

# Heparin-Induced Thrombocytopenia

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## 1. Introduction

Heparin is an effective anti-coagulant for the prevention of venous thromboembolism and for the treatment of venous thrombosis and pulmonary embolism (Girolami et al., 2003; Hirsh et al., 2004; Shantsila et al., 2009). It is often used for patients with unstable angina and acute myocardial infarction, and for patients who have undergone vascular surgery (Battistelli et al., 2010). The administration of heparin frequently induces a reduction in platelet counts. This phenomenon is called heparin-induced thrombocytopenia (HIT) and be classified as either type I or II. To avoid confusion between the syndromes, "HIT type I" has been changed to "non-immune heparin associated thrombocytopenia", and "HIT type II" is simply called "HIT".

The origin of non-immune heparin associated thrombocytopenia is not yet completely understood, though it is thought to be caused by heparin-induced platelet clumping (Fabris et al., 1983; Chong & Ismail, 1989). Thrombocytopenia of this type is mild (platelet count,  $>100 \times 10^9$  cells/L), not progressive, nor associated with bleeding or thrombosis (Salzman et al., 1980), and is independent of any immune reaction (Chong et al., 1993a; Burgess & Chong, 1997; Shantsila et al., 2009). It is characterized by a transitory, slight and asymptomatic reduction in platelet count, occurring during the first 1-2 days of heparin administration. This phenomenon gradually resolves without interruption of heparin administration, and platelet counts gradually rises to pre-treatment levels within a few days without special treatment. Non-immune heparin associated thrombocytopenia may be related to the direct binding of heparin to platelet membranes (Salzman et al., 1980; Fabris et al., 1983; Chong & Ismail, 1989).

In this view, the term HIT refers only to HIT type II. HIT is associated with a heparin-related immune reaction. It is a prothrombotic disease initiated by administration of heparin, and is related to antibody-mediated platelet activation causing thrombin generation and thrombotic complications. Thrombocytopenia is common in hospitalized patients, occurring in up to 58% of critically ill individuals, and can be caused by a variety of factors (Strauss et al., 2002). HIT, which is associated with significant morbidity and mortality if unrecognized, can be regarded as a very severe side effect of a drug, (Chong, 1992; Aster, 1995; Chong, 2003). Unfortunately, HIT often remains unrecognized, undiagnosed, and untreated, a problem that can be rectified with increased awareness and a high degree of suspicion for HIT. Current treatment recommendations are based on recent advances in research on the pathophysiology and the natural history of HIT (Jang & Hursting, 2005). HIT should be considered a clinicopathologic syndrome (Warkentin et al., 1998; Warkentin, 2002, 2003)

since its diagnosis is based on both clinical criteria, such as thrombocytopenia and thrombosis, and laboratory data, such as platelet count dynamics and the detection of HIT antibodies (Shantsila et al., 2009).

However, HIT is often difficult to diagnose because of the following phenomena: 1) nonimmune heparin-associated thrombocytopenia occurs in 10-30% of patients receiving heparin (Blank et al., 2002); 2) HIT antibody seroconversion is observed in the absence of thrombocytopenia or other clinical sequelae; and 3) enzyme-linked immunosorbent assay (ELISA) detects both clinically irrelevant (non-pathogenic) and clinically relevant (pathogenic) antibodies (Shantsila et al., 2009). Seroconversion of anti-HIT antibody without thrombocytopenia or other clinical sequela is not considered HIT, whereas a diagnosis of HIT can be made when anti-HIT antibody formation is accompanied by an otherwise unexplained platelet count fall, or by skin lesions at heparin injection sites or acute systemic reactions after intravenous injection of heparin (Bartholomew et al., 2005). Furthermore, HIT formation may also be related to the occurrence of venous limb gangrene occurring in HIT patients treated with oral anticoagulants (Warkentin, 1996a). A mnemonic device, the “4 Ts” of HIT, has been developed to remember the salient clinical features of HIT, thus facilitating patient assessment and HIT diagnosis (Warkentin & Heddle, 2003): the degree of Thrombocytopenia, the Timing of the platelet fall, the presence of Thrombosis or other sequelae, and oTher potential causes of thrombocytopenia (Lillo-Le Louët et al., 2004; Denys et al., 2008; Gruel et al., 2008).

Despite the utility of this memory device, severe morbidity and mortality in HIT patients persists because of lack of awareness accompanied by a delayed diagnosis. Thus, we have developed the following chapter to provide an overview of HIT, focusing particularly on the epidemiology, pathophysiology, diagnosis, laboratory evaluation, and treatment.

## 2. Overview of HIT

HIT type II, namely, HIT, is immune-mediated and associated with a risk of thrombosis. It develops in approximately 5-10% of patients treated with heparin and is characterized by a significant reduction in platelets (levels fall by 30% or more), generally after the fifth day of therapy (Warkentin et al., 1998; Warkentin, 2002). Although, this phenomenon is usually resolved by therapy within 5-15 days, some cases may require months of treatment (Chong, 1992; Warkentin et al., 1998; Warkentin, 2002).

Platelet count monitoring is recommended for heparin-treated patients in whom the risk of HIT is high (e.g., postoperative patients) or intermediate (e.g., medical or obstetric patients receiving a prophylactic dose of heparin or postoperative patients receiving antithrombotic prophylaxis) (Chong et al., 1993a; Jang & Hursting, 2005). Shantsila et al. (2009) have recommended measurement of platelet counts before, and 24 h after, initiation of heparin therapy in patients who have received heparin within the past 100 days. Platelet counts should also be performed every 2-3 days in intermediate-risk patients, every other day in high-risk patients, and immediately in patients with systemic, cardiorespiratory, or neurologic symptoms that occur within 30 min after an intravenous injection of heparin.

HIT should be suspected when thrombocytopenia ( $<15\text{-}20 \times 10^9$  cells/L, or a  $>50\%$  decrease in platelet count) occurs during heparin administration, typically 5-14 days after initiation of heparin administration (Warkentin & Kelton, 2001a); however, very severe

thrombocytopenia (platelet count  $<15-20 \times 10^9$  cells/L) is generally not due to HIT. Routine platelet count monitoring for HIT may be appropriate in at least some clinical situations, and it may be reasonable to stratify the intensity of need for platelet count monitoring in relation to the risk of HIT.

Although it is difficult to predict which heparin-exposed patients will develop HIT, one consistent factor is a property of heparin preparation. HIT antibodies occur at higher levels in patients given bovine unfractionated heparin (UFH) than in those treated with porcine UFH or low molecular weight heparin (LMWH) (Green et al., 1984; Bailey et al., 1986; Monreal et al., 1989; Rao et al., 1989; Warkentin et al., 1995; Warkentin et al., 2003a; Lee & Warkentin, 2004; Denys et al., 2008; Gruel et al., 2008).

HIT antibody formation is also influenced by the medical circumstances in which heparin is administered. For example, approximately 20% of heparin-exposed patients develop HIT antibodies after orthopedic surgery, while as many as 70% develop antibodies after receiving a cardiopulmonary bypass (CPB) (Amiral et al., 1996a). However, it can be difficult to compare results across studies because of differences in duration and route of anticoagulant administration, as well as patient group composition (Lee et al., 2004).

### 3. Epidemiology

There is a need for studies on HIT incidence. The work that has been done is sufficient, and they have so many differences that it is difficult to compare results across studies in order to uncover broader trends. Most HIT studies are retrospective, and differ with respect to patient characteristics, type of heparin preparation, duration of therapy, definition of thrombocytopenia, and laboratory tests used for confirm thrombocytopenia diagnosis (Schmitt & Adelman, 1993; Magnani, 1993; Chong, 2003). Despite these problems, it is possible to draw some general conclusions about HIT epidemiology.

There are 3 characteristic profiles in the timing of the onset of thrombocytopenia. Approximately 70% of HIT patients are classified as "typical-onset HIT". In these patients, platelet counts begin to decrease (seroconversion and initial platelet count fall) within 5-10 days after beginning heparin treatment (Warkentin & Kelton, 2001a). Approximately 25-30% of HIT patients are classified as having "Rapid-onset HIT", which occurs when platelet counts fall abruptly within 24 h of starting heparin therapy (Warkentin & Kelton, 2001a; Lubenow et al., 2002). "Rapid-onset HIT" is strongly associated with recent heparin exposure, usually within the preceding 100 days (Warkentin & Kelton, 2001a; Lubenow et al., 2002). Because of the previous exposure, patients already have circulating HIT antibodies, which cause platelet counts to fall quickly once heparin is re-administered. The last category of HIT, affecting approximately 3-5% of HIT patients, is "delayed-onset HIT" in which the onset of thrombocytopenia begins several days after completion of heparin treatment (Warkentin & Kelton, 2001b; Rice et al., 2002; Warkentin & Bernstein, 2003; Warkentin, 2004a). This type of syndrome is often clinically severe, as it is associated with high-titer, platelet-activating HIT antibodies that do not require ongoing heparin administration to exert their pathogenic effect (Rice et al., 2002). Furthermore, this type may occur in patients exposed to minimal amounts of heparin, although it has also been observed in patients exposed to large amounts of heparin during coronary artery bypass grafting (Rice et al., 2002; Jackson et al., 2006). Rarely, patients who received intravenous heparin develop acute inflammatory or cardiorespiratory symptoms and signs within 30 min (Warkentin, 2007).

HIT severity is mainly determined by the extent of thrombotic complications (Girolami et al, 2003). Many HIT patients develop thrombosis after receiving as an antithrombotic prophylaxis (Warkentin, 1996a; Wallis et al., 1999). The thrombotic event is frequently a worsening of pre-existing thrombosis in heparin-treated HIT patients, and this worsening of thrombosis may cause a new thromboembolic complication (Magnani, 1993; Warkentin & Kelton, 1990, 1996; Nands et al., 1997); this has been observed in approximately 20% of cases (Warkentin, 2004a). Development of a new thrombus or extension of an existing thrombus during treatment with prophylactic or therapeutic UFH or LMWH should always raise a suspicion of HIT (Shantsila et al., 2009). The first event to be associated with HIT was arterial thrombosis, which seems to be more frequent in patients with cardiovascular disease (Nands et al., 1997); venous complications may be common in patients undergoing post-surgical prophylaxis (Rhodes et al., 1973; Magnani, 1993; Warkentin & Kelton, 1996). In most cases, arterial complications manifest as thromboses of the large vessels, leading to gangrene and limb amputation, stroke, myocardial infarction, and cardiac thrombosis (Warkentin & Kelton, 1990; Boshkov et al., 1993; Fondu, 1995; Chong, 1995).

