIT-II syndrome was diagnosed, whereupon heparin was discontinued and therapy changed to administration of a heparinoid (200 IU/h, Orgaran, Organon, The Netherlands). Systemic thrombolytic therapy with 100 mg tissue plasminogen activator was also administered, and 70 g IgG (Sandoglobulin, Sandoz, Germany) was given intravenously. Despite this the patient died on day 28 with multiorgan system failure.

Case 2

In a 71-year-old woman, humeral fracture of the humerus was treated by intramedullary nailing. Preventive anticoagulation was started 3 days preoperatively with 5,000 IU heparin s.c. three times daily. On day 19 a deep venous thrombosis of the right leg occurred. In the next few days the patients platelet count dropped to 28×10^9/l. The mental status of the patient deteriorated, and there was a small subdural haemorrhage and mild cerebral oedema shown on cranial CT. The patient's course was complicated by generalized seizures; a second cranial CT revealed thrombosis of the venous sinuses. TEE showed a long thrombus attached to the wall of the ascending aorta. HIT-II syndrome was diagnosed, and systemic anticoagulation changed to the heparinoid Orgaran (200 IU/h). Despite a slight increase in the platelet count, the patient died on day 34 of fulminating pulmonary thrombotic occlusion.

Case 3

A 68-year-old woman underwent knee joint replacement. Heparin was administered three times daily (5000 IU s.c.). After 2 weeks she complained of dyspnoea and paresthesiae in both arms. On day 21 the patient developed respiratory insufficiency with shock, and her platelet count dropped to 24×10^9/l. TEE revealed a significant enlargement of the right ventricle and right atrium, with nonocclusive thrombus formation in the right and left atria; the right central pulmonary artery appeared to be totally occluded. On the next day, the right ventricle was seen by echocardiography to have become smaller again, suggesting regression of the thrombosis of the pulmonary artery. Therefore, thrombolytic therapy with rt-PA (100 mg/2 h, Actilyse, Thomae, Germany) was given immediately. Subsequent TEE showed a slight reduction of the right atrial and ventricular diameters. Despite this, the patient died on day 22.

General comments

There was no history of coagulative disorders in any of these patients. Thrombocytopenia from other causes, such as drugs, disseminated intravascular coagulation or septicaemia was excluded. In addition the presence of anti-platelet antibodies was demonstrated by the heparin-induced platelet activation assay [24]. In vitro cross-reaction to low-molecular-weight heparin was found, but was ruled out for Orgaran.

Materials and methods

For histomorphological evaluation the thrombotic material was stained with haematoxylin-eosin, elastic-van Gieson, azan, Ladewig and Congo red. Investigations with antibodies against IgG, IGM and fibrinogen were also performed.

Pathological findings

In case 1 the autopsy revealed extensive thrombosis of the following arterial and venous vessels: the aortic arch (3 cm distal to the aortic valve, a 6-cm long white thrombus with extension to both subclavian arteries); the abdominal aorta (large 10 cm white thrombus between diaphragm and 3 cm distal to the renal artery); the left common and internal iliac arteries; the inferior vena cava; both common iliac veins; both deep and superficial femoral veins; the left pulmonary artery (with occlusion of the branches to the upper and lower lobes).

In case 2 both arterial and venous thrombi were found, the vessels affected including the abdominal aorta (distal to the origin of the renal artery, thrombus 2.5 cm long, attached to the wall); the cerebral venous sinuses (with cerebral oedema); the right deep femoral vein; and the left pulmonary artery (complete occlusion).

In case 3 the autopsy confirmed the presence of white thrombi in the following vessels: abdominal aorta (5-cm white thrombus with occlusion of the coeliac trunk and superior mesenteric artery); the left axillary artery (two white thrombi of 1.5 cm in length); the right axillary artery (5 cm, with 75–80% occlusion); the right renal artery (0.5-cm white thrombus); the right deep femoral vein; the right superficial femoral vein (two thrombi, 3.5 cm and 5 cm in length); the right popliteal vein (12 cm); the right pulmonary artery (complete occlusion); the left pulmonary artery (3.5 cm distal to the pulmonary valve, a 1-cm white thrombus causing complete occlusion of the lumen).

