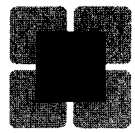

PATHOLOGY FEATURE
WILLIAM R. HART, MD, EDITOR

# Heparin-induced thrombocytopenia

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■ Thrombocytopenia is a lesser known but significant complication of heparin therapy. Heparin-dependent, antibody-mediated thrombocytopenia may be accompanied by hemorrhagic or thrombotic sequelae. Thrombosis is more frequent and is associated with significant morbidity and mortality. Diagnostic laboratory findings include a variable reduction in the platelet count during heparin administration and the presence of heparin-dependent platelet-associated antibodies that can induce in vitro platelet aggregation. Discontinuing heparin is the primary treatment, although other therapies may be used. This review discusses clinical features, pathophysiology, laboratory findings, and treatment alternatives.

□INDEX TERMS: HEPARIN; THROMBOCYTOPENIA □CLEVE CLIN J MED 1989; 56:483–490

HEPARIN is one of the most efficient and widely used anticoagulants for both prophylactic and therapeutic purposes, especially when immediate anticoagulation is indicated. It is, however, frequently associated with subclinical and clinically significant bleeding. This important drug is also associated with two other complications that are less well-known but of equal clinical significance: thrombocytopenia (heparin-induced thrombocytopenia [HIT]) and, paradoxically, thrombotic disease (heparin-induced thrombosis-thrombocytopenia syndrome [HITTS]). Clinical features, pathophysiology, laboratory findings, treatment alternatives, and the relationship between these two entities are detailed in this review.

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## GENERAL CONSIDERATIONS

The ability of heparin to induce thrombocytopenia in animals and humans has been known for many years.[1–3] Two distinct forms of this phenomenon are now recognized, based on time of onset, duration, severity, and the mechanism by which the thrombocytopenia is induced.

One form is characterized by a transient slight decline in the platelet count immediately following intravenous heparin administration. It is of such short duration that it is rarely detected and is of no clinical significance. It is caused by a nonimmune mechanism whereby heparin directly induces reversible agglutination/aggregation, trapping, or sequestration of platelets in vivo.[1–4]

The other type, which is associated with HIT and HITTS, is immune-mediated and has a delayed onset. It develops 5–10 days after initiation of heparin therapy and usually persists until the drug is discontinued. This form causes mild to severe thrombocytopenia, which may be associated with hemorrhagic and thrombotic sequelae.[5–10]

HIT and HITTS (the latter also known as the "white clot syndrome"[11–12]) are frequently reported in the medi-

**TABLE 1**
SUMMARY OF CHARACTERISTICS OF HEPARIN-INDUCED THROMBOCYTOPENIA

| |
|---|
| Incidence |
| ≤5% of patients exposed to heparin |
| Predisposing risk factors |
| History of heparin-induced thrombocytopenia |
| No other definitive risk factors known |
| Symptoms |
| Asymptomatic—majority of patients |
| Hemorrhagic manifestations of thrombocytopenia—petechiae, ecchymosis, epistaxis, or bleeding from venipuncture sites |
| Thrombotic manifestations—arterial occlusions (especially distal aorta and lower extremities), pulmonary emboli, or extension of a venous thrombosis |
| Incidence of hemorrhagic and thrombotic sequelae is unknown |
| Pathophysiology |
| Heparin-dependent—antibody-mediated platelet activation |
| Thrombocytopenia and thrombotic complications secondary to irreversible in vivo platelet aggregation |
| Laboratory findings |
| Platelet count declines to $<150 \times 10^9$/L or reduction of $>50 \times 10^9$/L from baseline while on heparin therapy |
| Thrombocytopenia develops between day 2–22 of therapy (median 8–10 days) |
| Nadir of thrombocytopenia within 1–5 days from onset of decline (usually $<75 \times 10^9$/L) |
| Recovery interval after cessation of heparin ranges 1–25 days (median 4–6 days) |
| Spontaneous recovery while on heparin may be seen |
| Diagnostic laboratory studies |
| Daily or every other day platelet counts while on heparin |
| In vitro platelet aggregation studies—highly specific (acute and convalescent studies may be required) |
| Platelet-associated immunoglobulin (PAIg) assay—sensitive but not specific |
| Treatment alternatives |
| Discontinue heparin |
| Use alternate anticoagulants |
| Antiplatelet or platelet inhibitory agents if heparin must be used |

cal literature as two distinct entities but are in fact the same process. Laboratory and clinical expressions range from mild to severe thrombocytopenia that may be asymptomatic or accompanied by hemorrhagic or thrombotic complications. These two entities will herein be discussed in terms of HIT, the more general process. The characteristics of HIT are summarized in *Table 1*.

## CLINICAL CHARACTERISTICS

### Incidence

The overall incidence of HIT (symptomatic or asymptomatic) is not well defined because no uniform criteria or standard diagnostic laboratory methods have been used in such studies. The incidence has been reported to be as high as 24% and 31% in two studies,[13,14] while ranging from 0% to 14% in most others.[15–22]

A more reasonable incidence of 3%–5% has been suggested[9]; however, most prospective studies fail to consider patients exhibiting a significant decline in platelet count from the baseline to a level that is still either within the normal range or above the lower limit established to define HIT (frequently $<100 \times 10^9$/L). This group of patients has been examined by other investigators[20,21,23,24] and if included could result in a significantly increased overall incidence.

