Heparin-induced thrombocytopenia in patients treated with unfractionated heparin: prevalence of thrombosis in a 1 year follow-up

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Key words:
Heparin-induced thrombocytopenia; Unfractionated heparin; IgG antibodies; Thrombosis.

Background. **Patients with unstable angina are usually treated with unfractionated heparin and aspirin, but very little is known about the prevalence of heparin-induced antibodies and their relation to thrombotic complications some time after the acute phase of unstable angina. The aim of the present study was to establish the prevalence of heparin-induced thrombocytopenia and the prevalence of heparin-dependent platelet-reactive antibodies in patients treated with unfractionated heparin and the occurrence of thrombosis in a 1 year follow-up.**

Methods. **Patient population included 124 consecutive patients with unstable angina treated with unfractionated heparin for almost 5 days. The prevalence of heparin-dependent platelet-reactive antibodies using an ELISA assay was measured before the beginning of heparin therapy and after 7 and 40 days. The platelet count was measured at the same time and the presence of thrombotic occurrences was checked. Clinical follow-up lasted 1 year.**

Results. **At baseline no one patient was positive for heparin-induced antibodies. On day 6, 38 patients (30%) produced a positive heparin-induced antibody result and 30 patients (24%) had an intermediate result. The majority of patients (74%) who developed antibodies became positive after 6 days of heparin therapy. The combined incidence of death, myocardial infarction, recurrent angina, urgent revascularization and stroke was 66% in patients with antibodies and 44% in patients without antibodies during a 1 year follow-up. The incidence of combined primary end points was statistically higher in patients positive for antibodies. The log-rank test was statistically significant ($\chi^2 = 4.39$, $p < 0.01$).**

Conclusions. **No one patient developed a clinical evidence of thrombocytopenia. Nevertheless thrombotic events during follow-up were more common in patients who developed heparin-induced antibodies. These patients need a more accurate evaluation and surveillance after hospital discharge.**

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Introduction

Heparin is effective in the prevention and treatment of venous thrombosis and pulmonary embolism. The drug is administered to almost all patients during cardiac catheterization, angioplasty and cardiopulmonary bypass surgery and is used in the treatment of unstable angina and myocardial infarction and in the prevention of coronary artery rethrombosis after thrombolysis.

Heparin treatment is usually strictly monitored to maintain the ratio of the patient's activated partial thromboplastin time (APTT) within a definite range of approximately 1.5 to 2.5. Despite its benefits, the use of heparin has some serious side effects. One of these is heparin-induced thrombocytopenia (HIT). HIT is a drug-induced immunoglobulin-mediated thrombocytopenic disorder[1,2] that is important for several reasons. The most dangerous form of HIT develops after 5 to 10 days of heparin therapy and is classified as HIT type II[2]. It is a relatively common drug-induced immunohematologic adverse reaction, and it is frequently accompanied by life-threatening thrombotic complications. Heparin can therefore cause the same devastating complications that it is meant to prevent. It is now generally accepted that HIT type II is caused by an immunoglobulin, most likely IgG, that becomes detectable 5 or more days after exposure to heparin[3]. This pathogenetic IgG activates platelets in the presence of pharmacologic concentrations of heparin[4].

Patients hospitalized because of unstable angina are now usually treated with heparin and aspirin, but very little is known about the prevalence of heparin-induced antibodies and their relation to thrombotic events a long time after the acute phase of unstable angina. The aim of the present study was to establish the prevalence of

HIT type II and the prevalence of heparin-dependent platelet-reactive antibodies using an enzyme-linked immunosorbent assay (ELISA) in 124 patients with unstable angina treated with heparin for almost 5 days. Our data suggest that antibodies are more common than has been previously thought and that the occurrence of thrombosis in a 1 year follow-up is more frequent in patients with a positive ELISA than in those with a negative ELISA.

