

## CLINICAL PLATELET DISORDERS

## Heparin-induced thrombocytopenia

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**Heparin-induced thrombocytopenia (HIT) is an immune complication of heparin therapy caused by antibodies to complexes of platelet factor 4 (PF4) and heparin. Pathogenic antibodies to PF4/heparin bind and activate cellular FcγRIIA on platelets and monocytes to propagate a hypercoagulable state culminating in life-threatening thrombosis. It is now recognized that anti-PF4/**

**heparin antibodies develop commonly after heparin exposure, but only a subset of sensitized patients progress to life-threatening complications of thrombocytopenia and thrombosis. Recent scientific developments have clarified mechanisms underlying PF4/heparin immunogenicity, disease susceptibility, and clinical manifestations of disease. Insights from clinical**

**and laboratory findings have also been recently harnessed for disease prevention. This review will summarize our current understanding of HIT by reviewing pathogenesis, essential clinical and laboratory features, and management. (Blood. 2017;129(21):2864-2872)**

## Introduction

Unfractionated heparin (UFH) celebrated its centennial from discovery in 2016.<sup>1</sup> In recognizing this historical milestone, it is fitting to acknowledge heparin's continued relevance to modern medicine. None of the newer anticoagulants, whether oral or parenteral, combine heparin's unique pharmacologic properties, including a rapid onset of action, ability to inhibit multiple coagulant proteins, ease of monitoring, and reversibility. Such favorable properties will continue to keep UFH and its derivatives, the low-molecular-weight heparins (LMWHs), at the therapeutic forefront for a number of indications where alternative therapies are currently lacking (cardiac surgery), unsuitable (mechanical valves), or untested (cancer associated thrombosis and antiphospholipid antibody syndrome). Thus, as a drug class, the heparin family (UFH and LMWH) is likely to remain in clinical use for the foreseeable future.

Heparin-induced thrombocytopenia (HIT) is heparin's most clinically relevant nonhemorrhagic complication. It is an immune complication caused by antibodies directed to complexes containing heparin and an endogenous platelet protein, platelet factor 4 (PF4). Although the immune reaction is common (8% to 50%),<sup>2-4</sup> clinical complications of thrombocytopenia and thrombosis are far less frequent, affecting ~0.2% to 3% of patients exposed to the drug in various clinical settings.<sup>2,5,6</sup> This review will summarize our current understanding of the clinical syndrome from disease pathogenesis to its treatment.

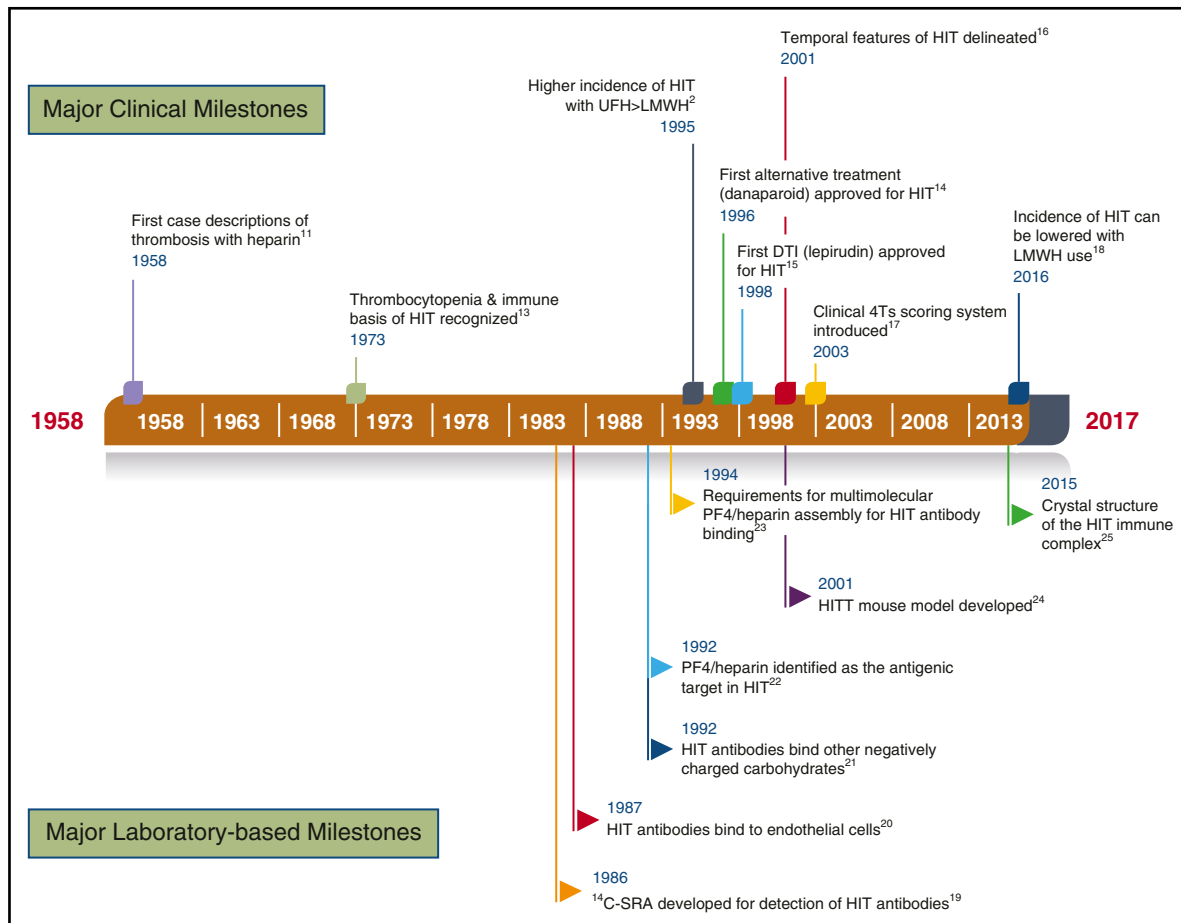
## Historical perspective

Acute thrombocytopenia occurring within minutes of heparin therapy was first described in animals in 1942<sup>7</sup> and later observed in humans<sup>8</sup> as a transient, nonimmune complication of heparin therapy. This pharmacologic effect of heparin, referred to as type I HIT<sup>9</sup> is caused by the agglutinating effects of heparin on platelets.<sup>10</sup> The more serious,

immune complication of HIT (type II) was first reported in 1958 by 2 surgeons, Weismann and Tobin, who summarized a case series of 10 patients at their institution who developed paradoxical thrombosis while receiving heparin therapy.<sup>11</sup> Similar findings were noted a few years later by Roberts et al<sup>12</sup> who described another case series of 11 patients and postulated that this clinical syndrome may be "the result of an antigen-antibody mechanism." By 1973, Rhodes et al succinctly described salient clinical features of this disease through systematic investigation of 2 patients with HIT, noting the following: (1) concurrent manifestations of thrombocytopenia in addition to thrombosis during heparin therapy, (2) improvement in platelet counts after discontinuation of heparin therapy, (3) demonstration of a heparin-dependent antibody using platelet aggregation assays in the acute phase, and (4) recurrent thrombocytopenia with heparin rechallenge during the acute phase of illness.<sup>13</sup> Since these initial descriptions, major clinical<sup>2,14-18</sup> and laboratory<sup>19-25</sup> discoveries over the ensuing decades have further refined our understanding of the disease. A time line of some of the landmark studies, in the opinion of the author, is provided in Figure 1. For a detailed historical perspective on HIT, the reader is referred to the excellent review by Kelton and Warkentin.<sup>26</sup>

## Pathogenesis of HIT: antigen, antibody, and thrombotic mechanisms

HIT is caused by antibodies that recognize complexes of PF4 and heparin.<sup>22</sup> Once antibodies form, a subset of patients develops a profound hypercoagulable state caused by cellular-activating antibodies that promote thrombin generation. This section summarizes our current understanding of the HIT antigen, triggers for PF4/heparin antibody formation, and mechanisms of thrombosis.