### Symptoms

The vast majority of patients reported in prospective studies are asymptomatic regardless of the severity of the thrombocytopenia. Those who do have hemorrhagic or thrombotic complications usually have severely depressed platelet counts ($<50 \times 10^9$/L), but thrombotic complications have been reported with slightly decreased or normal platelet counts.[9,12,25–27]

Hemorrhagic complications may be difficult to differentiate from those caused by anticoagulation alone, and include those frequently associated with thrombocytopenia: ecchymosis, petechiae, epistaxis, hematuria, hemoptysis, bleeding from venipuncture sites, and, rarely, intracerebral hemorrhage.[7,9,28,29]

Thrombotic complications apparently are more common than hemorrhagic sequelae, having a reported incidence as high as 50%.[30,31] The development of thrombosis invariably coincides with a declining platelet count and may be preceded by a transient increase in heparin requirements (heparin resistance).[28,30] This may be

manifest as either de novo or recurrent arterial or pulmonary emboli (frequently multiple), or as an extension of a pre-existing venous thrombosis.[7,9]

Arterial occlusions are more common, frequently involving the distal aorta and lower extremities,[7] as well as causing ischemic strokes.[32] Because they are associated with significant morbidity and mortality, early recognition is crucial. HIT should be suspected whenever a thromboembolic event occurs in a patient on heparin.

### Laboratory findings and related observations

The diagnostic hallmark of this disease is of course thrombocytopenia that develops during heparin therapy. How thrombocytopenia is defined, however, has significant diagnostic and prognostic implications. Most studies have defined HIT as the development of thrombocytopenia with a platelet count of <100 or <150 × $10^9$/L. The latter criterion appears most reasonable. However, since several series have documented HIT in patients with declines in platelet counts to a final value of >100 × $10^9$/L,[20,21,23,24] an additional or alternative criterion should be a decrease of 50 × $10^9$/L or more from the baseline platelet count.

The platelet decline usually occurs 2–22 days after initiation of heparin therapy, with a median of 8–10 days. The nadir is reached within 1–5 days from onset of the platelet decline and has a wide range, 2–312 × $10^9$/L, with the majority <75 × $10^9$/L. The recovery of the platelet count to >100 or >150 × $10^9$/L after discontinuing the heparin is progressive, usually ranging from 1 to 25 days with a median of 4–6 days.[7,9,12–14,17,18,20,22,24,27–30,33] Severe thrombocytopenia may develop suddenly, within a few minutes or up to three days, after heparin is restarted in patients who have received heparin within the last two months. Such patients are at high risk for developing thrombosis.[26,27,29,31,34,35] Spontaneous recovery of the platelet count to pre-heparin levels, despite continued heparin therapy, has been reported in several studies but is usually associated with mild thrombocytopenia.[9,13,17–19,21]

A history of HIT is the only recognized predisposing risk factor. Unfortunately, the outcome of the HIT syndrome is not possible to predict, although a recent study found that patients receiving therapeutic doses of heparin were at higher risk for developing complications.[10]

## PATHOPHYSIOLOGY

### Mechanisms

The mechanism primarily responsible for delayed-onset HIT is immune-mediated in vivo platelet aggregation. This has been established by a number of studies that have demonstrated, directly or indirectly, the presence of platelet-associated immunoglobulin, which is usually IgG but may also be IgA or IgM.[23,28,35–42] The antigen responsible for eliciting the immune response is thought to be composed of both heparin and a platelet surface protein that initially forms a heparin-platelet complex.[43] This antigenic complex stimulates production of an antibody having specificity for the heparin molecule, the platelet protein, and/or the heparin-platelet complex. This mechanism supports observations in which IgG from the plasma of HIT patients displayed direct reactivity with heparin[23] and platelets,[39] and caused direct platelet aggregation in vitro in the absence of heparin.[41] Recently, Cines et al[42] reported data supporting the role of IgG-heparin immune complexes mediating platelet and endothelial cell injury. The latter resulted in the elaboration of a procoagulant that could be an etiologic factor in the development of thrombotic complications.