In each case, autopsy confirmed the presence of white thrombi predominantly in large arterial and venous ves-

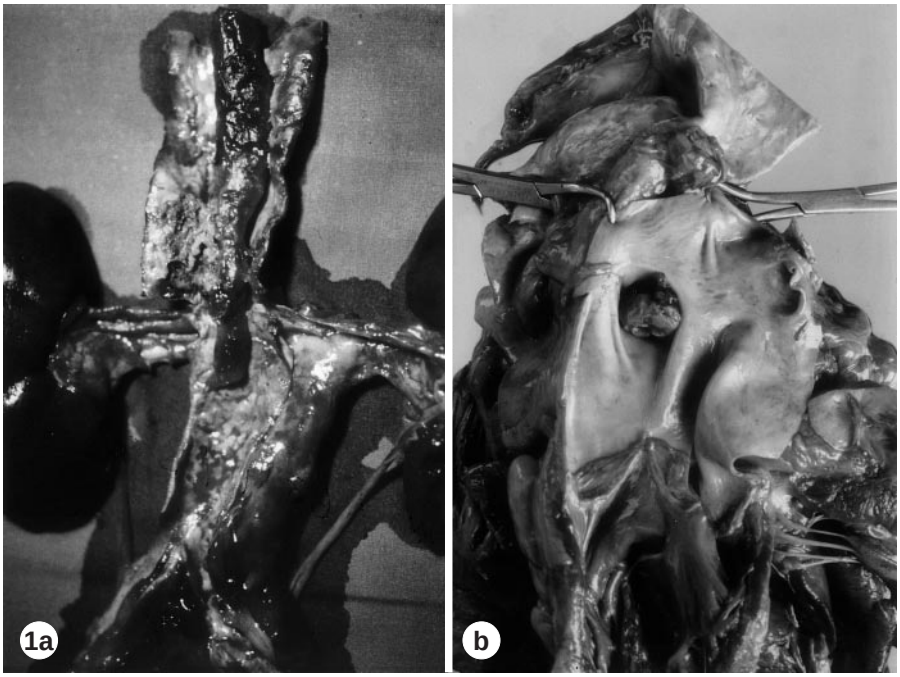


Fig. 1a, b Grossly, HIT-II thrombi are found predominantly in the great vessels. **a** Abdominal aorta with a 10-cm white clot localized between the diaphragm and 3 cm distal to the renal artery (case 1). **b** White clot causing complete occlusion of the right pulmonary artery (case 3)