**Figure 1. A time line of key clinical and laboratory-based observations in HIT.** Important clinical developments are provided above the time band, and major research observations are noted below. DTI, direct thrombin inhibitor; HITT, HIT with thrombosis; SRA, serotonin release assay.

### The HIT antigen: PF4/heparin complex

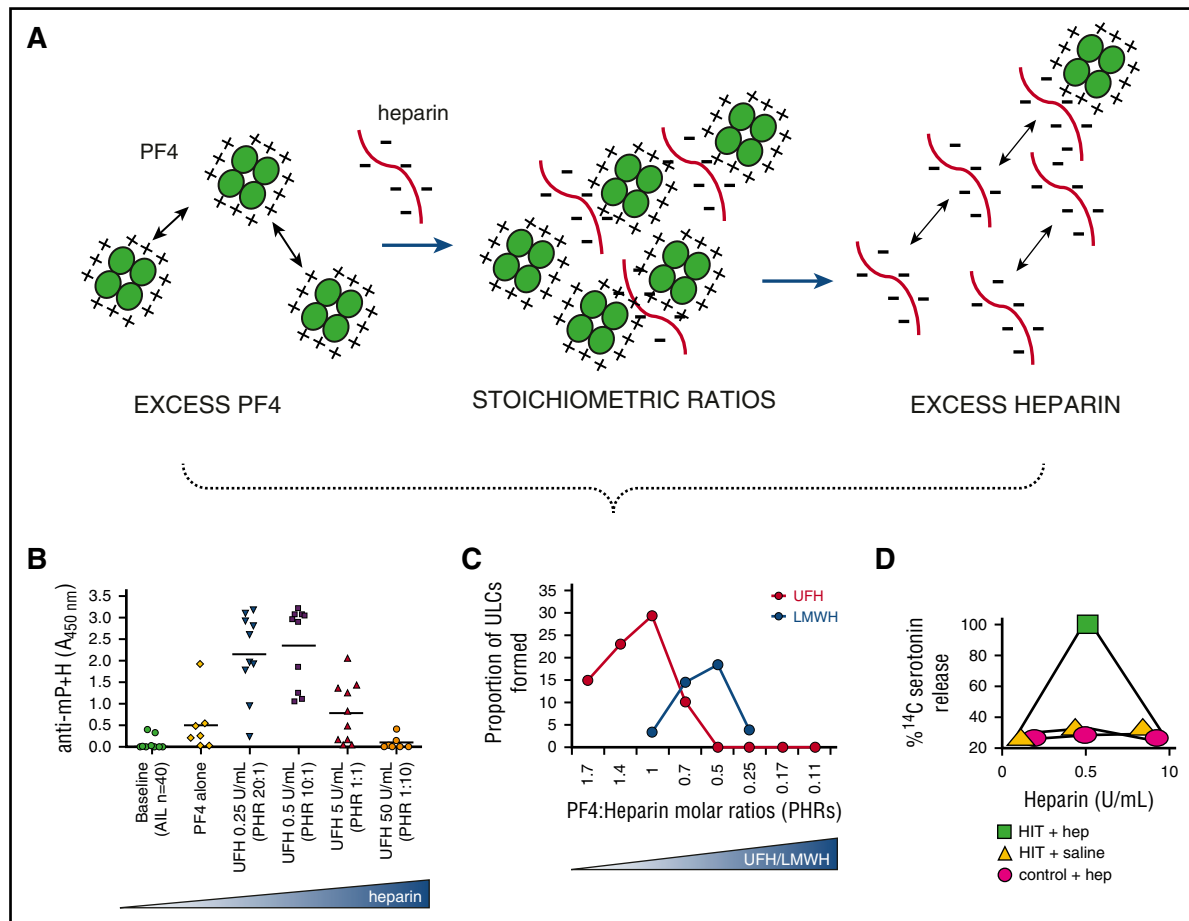
PF4 is a positively charged platelet protein stored in platelet  $\alpha$ -granules and released in high quantities at sites of platelet activation.<sup>27</sup> Released PF4 binds to negatively charged glycosaminoglycans (GAGs) on nearby endothelial cells, displaces bound antithrombin,<sup>28</sup> and renders the local milieu prothrombotic. Because of the higher affinity of PF4 for heparin relative to other GAGs (heparin > heparan sulfate > dermatan sulfate > chondroitin 6-sulfate > chondroitin 4-sulfate),<sup>29</sup> infusions of heparin displace released PF4 from vascular or other cell-binding sites into the circulation allowing for formation of immunogenic PF4/heparin complexes.

PF4 and heparin interactions are exclusively charge dependent.<sup>30</sup> Optimal PF4/heparin complex formation occurs when the 2 compounds are present at certain molar amounts associated with charge neutralization.<sup>31-33</sup> Changes in molar amounts of PF4 or heparin leading to excess of one component or another results in charge imbalance and increased repulsive forces that affect complex assembly. These fundamental chemical properties of PF4 and heparin result in the appearance of a “bell-shaped” curve of reactivity corresponding to heparin-dependent reactivity in laboratory assays,<sup>19,23</sup> differences in complex formation between UFH and LMWH,<sup>31,34</sup> and in vivo immunogenicity<sup>33</sup> (Figure 2). Recent structural studies have additionally identified critical thermodynamic<sup>35,36</sup> and essential structural features of the antigenic complex that govern chemical interactions.<sup>25</sup>

### The HIT antibody

It is now recognized that the immune response to PF4/heparin occurs far more frequently than clinical manifestations of thrombocytopenia or thrombosis.<sup>6,37,38</sup> The incidence of antibody formation in general medical and surgical patients treated with UFH is 8% to 17%,<sup>37</sup> for LMWH and fondaparinux is 2% to 8%,<sup>6,39,40</sup> and approaches ~50% in patients undergoing cardiac surgery.<sup>4,38,41</sup> Several theories have been advanced to explain this unusually high frequency of anti-PF4/heparin antibodies. PF4 binds effectively to bacterial walls of gram-positive and gram-negative bacteria,<sup>42-44</sup> and bacterial infection, if accompanied by platelet activation and PF4 release, may provide a sufficient priming stimulus for an immune response upon subsequent heparin exposure.<sup>42</sup>

Our laboratory's investigations have shown a more direct role for PF4/heparin complexes in dysregulating innate immunity.<sup>45</sup> In vitro studies and ex vivo studies of heparinized patients demonstrate nearly exclusive binding of PF4/heparin complexes to B cells. Further evaluation revealed that B-cell binding is mediated by C3 activation by PF4/heparin ULCs and subsequent binding of complement-opsonized antigen to B cells via complement receptor 2/CD21.<sup>45</sup> As binding of complement-coated antigen to CD21 enhances its immunogenicity by 1000- to 10 000-fold,<sup>46</sup> it is likely that direct complement activation by PF4/heparin complexes<sup>45</sup> contribute to subsequent antibody production. Other investigators have described findings of autoreactive B cells secreting anti-PF4/heparin immunoglobulin M (IgM) in human cord blood and peripheral blood of healthy subjects.<sup>44,47</sup> These latter