The primary interaction between platelet and heparin molecule may be explained by the presence of platelet-associated surface proteins/receptors that directly bind heparin, such as glycoprotein Ib, glycoprotein IIb/IIIa, glycoprotein V, G4, G17, G25, factor VIII-related antigen, fibronectin, fibrinogen, platelet factor 4, and thrombospondin.[39,44–47] In fact, Chong and Castaldi[45] have found glycoprotein IIb/IIIa to be required for this interaction. This heparin-platelet-protein interaction may in part be related to molecular charge since heparin is a polyglycosaminoglycan that is highly negatively charged. Thus, after binding to platelet membrane proteins, new antigenic sites may be exposed or existing sites may become more accessible or recognizable to the immune system.[48] This was demonstrated by Lynch and Howe,[39] who found that immune binding of platelet-directed antibody was greatly enhanced in the presence of heparin. Furthermore, the plasma or sera from HIT patients will not induce aggregation of all donor platelets in the presence of heparin,[49,50] suggesting a lack of antibody specificity and/or variable expression of platelet antigens. Pfueller and David[49] in 1986 concluded that the antibodies were directed against platelet antigens that were differently expressed in different individuals, possibly due to an alteration of the antigen by heparin and/or other aggregating agents (i.e. epinephrine), and that Fc receptor binding was not involved in the process. It is well known that heparin alone can directly induce aggregation[4,51,52] and can enhance or potentiate other activating agents (i.e. ADP and epinephrine).[4,21,53,54] Therefore, these substances may induce

platelet membrane alterations that facilitate or enhance antibody binding.

Thrombocytopenia that results from the interaction of heparin with platelets is presumably secondary to in vivo platelet aggregation and platelet injury. Antibody-induced platelet aggregation is ADP-dependent and mediated by the arachidonic acid pathway with synthesis of thromboxane $A_2$, as evidenced by inhibition with aspirin, dipyridamole, and apyrase (an ADP antagonist).[35,53,55,56] Some patients also demonstrate IgG-mediated complement activation with immune platelet injury.[23,35,40,42] Whether activation of the complement system adversely affects the severity of the thrombocytopenia and/or predisposes the patients to thrombotic events via enhanced platelet aggregation or endothelial cell injury is unclear.

### Mode of administration

The mode of heparin administration appears to be unrelated to the development or incidence of HIT, since it has been associated with all forms of administration: subcutaneous, continuous infusion, bolus injection, and even heparin flushes of intravenous catheters.[8,31,57–59] This last form of administration, however, deserves special attention for two reasons. First, the development and severity of HIT is not dose dependent,[17,28] although dose-dependent aggregation has been observed during in vitro testing.[17,23,60,61] Second, HIT should not be discounted as an etiologic mechanism for thrombocytopenia occurring in a patient receiving only small amounts of heparin.

### Duration of therapy

Although the mode of administration is not an important etiologic factor, the duration of therapy could be an important determinant of whether an immunologic response will be elicited. Therefore, the longer the therapy, the greater the likelihood of HIT in a "predisposed" individual.[10,17] For this reason some advocate limiting heparin therapy to five days.[22]

### Type of heparin

Numerous studies have evaluated the effect of different heparin preparations on the incidence and course of HIT. Eika[51] first noted considerable difference among several heparin preparations in their ability to induce in vitro platelet aggregation. The cause of this variation has been postulated to be the heparin moiety, protein contaminants, and preservatives. But it is now known that the heterogeneity of the mucopolysaccharide component of the heparin molecule predominantly determines its platelet-aggregating activity[17,26]

Bovine-lung heparin has a greater anionic charge density due to a higher degree of sulfation and contains proportionately more high-molecular-weight (HMW) species than porcine mucosal heparin. Salzman et al[4] have shown that HMW heparin fractions are more effective mediators of platelet aggregation. In this regard, several prospective in vivo studies have found that bovine-lung heparin is associated with a higher incidence of HIT.[4,8,17,20,22,36] Presumably this is related to an increased ability of the heparin to bind to the platelet surface and increased antigenicity attributable to differences in its molecular structure relative to porcine-mucosal heparin. In addition, Stead et al[26] have reported significant differences in the ability of different lots of beef-lung heparin to induce platelet activation in vitro. Again, this was attributed to differences in the mucopolysaccharide content of the preparations.

The low-molecular-weight (LMW) heparin fraction is the predominant molecular moiety in porcine-mucosal heparin and is available as a purified product in Europe. It appears to be less reactive with platelets in vitro and less antigenic than bovine heparin[4,17,20,56] and therefore a more desirable therapeutic agent. However, in a recent prospective comparative study, Bailey et al[22] found no significant difference in the incidence of HIT when comparing a purified bovine-lung heparin preparation with porcine-mucosal heparin.

## DIAGNOSTIC LABORATORY METHODS

The clinical diagnosis of HIT is basically one of exclusion. Therefore, other causes of thrombocytopenia, such as disseminated intravascular coagulation, hypersplenism, uremia, bone marrow hypoplasia, or other drugs, should be considered first and eliminated from the differential diagnoses. Bone marrow examination is usually unnecessary but if performed will invariably demonstrate normal or increased megakaryopoiesis, consistent with peripheral consumption or sequestration of platelets.[7,18,28,30]

### Monitoring heparin therapy

Monitoring the platelet count only when heparin treatment lasts five days or longer has been recommended in some reviews because severe thrombocytopenia secondary to an immune mechanism is more likely to develop after this period of primary exposure. This approach is probably acceptable for many patients; however, as previously noted, significant and persistent thrombocytopenia with complications can develop early