Approximately 10-20% of patients who develop HIT while receiving subcutaneous injections of heparin experience skin lesions, ranging from painful erythematous plaques to skin necrosis, at the injection sites (Warkentin, 2004a; Chong, 1995). Skin lesions have been observed in patients without thrombocytopenia but with circulating HIT antibodies (Warkentin, 1996b). Occasionally, HIT associated with intravenous heparin injection also manifests as an acute systemic reaction developing within 5-30 min after heparin administration (Ansell et al., 1986; Popov et al., 1997; Warkentin, 2004a).

Estimates of HIT prevalence among heparin-treated patients differ depending on heparin type. Fabris et al. (2000) found that laboratory-confirmed HIT occurs in approximately 2% of patients receiving UFH. Among patients given therapeutic doses of bovine UFH, HIT has been observed in approximately 5% of patients. This is a much higher rate than that observed in patients given porcine UFH (1%). Prophylactic doses of porcine heparin have been reported to cause HIT in <1% of patients (Warkentin & Kelton, 1990). However, risk of HIT is relatively low in medical and obstetric patients receiving LMWH (Fabris et al., 2000). For instance, Warkentin (2004a) reported that HIT was observed in 2.7% of patients treated with subcutaneous UFH injection, but in no patients receiving LMWH; further, thrombotic complications were more frequent in the former group (88.9%) than in the latter (17.8%). Both the bovine/porcine UFH and the LMWH data were collected from medical patients receiving LMWH or UFH as "flushes", e.g., oncology patients with indwelling catheters (Mayo et al., 1990; Kadidal et al., 1999). A randomized controlled trial that compared use of porcine UFH with LMWH after hip replacement surgery found that HIT was significantly less common among patients who received the latter treatment (Lee & Warkentin, 2004; Warkentin, 2004a).

#### 4. Pathophysiology

Francis and colleagues have reported that HIT antibody formation in cardiac surgery patients who received porcine UFH was significantly lower than those with bovine UFH (Francis et al., 2003). The IgG fraction of HIT patients serum has been found to cause *in vitro* platelet aggregation in the presence of therapeutic amounts of heparin (Rhodes et al., 1973), indicating that HIT has an immunologic etiology (Amiral et al., 1998; Warkentin et al., 2000).

Green *et al.* (1978) reported that immunoglobulin-heparin complexes form only in the presence of platelets (Green *et al.*, 1978), and several platelet proteins have been proposed as the receptors of heparin-dependent antibodies (Lynch & Howe, 1985). Multiple studies have found that the pro-aggregating effect of heparin depends on the degree of sulfation and the molecular weight of the heparin (Geinacher *et al.*, 1992; Geinacher *et al.*, 1993; Kelton *et al.*, 1994) and is mediated by the release of platelet alpha-granules (Gruel *et al.*, 1993), which contain platelet factor 4 (PF4), a small, positively charged molecule produced by megakaryocytes. Although its biological function is unknown, it has been identified as the main co-factor of heparin (Amiral *et al.*, 1992; Gentilini *et al.*, 1999); it also binds to endothelial-surface glycosaminoglycans, *e.g.*, heparin sulfate (Visentin *et al.*, 1994; Cines *et al.*, 2007). Normal blood levels of PF4 are very low, as it is only released into circulation following weak platelet activation. However, PF4 levels may be high in specific clinical circumstances, such as prosthetic hip or cardiac surgery, which are associated with platelet activation (Greinacher *et al.*, 1994a). When heparin binds with PF4, it undergoes a conformational change and becomes immunogenic, leading to the generation of anti-heparin/PF4 antibodies, namely, HIT antibodies (Suh *et al.*, 1998; Ziporen *et al.*, 1998). The PF4/heparin ratio is important for the constitution of the multimolecular antigenic complex, and the optimal PF4/heparin ratio has been reported to range from 4-6:1 (Kelton *et al.*, 1994; Visentin *et al.*, 1994; Amiral *et al.*, 1995; Cines *et al.*, 2007). The immunogenicity of heparin-PF4 conjugates may form the biological basis for differences in immunogenicity between bovine and porcine sources of heparin: Bovine lung heparin has longer polysaccharide chains and a higher degree of sulfation, which could increase reactivity with PF4 (Boshkov *et al.*, 1993).

HIT antibodies activate platelets and stimulate an immunomediated endothelial lesion, followed by the appearance of thrombocytopenia and/or thrombosis (Fondu, 1995; Greinacher, 1995; Warkentin *et al.*, 1998; Cines *et al.*, 2007). Platelet activation is primarily caused by binding between the immunocomplex and FcγRIIa (CD32) receptors (Adelman *et al.*, 1989; Anderson *et al.*, 1995; Baglin, 2001), leading to degranulation and the release of pro-coagulant substances (*e.g.*, serotonin, histamine, and adenosine diphosphate), thromboxane biosynthesis, an influx of Ca<sup>2+</sup>, and the generation of highly pro-thrombotic phospholipid microparticles (Chong *et al.*, 1981; Chong *et al.*, 1989a; Warkentin & Kelton, 2001a). The immunocomplex can bind to Fc receptors on the surfaces of monocytes, neutrophils, and endothelial cells, and the binding of the immunocomplex to so many types of cells can contribute to the profound thrombin generation seen in patients with HIT. Thrombin generation can be enhanced by HIT activation of monocytes and endothelial cell tissue factor on the surface of monocytes and endothelial cells (Visentin *et al.*, 1994; Warkentin, 1999; Newman & Chong, 2000; Pouplard *et al.*, 2001; Arepally & Mayer, 2001). These thrombotic processes may lead to a hypercoagulable state, thus increasing the risk of severe and extensive thromboembolic complications in many patients. Additional activation of platelets by thrombin and other released agonists results in a further increase in the numbers of FcγRIIa receptors on the platelet surface, facilitating even more platelet activation (Chong *et al.*, 1993b; Anderson *et al.*, 1995). However, some reports have indicated that platelet activation can be blocked by the FcγRIIa receptor-specific monoclonal antibody (IV.3) (Kelton *et al.*, 1988; Visentin *et al.*, 1994).

While IgG-class HIT antibody can be detected in most HIT patients, IgA and IgM HIT antibodies can be found in only a small portion of patients (Suh *et al.*, 1997; Amiral *et al.*,

1996b). Given that IgA and IgM antibodies do not activate platelets in the presence of heparin *in vitro*, and that they are unable to bind FcγRIIa, their presence in HIT patients could simply be coincidental, though IgM and IgA are not able to bind to FcγRIIa (Amiral et al., 1996a; Amiral et al., 1996b; Amiral et al., 1996c; Blank et al., 1997). These data suggest that platelet activation occurs independently of the IgG FcγRIIa receptor. After heparin administration is interrupted, the HIT antibody gradually disappears; laboratory tests for HIT antibodies are usually “negative” or “weakly positive” at 100 days (Shantsila et al., 2009). For HIT to develop at this point, the HIT antibody would need to form again (Lubenow et al., 2002; Rice et al., 2002). Harris et al. (2008) have reported an association between the PLA2 polymorphism of glycoprotein IIIa and the risk of thrombosis in patients with HIT antibodies.

## 5. Diagnosis of HIT

The diagnosis of HIT should be based both on clinical criteria, such as the presence of thrombocytopenia and thrombosis, and laboratory data, such as platelet count dynamics and detection of HIT antibodies. However, it may be difficult to establish a general diagnostic protocol, given the lack of a readily accessible standard laboratory test and the frequent detection of elevated anti-HIT antibody levels in heparin-treated patients that display no clinical features of the disease (Arepally & Ortel, 2006).

In the majority of patients with HIT, thrombocytopenia is defined as an otherwise unexplained >50% drop in the platelet count (Warkentin et al., 2008a); thrombocytopenia is generally of moderate severity, and median platelet count is approximately  $50\text{--}60 \times 10^9$  platelets/L (Jackson et al., 2006). In HIT patients, platelet counts are generally  $< 20 \times 10^9$  platelets/L. Clinical presentation of HIT in patients with profound thrombocytopenia can be rapidly progressive and include the development of disseminated intravascular coagulation and microvascular thrombotic complications (Ortel, 2009). Patients are likely to suffer from HIT when their platelet counts drop to less than 50% of normal levels and when they present with thrombosis or skin lesions at heparin injection sites (Jackson et al., 2006). Generally, platelet count decreases and/or thrombotic events begin 5–10 days after the initiation of heparin therapy in heparin-hypersensitive individuals, and platelet count in thrombocytopenic patients may not return to initiation levels until several days later (Warkentin & Kelton, 2001a). In patients who have undergone operation, the expected pattern would show a return of normal platelet count immediately after surgery, followed by an unexpected drop (Warkentin, 2003). Persistent thrombocytopenia following cardiac bypass surgery is usually not a result of HIT, but instead may stem from other causes, such as postoperative complications. However, postoperative thrombocytopenia lasting for >5 days without an apparent alternative cause may be the result of HIT (Lillo-Le et al., 2004).