**Figure 2. Importance of PF4/heparin ultralarge complexes (ULCs) in HIT pathogenesis.** (A) PF4, a positively charged protein, binds to negatively charged heparin through electrostatic interactions to form ULCs that govern HIT biology. At concentrations of PF4 or heparin excess, repulsive forces from excess charge predominate and are not permissive for complex formation. At stoichiometric ratios associated with charge neutralization, ULCs form capable of biological effects associated with in vivo immunogenicity<sup>33</sup> (B), differential clinical effects of UFH and LMWH<sup>31</sup> (C), and heparin-dependent reactivity in laboratory assays<sup>19</sup> (D).

studies suggest that inflammation and/or infection transiently lift peripheral tolerance mechanisms to allow proliferation of autoreactive B cells.<sup>47,48</sup>

### Mechanisms of HIT thrombosis

Once an immune response occurs, a subset of seropositive patients experience thrombocytopenia and progress to life-threatening complications of thrombosis. The principal cellular target for HIT antibodies is platelets, which express FcγRIIA receptors.<sup>49</sup> These findings were confirmed in a murine model by Reilly et al, who demonstrated the requirements for anti-PF4/heparin antibodies and cellular FcγRIIA for disease manifestations.<sup>24</sup> Binding of HIT antibodies to platelet FcγRIIA elicits platelet activation via intracellular signaling involving the spleen tyrosine kinase (Syk<sup>50</sup>) and release of procoagulant microparticles.<sup>51</sup>

Platelet activation in HIT is also accompanied by intense thrombin generation.<sup>52</sup> The mechanisms underlying thrombin generation in HIT are not fully understood, but recent studies indicate that cellular activation of monocyte FcγRIIA promotes tissue factor expression, which in turn facilitates platelet activation via thrombin.<sup>53-55</sup> Studies by Tutwiler et al recently provided experimental proof of this amplification loop.<sup>56</sup> Using in vitro and in vivo studies, these investigators showed that HIT antibodies triggered dual activation of platelets and monocytes via cellular FcγRIIA. They further showed that depleting monocytes

and/or blocking monocyte FcγRIIA diminished monocyte-derived thrombin, which provided a second signal for platelet activation via protease-activated receptor 1 (PAR-1).<sup>56</sup> These findings further underscore the vital contributions of varied cellular FcγRIIA in HIT pathogenesis.

### Epidemiology of HIT

Anti-PF4/heparin antibodies are extremely uncommon in healthy individuals (0.3% to 0.5%) as assessed in large studies of blood bank donors<sup>42,57</sup>. Recent studies suggest that autoantibodies to PF4/heparin can develop in the context of surgical inflammation.<sup>40</sup> In addition, infection and surgical inflammation can also give rise to “spontaneous HIT,” a rare clinical variant occurring without antecedent heparin exposure.<sup>58,59</sup> In reports of spontaneous HIT, complications of unexplained thrombocytopenia and/or thrombosis are accompanied by laboratory evidence of high-titer platelet activating anti-PF4/heparin antibodies.<sup>58,59</sup> Clinical and laboratory features of several case reports to date are summarized in Table 1.

The vast majority of patients with HIT, however, develop clinical disease in the wake of heparin exposure. The incidence of disease varies by clinical setting, heparin formulation (UFH vs LMWH), and to some extent, drug dose (prophylactic vs therapeutic). Adults receiving UFH

**Table 1. Summary of case reports of spontaneous HIT**

Patient	Age	Sex	Recent hospitalization (<3 mo)	Antecedent illness	Platelet count at presentation	Thrombosis at presentation	IgG EIA	SRA	Outcome	Reference
1	69	M	No	Five-day course of ampicillin for periodontitis and intra-articular injection methylprednisolone 8 d prior	17	Bilateral iliac femoral artery thromboses	2.03	Positive	Death	129
2	40	F	No	I&D of groin cysts	297→175K (hours)→59K within 24 h of UFH	Right MCA thrombosis/femoral artery thrombosis	2.91	Positive	Survived	129
3	24	F	No	2 wk h/o URI; 1 wk h/o pneumonia	301K→62 after 2 doses LMWH		2.62	Positive	Survived	129
4	69	F	No	Right total knee replacement 7 d prior	205K→124K over 7 d	Cardiac arrest; bilateral adrenal hemorrhage/DIC	1.89	Positive	Death	130
5	62	M	No	None	65 × 10 <sup>9</sup> /L	Left MCA thrombosis	1.06	Positive	Survived	58
6	54	F	Yes	Right shoulder hemiarthroplasty 15 d prior	61	Left posterior cerebral artery thrombosis and left vertebral artery thrombosis	2.87	Positive	Survived	58
7	61	F	None	Right total knee replacement 7 d prior	251K→29 by postoperative day 9	Right greater saphenous vein thrombosis and nonocclusive left popliteal and femoral DVTs	2.3	Positive	Survived	131

DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis; EIA, enzyme immunoassay; F, female; h/o, history of; I&D, incision and drainage; K, thousand; M, male; MCA, middle cerebral artery; RLE, right lower extremity; URI, upper respiratory infection.

or LMWH for medical or general surgical indications are at higher risk for HIT<sup>6</sup> than pediatric<sup>60</sup> or obstetric patients.<sup>61,62</sup> These population differences are attributed to the existence of preexisting vascular disease and alterations in basal levels of platelet activation and circulating PF4. Other minor influences on HIT epidemiology have been described, including biological source of heparin (bovine > porcine<sup>63</sup>), route (intravenous > subcutaneous<sup>64</sup>), and sex (females > males).<sup>65</sup>

The differential immunogenicity and incidence of HIT with UFH and LMWH were first noted in a study by Warkentin et al in 1995.<sup>2</sup> In this study of ~700 orthopedic patients randomized to receive prophylactic dose UFH or LMWH, they noted a lower incidence of HIT (2.7% UFH vs 0% LMWH;  $P = .0018$ ) and platelet activating antibodies (7.8% UFH vs 2.2% LMWH;  $P = .02$ ).<sup>2</sup> Subsequent studies and a meta-analysis of ~7300 patients have confirmed nearly a 10-fold lower risk of HIT with use of prophylactic dose LMWH as compared with UFH.<sup>66</sup> This differential effect, however, is not seen at therapeutic doses of UFH and LMWH. In a meta-analysis of studies using these drugs at therapeutic doses, the incidence of clinical HIT appeared to be comparable (UFH 1.5% vs LMWH 1.2%).<sup>67,68</sup> Differences in the immunogenicity of prophylactic vs therapeutic doses of LMWH could potentially be explained by LMWH concentrations required to form immunogenic ULCs (Figure 2; see Rauova et al<sup>31</sup>).