TABLE 2
PLATELET AGGREGOMETRY METHOD FOR THE DETECTION OF HEPARIN-INDUCED PLATELET ANTIBODY

| |
|---|
| Prepare platelet-poor plasma (PPP) from citrated whole blood from patient |
| Prepare platelet-rich plasma (PRP) and PPP from citrated whole blood from normal type-compatible donor |
| Control and test mixtures for aggregation (final platelet concentration in each mixture should be $250 \times 10^9$/L in a total volume of 0.45 mL) |
| Mixture A: 0.25 mL PRP + 0.20 mL *normal* PPP + 0.05 mL saline |
| Mixture B: 0.25 mL PRP + 0.20 mL *normal* PPP + 0.05 mL heparin (heparin concentration 0.3–0.5 U/mL) |
| Mixture C: 0.25 mL PRP + 0.20 mL *patient* PPP + 0.05 mL saline |
| Mixture D: 0.25 mL PRP + 0.20 mL *patient* PPP + 0.05 mL heparin (heparin concentration 0.3–0.5 U/mL) |
| Blank the platelet aggregometer with 0.5 mL *normal* PPP for Mixtures A and B, and 0.5 mL *patient* PPP for Mixtures C and D |
| Each mixture should be tested for aggregation for 15 minutes |
| Following the 15-minute test period add 0.05 mL of ADP (2 μg/mL) to each mixture that has not aggregated to ensure platelet aggregability |
| If aggregation occurs with the mixture of *normal* PRP and *normal* PPP, either before or after the addition of heparin, follow these corrective actions: |
| Review PRP + PPP mixture procedure to ensure that proper mixing was done |
| Review heparin preparation to assure correct concentration was achieved |
| Use a new normal donor if this problem is not corrected with the above actions |
| If the normal platelets fail to aggregate with ADP, follow these corrective actions: |
| Review preparation of ADP concentration |
| Check expiration date of stock ADP solution |
| Check to make sure a stir bar was added to the platelet mixture in the cuvette and that the stirrer was on |
| Use a new normal donor if this problem is not corrected with the above actions |
| A heparin-induced platelet antibody is indicated by an aggregating response in Mixture D of ≥20% increase in light transmittance, with no aggregation in Mixtures A, B, or C |
| This study may also be performed using *patient* PRP, if the platelet count is $>100 \times 10^9$/L, by substituting *patient* PRP and PPP in Mixtures C and D, and omitting Mixtures A and B |

in the course of therapy,[16] especially in patients who have been recently exposed to heparin (a part of the medical history that can be easily overlooked). Therefore, in view of the insidious nature and life-threatening potential of this disorder, a vigilant approach is recommended. A baseline platelet count should be obtained before initiating therapy and should be repeated either daily or every other day thereafter until the heparin is discontinued. A decline in the platelets on two consecutive days should alert the clinician to the possibility that this process is evolving.

## Diagnosis of HIT

A variety of laboratory tests have been developed and used to confirm the diagnosis of HIT, however, many are not applicable in a routine clinical laboratory or are insensitive and nonspecific. The most frequently used methods determine the presence of heparin-dependent platelet-associated immunoglobulin using an ELISA technique,[62] radioimmunoassay,[63] or platelet aggregometry.[60,64] The latter method will be discussed in detail since it is widely used, highly specific,[65] and can provide a reliable result within a reasonably short period of time.

Platelet aggregometry is a method that can qualitatively detect a proaggregating factor (i.e. antibody) in the plasma and/or on the platelet surface by inducing platelet aggregation when heparin is added. Briefly, this procedure utilizes a platelet aggregometer and either platelet-rich plasma (PRP) (if the whole blood platelet count is $>100 \times 10^9$/L) or platelet-poor plasma (PPP) (if the whole blood platelet count is $<100 \times 10^9$/L) prepared from citrated whole blood. When patient PPP is used, target platelets are obtained from PRP from a normal donor of the same ABO blood type. Heparin is then added to the patient PRP or patient PPP plus donor PRP to reach a final concentration in the cuvette of 0.3–0.5 U/mL (the normal therapeutic range in vivo). Controls are also prepared to exclude spontaneous platelet aggregation, nonspecific aggregation of donor platelets by heparin, and the aggregation of donor platelets by patient plasma alone. The controls and patient mixtures are monitored in the aggregometer for 15 minutes. A positive test is denoted by an aggregatory response of ≥ 20% (increase in light transmittance from the baseline). Finally, to assure platelet aggregability ADP is added to all mixtures that did not aggregate during the 15-min period. Heparin from the same source (bovine or porcine) and lot number as that administered to the patient therapeutically should be used for the in vitro testing because of observed differences in the ability of the two preparations to induce aggregation.[64] This procedure is summarized in *Table 2*.