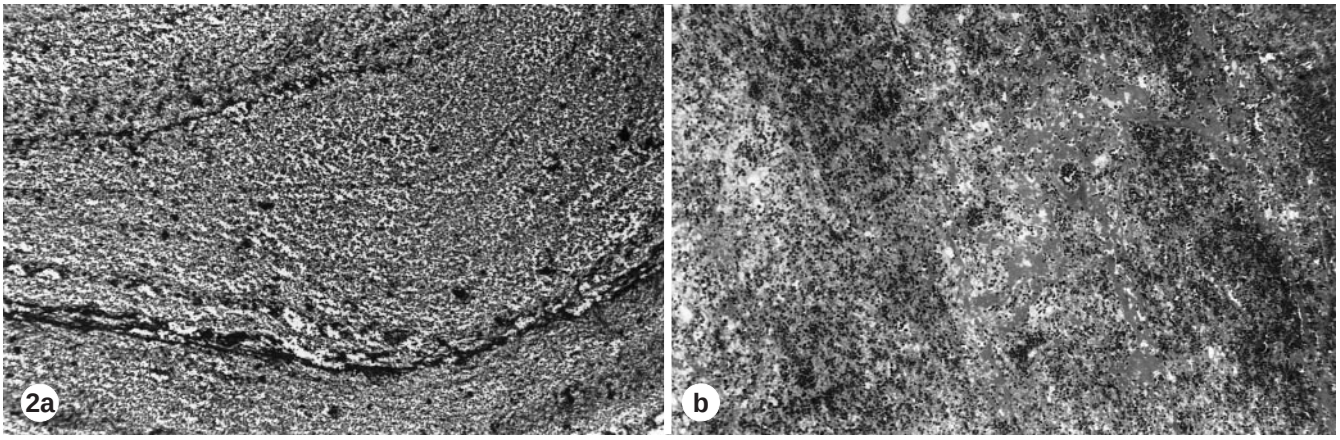


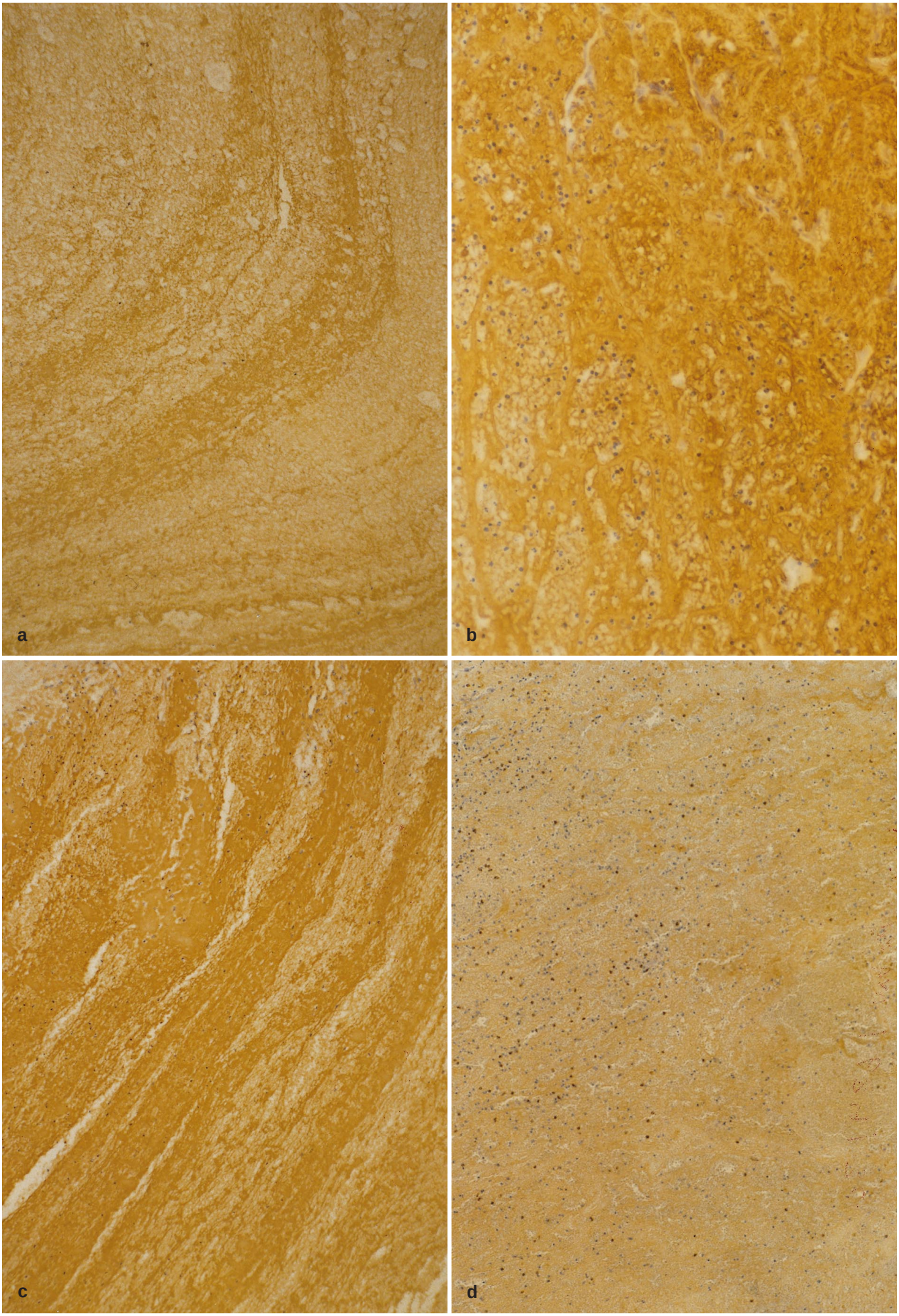
Fig. 2a, b Histomorphology of thrombi. **a** Heparin-induced thrombus is similar to onion skin in structure, with markedly decreased fibrin content resulting in a loose and wide-meshed fibrin web and a decreased number of leucocytes and lymphocytes. **b** Mural thrombi showing a large amount of fibrin with entrapped erythrocytes, leucocytes and thrombocytes. Haematoxylin-eosin, original magnification × 80