Methods

A total of 124 consecutive patients with unstable angina were enrolled in the study between December 1994 and December 1996. The inclusion criteria were: prolonged anginal pain or repetitive episodes of angina at rest or during minimal exercise in the previous 12 hours, new transient or persistent ST-T ischemic changes on ECG (ST segment elevation or depression • 0.1 mV, T wave inversion • 0.3 mV in 3 or more limb leads or 4 or more precordial leads excluding V_1), or an elevation of plasma levels of creatine kinase (CK) and of the CK-MB fraction. Exclusion criteria were: ST segment elevation lasting more than 20 min; thrombolysis in the previous 48 hours; coronary angioplasty within the previous 6 months; bypass surgery within the previous month; a history of platelet disorder or thrombocytopenia, active bleeding or high risk of bleeding; or stroke within the previous year. Patients who had serum creatinine values > 2.5 mg/dl or a platelet count < 150 000/mm^3 were also excluded. Written informed consent was obtained from all patients.

All patients were treated with 100 mg of aspirin daily. The choice of antianginal therapy was left to the discretion of the treating physician. The study drug was infused for at least 5 days.

Serologic assay for heparin-dependent IgG antibodies. We used an ELISA method to detect heparin-dependent IgG antibodies.

Plasma samples were tested with the use of Asserachrom H PF4 ELISA kits (Diagnostica STAGO, Asnieres, France). All reagents were included in the kit with the exception of 3 mol/l sulfuric acid (JT Baker Chemical, Phillipsburg, NJ, USA). Solutions were reconstituted, and the ELISA protocol was followed according to the manufacturer's directions. Each assay included a known positive control (heparin/platelet factor 4-PF4 antibody positive) and negative control (no heparin/PF4 antibodies) run in parallel. The results were interpreted according to the manufacturer's recommendations: a negative result was defined as an A492nm of < 0.25, an intermediate result was defined as an A492nm of < 0.25 and < 0.5, and a positive result was defined as an A492nm of < 0.5. These cut-off values recommended by the manufacturer are based on studies of the PF4/heparin ELISA in patients with established HIT and various control populations[5]. Absorbances of 0.5 are > 8 standard deviations above the values obtained from healthy normal subjects and are characteristically seen in patients with HIT[5]. Samples that generate absorbances in the intermediate range of detection (• 0.25 to < 0.5) are < 8 standard deviations necessary for a positive result and > 2 standard deviations above those of healthy normal subjects[5].

End points. The primary end point was a composite of death from any cause, new myocardial infarction, or refractory ischemia within 7 days of drug therapy. Rehospitalization for unstable angina was also counted in the composite primary end point when occurring at 7 and 30 days, then at 6 and 12 months.

Predefined secondary end points included the three components of the primary end point as separate measures, and a composite of death and myocardial infarction.

Myocardial infarction was defined as a new episode of chest pain, lasting at least 20 min, with new ST-T changes, new Q waves (> 0.03 s in duration in 2 or more leads) or both and a rise in the serum CK level to 2 times the normal upper limit with elevated CK-MB values. Refractory ischemic conditions included the following three sets of signs and symptoms: chest pain • 20 min in duration, or two episodes of chest pain, each lasting • 10 min, within a 1 hour period, with transient ST-T changes while the patient was receiving medical therapy which was adjusted according to heart rate and blood pressure; recurrent ischemia with pulmonary edema or hypotension; or repetitive chest pain (three or more episodes, each lasting • 5 min) necessitating intraaortic counterpulsation, urgent intervention, or both, within 12 hours.

Statistical analysis. Continuous variables are presented as mean ± 1 SD. The primary end point was analyzed with the use of Cox regression analysis to calculate the odds ratios and 95% confidence intervals (CI). The cumulative proportion of patients who had an event over time was estimated by the Kaplan-Meier method. Cumulative incidence curves were compared between groups by the Mantel-Haenszel test. A p value of < 0.05 was considered statistically significant.

Results

A total of 124 patients were enrolled in the treatment. Clinical characteristics of patients are reported in table I. The frequency of a positive ELISA did not vary with patient age, sex, previous cardiac disease, extension and gravity of ECG alteration, intravascular catheters, or extension of coronary involvement. At baseline, before starting heparin infusion no one patient tested positive for heparin-induced antibodies. On day 6, 38 patients (30%) tested positive for antibodies and 30 (24%) tested intermediate. Furthermore, 68 patients (54%) had an

Table I. Clinical and laboratory findings of patients included in the study.