The differential immunogenicity of prophylactic dose UFH and LMWH can be harnessed for disease prevention.<sup>18,69</sup> Investigators at Sunnybrook Health Sciences Center in Toronto implemented an “Avoid Heparin” campaign where they systematically replaced UFH with LMWH for thromboprophylaxis and/or treatment and removed UFH from catheter flushes and nursing units. By doing so, investigators noted a dramatic reduction after implementation of this program with respect to suspected HIT (85.5 vs 49.0 cases per 10 000 admission;  $P < .001$ ), positive HIT enzyme-linked immunosorbent assay (16.5 vs 6.1 cases per 10 000 admission;  $P < .001$ ), and adjudicated cases of HIT/HITT (10.7/4.6 vs 2.2/0.4 cases per 10 000 admission;  $P < .001$ ).<sup>18</sup>

## Clinical features of HIT

The clinical diagnosis of HIT rests on demonstrating thrombocytopenia and/or thrombosis in temporal association with heparin therapy while excluding other causes of thrombocytopenia. Such an exercise is rarely straightforward in hospitalized patients who are often on heparin thromboprophylaxis<sup>5</sup> and have competing causes of thrombocytopenia (infection, other medications, intravascular devices, and surgery).<sup>70,71</sup> The following section will summarize the essential diagnostic features of HIT (thrombocytopenia, thrombosis, and temporal features) and discuss clinical scoring systems that have been developed to assist clinicians in disease risk stratification.

### Thrombocytopenia

The cardinal manifestation of HIT is the occurrence of thrombocytopenia, which occurs in ~95% patients in temporal association with heparin therapy.<sup>72</sup> Thrombocytopenia can manifest either as an absolute drop in platelet count ( $<150 \times 10^9/L$ ) or a relative decline of 30% to 50% from baseline platelet counts.<sup>72</sup> Absolute thrombocytopenia in HIT is often moderate ( $50\text{--}70 \times 10^9/L$ ) and typically not associated with bleeding complications. Severe thrombocytopenia ( $<20 \times 10^9/L$ ) can occur as a manifestation of fulminant thrombotic disease<sup>72</sup> and consumptive coagulopathy.<sup>73</sup> Patients who only manifest an isolated thrombocytopenia (isolated HIT) remain at high risk for subsequent thrombosis (20% to 50%) from circulating antibodies capable of binding endogenous PF4/GAG complexes.<sup>74,75</sup> With unusual presentations of HIT, such as heparin-induced skin necrosis, thrombotic complications can occur in the absence of thrombocytopenia.<sup>76</sup>

### Thrombosis

Thrombosis is the most severe complication of HIT and contributes to disease morbidity and mortality.<sup>64,75</sup> In most patients, thrombotic complications occur concurrently with thrombocytopenia<sup>72</sup> and can

affect any vascular bed. Venous thromboses predominate, particularly at sites of vascular injury from catheters.<sup>77</sup> Atypical presentations, such as bilateral adrenal hemorrhage,<sup>78</sup> venous limb gangrene, and skin necrosis should prompt diagnostic consideration of HIT.<sup>76</sup>

Presently, it is not known why some seropositive patients remain asymptomatic while others progress to severe disease. There are no differences in traditional thrombophilic markers such as protein C, protein S, antithrombin, or factor V Leiden.<sup>79,80</sup> Although several studies have shown no major role for the common platelet FcγRIIA polymorphisms (FcγRIIAH131R)<sup>81</sup> and risk for HIT, one recent study challenges these earlier observations based on new biological findings. These investigators noted a higher risk of thrombosis in individuals homozygous for the 131-RR genotype<sup>82</sup> because of the inability of endogenous monomeric IgG<sub>2</sub> (which binds the H-, but not the R-isoform) to effectively compete with HIT immune complexes for binding platelet FcγRIIA.<sup>82</sup>

Clinical studies indicate that certain serologic features, such as IgG isotype,<sup>83</sup> capacity to activate platelets,<sup>84</sup> and high antibody levels (as measured by optical density [OD] and/or titer<sup>84-86</sup>) are associated with thrombotic risk. The contribution of IgA or IgM isotypes to thrombosis in HIT<sup>87,88</sup> is likely subordinate to established pathogenic mechanisms directly linking IgG-mediated activation of platelet and monocyte FcγRIIA receptors to thrombin generation.<sup>24,56</sup> Severity of thrombocytopenia serves as another clinical correlate of thrombotic risk, as patients with marked thrombocytopenia (>90% decline from baseline counts) have greater than eightfold risk of thrombosis as compared with patients with <30% decline.<sup>72</sup>

### Temporal features

The most important diagnostic element of HIT is timing of complications relative to heparin therapy. In heparin-naïve individuals, PF4/heparin antibodies become detectable at a median of 4 days from start of heparin therapy.<sup>89</sup> Clinical manifestations of thrombocytopenia and/or thrombosis develop 5 to 14 days after initial heparin therapy,<sup>72,89</sup> and on average ~2 days (range 1-5 days) after antibody detection.<sup>89</sup> In patients with recent heparin exposure (<100 days), thrombocytopenia occurs precipitously within 24 hours of drug reexposure because of circulating anti-PF4/heparin antibodies. Another rare clinical variant called “delayed-onset HIT” is recognized days to weeks after heparin discontinuation. Although thrombocytopenia likely occurs in the expected time frame after heparin exposure, there is a delay in manifestations of thrombosis days to weeks after discharge. If unrecognized, patients may be inadvertently reexposed to heparin leading to significant morbidity and mortality.<sup>90,91</sup> Delayed-onset HIT is caused by antibodies that are cross-reactive with PF4/GAG complexes found on cell surfaces and can trigger heparin-independent platelet activation in functional assays.<sup>90</sup>

The median time to antibody clearance is 50 days for platelet activation assays and 85 to 90 days by immunoassays.<sup>16,92</sup> Immunoassays may remain positive in ~35% of patients for up to 1 year, with levels decreasing over time.<sup>92</sup> Platelets rebound to the normal range within 1 week of discontinuation in ~65% of patients.<sup>93</sup> Even with platelet count recovery, patients remain at risk for thrombosis for 4 to 6 weeks after diagnosis<sup>74</sup> because of circulating anti-PF4/heparin antibodies.<sup>16</sup> Presently, there is no compelling laboratory or clinical evidence for immunologic memory in patients with HIT.<sup>16,94-96</sup> Patients who are reexposed to the drug months to years after antibody disappearance often do not manifest anamnestic responses, and seroconversion risk appears similar to de novo heparin exposure.<sup>16,95,96</sup> In a small study of 17 patients with HIT who are reexposed to heparin for cardiac surgery, a higher proportion of patients developed anti-PF4/heparin antibodies (65%)

relative to the incidence described in the literature (~27% to 51%).<sup>4,38,41,97</sup>

### Clinical scoring systems

Clinical scoring tools have been developed to aid clinicians in ascertaining disease. The 4Ts clinical scoring system, developed by Warkentin is the simplest and most widely used risk assessment tool for HIT.<sup>98-101</sup> The 4Ts incorporates essential features of disease described previously (timing of heparin therapy, complications of thrombocytopenia and thrombosis, and exclusion of other causes) and categorizes disease likelihood based on the cumulative score (low risk: 0-3; intermediate risk: 4-5; high risk: 6-8). A recent meta-analysis of studies confirmed that the clinical utility of the 4Ts is the greatest for excluding HIT. A low 4Ts score (≤3) carries a high negative predictive value (0.998; 95% confidence interval, 0.970-1.000).<sup>99</sup> Intermediate (4Ts: 4-5) and high (4Ts: >6) scores on the other hand performed poorly in predicting disease because of significant inter-operator variability.<sup>99</sup> A recent prospective study utilizing the 4Ts suggests that assignment of low 4T scores can also be subject to operator inexperience, resulting in misclassification in ~2% of cases.<sup>101</sup>

The HIT expert probability score is another clinical scoring system developed by Cuker and colleagues utilizing 8 clinical features and broad expert opinion to assign clinical significance.<sup>102</sup> In initial descriptions, when compared with the 4Ts score, the HIT expert probability score demonstrated improved correlation with serologic HIT testing and improved inter-observer agreement.<sup>102</sup> This scoring system is currently undergoing prospective validation.