sels, regardless of the degree of preexisting arteriosclerosis (Fig. 1).

In each case, the heparin-induced thrombi had a structure reminiscent of onion skin, and most lacked entrapped leucocytes and lymphocytes (Fig. 2a). In comparison with thrombi from patients not suffering from HIT-II syndrome (Fig. 2b), heparin-induced thrombi had a markedly decreased fibrin content (Fig. 2a). Immunohistochemical staining for fibrinogen (DAKO, Hamburg, Germany) was weak in the HIT-II thrombi (Fig. 3a), while IgG and IgM staining (DAKO, Hamburg, Germany) was strong (Fig. 3c). This staining pattern was reversed in thrombi from patients not suffering from HIT-II syndrome (Fig. 3b, d). Endothelial cells from the occluded vessel showed no ulcerative or arteriosclerotic changes. The age of heparin-induced thrombi cannot be estimated, as they contain thrombocytes alone and have hardly any fibrin webbing. In Table 1 the differences between heparin-induced thrombi and those of other origins are listed.

Table 1 Morphological and immunohistochemical characteristics of heparin-induced thrombi and thrombi of other origins

Feature	Heparin-induced thrombi	Thrombi of other origins, e.g. mural thrombi
Structure	Lost of fibrin webbing Wide-meshed pattern Layerlike structure Similar to onion skin	Fibrin webbing Similar to coral colony
Cell content	Thrombocytes	Thrombocytes Erythrocytes Leucocytes Few lymphocytes
Fibrin content	(+)	+++
IgG/IgM content	++ – +++	(+)
Color	Whitish	White to greyish Grey-red


a
b
c
d

Discussion

In each of the three cases, autopsy revealed extensive thrombosis of the arterial and venous vessels, predominantly of the great vessels. Simultaneous thrombosis of the central arterial and venous vessels is the main difference from thrombosis of other aetiologies, where the thrombosis takes place preferentially in the peripheral vascular system. This uncommon distribution pattern is also seen in hyperhomocysteinaemia and in the antiphospholipid syndrome.

In cases of inflammatory, allergic, traumatic or degenerative endothelial denudation, mural thrombi develop. They occur classically in arteriosclerotic lesions of the great arteries. Coagulation of the entire blood column is found where there is reduction of the blood flow, turbulence or stasis, often in the deep veins of the leg and pelvis as a result of right heart failure of immobility and together with superficial varicosi ties. Thrombi caused by changes in blood composition (polycythaemia vera, hyperglobulinaemia, haemoconcentration or thrombocytosis) show the same pattern with both the arterial and venous systems affected. In disseminated intravascular coagulation thrombi are predominantly peripheral.

Histologically the HIT-II thrombi have a structure reminiscent of onion skin, suggesting an event that has taken place over some time. Fibrin is markedly diminished in HIT-II thrombi, and organization seems to be lost owing to the lack of fibrin webbing. The wide-meshed pattern may cause the loss of a filter function, leading to decreased leucocyte and lymphocyte content.