Several diagnostic algorithms have been developed to provide a more systematic approach to the diagnosis of HIT. Patients can be assigned an HIT score using the “4 Ts,” an assessment protocol and memory device focused on the salient clinical features of HIT: degree of Thrombocytopenia (maximum points for a platelet count fall of >50% or a nadir of  $20\text{--}100 \times 10^9$ /L), the Timing of the fall in platelet count (maximum points for an onset of 5–10 days after initiation of heparin treatment or within 1 day if there has been recent heparin exposure), the presence of Thrombosis or other sequelae (maximum points for new thrombosis, skin lesions, or acute systemic reactions), and oTher causes of

thrombocytopenia excluded (maximum points for no other cause event) (Warkentin, 2003, Warkentin et al., 2003b, Bryant et al., 2008). The “4 Ts” is useful for following the recommendation of Warkentin and Heddle, who suggest the employment of a clinical decision-making model to establish a pretest probability for HIT in patients who receive UFH or LMWH (Warkentin & Heddle, 2003). A diagnostic score for HIT after cardiopulmonary bypass surgery has also been proposed (Lillo-Le et al., 2004). Other studies have demonstrated the usefulness of combining the 4Ts score with laboratory testing when diagnosing HIT (Lillo-Le et al., 2004; Denys et al., 2008; Gruel et al., 2008); laboratory tests can also be used independently for confirming a clinical diagnosis.

### 5.1 Laboratory testing

Laboratory testing is necessary to confirm the diagnosis of HIT, and is most helpful in these patients clinically assessed to be at intermediate high clinical risk of HIT (Arepally & Ortel, 2006). Patients who have undergone cardiopulmonary bypass surgery frequently have elevated antiheparin/PF4 antibody levels; among these individuals, testing should not be used to “screen” patients for HIT or evaluate patients assessed to have a low pre-test probability for HIT (Warkentin et al., 2008a). Although a lot of laboratory tests are available for detection of heparin-PF4 antibody, these tests have several advantages and disadvantages. Blood sampling for the detection of HIT antibodies was performed in patients with clinically suspected HIT on days 5 to 14 following the initiation of heparin therapy (Warkentin et al., 2008a). Although HIT antibodies are detectable in the blood for several weeks after heparin administration, discontinuation of heparin administration, samples should be collected as soon as possible because antibody levels can decrease quite rapidly.

The first test developed for diagnosing HIT was the platelet aggregation test, which uses citrated platelet-rich plasma (PRP) and standard platelet aggregometry (Warkentin, 2004b). The platelet aggregation test is able to provide results quickly (Kelton et al, 1988), although results of this test is more influenced by heparin concentrations and donor platelet variability compared to those of <sup>14</sup>C-serotonin release assay (SRA) (Warkenin & Kelton, 1990; Chong, 1992, 1995). Accordingly, to increase sensitivity and specificity, test conditions need to be optimized– instance, by washed platelets (Chong et al., 1993a; Greinacher et al., 1994a; Pouplard et al., 1999a). Washed platelet activation assays, such as the platelet SRA, (Sheridan et al., 1986; Warkentin et al., 1992; Warkentin, 2001; Price E et al., 2007), and the heparin-induced platelet activation test (Greinacher et al., 1991; Greinacher et al., 1994a) are used by a few reference laboratories. In these assays, it is necessary to use of apyrase in a wash step for maintenance of platelet reactivity to HIT antibodies, and to resuspend in a calcium- and magnesium-containing buffer (Polgár et al., 1998; Warkentin, 2001). The major limitation of this method is its technically demanding nature (Warkentin, 2000), which limits its use to a few reference laboratories. In most clinical laboratories, immunological tests such as ELISA are used because they are easy to perform, have a rapid turnaround time, and are highly sensitive (Price et al., 2007). There are 2 PF4-dependent antigen assays that are commercially available for detecting HIT antibodies (Amiral et al., 1992; Collins et al., 1997; Warkentin, 2000; Warkentin et al., 2001): the Asserachrom (Stago, Asnières, France), which detects antibodies that react with PF4-heparin complexes, and the GTI-PF4 (GTI, Brookfield, WI, USA), which detects antibodies that react with PF4 bound to polyvinyl sulfonate.

Prospective studies have shown that, among HIT antibody classes, only HIT-IgG antibodies have very high sensitivity for diagnosing clinical HIT (Warkentin et al., 2000; Lindhoff-Last et al., 2001). Detection of PF4-heparin antibodies is performed as followed. Unbound material is removed, and a chromogen is added to label the bound conjugate, producing a yellow color, read at 405 nm. The amount of yellow produced at the end point, as indicated by the optical density (OD), is proportional to the amount of antibody present. A positive result is reported if OD reading is 0.400 or more. Higher ELISA OD results have been shown to significant correlation of the serotonin release assay results and an increased risk for thrombosis in patients with HIT (Warkentin et al., 2008b). Furthermore, the ELISA results are most useful when combined with a clinical scoring system (Janatpour et al., 2007). Zwicker et al (2004) have reported that higher ELISA OD measurements correlated significantly with thrombosis, and patients with isolated HIT (HIT in the absence of thrombosis) and an OD level of 1.0 or more had a 6-fold increased risk of thrombosis compared with patients who had OD levels between 0.4 and 0.99 (Zwicker et al, 2004). The sensitivity of the ELISA for PF4-heparin antibodies is greater than functional assays (Greinacher et al., 1994b; Amiral et al., 1995; Pouplard et al., 1999b), though a “positive” result may not denote the same magnitude of thrombotic risk.

## 5.2 Laboratory data from 2 potential HIT patients

We examined 2 patients who experienced thrombocytopenia after being given UFH during percutaneous transluminal coronary angioplasty (PTCA) and cardiac surgery. For both individuals, we tested for HIT by measuring platelet aggregability and quantifying levels of both anti-heparin-PF4 complex antibody (anti-HIT antibody) and thrombin-antithrombin III complex (TAT). Platelet aggregation in response to 0.2 µg/mL collagen was measured using Born’s turbidimetric methods (Born GVR, 1962), and quantified by light transmission, as previously reported (Toyohira et al., 1995; Kariyazono et al., 1997; Nakamura et al., 1999); platelet aggregation activity was evaluated as percent maximum aggregation (MA). First samples were prepared by incubating the PRP of suspected HIT patients with UFH (0.2 IU/mL), and second samples were prepared by separately adding plasma from the 2 patients to the PRP of healthy volunteers at a ratio of 1:1, then adding UFH (0.2 IU/mL). We then used a commercial ELISA kit (GTI Diagnostics, Waukesha, WI, NJ, USA) to measure anti-heparin antibodies in these samples.

As shown in Figure 1, platelet aggregation was much higher in the first set of sample than in the first control sample (without UFH); in other words, heparin had a strong positive effect on aggregation. As a result, MA of second sample was 68% (Figure 2), and showed strong aggregation. The ELISA results indicated significantly higher OD values for the 2 potential HIT patients than in the healthy volunteers. Furthermore, remarkably high levels of circulating TAT and TNF-alpha were found in the plasma of the suspected HIT patients. Our laboratory data indicate the likelihood that anti-HIT antibodies were present in the plasma of both patients. Furthermore, these data demonstrate the marked acceleration of blood coagulation in these patients, suggesting an increased risk of thrombosis.

These findings support previous reports that many HIT patients are hypercoagulable and have greatly elevated levels of TAT (Warkentin et al., 1997; Greinacher et al., 2000); furthermore, this helps explain the strong relationship between HIT and venous or arterial thrombosis (Warkentin & Kelton, 2001a).

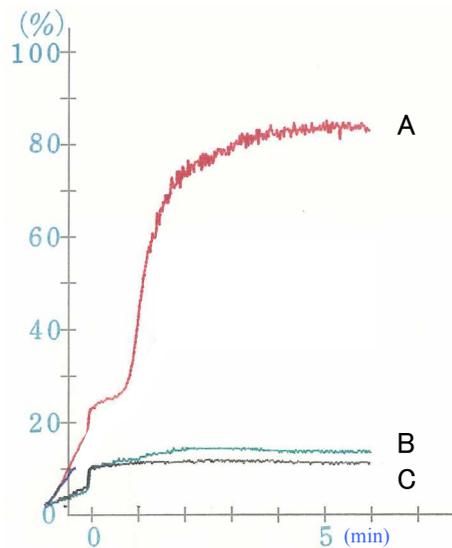


Fig. 1. Platelet aggregation was stimulated by 0.2  $\mu\text{g}/\text{mL}$  collagen. In case A's PRP incubated without UFH (first control sample) (C). In healthy volunteer's PRP incubated with UFH (second control sample) (B). In case A's PRP incubated with UFH (A).

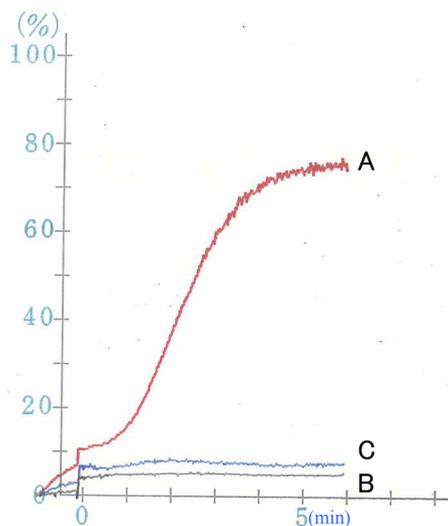


Fig. 2. Anti-heparin antibody was detected by turbidimetric method. Collagen (0.2  $\mu\text{g}/\text{mL}$ ) was used as agonist. In case A's PRP incubated without UFH (first control sample) (C). In healthy volunteer's PRP was mixed with case A's plasma following incubation without UFH (third control sample) (B). In healthy volunteer's PRP was mixed with case A's plasma following incubation with UFH (A).

Given these findings, we propose a model for the pathophysiological mechanism of HIT (Figures 3 and 4). This model helps explain observations that the hypercoagulable state, coupled with endothelial cell dysfunction due to injury from heparin antibody, activated platelets, leukocytes, platelet microparticles, atherosclerosis or medical intervention, can lead to arterial thrombosis (Walenga et al., 2000). Furthermore, the model is supported by the report that anti-HIT antibodies bind to and directly activate microvascular endothelial cells, whereas binding to and activating macrovascular endothelial cells requires pre-activation by platelets or TNF-alpha (Walenga et al., 2004).