### Laboratory diagnosis of HIT

A diagnosis of HIT cannot be made without laboratory evidence of anti-PF4/heparin antibodies. Laboratory assays detect the presence of HIT antibodies using platelet activation assays (“functional”) or by immunoassays. Platelet activation assays, such as the <sup>14</sup>C-SRA,<sup>19</sup> platelet aggregation,<sup>103</sup> and more recent flow-based platelet activation assays<sup>104,105</sup> detect antibodies capable of binding and cross-linking platelet FcγRIIA. In stringently defined cases of HIT, the functional assays offer high specificity (>95%)<sup>39</sup> and PPVs (89% to 100%) but lower sensitivity (56% to 100%).<sup>39,106</sup> The reduced sensitivity of assays depends on the functional end points of the assay (light transmission < flow based < radioactive signals). The functional assays are technically more challenging and thus not routinely performed at most medical centers.

Immunoassays, on the other hand, measure the presence of anti-PF4/heparin antibodies using a variety of antibody capture platforms (enzyme-linked immunosorbent assay, particle gel, immunoturbidimetric etc.). For detailed information on performance characteristics of HIT immunoassays, the reader is referred to recent comprehensive reviews on this topic.<sup>107,108</sup> Advantages of immunoassays are technical simplicity and high sensitivity (>99%). However, immunoassays lack specificity (30% to 70%) for the diagnosis of HIT<sup>109</sup> because of the occurrence of asymptomatic seroconversions. Specificity of immunoassays can be increased through detection of IgG antibodies and numerical quantification of OD and/or titers. A number of studies have shown increased correlation between high ODs with platelet activating antibodies, clinical HIT and thrombotic risk.<sup>86,110</sup>

For most patients suspected of HIT (high or intermediate 4Ts), combining clinical scoring data with immunoassay results offers greater diagnostic utility than either approach alone and often, can bypass the

need for functional assays. Retrospective<sup>111</sup> and prospective studies<sup>101,112</sup> have shown that for patients with a high clinical suspicion of HIT by the 4Ts (eg, >6), whose immunoassays confirm anti-PF4/heparin antibodies, the likelihood of positive functional assays is sufficiently high (83% to 95%) that additional platelet activation assays become unnecessary. For patients in the “intermediate” category (4Ts = 4-5) of clinical suspicion for HIT, a negative immunoassay virtually eliminates the possibility of HIT reducing pretest probabilities from 7% to 14% to posttest probabilities of ~0% to 0.4%.<sup>111</sup> A positive immunoassay for the intermediate risk group, however, increases the posttest probability of HIT to 40% to 64%.<sup>111</sup> It is in this category of patients where additional functional assays may be informative.

## Treatment of HIT

Management of HIT begins at time of disease suspicion, and includes discontinuation of all sources of heparin and administration of one of the parenteral alternative agents described subsequently. Selection of a parenteral agent is largely based on drug availability and patient comorbidities (renal or hepatic dysfunction). In the United States, intravenous direct thrombin inhibitors are preferred agents in the critically ill population who often require multiple procedures and may be at higher bleeding risk. Overlap with a vitamin K antagonist (VKA) should be initiated only after platelet counts recover to a stable baseline or greater than  $150 \times 10^9/L$ .<sup>113</sup> Initiation of VKA prior to platelet count recovery is contraindicated because of heightened risk for venous limb gangrene from the combined effects of VKA and anti-PF4/heparin antibodies on protein C reduction.<sup>114,115</sup> Additionally, if a patient is being treated with a VKA at time of diagnosis, reversal with vitamin K is recommended to lower the risk of venous limb gangrene and warfarin-induced skin necrosis.<sup>113</sup>

Duration of anticoagulation therapy for HIT is based on concurrence of thrombosis. American and British guidelines recommend therapeutic dose anticoagulation for 4 weeks in patients with isolated HIT and 3 months for HIT patients with thrombosis.<sup>113,116</sup> Prospective data are lacking on the best approach for managing future heparin exposure in patients with a confirmed diagnosis of HIT. Guidelines recommend limiting heparin reexposure in patients with a prior history of HIT, except in seronegative patients who require cardiac surgery.<sup>113,116</sup> For a more comprehensive discussion on the management of patients with a prior history of HIT, the reader is referred to a recent review by Warkentin on this topic.<sup>59</sup>

The following section briefly summarizes parenteral and emerging therapies for HIT. For more in-depth reviews of pharmacology, dosing and monitoring guidelines of the parenteral anticoagulants used in the treatment of HIT, the reader is referred to recent reviews.<sup>113,117</sup>

### Parenteral anticoagulants

Danaparoid is a mixture of naturally occurring GAGs (heparan sulfate, dermatan, and chondroitin) sulfate and was the first alternative agent available for the treatment of HIT. Although the drug has not been available in the US market since 2002, it is currently approved in several countries outside the United States (Europe, Canada, Japan, and Australia) for treatment of HIT. It is the only agent that has been compared with an alternative agent (dextran 70) in a prospective randomized trial.<sup>118</sup> Fifty-six percent of patients treated with danaparoid experienced complete clinical recovery from thromboembolic events as compared with 14% randomized to dextran 70 treatment ( $P = .02$ ).<sup>118</sup>

Argatroban, a small, synthetic reversible inhibitor of thrombin, is approved for treatment of HIT (argatroban) based on 2 multicenter trials comparing argatroban with historical controls. In these studies of HIT patients with and without thrombosis, argatroban therapy resulted in a reduced composite end point of death, amputation, and thrombosis (26% to 44.0%) as compared with historical controls (39% to 56%).<sup>75,119</sup> Argatroban is hepatically cleared, and dose reductions are essential in patients with liver disease or who are critically ill with multiorgan failure. Argatroban dosing nomograms have been developed for use in special intensive care unit populations.<sup>120</sup> Argatroban prolongs the prothrombin time at therapeutic doses, which must be taken into consideration at time of transition to warfarin therapy.

Bivalirudin is also a synthetic thrombin inhibitor that is cleared by plasma proteases and partially cleared by renal excretion. Bivalirudin is approved in the United States for patients who are undergoing percutaneous cardiac intervention and who either have HIT or are at risk for developing HIT. Although medical treatment of HIT is not a US Food and Drug Administration–approved indication, bivalirudin is routinely used for this purpose in the United States. Bivalirudin prolongs the prothrombin time, but to a lesser extent than argatroban.