	Total population	Antibody positive	Antibody negative
Clinical characteristics			
No. patients	124	38	86
Mean age (years)	65 – 10	67 – 8	64 – 11
Female/male	37/87	8/30	29/57
Hypertension	65	31	34
Diabetes	53	32	21
Laboratory findings			
Lowest platelet count (mm^3)	200 000	198 000	216 000
ALT	25 – 11	23 – 10	26 – 12
AST	27 – 12	25 – 10	29 – 12

increase in antibody titer measured as an increase in absorbance.

There was no difference in the mean platelet count between the antibody-positive and antibody-negative groups at baseline and after 7 days (baseline 267 ± 65 vs 251 ± 55 mm^3; seventh day 223 ± 31 vs 245 ± 38 mm^3).

The combined incidence of death, myocardial infarction, recurrent angina, urgent revascularization and stroke was 66% in patients with antibodies and 44% in patients without antibodies during a 1 year follow-up. The combined incidence of primary end points was statistically higher in patients who were antibody positive. The log-rank test was statistically significant ($c^2 = 4.39$, $p < 0.01$; Fig. 1). The rate of cardiac death was 13% in the group with antibodies and 8% in patients without antibodies (odds ratio 1.4, 95% CI -23 to +25%), and the rate of non-fatal myocardial infarction was 12% in patients with antibodies and 5% in patients without antibodies (odds ratio 2.3, 95% CI -18 to +16%). Recurrent angina was present in 12% of the patients who were antibody positive and in 10% of the patients without antibodies (odds ratio 1.0, 95% CI -13 to +17%). The incidence of urgent revascularization was 22% in patients with antibodies and 12% in patients without antibodies (odds ratio 2.0, 95% CI -40 to +41%). The occurrence of stroke was 7% in patients with antibodies and 5% in patients without antibodies (odds ratio 1.3, 95% CI -25 to +26%).

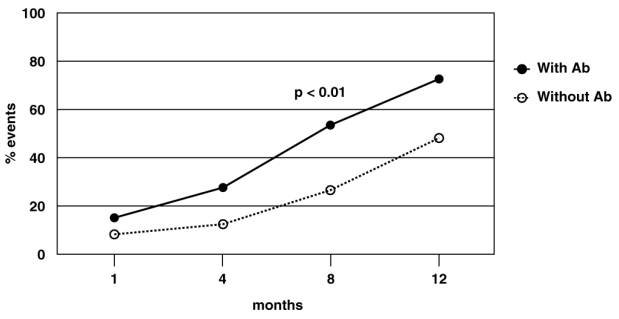

Figure 1. *Cumulative frequency of events in patients with and patients without heparin-induced antibodies (Ab).*

Discussion

In the present study we have evaluated the prevalence of heparin PF4 antibodies in patients before and after 5 days of treatment with heparin administered for unstable angina. A number of patients developed antibodies specific to heparin-PF4 complexes after treatment with the drug. At baseline we did not find any patients with a positive test for heparin-induced antibodies. The majority of patients (74%) who developed antibodies became positive after 6 days of heparin therapy and a minority of patients became positive at 40 days.

Fifty-four percent of patients were positive or intermediately positive for antibodies. This prevalence was quite high but is comparable to results reported by others[6]. These findings suggest that heterogeneous mucopolysaccharide heparin is considerably more immunogenic than has been previously appreciated[6]. The increase in antibody titer at 40 days is probably due to an increase in absorbance as an anamnestic response in previously sensitized individuals, since the addition of heparin or PF4 antibodies to samples that were negative had no effect on the ELISA results[7].