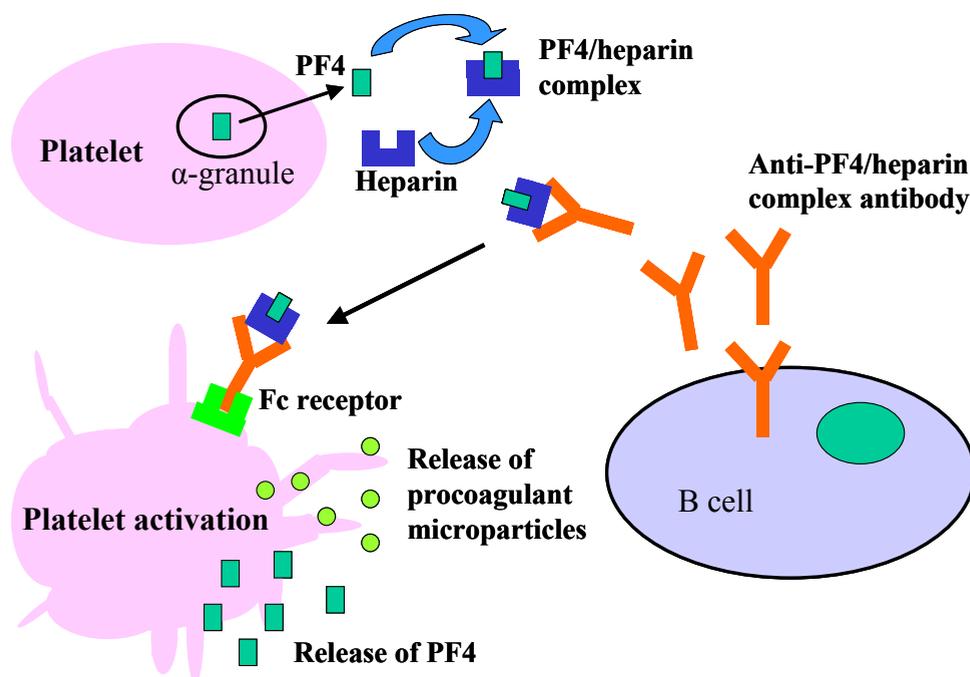


Fig. 3. Pathophysiological mechanism of HIT (1). Stage 1; binding of heparin to PF4 induces the formation of a neoepitope. Stage 2; an immune response against the PF4/heparin complex induces antibody formation. Stage 3; the complex of PF4/heparin and specific antibody associates with platelets via binding of the antibody Fc part to the platelet immunoglobulin receptor Fc $\gamma$ RIIa (CD32), representing the major stage in platelet activation in HIT. Stage 4; activated platelets shed procoagulant microparticles which enhance thrombin generation.

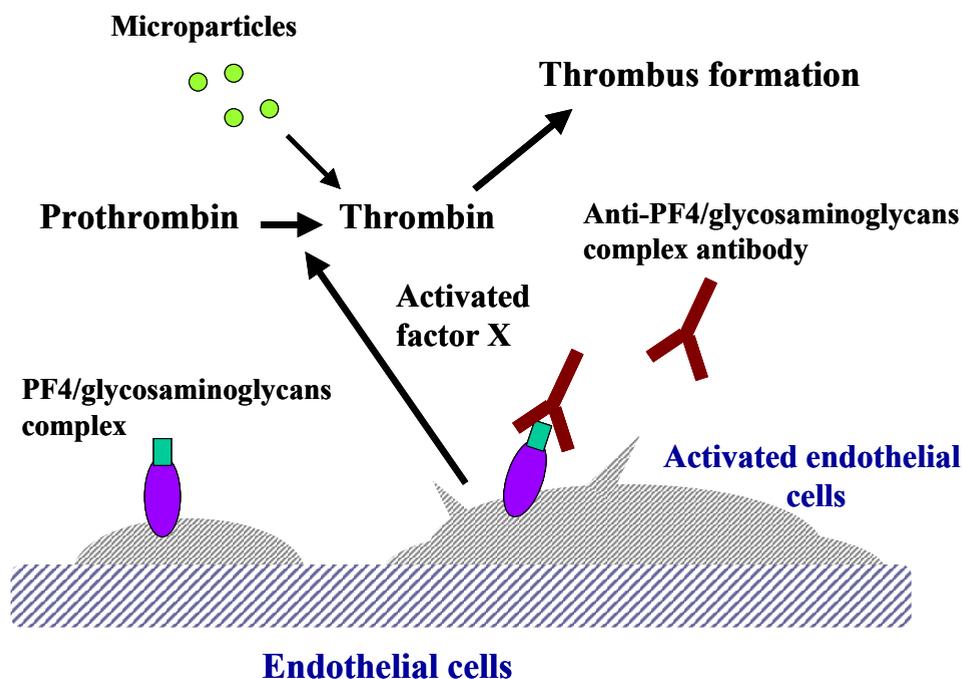


Fig. 4. Pathophysiological mechanism of HIT (2). Stage 5; binding of released PF4 to heparan sulfate of endothelial cell induces the formation of PF4/glycosaminoglycans complex on the surface of endothelial cell. Stage 6; PF4/glycosaminoglycans complex antibody binds to PF4/glycosaminoglycans complex on the surface of endothelial cell, and activates endothelial cell. Stage 7; activation of endothelial cells lead to the release of activated factor X. Stage 8; thrombin generation induces thrombus formation.

## 6. Management of HIT

All heparins must be avoided when treating patients suspected to have HIT; even exposure to very low amounts of heparin through heparin-coated catheters and heparin flushes used to maintain intravenous lines may trigger HIT (Laster et al., 1989). Likewise, preparations of LMWH can induce severe HIT, though they do so less frequently than UFH (Warkentin et al., 2003a; Warkentin & Greinacher, 2003; Walenga et al., 2004). Further, the cross-reactivity rate between heparin and LMWH is so high that LMWH is not recommended for the treatment of HIT patients (Chong, 2003; Warkentin et al., 2008a). Alternative anticoagulant therapy must be initiated immediately, both for HIT patients diagnosed with thrombocytopenia alone or with thromboembolism (Warkentin & Greinacher, 2004). In patients in whom treatment was delayed until an HIT diagnosis could be confirmed by laboratory tests, the incidence of new thrombosis was approximately 10-fold higher than in individuals treated promptly with a direct thrombin inhibitor (Greinacher et al., 2000).

Marked decreases of platelet counts in HIT patients require anticoagulation with an effective antithrombotic drug that does not cross-react *in vivo* with the circulating anti-heparin/PF4

antibodies. There are currently 3 thrombin inhibitors available for patients with HIT: lepirudin, argatroban, and bivalirudin, all of which directly bind and inactivate thrombin. Prospective cohort studies have been performed to investigate the efficacy and major bleeding endpoints for lepirudin (Greinacher et al., 1999a; Greinacher et al., 1999b; Lubenow et al., 2004; Lubenow et al., 2005) and argatroban (Lewis et al., 2001; Lewis et al., 2003) in patients with HIT complicated by thrombosis. When respective historical control data were taken into consideration, composite end point risk rates and new thrombosis risk rates were 0.48 and 0.28 for lepirudin, respectively, and 0.75 and 0.45 for argatroban, respectively. The corresponding absolute event rates were 19.2% (lepirudin) and 42.3% (argatroban) for the composite end point, and 7.0% (lepirudin) and 15.5% (argatroban) for new thrombosis. Patients who received lepirudin were less likely to require amputation than those who were given argatroban. Fatal bleeding after treatment with lepirudin has been found to range from 1.2% of patients (in a prospective study) to 3.9% of patients (in a retrospective observational study) (Lubenow et al., 2005; Tardy et al., 2006). Danaparoid and fondaparinux may also be used to manage HIT (Warkentin et al., 2008a). When abrupt decreases in platelet count (to  $<10 \times 10^9$  clls/L) are observed in patients who underwent angioplasty and were treated with a combination of heparin and glycoprotein IIb/IIIa antagonist, the recommended antagonist is always glycoprotein IIb/IIIa (Shantsila et al., 2009)

### 6.1 Lepirudin

Lepirudin, a recombinant form of hirudin, is a direct, specific, and irreversible inhibitor of thrombin that is administered via intravenous injection (Greinacher & Lubenow, 2001). The biggest drawback of Lepirudin is that it must be accompanied by the strict laboratory monitoring: activated partial thromboplastin time (aPTT) monitoring should be performed at 4-h intervals until it is clear that patients have reached a steady state within the normal range of values (Warkentin et al., 2008b). Moreover, patients should be informed that they have received lepirudin, since fatal anaphylactic reactions have been reported in patients re-exposed to lepirudin during a second round of intravenous treatment (Greinacher et al., 2003). Although similar bleeding rates have been observed in lepirudin-treated patients and historical control subjects, this drug has been found to significantly reduce the combined end point of death, limb amputation, and new thromboembolic complications in patients with HIT associated thrombosis (Greinacher et al., 1999a; Greinacher et al., 1999b).

### 6.2 Argatroban

Argatroban is a synthetic L-arginine-derived direct thrombin inhibitor that reversibly binds to the thrombin active site. Argatroban is administered by intravenous injection, with dose adjustment to maintain aPTT at 1.5-3.0 times the baseline value (Gosselin et al., 2004). It has been reported that, in HIT patients without thrombosis, treatment with argatroban produces a significant reduction in the composite end point, such as all-cause death, all-cause limb amputation, and new thrombosis at 37 days (Lewis et al., 2001; Lewis et al., 2003). In HIT patients who underwent lower extremity revascularization, the composite end point of deaths, urgent revascularization, and limb amputations developed in 25% of patients treated with argatroban, and 6% of patients had major bleeding episodes (Baron et al., 2008). Restoration of platelet counts is universally observed within 6-7 days of the initiation of

argatroban therapy (Bartholomew et al., 2007). Some precautions are required when argatroban infusion is overlapped by warfarin anticoagulant therapy, although argatroban causes a further substantial increase in the international normalized ratio (INR). In addition, careful thought should be given to the participation of direct thrombin inhibitor to the patient's INR.