Fondaparinux, a synthetic pentasaccharide LMWH<sup>121</sup> is another anticoagulant that is being increasingly used off-label for the management of HIT.<sup>122</sup> Fondaparinux does not cross-react with HIT antibodies, but has been rarely implicated as a cause of HIT in case reports.<sup>123</sup> In a retrospective study of 133 patients receiving fondaparinux for HIT, thrombosis and/or bleeding complications, however, did not significantly differ from propensity matched controls on DTI.<sup>124</sup> Some case series document increased bleeding rates (~10% to 22%) with this agent<sup>125,126</sup> and its use must be carefully monitored in patients with renal compromise.

### Emerging therapies

Direct oral anticoagulants (DOACs): Only a few case reports and/or case series have reported on the safety and efficacy of several DOACs in HIT. Rivaroxaban was studied in a small multicenter single arm prospective study, which was closed early because of poor enrollment.<sup>127</sup> In this small cohort of 22 HIT patients, rivaroxaban appeared to be safe and effective without occurrence of new thrombosis. In another case series, a heterogeneous group of 11 patients with HIT were treated with rivaroxaban, apixaban, or dabigatran. In these patients, there were no recurrent arterial or venous thrombosis or bleeding complications.<sup>128</sup> Presently, there is insufficient clinical information to recommend use of DOACs in the management of HIT.

## Conclusion

HIT remains a commonly encountered iatrogenic complication of heparin therapy in hospitalized patients. In the nearly 6 decades since the initial descriptions of the disease, there have been major advances in understanding the pathogenesis of HIT, its varied clinical complications and treatment. Emerging anticoagulant therapies hold promise not only for the treatment of HIT but also in reducing its incidence.

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## Authorship

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receives royalties from the University of New Mexico, Science and Technology Corporation for exclusive licensing of the monoclonal antibody KKO.

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## References

1. Wardrop D, Keeling D. The story of the discovery of heparin and warfarin. *Br J Haematol*. 2008; 141(6):757-763.
2. Warkentin TE, Levine MN, Hirsh J, et al. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *N Engl J Med*. 1995; 332(20):1330-1336.
3. Trossaert M, Gaillard A, Commin PL, Amiral J, Vissac AM, Fressinaud E. High incidence of anti-heparin/platelet factor 4 antibodies after cardiopulmonary bypass surgery. *Br J Haematol*. 1998;101(4):653-655.
4. Pouplard C, May MA, Iochmann S, et al. 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*. 1999; 99(19):2530-2536.
5. Smythe MA, Koerber JM, Mattson JC. The incidence of recognized heparin-induced thrombocytopenia in a large tertiary care, teaching hospital. *Chest*. 2007;131(6): 1644-1649.
6. Warkentin TE, Sheppard JA, Horsewood P, Simpson PJ, Moore JC, Kelton JG. Impact of the patient population on the risk for heparin-induced thrombocytopenia. *Blood*. 2000;96(5): 1703-1708.
7. Copley AL, Robb TP. Studies on platelets: III. The effect of heparin in vivo on the platelet count in mice and dogs. *Am J Clin Pathol*. 1942;12(11): 563-570.
8. Fidler E, Jaques LB. The effect of commercial heparin on the platelet count. *J Lab Clin Med*. 1948;33(11):1410-1423.
9. Chong BH, Berndt MC. Heparin-induced thrombocytopenia. *Blood*. 1989;58(2):53-57.
10. Chong BH, Ismail F. The mechanism of heparin-induced platelet aggregation. *Eur J Haematol*. 1989;43(3):245-251.
11. Weismann RE, Tobin RW. Arterial embolism occurring during systemic heparin therapy. *AMA Arch Surg*. 1958;76(2):219-227.
12. Roberts B, Rosato FE, Rosato EF. Heparin—a cause of arterial emboli? *Surgery*. 1964;55: 803-809.
13. Rhodes GR, Dixon RH, Silver D. Heparin induced thrombocytopenia with thrombotic and hemorrhagic manifestations. *Surg Gynecol Obstet*. 1973;136(3):409-416.
14. US Food and Drug Administration, Center for Drug Evaluation and Research. Approval package for: 020430, Organon injection (danaparoid sodium injection). [http://www.accessdata.fda.gov/drugsatfda\\_docs/nda/96/020430ap-1.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/nda/96/020430ap-1.pdf). Accessed 11 December 2016.
15. US Food and Drug Administration, Center for Drug Evaluation and Research. Approval letter: 020807 (Refudan). [http://www.accessdata.fda.gov/drugsatfda\\_docs/nda/98/020807\\_Refudan\\_Approv.pdf](http://www.accessdata.fda.gov/drugsatfda_docs/nda/98/020807_Refudan_Approv.pdf). Accessed 11 December 2016.
16. Warkentin TE, Kelton JG. Temporal aspects of heparin-induced thrombocytopenia. *N Engl J Med*. 2001;344(17):1286-1292.
17. Warkentin TE, Heddle NM. Laboratory diagnosis of immune heparin-induced thrombocytopenia. *Curr Hematol Rep*. 2003;2(2):148-157.
18. McGowan KE, Makari J, Diamantouros A, et al. Reducing the hospital burden of heparin-induced thrombocytopenia: impact of an avoid-heparin program. *Blood*. 2016;127(16):1954-1959.