The use of patient PRP in the platelet aggregometry test has been reported to be associated with better re-

sponses[9] and the ability to demonstrate the presence of the antibody longer.[21] The optimal time to perform platelet aggregometry testing is debatable since a positive response depends upon the concentration of antibody. Reports on this aspect of testing conflict[18,23,26,41]; it may be necessary to test patients after stopping the heparin for 24 hours.[9] This test appears to be uniformly positive during the recovery phase, after the heparin has been discontinued, when the platelet count is returning or has returned to normal. Heparin-dependent platelet aggregation may be detectable for up to three months after cessation of therapy.[9]

Patients who have negative aggregometry testing while thrombocytopenic but have clinical features consistent with HIT should be considered to have the disorder and treated appropriately. This discrepancy is probably most often due to a low level of antibody, but Chong et al[66] have also described patients in whom a qualitative platelet defect precludes the platelets' ability to aggregate.

## TREATMENT

The most efficacious treatment for HIT is simply to discontinue heparin therapy. Although sound in principle, this approach may not be medically advisable in the face of recent thromboembolic disease. Therefore, alternate therapeutic approaches have been tried with variable success. These may be divided into two categories: replacement of heparin with a substitute anticoagulant or use of anti-platelet or platelet-inhibitory agents in conjunction with heparin.

### Substitute anticoagulants

Coumadin is obviously an acceptable substitute anticoagulant. Its only drawback is the inherent time delay in effecting anticoagulation. This may or may not be an important factor in patients with HIT, depending on the severity of the thrombocytopenia and whether it is associated with thrombotic complications. Certainly the risk of developing complications while continuing heparin during the induction of coumadin anticoagulation must be weighed carefully. Unfortunately, there are no guidelines to assist in making this decision, but it is probably advisable to begin coumadin as soon as a presumptive diagnosis of HIT has been made.

An alternative approach is to replace the offending heparin with a different heparin, such as LMW heparin, or porcine for bovine and vice versa.[20] However, cross-sensitivity does exist to bovine and porcine heparins[67] so substitution may be ineffective and contraindicated.[41] LMW heparin, although unavailable in the United States, has been effective in some HIT patients,[9,58,68] but cross-sensitivity with standard heparin preparations has also been observed.[69] In vitro platelet aggregation studies are valuable in predicting in vivo response to an alternate heparin but exceptions have been reported.[70]

Another product, also unavailable in the United States, that appears promising as a substitute agent is Org 10172 (Organon International, Oss, The Netherlands), a heparinoid. This agent has been used successfully in one patient with HIT in West Germany and was recently found not to induce platelet aggregation in vitro using the plasma from 13 HIT patients who demonstrated cross-sensitivity to porcine, bovine, and LMW heparins.[27,71]

### Anti-platelet therapy

Anti-platelet agents, such as aspirin and dipyridamole, have been shown to block heparin-induced platelet aggregation in vitro.[27,45,72] They have been used successfully in patients undergoing cardiopulmonary bypass for open-heart surgery,[73–75] but in other reports their efficacy has been questioned.[7,31]

The major drawback to these agents is that hemostatic function is permanently impaired in the circulating platelets and that normal platelet activity is restored only after seven to 10 days, or after platelet transfusions, assuming the drug is no longer present in the circulation. Therefore, this form of therapy must be carefully considered since primary hemostasis may be impaired for several days.

An investigational drug, Iloprost (Berlex Laboratories, Inc., Cedar Knolls, New Jersey), has been used without complication in several patients with HIT who have undergone open-heart surgery.[76,77] This is a stable prostacyclin analogue ($PGI_2$) given intravenously and having a half-life of approximately 15–30 minutes. It will immediately inhibit platelet aggregability, but when discontinued, normal platelet function is quickly restored. Its major side effect is hypotension (dose-dependent), which limits its clinical utility.

Other therapeutic modalities tried with apparent success include: plasmapheresis,[78] fibrinolytic therapy using streptokinase,[79] and LMW dextran, either alone or in combination with anti-platelet agents.[9,28,67] Platelet transfusions are ineffective and contraindicated with continued heparin administration since they may contribute to the extension or formation of thrombi.[34,37]

SUMMARY

HIT is a clinically significant entity having a poorly defined incidence of approximately 3%–5%. The thrombocytopenia is heparin-dependent and antibody-mediated but unrelated to the heparin dose or route of administration. The severity of platelet reduction is variable, ranging from a level still within the normal range to severe thrombocytopenia.

Most patients with HIT are asymptomatic regardless of the severity of the thrombocytopenia, but some will develop hemorrhagic or thrombotic complications, especially arterial occlusions. The latter appear to be more common and are associated with significant morbidity and mortality. Therefore, early recognition is of utmost importance.

Platelet counts should be obtained prior to initiating heparin therapy and daily or every other day thereafter until the heparin is discontinued. If HIT is suspected, a test to detect the heparin-dependent platelet antibody should be performed. Platelet aggregometry is frequently used and can be easily and rapidly performed in any laboratory having a standard platelet aggregometer.

The treatment of HIT primarily consists of immediate cessation of heparin administration, but if continued anticoagulation is required, substitute anticoagulants or anti-platelet or platelet-inhibitory agents may be tried.