### 6.3 Danaparoid

Danaparoid is approved as an alternative anticoagulant for HIT in many countries. Danaparoid, a heparinoid with predominantly anti-Xa activity and some anti-IIa activity, is a mixture of three glycosaminoglycans such as heparin sulfate, dermatan sulfate, and chondroitin sulfate. Danaparoid presents its anticoagulant effect by catalyzing the inactivation of factor Xa in the presence of antithrombin, and has a unique property of specific suppression of HIT antibody-induced platelet activation that is not observed with other drugs used for HIT treatment (Chong et al., 1989b). Tardy-Poncet et al. reported that major bleeding episodes in those patients treated with danaparoid were significantly fewer compared with those treated with lepirudin (Tardy-Poncet et al., 1999). Among the drugs used for the treatment of HIT, danaparoid is the only drug whose efficacy and safety have been confirmed by a prospective randomized controlled study (Chong et al., 2001).

### 6.4 Bivalirudin

Bivalirudin, which is not approved as an HIT treatment, is a 20-amino acid polypeptide with sequence homology to hirudin. Campbell et al. (2000) reported 94% procedural success in a series of 17 percutaneous coronary intervention patients who were given bivalirudin as a treatment for HIT (Campbell et al., 2000). In the Anticoagulant Therapy with Bivalirudin to Assist in the Performance of Percutaneous Coronary Intervention in Patients with HIT (or ATBAT) multicenter open-label trial (Mahaffey et al., 2003), clinical percutaneous coronary intervention success, defined as the absence of death, emergency bypass surgery, or Q-wave infarction, was achieved in 96% of patients treated with bivalirudin; further, patients displayed a low rate of bleeding. Cumulatively, these results indicate that bivalirudin therapy is safe and effective during percutaneous coronary intervention.

### 6.5 Fondaparinux

Fondaparinux, a synthetic pentasaccharide with potent indirect anti-Xa inhibitor properties, is used only a limited number of patients with HIT. The generation of HIT-related antigen depends on the polysaccharide chain length (Amiral et al., 1995). Because patients have a very low risk of developing HIT while receiving fondaparinux (Warkentin et al., 2005), platelet count monitoring is not needed during administration of this drug. Despite these advantages, fondaparinux cannot be recommended for in the treatment of HIT until there are more data demonstrating its efficacy and safety (Rota et al., 2008; Lobo et al., 2008).

## 7. Conclusion

Heparin is one of the most widely used and valuable anticoagulants for the treatment and prophylaxis of thrombotic complications. However, HIT is not only a common but also a

serious complication of heparin therapy with a high rate of morbidity and mortality. Its prompt clinical and laboratory recognition is necessary to stop heparin administration immediately and start an alternative anticoagulant. HIT antibody test results must be interpreted in the appropriate context of the available clinical information. Furthermore, additional diagnostic information is available as a result of considering the magnitude of a given positive test result. The diagnosis of HIT should be clearly recorded in the patient's notes and marked as a serious allergy.

## 8. References

- Adelman B, Sobel M, Fujimura Y, Ruggeri ZM, & Zimmerman TS. (1989). Heparin-associated thrombocytopenia: observations on the mechanism of platelet aggregation. *J Lab Clin Med*, 113, 2, (Feb 1989), pp.204-210.
- Amiral J, Bridey F, Dreyfus M, Vissoc AM, Fressinaud E, et al. (1992). Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia. *Thromb Haemost*, 68, 1, (Jun 1992), pp.95-96.
- Amiral J, Bridey F, Wolf M, Boyer-Neumann C, Fressinaud E, et al. (1995). Antibodies to macromolecular platelet factor 4-heparin complexes in heparin-induced thrombocytopenia: a study of 44 cases. *Thromb Haemost*, 73,11, (Jan 1995), pp.21-28.
- Amiral J, Marfaing-Koka A, Poncz M, & Meyer D. (1998). The biological basis of immune heparin-induced thrombocytopenia. *Platelets*, 9, 2, (1998), pp.77-91.
- Amiral J, Marfaing-Koka A, Wolf M, Alessi MC, et al. (1996c). Presence of autoantibodies to interleukin-8 or neutrophil-activating peptide-2 in patients with heparin-associated thrombocytopenia. *Blood*, 88, 2, (Jul 1996), pp.410-416.
- Amiral J, Peynaud-Debayle E, Wolf M, Bridey F, Vissac AM, et al. (1996a). Generation of antibodies to heparin-PF4 complexes without thrombocytopenia in patients treated with unfractionated or low-molecular-weight heparin. *Am J Hematol*, 52, 2, (Jun 1996), pp.90-95.
- Amiral J, Wolf M, Fischer A, Boyer-Neumann C, Vissac A, et al. (1996b). Pathogenicity of IgA and/or IgM antibodies to heparin-PF4 complexes in patients with heparin-induced thrombocytopenia. *Br J Haematol*, 92,4, (Mar 1996), pp.954-959.
- Anderson CL, Chacko GW, Osborne JM, & Brandt JT. (1995). The Fc receptor for immunoglobulin G (Fc gamma RII) on human platelets. *Semin Thromb Hemost*, 21, 1, (1995), pp.1-9.
- Ansell JE, Clark WP Jr, Compton CC. (1986). Fatal reactions associated with intravenous heparin. *Drug Intell Clin Pharm*, 20, 1, (Jun 1986), pp.74-75.
- Arepally GM, & Mayer IM. (2001). Antibodies from patients with heparin-induced thrombocytopenia stimulate monocytic cells to express tissue factor and secrete interleukin-8. *Blood*. 98, 4, (Aug 2001), pp.1252-1254.
- Arepally GM, & Ortel TL. (2006). Clinical practice. Heparin-induced thrombocytopenia. *N Engl J Med*, 355, 8, (Aug 2006), pp.809-817.
- Aster RH. (1995). Heparin-induced thrombocytopenia and thrombosis. *N Engl J Med*, 332, 20, (May 1995), pp. 1374-1376.

- Baglin TP. (2001). Heparin induced thrombocytopenia thrombosis (HIT/T) syndrome: diagnosis and treatment. *J Clin Pathol*, 54, 4, (Apr 2001), pp.272-274.
- Bailey RT Jr, Ursick JA, Heim KL, Hilleman DE, & Reich JW. (1986). Heparin-associated thrombocytopenia: a prospective comparison of bovine lung heparin, manufactured by a new process, and porcine intestinal heparin. *Drug Intell Clin Pharm*, 20, 5, (May 1986), pp.374-378.
- Baron SJ, Yeh RW, Cruz-Gonzalez I, Healy JL, Pomerantsev E, et al. (2008). Efficacy and safety of argatroban in patients with heparin induced thrombocytopenia undergoing endovascular intervention for peripheral arterial disease. *Catheter Cardiovasc Interv*, 72, 1, (Jul 2008), pp.116-120.
- Bartholomew JR, Begelman SM, & Almahameed A. (2005). Heparin-induced thrombocytopenia: principles for early recognition and management. *Cleve Clin J Med*, 72, (Suppl 1), (Apr 2005), pp.S31-S36.
- Bartholomew JR, Pietrangeli CE, & Hursting MJ. (2007). Argatroban anticoagulation for heparin-induced thrombocytopenia in elderly patients. *Drugs Aging*, 24, 6, (Jun 2007), pp.489-499.
- Battistelli S, Genovese A, & Gori T. (2010). Heparin-induced thrombocytopenia in surgical patients. *Am J Surg*, 199, 1, (Jan 2010), pp.43-51.
- Blank M, Cines DB, Arepally G, Eldor A, Afek A, et al. (1997). Pathogenicity of human anti-platelet factor 4 (PF4)/heparin in vivo: generation of mouse anti- PF4/heparin and induction of thrombocytopenia by heparin. *Clin Exp Immunol*, 108, 2, (May 1997), pp.333-339.
- Blank M, Shoenfeld Y, Tavor S, Praprotnik S, Boffa MC, et al. (2002). Anti-platelet factor 4/heparin antibodies from patients with heparin-induced thrombocytopenia provoke direct activation of microvascular endothelial cells. *Int Immunol*, 14, 2, (Feb 2002), pp.121-129.
- Born GVR. (1962). Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature*, 194, (Jun 1962), pp.927-929.
- Boshkov LK, Warkentin TE, Hayward CP, Andrew M, & Kelton JG. (1993). Heparin-induced thrombocytopenia and thrombosis: clinical and laboratory studies. *Br J Haematol*. 84, 2, (Jun 1993), pp.322-328.
- Bryant A, Low J, Austin S, & Joseph J. (2008). Timely diagnosis and management of heparin-induced thrombocytopenia in a frequent request, low incidence single centre using clinical 4T's score and particle gel immunoassay. *Br J Haematol*, 143, 5, (Dec 2008), pp.721-726.
- Burgess JK, & Chong BH. (1997). The platelet proaggregating and potentiating effects of unfractionated heparin, low molecular weight heparin and heparinoid in intensive care patients and healthy controls. *Eur J Haematol*, 58, 4, (Apr 1997), pp.279-285.
- Campbell KR, Mahaffey KW, Lewis BE, Weitz JI, Berkowitz SD, et al. (2000). Bivalirudin in patients with heparin-induced thrombocytopenia undergoing percutaneous coronary intervention [abstract]. *J Invasive Cardiol*, 12, (Suppl F), (Dec 2000), pp.14F-19F.