Mural thrombi are often referred to as platelet clots, are white to greyish colour and have a lamellated surface. In thrombi formed in diminished blood flow the findings are aggregation of erythrocytes (causing the red colour) and few leucocytes. These histomorphological phenomena allowed us to differentiate HIT thrombi from thrombi of other origins.

The commonly accepted theory of the aetiology of heparin-induced thrombi assumes an immunological process. Manifestation of the HIT-II syndrome after 6–14 days and evidence of heparin-induced anti-platelet antibodies in IgG and IgM fractions support this theory [2, 21, 25, 26]. Heparin seems to act as a hapten which, in complexes with platelet factor IV, induces the synthesis of anti-platelet antibodies. These bind to the Fc-receptors of thrombocytes, which thus become activated and release more platelet factor IV. In addition to this, Amiral et al. [27] demonstrated in a recent study that HIT-II syndrome can also be induced by IgA and IgM antibodies. This shows that platelet Fc-receptors are not necessarily involved in the pathophysiology of this syndrome, indicating the existence of other binding sites apart from the Fc-receptor that may be stimulated by immunoglobulins to release platelet factor IV. The antibody binds to soluble heparin and heparin-like molecules in addition to glycosaminoglycans localized on the surface of endothelial cells [3, 22, 23, 28]. Endothelial cells have differing surface morphology depending on the localization and type of the vessels, which may explain the different thrombusting patterns.

Our cases provide evidence that clotting in the HIT-II syndrome occurs regardless of the degree of preexisting arteriosclerosis. The mechanism may explain platelet aggregation without previous endothelial cell damage or denudation, and this finding and the decreased amount of fibrin demonstrated contradict the hypothesis that in the HIT-II syndrome the aggregation of thrombocytes is caused by activation of the coagulation system [15, 16, 29, 30]. The genesis of mural thrombus is thus less likely. It seems more likely that in the days before a thromboembolism appears, antigen–antibody reactions take place on the endothelial surface [1]. The antigen–antibody complexes mediated by a specific receptor in combination with thrombocytes create aggregated accumulations appearing as thrombi. The marked local enhancement of IgG and IgM supports this theory.

After cessation of heparin in HIT-II syndrome the platelet count may return to normal values within a few days [31], with a reduction in the risk of further thromboembolic events [2, 15]. Nevertheless, each of our three patients developed a progressive and ultimately lethal thrombosis. In case 1 no in vitro testing for cross-reactivity was performed, since the relationship of cross-reactivity to low-molecular-weight heparin was not fully understood in 1993 and Orgaran was not available for routine use at that time. In cases 1 and 2 the patients died after a recurrent thrombosis 11 days and 5 days, respectively, after heparin therapy was stopped. The persistent risk of thromboembolism was explained as due to persistent antibodies and sufficient endogenous heparin production. The fact that thromboembolic complications after cessation of heparin therapy are not found in all patients must be due to individual differences relating to the immune system and the quantity of endogenously produced heparin. Further investigations are necessary to determine whether arterial endothelial cells produce more or less heparan sulfate than venous endothelial cells.

The reduced fibrin content of heparin-induced clots in general may contribute to the almost complete unresponsiveness to anticoagulants and thrombolytic agents. In some patients, treatment with high-doses of immunoglobulins (IgG preparations) and prednisolone has produced an improvement in the clinical picture [32–35]. Immunomodulation seems to be a hopeful alternative therapy in HIT-II syndrome.

◀ **Fig. 3** Immunohistological staining with antibodies **a**, **b** against fibrinogen and **c**, **d** against immunoglobulin IgG. Heparin-induced clot shows **a** a markedly decreased fibrin content and **c** a high amount of IgG. Mural thrombi show **b** strong staining for fibrin and **d** weak staining for IgG. Original magnification × 100

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