19. Sheridan D, Carter C, Kelton JG. A diagnostic test for heparin-induced thrombocytopenia. *Blood*. 1986;67(1):27-30.
20. Cines DB, Tomaski A, Tannenbaum S. Immune endothelial-cell injury in heparin-associated thrombocytopenia. *N Engl J Med*. 1987;316(10): 581-589.
21. Greinacher A, Michels I, Mueller-Eckhardt C. Heparin-associated thrombocytopenia: the antibody is not heparin specific. *Thromb Haemost*. 1992;67(5):545-549.
22. Amiral J, Bridey F, Dreyfus M, et al. Platelet factor 4 complexed to heparin is the target for antibodies generated in heparin-induced thrombocytopenia. *Thromb Haemost*. 1992; 68(1):95-96.
23. Greinacher A, Potzsch B, Amiral J, Dummel V, Eichner A, Mueller-Eckhardt C. Heparin-associated thrombocytopenia: isolation of the antibody and characterization of a multimolecular PF4-heparin complex as the major antigen. *Thromb Haemost*. 1994;71(2): 247-251.
24. Reilly MP, Taylor SM, Hartman NK, et al. Heparin-induced thrombocytopenia/thrombosis in a transgenic mouse model requires human platelet factor 4 and platelet activation through FcγRIIA. *Blood*. 2001;98(8):2442-2447.
25. Cai Z, Yarovoï SV, Zhu Z, et al. Atomic description of the immune complex involved in heparin-induced thrombocytopenia. *Nat Commun*. 2015;6:8277.
26. Kelton JG, Warkentin TE. Heparin-induced thrombocytopenia: a historical perspective. *Blood*. 2008;112(7):2607-2616.
27. Zucker MB, Katz IR. Platelet factor 4: production, structure, and physiologic and immunologic action. *Proc Soc Exp Biol Med*. 1991;198(2): 693-702.
28. Marcum JA, McKenney JB, Rosenberg RD. Acceleration of thrombin-antithrombin complex formation in rat hindquarters via heparinlike molecules bound to the endothelium. *J Clin Invest*. 1984;74(2):341-350.
29. Handin RI, Cohen HJ. Purification and binding properties of human platelet factor four. *J Biol Chem*. 1976;251(14):4273-4282.
30. Bock PE, Luscombe M, Marshall SE, Pepper DS, Holbrook JJ. The multiple complexes formed by the interaction of platelet factor 4 with heparin. *Biochem J*. 1980;191(3):769-776.
31. Rauova L, Poncz M, McKenzie SE, et al. Ultralarge complexes of PF4 and heparin are central to the pathogenesis of heparin-induced thrombocytopenia. *Blood*. 2005;105(1):131-138.
32. Greinacher A, Gopinadhan M, Gunther J-U, et al. Close approximation of two platelet factor 4 tetramers by charge neutralization forms the antigens recognized by HIT antibodies. *Arterioscler Thromb Vasc Biol*. 2006;26(10): 2386-2393.
33. Suvarna S, Espinasse B, Qi R, et al. Determinants of PF4/heparin immunogenicity. *Blood*. 2007;110(13):4253-4260.
34. Greinacher A, Alban S, Omer-Adam MA, Weitschies W, Warkentin TE. Heparin-induced thrombocytopenia: a stoichiometry-based model to explain the differing immunogenicities of unfractionated heparin, low-molecular-weight heparin, and fondaparinux in different clinical settings. *Thromb Res*. 2008;122(2):211-220.
35. Brandt S, Krauel K, Gottschalk KE, et al. Characterisation of the conformational changes in platelet factor 4 induced by polyanions: towards in vitro prediction of antigenicity. *Thromb Haemost*. 2014;112(1):53-64.
36. Kreimann M, Brandt S, Krauel K, et al. Binding of anti-platelet factor 4/heparin antibodies depends on the thermodynamics of conformational changes in platelet factor 4. *Blood*. 2014; 124(15):2442-2449.
37. Amiral J, Peynaud-Debaille E, Wolf M, Bridey F, Vissac AM, Meyer D. Generation of antibodies to heparin-PF4 complexes without thrombocytopenia in patients treated with unfractionated or low-molecular-weight heparin. *Am J Hematol*. 1996;52(2):90-95.
38. Bauer TL, Arepally G, Konkle BA, et al. Prevalence of heparin-associated antibodies without thrombosis in patients undergoing cardiopulmonary bypass surgery. *Circulation*. 1997;95(5):1242-1246.
39. Warkentin TE, Cook RJ, Marder VJ, et al. Anti-platelet factor 4/heparin antibodies in orthopedic surgery patients receiving antithrombotic prophylaxis with fondaparinux or enoxaparin. *Blood*. 2005;106(12):3791-3796.
40. Bito S, Miyata S, Migita K, et al. Mechanical prophylaxis is a heparin-independent risk for anti-platelet factor 4/heparin antibody formation after orthopedic surgery. *Blood*. 2016;127(8): 1036-1043.
41. Visentin GP, Malik M, Cyganiak KA, Aster RH. Patients treated with unfractionated heparin during open heart surgery are at high risk to form antibodies reactive with heparin:platelet factor 4 complexes. *J Lab Clin Med*. 1996;128(4): 376-383.
42. Krauel K, Potschke C, Weber C, et al. Platelet factor 4 binds to bacteria, inducing antibodies cross-reacting with the major antigen in heparin-induced thrombocytopenia [published correction appears in *Blood*. 2011;117(21):5783]. *Blood*. 2011;117(4):1370-1378.
43. Krauel K, Weber C, Brandt S, et al. Platelet factor 4 binding to lipid A of Gram-negative bacteria exposes PF4/heparin-like epitopes. *Blood*. 2012;120(16):3345-3352.
44. Krauel K, Schulze A, Jouni R, et al. Further insights into the anti-PF4/heparin IgM immune response. *Thromb Haemost*. 2016;115(4): 752-761.
45. Khandelwal S, Lee GM, Hester CG, et al. The antigenic complex in HIT binds to B-cells via