- Chong BH, & Ismail F. (1989). The mechanism of heparin-induced platelet aggregation. *Eur J Haematol*, 43, 3, (Sep 1989), pp.245-251.
- Chong BH, Burgess J, & Ismail F. (1993a). The clinical usefulness of the platelet aggregation test for the diagnosis of heparin-induced thrombocytopenia. *Thromb Haemost*, 69, 4, (Apr 1993), pp.344-350.
- Chong BH, Grace CS, & Rozenberg MC. (1981). Heparin-induced thrombocytopenia: effect of heparin platelet antibody on platelets. *Br J Haematol*, 49, 4, (Dec 1981), pp.531-540.
- Chong BH, Ismail F, Cade J, Gallus AS, Gordon S, Chesterman CN. (1989a). Heparin-induced thrombocytopenia: studies with a new low molecular weight heparinoid, Org 10172. *Blood*, 73, 6, (May 1989), pp.1592-1596.
- Chong BH, Ismail F, Cade J, Gallus AS, Gordon S, et al. (1989b). Heparin-induced thrombocytopenia: studies with a new low molecular weight heparinoid, Org 10-172. *Blood*, 73, 6, (May 1989), pp.1592-1596.
- Chong BH, Pilgrim RL, Cooley MA, & Chesterman CN. (1993b). Increased expression of platelet IgG Fc receptors in heparin induced thrombocytopenia. *Blood*, 81, 4, (Feb 1993), pp.988-993.
- Chong BH. (1995). Heparin-induced thrombocytopenia. *Br J Haematol*, 89, 3, (1995), pp.431-439.
- Chong BH. (2003). Heparin-induced thrombocytopenia. *J Thromb Haemost*, 1, 7, (Jul 2003), pp.1471-1478.
- Chong BH. Heparin-induced thrombocytopenia. (1992). *Aust N Z J Med*, 22, 2, (Apr 1992), pp.145-152.
- Cines DB, Rauova L, Arepally G, Reilly MP, McKenzie SE, et al. (2007). Heparin-induced thrombocytopenia: an autoimmune disorder regulated through dynamic autoantigen assembly/disassembly. *J Clin Apheresis*, 22, 1, (Feb 2007), pp.31-36.
- Collins JL, Aster RH, Moghaddam M, Piotrowski MA, Strauss TR, et al. (1997). Diagnostic testing for heparin-induced thrombocytopenia (HIT): an enhanced platelet factor 4 complex enzyme linked immunosorbent assay (PF4ELISA). *Blood*, 90, (suppl), p.461a.
- Denys B, Stove V, Philippé J, & Devreese K. (2008). A clinical-laboratory approach contributing to a rapid and reliable diagnosis of heparin-induced thrombocytopenia. *Thromb Res*, 123, 1, (Jan 2008), pp.137-145.
- Fabris F, Fussi F, Casonato A, Visentin L, Randi M, et al. (1983). Smith MR, Girolami A. Normal and low molecular weight heparins: interaction with human platelets. *Eur J Clin Invest*, 13, 2, (Apr 1983), pp.135-139.
- Fabris F, Luzzatto G, Stefani PM, Girolami B, Cella G, et al. (2000). Heparin-induced thrombocytopenia. *Haematologica*, 85, 1, (Jun 2000), pp.72-81.
- Fondu P. (1995). Heparin-associated thrombocytopenia: an update. *Acta Clin Belg*, 50, 6, (1995), pp.343-357.
- Francis JL, Palmer GJ 3rd, Moroosse R, & Drexler A. (2003). Comparison of bovine and porcine heparin in heparin antibody formation after cardiac surgery. *Ann Thorac Surg*, 75, 1, (Jun 2003), pp.17-22.

- Gentilini G, Kirschbaum NE, Augustine JA, Aster RH, & Visentin GP. (1999). Inhibition of human umbilical vein endothelial cell proliferation by the CXC chemokine, platelet factor 4 (PF4), is associated with impaired downregulation of p21(Cip1/WAF1). *Blood*, 93, 1, (Jun 1999), pp.25-33.
- Girolami B, Prandoni P, Stefani PM, Tanduo C, Sabbion P, et al. (2003). The incidence of heparin-induced thrombocytopenia in hospitalized medical patients treated with subcutaneous unfractionated heparin: a prospective cohort study. *Blood*, 101, 8, (April 2003), pp.2955-2959.
- Gosselin RC, Dager WE, King JH, Janatpour K, Mahackian K, et al. (2004). Effect of direct thrombin inhibitors, bivalirudin, lepirudin, and argatroban, on prothrombin time and INR values. *Am J Clin Pathol*, 121, 4, (Apr 2004), pp.593-599.
- Green D, Harris K, Reynolds N, Roberts M, & Patterson R. (1978). Heparin immune thrombocytopenia: evidence for a heparin-platelet complex as the antigenic determinant. *J Lab Clin Med*, 91, 1, (Jun 1978), pp.167-175.
- Green D, Martin GJ, Shoichet SH, DeBacker N, Bomalaski JS, et al. (1984). Thrombocytopenia in a prospective, randomized, double-blind trial of bovine and porcine heparin. *Am J Med Sci*, 288, 2, (Sep 1984), pp.60-64.
- Greinacher A, & Lubenow N. (2001). Recombinant hirudin in clinical practice: focus on lepirudin. *Circulation*, 103, 10, (Mar 2001), pp.1479-1484.
- Greinacher A, Amiral J, Dummel V, Vissac A, Kiefel V, et al. (1994b). Laboratory diagnosis of heparin-associated thrombocytopenia and comparison of platelet aggregation test, heparin-induced platelet activation test, and platelet factor 4/heparin enzyme-linked immunosorbent assay. *Transfusion*, 34, 5, (May 1994), pp.381-385.
- Greinacher A, Eichler P, Lubenow N, Kwasny H, & Luz M. (2000). Heparin-induced thrombocytopenia with thromboembolic complications: meta-analysis of 2 prospective trials to assess the value of parenteral treatment with lepirudin and its therapeutic aPTT range. *Blood*, 96, 3, (Aug 2000), pp.846-851.
- Greinacher A, Janssens U, Berg G, Böck M, Kwasny H, et al. (1999a). Lepirudin (recombinant hirudin) for parenteral anticoagulation in patients with heparin-induced thrombocytopenia: Heparin-Associated Thrombocytopenia Study (HAT) investigators. *Circulation*, 100, 6, (Aug 1999), pp.587-593.
- Greinacher A, Lubenow N, & Eichler P. (2003). Anaphylactic and anaphylactoid reactions associated with lepirudin in patients with heparin-induced thrombocytopenia. *Circulation*, 108, 17, (Oct 2003), pp.2062-2065.
- Greinacher A, Michels I, & Mueller-Eckhardt C. (1992). Heparin-associated thrombocytopenia: the antibody is not heparin specific. *Thromb Haemost*, 67, 5, (May 1992), pp.545-549.
- Greinacher A, Michels I, Kiefel V, Mueller-Eckhardt C. (1991). A rapid and sensitive test for diagnosing heparin-associated thrombocytopenia. *Thromb Haemost*, 66, 6, (Dec 1991), pp.734-736.
- Greinacher A, Michels I, Liebenhoff U, Presek P, & Mueller-Eckhardt C. (1993). Heparin-associated thrombocytopenia: immune complexes are attached to the platelet membrane by the negative charge of highly sulphated oligosaccharides. *Br J Haematol*, 84, 4, (Aug 1993), pp.711-716.

- Greinacher A, Pötzsch B, Amiral J, Dummel V, Eichner A, et al. (1994a). Heparin-associated thrombocytopenia: isolation of the antibody and characterization of a multimolecular PF4-heparin complex as the major antigen. *Thromb Haemost*, 71, 2, (Feb 1994), pp.247-251.
- Greinacher A, Völpel H, Janssens U, Hach-Wunderle V, Kemkes-Matthes B, Eichler P, et al. (1999b). Lepirudin provides safe and effective anticoagulation in patients with heparin-induced thrombocytopenia: a prospective study. *Circulation*, 99, 1, (Jan 1999), pp.73-80.
- Greinacher A. (1995). Antigen generation in heparin-associated thrombocytopenia: the nonimmunologic type and the immunologic type are closely linked in their pathogenesis. *Semin Thromb Hemost*, 21, 1, (1995), pp.106-116.
- Gruel Y, Boizard-Boval B, & Wautier JL. (1993). Further evidence that alpha-granule components such as platelet factor 4 are involved in platelet-IgG-heparin interactions during heparin-associated thrombocytopenia. *Thromb Haemost*, 70, 2, (Aug 1993), pp.374-375.
- Gruel Y, Régina S, & Pouplard C. (2008). Usefulness of pretest clinical score (4Ts) combined with immunoassay for the diagnosis of heparin-induced thrombocytopenia. *Curr Opin Pulm Med*, 14,5, (Sep 2008), pp.397-402.
- Harris K, Nguyen P, & Van Cott EM. (2008). Platelet PLA2 polymorphism and the risk for thrombosis in heparin-induced thrombocytopenia. *Am J Clin Pathol*, 129, 2, (Feb 2008), pp.282-286.
- Hirsh J, Heddle N, & Kelton JG. (2004). Treatment of heparin-induced thrombocytopenia: a critical review. *Arch Intern Med*, 164, 4, (Feb 2004), pp.361-369.
- Jackson MR, Neilson WJ, Lary M, Baay P, Web K, & ET AL. (2006). Delayed-onset heparin-induced thrombocytopenia and thrombosis after intraoperative heparin anticoagulation: four case reports. *Vasc Endovasc Surgery*, 40, 11, (Jun-Feb 2006), pp.67-70.
- Janatpour KA, Gosselin RC, Dager WE, Lee A, Owings JT, et al. (2007). Usefulness of optical density values from heparin-platelet factor 4 antibody testing and probability scoring models to diagnose heparin-induced thrombocytopenia. *Am J Clin Pathol*, 127, 3, (Mar 2007), pp.429-433.
- Jang IK, & Hursting MJ. (2005). When heparins promote thrombosis: review of heparin-induced thrombocytopenia. *Circulation*, 111, 20, (May 2005), pp.2671-2683.
- Kadidal VV, Mayo DJ, & Horne MK. (1999). Heparin-induced thrombocytopenia (HIT) due to heparin flushes: a report of three cases. *J Intern Med*. 246, 3, (Sep 1999), pp.325-329.
- Kariyazono H, Nakamura K, Shinkawa T, Moriyama Y, Toyohira H, et al. (1997). Inhibitory effects of antibiotics on platelet aggregation in vitro. *Hum Exp Toxicol*, 16, 11, (Nov 1997), pp.662-666.
- Kelton JG, Sheridan D, Santos A, Smith J, Steeves K, et al. (1988). Heparin-induced thrombocytopenia: laboratory studies. *Blood*, 72, 3, (Sep 1988), pp.925-930.
- Kelton JG, Smith JW, Warkentin TE, Hayward CP, Denomme GA, & Horsewood P. (1994). Immunoglobulin G from patients with heparin-induced thrombocytopenia binds