There was no difference in mean platelet count between the antibody-positive and antibody-negative groups. An analysis of platelet counts in a postoperative orthopedic patient population receiving heparin revealed that about 29% of patients developed an early decrease in platelet count to less than 150 ´ 10/l[5]. In a recent study the prevalence of PF4 antibodies was 83% among the patients who received heparin before cardiopulmonary bypass and the authors did not find any difference in the mean platelet count between the antibody-positive and antibody-negative groups. Low titers of antiheparin PF4 antibodies have been detected in about 20% of patients receiving heparin for medical therapy who were not thrombocytopenic[7]. The dicotomy between thrombocytopenia and the presence of PF4 antibodies is now generally observed. Prospective studies on HIT have reported a small number of patients that have developed thrombotic complications[1]. The frequency of thrombosis varies with the baseline risk of thrombosis in the studied population[1,2]. Venous and arterial

thrombotic complications are the most common sequelae of HIT. In this study there was a higher prevalence of thrombotic events in patients who were antibody-positive: log-rank tests showed a higher incidence of the combined end point of death, non-fatal myocardial infarction, recurrent angina, urgent revascularization and stroke. No statistical difference was noted in individual end points. A dissociation between combined and single end points is not rare. In this study the result may be due to the fact that one sample size was relatively small. Therefore a much larger sample size would have been required to determine whether a relation exists between the *in vitro* test results and clinical adverse events.

The absence of deep venous thrombosis is puzzling: venous thrombotic complications, especially proximal deep vein thrombosis and pulmonary embolism, are the most commonly observed complications of HIT, in a prospective study the relative risk was 27.0 and 93.4 respectively[8]. In the present study it can be hypothesized that the coronary artery disease observed in the majority of patients has played a role in the localization of the thrombotic phenomenon. Combined thrombotic events were more common in all the 1 year follow-up in patients with heparin-induced PF4 antibodies. It has been observed that about 10% of patients with unstable angina treated with heparin experienced recurrent angina after the interruption of heparin treatment[9]. A beneficial effect can be induced by platelet stabilization. New drugs such as tirofiban, acting on the final step in platelet aggregation can be useful in plaque stabilization[10].

In conclusion, in the present study thrombotic events at follow-up were more common in patients who developed heparin-induced antibodies compared to patients who did not. These patients need a more accurate evaluation and surveillance after hospital discharge.

References

1. Warkentin TE, Chong BH, Greinnacher A. Heparin induced thrombocytopenia: toward consensus. Thromb Haemost 1998; 79: 1-7.
2. Brieger DB, Mak KH, Kottke-Marchant K, Topol EJ. Heparin-induced thrombocytopenia. J Am Coll Cardiol 1998; 31: 1449-59.
3. Chong BH, Piney WR, Castaldi PA. Heparin-induced thrombocytopenia: association with thrombotic complications with heparin-dependent IgG antibody that induces thromboxane synthesis and platelet aggregation. Lancet 1982; 2: 1246-9.
4. Bauer TL, Arepally G, Konkle BA, et al. Prevalence of heparin associated antibodies without thrombosis in patients undergoing cardiopulmonary bypass surgery. Circulation 1997; 95: 1242-6.
5. Amiral J, Bridey F, Wolf M, et al. Antibodies to macromolecular platelet factor 4-heparin complexes in heparin-induced thrombocytopenia: a study of 44 cases. Thromb Haemost 1995; 73: 21-8.
6. Visentin GP, Malik MI, Cyganiak KA, Aster RH. Patients treated with unfractionated heparin during open heart surgery are at high risk to form antibodies reactive with heparin-platelet factor 4 complexes. J Lab Clin Med 1996; 128: 376-83.
7. Cines DB, Kaywin P, Bina M, Tomaski A, Schreiber AD. Heparin-associated thrombocytopenia. N Engl J Med 1980; 303: 783-5.
8. Warkentin TE, Levine MN, Hirsh J, et al. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. N Engl J Med 1995; 332: 1330-5.
9. Gruel Y, Lang M, Darnige L. Fatal effect of re-exposure to heparin after previous heparin-associated thrombocytopenia and thrombosis. Lancet 1990; 336: 1077-8.
10. PRISM-PLUS Investigators. Inhibition of the platelet glycoprotein IIb/IIIa receptor with tiofibran in unstable angina and non-Q wave myocardial infarction. The Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms Study Investigators. N Engl J Med 1998; 38: 1488-97.