REFERENCES

1. Copley AL, Robb TP. The effect of heparin in vivo on the platelet count in mice and dogs. Am J Clin Pathol 1942; **12**:563–570.
2. Fidlar E, Jacques LB. The effect of commercial heparin on the platelet count. J Lab Clin Med 1948; **33**:1410–1423.
3. Gollub S, Ulin AW. Heparin-induced thrombocytopenia in man. J Lab Clin Med 1962; **59**:430–435.
4. Salzman EW, Rosenberg RD, Smith MH, Lindon JN, Favreau L. Effect of heparin and heparin fractions on platelet aggregation. J Clin Invest 1980; **65**:64–73.
5. Rhodes GR, Dixon RH, Silver D. Heparin induced thrombocytopenia with thrombotic and hemorrhagic manifestations. Surg Gynecol Obstet 1973; **136**:409–416.
6. Curry N, Bardana EJ, Pirofsky B. Heparin sensitivity: report of a case. Arch Intern Med 1973; **132**:744–745.
7. Rector TS, Cipolle RJ, Seifert RD, Ramirez-Lassepas M. Characteristics of heparin-associated thrombocytopenia. Am J Hosp Pharm 1979; **36**:1561–1565.
8. Godal HC. Report of the International Committee on Thrombosis and Haemostasis: thrombocytopenia and heparin. Thromb Haemost 1980; **43**:222–224.
9. Arthur CK, Isbister JP, Aspery EM. The heparin induced thrombosis—thrombocytopenia syndrome (H.I.T.T.S.): a review. Pathology 1985; **17**:82–86.
10. Laster J, Cikrit D, Walker N, Silver D. The heparin-induced thrombocytopenia syndrome: an update. Surgery 1987; **102**:763–770.
11. Towne JB, Bernhard VM, Hussey C, Garancis JC. White clot syndrome: peripheral vascular complications of heparin therapy. Arch Surg 1979; **114**:372–377.
12. Chang JC. White clot syndrome associated with heparin-induced thrombocytopenia: a review of 23 cases. Heart Lung 1987; **16**:403–407.
13. Nelson JC, Lerner RG, Goldstein R, Cagin NA. Heparin-induced thrombocytopenia. Arch Intern Med 1978; **138**:548–552.
14. Bell WR, Tomasulo PA, Alving BM, Duffy TP. Thrombocytopenia occurring during the administration of heparin: a prospective study in 52 patients. Ann Intern Med 1976; **85**:155–160.
15. Malcolm ID, Wigmore TA. Thrombocytopenia induce by low-dose subcutaneous heparin (letter). Lancet 1978; **1**:444.
16. Gallus AS, Goodall KT. Heparin-associated thrombocytopenia: a prospective study. Presented at the Seventeenth Congress of the International Society and Haematology, Paris, July 24–28, 1978.
17. Bell WR, Royall RM. Heparin-associated thrombocytopenia: a comparison of three heparin preparations. N Engl J Med 1980; **303**:902–906.
18. Ansell J, Slepchuk N Jr, Kumar R, Lopez A, Southard L, Deykin D. Heparin induced thrombocytopenia: a prospective study. Thromb Haemost 1980; **43**:61–65.
19. Olin J, Graor R. Heparin-associated thrombocytopenia (letter). N Engl J Med 1981; **304**:609.
20. Powers PJ, Kelton JG, Carter CJ. Studies on the frequency of heparin-associated thrombocytopenia. Thromb Res 1984; **33**:439–443.
21. Green D, Martin GJ, Shoichet SH, DeBacker N, Bomalaski JS, Lind RN. Thrombocytopenia in a prospective, randomized, double-blind trial of bovine and porcine heparin. Am J Med Sci 1984; **288**:60–64.
22. Bailey RT Jr, Ursick JA, Heim KL, Hilleman DE, Reich JW. Heparin-associated thrombocytopenia: a prospective comparison of bovine lung heparin, manufactured by a new process, and porcine intestinal heparin. Drug Intell Clin Pharm 1986; **20**:374–378.
23. Cines DB, Kaywin P, Bina M, Tomaski A, Schreiber AD. Heparin-associated thrombocytopenia. N Engl J Med 1980; **303**:788–795.
24. Schwartz KA, Royer G, Kaufman DB, Penner JA. Complications of heparin administration in normal individuals. Am J Hematol 1985; **19**:355–363.
25. Phelan BK. Heparin-associated thrombosis without thrombocytopenia. Ann Intern Med 1983; **99**:637–638.
26. Stead RB, Schafer AI, Rosenberg RD, Handin RI, Josa M, Khuri SF. Heterogeneity of heparin lots associated with thrombocytopenia and thromboembolism. Am J Med 1984; **77**:185–188.
27. Makhoul R, Greenberg CS, McCann RL. Heparin-associated thrombocytopenia and thrombosis: a serious clinical problem and potential solution. J Vasc Surg 1986; **4**:522–528.
28. Rhodes GR, Dixon RH, Silver D. Heparin induced thrombocytopenia: eight cases with thrombotic-hemorrhagic complications. Ann Surg 1977; **186**:752–758.
29. Kapsch DN, Adelstein EH, Rhodes GR, Silver D. Heparin-induced thrombocytopenia, thrombosis and hemorrhage. Surgery 1979; **86**:148–155.
30. Ansell J, Deykin D. Heparin-induced thrombocytopenia and recurrent thromboembolism. Am J Hematol 1980; **8**:325–332.
31. Rice L, Huffman DM, Levine ML, Udden MM, Waddell CC, Luper WE. Heparin-induced thrombocytopenia/thrombosis syndrome: clinical manifestation and insights (abst). Blood 1986; [Suppl] 68-339a.
32. Atkinson JLD, Sundt TM Jr, Kazmier FJ, Bowie EJW, Whisnant JP. Heparin-induced thrombocytopenia and thrombosis in ischemic stroke. Mayo Clin Proc 1988; **63**:353–361.
33. Alving BM, Shulman NR, Bell WR, Evatt BL, Tack KM. In vitro studies of heparin-associated thrombocytopenia Thromb Res 1977; **11**:827–834.
34. Cimo PL, Moake JL, Weinger RS, Ben-Menachem Y, Khalil KG. Heparin-induced thrombocytopenia: association with a platelet aggregating factor and arterial thrombosis. Am J Hematol 1979; **6**:125–133.
35. Chong BH, Pitney WR, Castaldi PA. Heparin-induced thrombocytopenia: association of thrombotic complications with heparin-dependent IgG antibody that induces thromboxane synthesis and platelet aggregation. Lancet 1982; **2**:1246–1249.