- to a complex of heparin and platelet factor 4. *Blood*, 83, 11, (Jun 1994), pp.3232-3239.
- Laster JL, Nichols WK, & Silver D. (1989). Thrombocytopenia associated with heparin-coated catheters inpatients with heparin-associated antiplatelet antibodies. *Arch Intern Med*, 149, 10, (Oct 1989), pp.2285-2287.
- Lee DH, & Warkentin TE. (2004). Frequency of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A. eds. *Heparin-induced thrombocytopenia*. 3<sup>rd</sup> ed. New York, NY: Marcel Dekker, pp.107-148
- Lewis BE, Wallis DE, Berkowitz SD, Matthai WH, Fareed J, et al. (2001). Argatroban anticoagulant therapy in patients with heparin-induced thrombocytopenia. *Circulation*, 103, 14, (Apr 2001), pp.1833-1843.
- Lewis BE, Wallis DE, Leya F, Hursting MJ, & Kelton JG; Argatroban-915 Investigators. (2003). Argatroban anticoagulation in patients with heparin-induced thrombocytopenia. *Arch Intern Med*, 163, 15, (Aug 1003), pp.1849-1856.
- Lillo-Le Louët A, Boutouyrie P, Alhenc-Gelas M, Le Beller C, Gautier I, et al. (2004). Diagnostic score for heparin-induced thrombocytopenia after cardiopulmonary bypass. *J Thromb Haemost*, 2, 11, (Nov 2004), pp.1882-1888.
- Lindhoff-Last E, Gerdson F, Ackermann H, & Bauersachs R. (2001). Determination of heparin-platelet 4-IgG antibodies improves diagnosis of heparin-induced thrombocytopenia. *Br J Haematol*, 113, 4, (Jun 2001), pp.886-890.
- Lobo B, Finch C, Howard A, & Minhas S. (2008). Fondaparinux for the treatment of patients with acute heparin-induced thrombocytopenia. *Thromb Haemost*, 99, 1, (Jan 2008), pp.208-214.
- Lubenow N, Eichler P, Lietz T, & Greinacher A; Hit Investigators Group. (2005). Lepirudin in patients with heparin-induced thrombocytopenia: results of the third prospective study (HAT-3) and a combined analysis of HIT-1, HAT-2, and HAT-3. *J Thromb Haemost*, 3, 11, (Nov 2005), pp.2428-2436.
- Lubenow N, Eichler P, Lietz T, Farner B, Greinacher A. (2004). Lepirudin for prophylaxis of thrombosis in patients with acute isolated heparin-induced thrombocytopenia: an analysis of 3 prospective studies. *Blood*, 104, 10, (Nov 2004), pp.3072-7.
- Lubenow N, Kempf R, Eichner A, Eichler P, Carlsson LE, et al. (2002). Heparin-induced thrombocytopenia: temporal pattern of thrombocytopenia in relation to initial use or reexposure to heparin. *Chest*, 122, 1, (Jul 2002), pp.37-42.
- Lynch DM, & Howe SE. (1985). Heparin-associated thrombocytopenia: antibody binding specificity to platelet antigen. *Blood*, 66, 5, (Nov 1985), pp.1176-1181.
- Magnani HN. (1993). Heparin-induced thrombocytopenia (HIT): an overview of 230 patients treated with organan (Org 10172). *Thromb Haemost*, 70, 4, (Oct 1993), pp.554-561.
- Mahaffey KW, Lewis BE, Wildermann NM, Berkowitz SD, Oliverio RM, et al. (2003). The anticoagulant therapy with bivalirudin to assist in the performance of percutaneous coronary intervention in patients with heparin-induced thrombocytopenia (ATBAT) study. *J Invasive Cardiol*, 15, 11, (Nov 2003), pp.611-616.

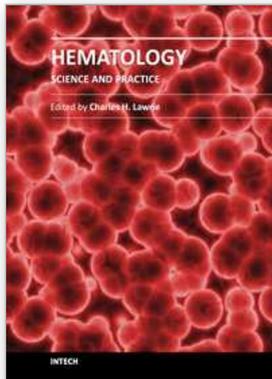
- Mayo DJ, Cullinane AM, Merryman PK, & Horne MK 3rd. (1990). Serologic evidence of heparin sensitization in cancer patients receiving heparin flushes of venous access devices. *Support Care Cancer*, 7, 6, (Nov 1990), pp.425-427.
- Monreal M, Lafoz E, Salvador R, Roncales J, & Navarro A. (1989). Adverse effects of three different forms of heparin therapy: thrombocytopenia, increased transaminases, and hyperkalaemia. *Eur J Clin Pharmacol*, 37, 4, (1989), pp.415-418.
- Nakamura K, Kariyazono H, Moriyama Y, Toyohira H, Kubo H, et al. (1999). Effects of sarpogrelate hydrochloride on platelet aggregation, and its relation to the release of serotonin and P-selectin. *Blood Coagul Fibrinolysis*, 10, 8, (Dec 1999), pp.513-519.
- Nand S, Wong W, Yuen B, Yetter A, Schmulbach E, et al. (1997). Heparin-induced thrombocytopenia with thrombosis: incidence, analysis of risk factors, and clinical outcomes in 108 consecutive patients treated at a single institution. *Am J Hematol*, 56, 1, (Sep 1997), pp.12-16.
- Newman PM, & Chong BH. (2000). Heparin-induced thrombocytopenia: new evidence for the dynamic binding of purified anti-PF4-heparin antibodies to platelets and the resultant platelet activation. *Blood*, 96, 1, (Jul 2000), pp.182-187.
- Ortel TL. (2009). Heparin-induced thrombocytopenia: when a low platelet count is a mandate for anticoagulation. *Hematology (Am Soc Hematol Educ Prog)*, pp.225-232.
- Polgár J, Eichler P, Greinacher A, Clemetson KJ. (1998). Adenosine diphosphate (ADP) and ADP receptor play a major role in platelet activation/aggregation induced by sera from heparin-induced thrombocytopenia patients. *Blood*, 91, 2, (Jun 1998), pp.549-554.
- Popov D, Zarrabi MH, Foda H, & Graber M. (1997). Pseudopulmonary embolism: acute respiratory distress in the syndrome of heparin-induced thrombocytopenia. *Am J Kidney Dis*, 29, 3, (Mar 1997), pp.449-452.
- Pouplard C, Amiral J, Borg JY, Laporte-Simitsidis S, Delahousse B, et al. (1999b). Decision analysis for use of platelet aggregation test, carbon 14-serotonin release assay, and heparin-platelet factor 4 enzyme-linked immunosorbent assay for diagnosis of heparin-induced thrombocytopenia. *Am J Clin Pathol*, 111, 5, (May 1999), pp.700-706.
- Pouplard C, Iochmann S, Renard B, Herault O, Colombat P, et al. (2001). Induction of monocyte tissue factor expression by antibodies to heparin-platelet factor 4 complexes developed in heparin-induced thrombocytopenia. *Blood*, 97, 10, (May 2001), pp.3300-3302.
- Pouplard C, May MA, Iochmann S, Amiral J, Vissac AM, et al. (1999a). Antibodies to platelet factor 4-heparin after cardiopulmonary bypass in patients anticoagulated with unfractionated heparin or a low-molecular-weight heparin : clinical implications for heparin-induced thrombocytopenia. *Circulation*, 99, 19, (May 1999), pp.2530-2536.
- Price E, Hayward C, Moffat K, Moore J, Warkentin T, et al. (2007). Laboratory testing for heparin-induced thrombocytopenia is inconsistent in North America: a survey of North American specialized coagulation laboratories. *Thromb Haemost*, 98, 6, (Dec 2007), pp.1357-1361.