36. Trowbridge AA, Caraveo J, Green JB, Amaral B. Heparin-related immune thrombocytopenia. Studies of antibody-heparin specificity. Am J Med 1978; **65:**277–283.
37. Babcock RB, Dumper CW, Scharfman WB. Heparin-induced immune thrombocytopenia. N Engl J Med 1976; **295:**237–241.
38. Chong BH, Grace CS, Rozenberg NC. Heparin-induced thrombocytopenia: Effects of heparin platelet antibody on platelets. Br J Haematol 1981; **49:**531–540.
39. Lynch DM, Howe SE. Heparin-associated thrombocytopenia: antibody binding specificity to platelet antigens. Blood 1985; **66:**1176–1181.
40. Huisee M-G, Guillin M-C, Bezeaud A, Toulemonde F, Kitzis M, Andreassian B. Heparin-associated thrombocytopenia. In vitro effects of different molecular weight heparin fractions. Thromb Res 1982; **27:**485–490.
41. Sandler RN, Seifer DB, Morgan K, et al. Heparin-induced thrombocytopenia and thrombosis: detection and specificity of a platelet-aggregating IgG. Am J Clin Pathol 1985; **83:**760–764.
42. Cines DB, Tomasaki A, Tannebaum S. Immune endothelial-cell injury in heparin-associated thrombocytopenia. N Engl J Med 1987; **316:**581–589.
43. Green D, Harris K, Reynold N, Roberts M, Patterson R. Heparin immune thrombocytopenia: evidence for a heparin-platelet complex as the antigenic determinant. J Lab Clin Med 1978; **91:**167–175.
44. Gogstad GO, Solum NO, Krutnes M-B. Heparin-binding platelet proteins demonstrated by crossed affinity immunoelectrophoresis. Br J Haematol 1983; **53:**563–573.
45. Chong BH, Castaldi PA. Heparin-induced thrombocytopenia: further studies of the effects of heparin-dependent antibodies on platelets. Br J Haematol 1986; **64:**347–354.
46. Stathakis NE, Mosesson MW. Interactions among heparin, cold insoluble globulin, and fibrinogen in formation of the heparin-precipitable fraction of plasma. J Clin Invest 1977; **60:**855–865.
47. Madaras F, Bell WR, Castaldi PA. Insolubilization of factor VIII by affinity chromatography. Haemostasis 1978; **7:**321–331.
48. Wolf H, Nowack H, Wick G. Detection of antibodies interacting with glycosaminoglycan polysulfate in patients treated with heparin or other polysulfated glycosaminoglycans. Int Arch Allergy Appl Immunol 1983; **70:**157–163.
49. Pfueller SL, David R. Different platelet specificities of heparin-dependent platelet aggregating factors in heparin-associated immune thrombocytopenia. Br J Haematol 1986; **64:**149–159.
50. Salem HH, Van Der Weyden MB. Heparin-induced thrombocytopenia. Variable platelet-rich plasma reactivity to heparin-dependent platelet aggregating factor. Pathology 1983; **15:**297–299.
51. Eika C. The platelet aggregating effect of eight commercial heparins. Scand J Haematol 1972; **9:**480–482.
52. McLean MR, Hause LL. Heparin-induced aggregation of normal human platelets (letter). Thromb Haemost 1981 **46:**570.
53. Thomson C, Forbes CD, Prentice CRM. The potentiation of platelet aggregation and adhesion by heparin *in vitro* and *in vivo*. Clin Sci 1973; **45:**485–494.
54. Ellison N, Edmunds LH Jr., Colman RW. Platelet aggregation following heparin and protamine administration. Anesthesiology 1978; **48:**65–68.
55. Carreras LO. Thrombosis and thrombocytopenia induced by heparin. Scand J Haematol 1980; **25** (suppl 36)**:**64–80.
56. Blockmans D, Bounameaux H, Vermylen J, Verstraete M. Heparin-induced thrombocytopenia platelet aggregation studies in the presence of heparin fractions or semi-synthetic analogues of various molecular weights and anticoagulant activities. Thromb Haemost 1986; **55:**90–93.
57. Hrushesky WJ. Subcutaneous heparin-induced thrombocytopenia. Arch Intern Med 1978; **138:**1489–1491.