- Rao AK, White GC, Sherman L, Colman R, Lan G, et al. (1989). Low incidence of thrombocytopenia with porcine mucosal heparin. A prospective multicenter study. *Arch Intern Med*, 6, (Jun 1989), 149, pp.1285-1288.
- Rhodes GR, Dixon RH, & Silver D. (1973). Heparin induced thrombocytopenia with thrombotic and hemorrhagic manifestations. *Surg Gynecol Obstet*, 136, 3, (Mar 1973), pp.409-416.
- Rice L, Attisha WK, Drexler A, & Francis JL. (2002). Delayed-onset heparin-induced thrombocytopenia. *Ann Intern Med*, 136, 3, (Feb 2002), pp.210-215.
- Rota E, Bazzan M, & Fantino G. (2008). Fondaparinux-related thrombocytopenia in a previous low-molecular-weight heparin (LMWH)-induced heparin-induced thrombocytopenia (HIT). *Thromb Haemost*, 99, 4, (Apr 2008), pp.779-781.
- Salzman EW, Rosenberg RD, Smith MH, Lindon JN, & Favreau L. (1980). Effect of heparin and heparin fractions on platelet aggregation. *J Clin Invest*, 65, 1, (Jan 1980), pp.64-73.
- Schmitt BP, & Adelman B. (1993). Heparin-associated thrombocytopenia: a critical review and pooled analysis. *Am J Med Sci*, 305, 4, (Apr 1993), pp.208-215.
- Shantsila E, Lip GY, & Chong BH. (2009). Heparin-induced thrombocytopenia. A contemporary clinical approach to diagnosis and management. *Chest*, 135, 6, (June 2009), pp.1651-1664.
- Sheridan D, Carter C, Kelton JG. (1986). A diagnostic test for heparin-induced thrombocytopenia. *Blood*, 67, 2, (Jan 1986), pp.27-30.
- Strauss R, Wehler M, Mehler K, Kreutzer D, Koebnick C, et al. (2002). Thrombocytopenia in patients in the medical intensive care unit: bleeding prevalence, transfusion requirements, and outcome. *Crit Care Med*, 30, 2, (Aug 2002), pp.1765-1771.
- Suh JS, Aster RH, & Visentin GP. (1998). Antibodies from patients with heparin-induced thrombocytopenia/thrombosis recognize different epitopes on heparin: platelet factor 4. *Blood*, 91, 1, (Feb 1998), pp.916-922.
- Suh JS, Malik MI, Aster RH, & Visentin GP. (1997). Characterization of the humoral immune response in heparin-induced thrombocytopenia. *Am J Hematol*, 54, 3, (Mar 1997), pp.196-201.
- Tardy B, Lecompte T, Boelhen F, Tardy-Poncet B, Elalamy I, et al. (2006). Predictive factors for thrombosis and major bleeding in an observational study in 181 patients with heparin-induced thrombocytopenia treated with lepirudin. *Blood*, 108, 5, (Sep 2006), pp.1492-1496.
- Tardy-Poncet B, Tardy B, Reynaud J, Mahul P, Mismetti P, et al. (1999). Efficacy and safety of danaparoid sodium (ORG 10172) in critically ill patients with heparin-associated thrombocytopenia. *Chest*, 115, 6, (Jun 1999), pp.1616-1620.
- Toyohira H, Nakamura K, Kariyazono H, Yamada K, Moriyama Y, et al. (1995). Significance of combined use of anticoagulants and antiplatelet agents in the early stage after prosthetic valve replacement. *Kyobu Geka*, 48, 9, (Aug 1995), pp.749-755.
- Visentin GP, Ford SE, Scott JP, & Aster RHJ. (1994). Antibodies from patients with heparin-induced thrombocytopenia/thrombosis are specific for platelet factor 4 complexed with heparin or bound to endothelial cells. *Clin Invest*, 93, 1, (Jun 1994), pp.81-88.

- Walenga JM, Jeske WP, & Messmore HL. (2000). Mechanisms of venous and arterial thrombosis in heparin-induced thrombocytopenia. *J Thromb Thrombolysis*, 10, (Suppl), pp.S13-S20.
- Walenga JM, Jeske WP, Prechel MM, & Bakhos M. (2004). Newer insights on the mechanism of heparin-induced thrombocytopenia. *Semin Thromb Hemost*, 30, (Suppl1), (Feb 2004), pp.S57-S67.
- Wallis DE, Workman DL, Lewis BE, Steen L, Pifarre R, et al. (1999). Failure of early heparin cessation as treatment for heparin-induced thrombocytopenia. *Am J Med*, 106, 6, (Jun 1999), pp.629-635.
- Warkentin TE, & Bernstein RA. (2003). Delayed-onset heparin-induced thrombocytopenia and cerebral thrombosis after a single administration of unfractionated heparin [letter]. *N Engl J Med*, 348, 11, (Mar 2003), pp.1067-1069.
- Warkentin TE, & Greinacher A. (2004). Heparin-induced thrombocytopenia: recognition, treatment, and prevention. *Chest*, 126, (suppl 3), (Sep 2004), pp.311S-337S.
- Warkentin TE, & Heddle NM. (2003). Laboratory diagnosis of immune heparin-induced thrombocytopenia. *Curr Hematol Rep*, 2, 2, (Mar 2003), pp.148-157.
- Warkentin TE, & Kelton JG. (1990). Heparin and platelets. *Hematol Oncol Clin North Am*, 4, 1, (Feb 1990), pp.243-264.
- Warkentin TE, & Kelton JG. (1996). A 14-year study of heparin-induced thrombocytopenia. *Am J Med*, 101, pp.502-507.
- Warkentin TE, & Kelton JG. (2001a). Temporal aspects of heparin-induced thrombocytopenia. *N Engl J Med*, 344, 17, (Apr 2001), pp.1286-1292.
- Warkentin TE, & Kelton JG. (2001b). Delayed-onset heparin-induced thrombocytopenia and thrombosis. *Ann Intern Med*, 135, 7, (Oct 2001), pp.502-506.
- Warkentin TE, Aird WC, & Rand JH. (2003b). Platelet-endothelial interactions: sepsis, HIT, and antiphospholipid syndrome. *Hematology (Am Soc Hematol Educ Prog)*, pp.497-519.
- Warkentin TE, Chong BH, & Greinacher A. (1998). Heparin-induced thrombocytopenia: towards consensus. *Thromb Haemost*, 79, 1, (Jun 1998), pp.1-7.
- Warkentin TE, Cook RJ, Marder VJ, Sheppard JA, Moore JC, et al. (2005). Anti-platelet factor 4/heparin antibodies in orthopedic surgery patients receiving antithrombotic prophylaxis with fondaparinux or enoxaparin. *Blood*, 106, 12, (Dec 2005), pp.3791-3796.
- Warkentin TE, Elavathil LJ, Hayward CP, Johnston MA, Russett JI, et al. (1997). The pathogenesis of venous limb gangrene associated with heparin-induced thrombocytopenia. *Ann Intern Med*, 127, 9, (Nov 1997), pp.804-812.
- Warkentin TE, Greinacher A, Koster A, & Lincoff AM; American College of Chest Physicians. (2008a). Treatment and prevention of heparin-induced thrombocytopenia. American College of Physicians evidence-based clinical practice guidelines (8th edition). *Chest*, 133, (suppl 6), (Jun 2008), pp.340S-380S.
- Warkentin TE, Hayward CP, Smith CA, Kelly PM, Kelton JG. (1992). Determinants of donor platelet variability when testing for heparin-induced thrombocytopenia. *J Lab Clin Med*, 120, 3, (Sep 1992), pp.371-379.

- Warkentin TE, Levine MN, Hirsh J, Horsewood P, Roberts RS, et al. (1995a). Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *N Engl J Med*, 332, 20, (May 1995), pp.1330-1335.
- Warkentin TE, Roberts RS, Hirsh J, & Kelton JG. (2003a). An improved definition of immune heparin-induced thrombocytopenia in postoperative orthopedic patients. *Arch Intern Med*, 163, 20, (Nov 2003), pp.2518-2524.
- Warkentin TE, Sheppard J, Moore J, Sigouin CS, & Kelton J. (2008b). Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. *J Thromb Haemost*, 6, 8, (Aug 2008). pp.1304-1312.
- Warkentin TE, Sheppard JA, Horsewood P, Simpson PJ, Moore JC, et al. (2000). Impact of the patient population on the risk for heparin-induced thrombocytopenia. *Blood*, 96, 5, (Sep 2000), pp.1703-1708.
- Warkentin TE. (1996a). Heparin-induced thrombocytopenia: IgG-mediated platelet activation, platelet microparticle generation, and altered procoagulant/anticoagulant balance in the pathogenesis of thrombosis and venous limb gangrene complicating heparin-induced thrombocytopenia. *Transfus Med Rev*, 10, 4, (Oct 1996), pp.249-258.
- Warkentin TE. (1996b). Heparin-induced skin lesions. *Br J Haematol*, 92, 2, (Feb 1996), pp.494-497.
- Warkentin TE. (1999). Generation of platelet-derived microparticles and procoagulation activity by heparin-induced thrombocytopenia IgG/serum and other IgG platelet agonists: a comparison with standard platelet agonists. *Platelets*. 10, 5, (1999), pp.319-326.
- Warkentin TE. (2000). Laboratory testing for heparin-induced thrombocytopenia. *J Thromb Thrombolysis*, 10, (suppl 1), (Nov 2000), pp.S35-S45.
- Warkentin TE. (2001). Laboratory testing for heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A. eds. Heparin-induced thrombocytopenia. 2nd ed. New York, NY: Marcel Dekker Inc; pp.231-269.
- Warkentin TE. (2002). Platelet count monitoring and laboratory testing for heparin-induced thrombocytopenia. *Arch Pathol Lab Med*, 126, 11, (Nov 2002), pp.1415-1423.
- Warkentin TE. (2003). Heparin-induced thrombocytopenia: pathogenesis and management. *Br J Haematol*, 121, 4, (May 2003), pp.535-555.
- Warkentin TE. (2004a). Clinical picture of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A. eds. Heparin-induced thrombocytopenia. 3rd ed. New York, NY: Marcel Dekker, pp.53-106.
- Warkentin TE. (2004b) Laboratory testing for heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A. eds. Heparin-induced thrombocytopenia. 3rd ed. New York, NY: Marcel Dekker, pp.271-311.
- Warkentin TE. (2007). Clinical picture of heparin-induced thrombocytopenia. In: Warkentin TE, Greinacher A. eds. Heparin-induced thrombocytopenia. 4th ed. New York, NY: InformaHealthcare USA, pp.21-66.
- Ziporen L, Li ZQ, Park KS, Sabnekar P, Liu WY, Arepally G, et al. (1998). Defining an antigenic epitope on platelet factor 4 associated with heparin-induced thrombocytopenia. *Blood*, 92, 9, (Nov 1998), pp.3250-3259.

Zwicker JI, Uhl L, Huang WY, Shaz BH, Bauer KA. (2004). Thrombosis and ELISA optical density values in hospitalized patients with heparin-induced thrombocytopenia. *J Thromb Haemost.* 12, 2, (Dec 2004), pp.2133-2137.



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Hematology encompasses the physiology and pathology of blood and of the blood-forming organs. In common with other areas of medicine, the pace of change in hematology has been breathtaking over recent years. There are now many treatment options available to the modern hematologist and, happily, a greatly improved outlook for the vast majority of patients with blood disorders and malignancies. Improvements in the clinic reflect, and in many respects are driven by, advances in our scientific understanding of hematological processes under both normal and disease conditions. Hematology - Science and Practice consists of a selection of essays which aim to inform both specialist and non-specialist readers about some of the latest advances in hematology, in both laboratory and clinic.

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