58. Vitoux J-F, Mathieu J-F, Roncato N, Fiessinger J-N, Aiach M. Heparin-associated thrombocytopenia treatment with low molecular weight heparin. Thromb Haemost 1986; **55:**37–39.
59. Heeger PS, Backstrom JT. Heparin flushes and thrombocytopenia (letter). Ann Intern Med 1986; **105:**143.
60. Deykin D, Hellerstein LJ. The assessment of drug-dependent and isoimmune antiplatelet antibodies by the use of platelet aggregometry. J Clin Invest 1972; **51:**3142–3153.
61. Beck EA. Effects of heparin on platelet reactivity in citrated plasma (letter). Thromb Haemost 1977; **38:**578–580.
62. Howe SE, Lynch DM. An enzyme-linked immunosorbent assay for the evaluation of thrombocytopenia induced by heparin. J Lab Clin Med 1985; **105:**554–559.
63. Cines DB, Schreiber AD. Immune thrombocytopenia: use of a Coombs antiglobulin test to detect IgG and C3 on platelets. N Engl J Med 1979; **300:**106–111.
64. Fratantoni JC, Pollet R, Gralnick HR. Heparin-induced thrombocytopenia: confirmation of diagnosis with in vitro methods. Blood 1975; **45:**395–401.
65. Kelton JG, Sheridan D, Brain H, Powers PJ, Turpie AG, Carter CJ. Clinical usefulness for a heparin-dependent platelet aggregating factor in patients with suspected heparin-associated thrombocytopenia. J Lab Clin Med 1984; **103:**606–612.
66. Chong BH, Grace CS, Rozenberg MC, Castaldi PA. Qualitative platelet defect in a case of heparin-induced thrombocytopenia. Scand J Haematol 1983; **30:**427–429.
67. Guay DRP, Richard A. Heparin-induced thrombocytopenia—association with a platelet aggregating factor and cross-sensitivity to bovine and porcine heparin. Drug Intell Clin Pharm 1984; **18:**398–401.
68. Copplestone A, Oscier DG. Heparin-induced thrombocytopenia in pregnancy (letter). Br J Haematol 1987; **65:**248.
69. Leroy J, Leclerc MH, Delahousse B, et al. Treatment of heparin-associated thrombocytopenia and thrombosis with low molecular weight heparin (CY 216). Semin Thromb Hemost 1985; **11:**326–329.
70. Vitoux J-F, Roncato M, Hourdebaigt P, Aiach M, Fiessinger J-N. Heparin-induced thrombocytopenia and pentosan polysulfate: treatment with a low molecular weight heparin despite in vitro platelet aggregation. Thromb Haemost 1985; **55:**294–295.
71. Harenberg J, Zimmermann R, Schwarz F, Kübler W. Treatment of heparin-induced thrombocytopenia with thrombosis by new heparinoid (letter). Lancet 1983; **1:**986–987.
72. Janson PA, Moake JL, Carpinito G. Aspirin prevents heparin-induced platelet aggregation *in vivo* (letter). Br J Haematol 1983; **53:**166–168.
73. Smith JP, Walls JT, Muscato MS, et al. Extracorporeal circulation in a patient with heparin-induced thrombocytopenia. Anesthesiology 1985; **62:**363–365.
74. Makhoul RG, McCann RL, Austin EH, Greenberg CS, Lowe JE. Management of patients with heparin-associated thrombocytopenia and thrombosis requiring cardiac surgery. Ann Thorac Surg 1987; **43:**617–621.
75. Long RW. Management of patients with heparin-induced thrombocytopenia requiring cardiopulmonary bypass (letter). J Thorac Cardiovasc Surg 1985; **89:**950.
76. Ellison N, Kappa JR, Fisher CA, Addonizio VP Jr. Extracorporeal circulation in a patient with heparin-induced thrombocytopenia (letter). Anesthesiology 1985; **63:**336–337.
77. Bell PI, Kappa JR, Leibowitz DA, Dole WP, Addonizio P. Prevention of heparin associated thrombocytopenia with Iloprost. Presented at the American Heart Association 60th Scientific Sessions, Anaheim, CA, November 16–19, 1987.
78. Vender JS, Matthew EB, Silverman IM, Konowitz H, Dau PC. Heparin-associated thrombocytopenia: alternative managements. Anesth Analg 1986; **65:**520–522.
79. Fiessinger JN, Aiach M, Roncato M, Debure C, Gaux J-C. Critical ischemia during heparin-induced thrombocytopenia. Treatment by intra-arterial streptokinase. Thromb Res 1984; **33:**235–238.