- complement and complement receptor 2 (CD21). *Blood*. 2016;128(14):1789-1799.
46. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science*. 1996;271(5247):348-350.
  47. Zheng Y, Wang AW, Yu M, et al. B-cell tolerance regulates production of antibodies causing heparin-induced thrombocytopenia. *Blood*. 2014;123(6):931-934.
  48. Zheng Y, Yu M, Podd A, et al. Critical role for mouse marginal zone B cells in PF4/heparin antibody production. *Blood*. 2013;121(17):3484-3492.
  49. Kelton JG, Sheridan D, Santos A, et al. Heparin-induced thrombocytopenia: laboratory studies. *Blood*. 1988;72(3):925-930.
  50. Reilly MP, Sinha U, Andre P, et al. PRT-060318, a novel Syk inhibitor, prevents heparin-induced thrombocytopenia and thrombosis in a transgenic mouse model. *Blood*. 2011;117(7):2241-2246.
  51. Warkentin TE, Hayward CP, Boshkov LK, et al. Sera from patients with heparin-induced thrombocytopenia generate platelet-derived microparticles with procoagulant activity: an explanation for the thrombotic complications of heparin-induced thrombocytopenia. *Blood*. 1994;84(11):3691-3699.
  52. Greinacher A, Eichler P, Lubenow N, Kwasny H, Luz M. 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*. 2000;96(3):846-851.
  53. Arepally GM, Mayer IM. Antibodies from patients with heparin-induced thrombocytopenia stimulate monocytic cells to express tissue factor and secrete interleukin-8. *Blood*. 2001;98(4):1252-1254.
  54. Kasthuri RS, Glover SL, Jonas W, et al. PF4/heparin-antibody complex induces monocyte tissue factor expression and release of tissue factor positive microparticles by activation of FcγRIIb. *Blood*. 2012;119(22):5285-5293.
  55. Pouplard C, Lochmann S, Renard B, et al. Induction of monocyte tissue factor expression by antibodies to heparin-platelet factor 4 complexes developed in heparin-induced thrombocytopenia. *Blood*. 2001;97(10):3300-3302.
  56. Tutwiler V, Madeeva D, Ahn HS, et al. Platelet transactivation by monocytes promotes thrombosis in heparin-induced thrombocytopenia. *Blood*. 2016;127(4):464-472.
  57. Hursting MJ, Pai P, McCracken JE, et al. Platelet factor 4/heparin antibodies in blood bank donors. *Am J Clin Pathol*. 2010;134(5):774-780.
  58. Warkentin TE, Basciano PA, Knopman J, Bernstein RA. Spontaneous heparin-induced thrombocytopenia syndrome: 2 new cases and a proposal for defining this disorder. *Blood*. 2014;123(23):3651-3654.
  59. Warkentin TE, Anderson JA. How I treat patients with a history of HIT. *Blood*. 2016;128(3):348-359.
  60. Obeng EA, Harney KM, Moniz T, Arnold A, Neufeld EJ, Trenor CC III. Pediatric heparin-induced thrombocytopenia: prevalence, thrombotic risk, and application of the 4Ts scoring system. *J Pediatr*. 2015;166(1):144-150.
  61. Fausett MB, Vogtlander M, Lee RM, et al. Heparin-induced thrombocytopenia is rare in pregnancy. *Am J Obstet Gynecol*. 2001;185(1):148-152.
  62. Chaudhary RK, Nepal C, Khanal N, Pathak R, Giri S, Bhatt VR. Management and outcome of heparin-induced thrombocytopenia in pregnancy: a systematic review. *Cardiovasc Hematol Agents Med Chem*. 2015;13(2):92-97.
  63. Francis JL, Palmer GJ III, Moroosse R, Drexler A. Comparison of bovine and porcine heparin in heparin antibody formation after cardiac surgery. *Ann Thorac Surg*. 2003;75(1):17-22.
  64. Ban-Hoefen M, Francis C. Heparin induced thrombocytopenia and thrombosis in a tertiary care hospital. *Thromb Res*. 2009;124(2):189-192.
  65. Warkentin TE, Sheppard J, Sigouin CS, Kohlmann T, Eichler P, Greinacher A. Gender imbalance and risk factor interactions in heparin-induced thrombocytopenia. *Blood*. 2006;108(9):2937-2941.
  66. Martel N, Lee J, Wells PS. Risk for heparin-induced thrombocytopenia with unfractionated and low-molecular-weight heparin thromboprophylaxis: a meta-analysis. *Blood*. 2005;106(8):2710-2715.
  67. Morris TA, Castrejon S, Devendra G, Gamst AC. No difference in risk for thrombocytopenia during treatment of pulmonary embolism and deep venous thrombosis with either low-molecular-weight heparin or unfractionated heparin: a metaanalysis. *Chest*. 2007;132(4):1131-1139.
  68. Stein PD, Hull RD, Matta F, Yaekoub AY, Liang J. Incidence of thrombocytopenia in hospitalized patients with venous thromboembolism. *Am J Med*. 2009;122(10):919-930.
  69. Greinacher A, Eichler P, Lietz T, Warkentin TE. Replacement of unfractionated heparin by low-molecular-weight heparin for postorthopedic surgery antithrombotic prophylaxis lowers the overall risk of symptomatic thrombosis because of a lower frequency of heparin-induced thrombocytopenia. *Blood*. 2005;106(8):2921-2922.
  70. Teo CP, Kueh YK. Incidence of thrombocytopenia in an acute care hospital. *Ann Acad Med Singapore*. 1989;18(4):379-381.
  71. Oliveira GB, Crespo EM, Becker RC, et al. Incidence and prognostic significance of thrombocytopenia in patients treated with prolonged heparin therapy. *Arch Intern Med*. 2008;168(1):94-102.
  72. Greinacher A, Farner B, Kroll H, Kohlmann T, Warkentin TE, Eichler P. Clinical features of heparin-induced thrombocytopenia including risk factors for thrombosis. A retrospective analysis of 408 patients. *Thromb Haemost*. 2005;94(1):132-135.
  73. Warkentin TE, Greinacher A. Management of heparin-induced thrombocytopenia. *Curr Opin Hematol*. 2016;23(5):462-470.
  74. Warkentin TE, Kelton JG. A 14-year study of heparin-induced thrombocytopenia. *Am J Med*. 1996;101(5):502-507.
  75. Lewis BE, Wallis DE, Leya F, Hursting MJ, Kelton JG, Argatroban I. Argatroban anticoagulation in patients with heparin-induced thrombocytopenia. *Ann Intern Med*. 2003;139(15):1849-1856.
  76. Warkentin TE, Roberts RS, Hirsh J, Kelton JG. Heparin-induced skin lesions and other unusual sequelae of the heparin-induced thrombocytopenia syndrome: a nested cohort study. *Chest*. 2005;127(5):1857-1861.
  77. Hong AP, Cook DJ, Sigouin CS, Warkentin TE. Central venous catheters and upper-extremity deep-vein thrombosis complicating immune heparin-induced thrombocytopenia. *Blood*. 2003;101(8):3049-3051.
  78. Ernest D, Fisher MM. Heparin-induced thrombocytopenia complicated by bilateral adrenal haemorrhage. *Intensive Care Med*. 1991;17(4):238-240.
  79. Boshkov LK, Warkentin TE, Hayward CP, Andrew M, Kelton JG. Heparin-induced thrombocytopenia and thrombosis: clinical and laboratory studies. *Br J Haematol*. 1993;84(2):322-328.
  80. Carlsson LE, Lubenow N, Blumentritt C, et al. Platelet receptor and clotting factor polymorphisms as genetic risk factors for thromboembolic complications in heparin-induced thrombocytopenia. *Pharmacogenetics*. 2003;13(5):253-258.
  81. Trikalinos TA, Karassa FB, Ioannidis JP. Meta-analysis of the association between low-affinity FcγRIIIb receptor gene polymorphisms and hematologic and autoimmune disease. *Blood*. 2001;98(5):1634-1636.
  82. Rollin J, Pouplard C, Sung HC, et al. Increased risk of thrombosis in FcγRIIIA 131RR patients with HIT due to defective control of platelet activation by plasma IgG2. *Blood*. 2015;125(15):2397-2404.
  83. Warkentin TE, Sheppard J-Al, Moore JC, Moore KM, Sigouin CS, Kelton JG. Laboratory testing for the antibodies that cause heparin-induced thrombocytopenia: how much class do we need? *J Lab Clin Med*. 2005;146(6):341-346.
  84. Alberio L, Kimmerle S, Baumann A, Taleghani BM, Biasiutti FD, Lammle B. Rapid determination of anti-heparin/platelet factor 4 antibody titers in the diagnosis of heparin-induced thrombocytopenia. *Am J Med*. 2003;114(7):528-536.
  85. Zwicker J, Uhl L, Huang WY, Shaz BH, Bauer KA. Thrombosis and ELISA optical density values in hospitalized patients with heparin-induced thrombocytopenia. *J Thromb Haemost*. 2004;2(12):2133-2137.
  86. Baroletti S, Hurwitz S, Conti NA, Fanikos J, Piazza G, Goldhaber SZ. Thrombosis in suspected heparin-induced thrombocytopenia occurs more often with high antibody levels. *Am J Med*. 2012;125(1):44-49.
  87. Amiral J, Wolf M, Fischer A, Boyer-Neumann C, Vissac A, Meyer D. Pathogenicity of IgA and/or IgM antibodies to heparin-PF4 complexes in patients with heparin-induced thrombocytopenia. *Br J Haematol*. 1996;92(4):954-959.
  88. Greinacher A, Juhl D, Strobel U, et al. Heparin-induced thrombocytopenia: a prospective study on the incidence, platelet-activating capacity and clinical significance of antiplatelet factor 4/heparin antibodies of the IgG, IgM, and IgA classes. *J Thromb Haemost*. 2007;5(8):1666-1673.
  89. Warkentin TE, Sheppard JA, Moore JC, Cook RJ, Kelton JG. Studies of the immune response in heparin-induced thrombocytopenia. *Blood*. 2009;113(20):4963-4969.
  90. Warkentin TE, Kelton JG. Delayed-onset heparin-induced thrombocytopenia and thrombosis. *Ann Intern Med*. 2001;135(7):502-506.
  91. Rice L, Attisha WK, Drexler A, Francis JL. Delayed-onset heparin-induced thrombocytopenia. *Ann Intern Med*. 2002;136(3):210-215.
  92. Mattioli AV, Bonetti L, Zennaro M, Ambrosio G, Mattioli G. Heparin/PF4 antibodies formation after heparin treatment: temporal aspects and long-term follow-up. *Am Heart J*. 2009;157(3):589-595.
  93. Wallis DE, Workman DL, Lewis BE, Steen L, Pifarre R, Moran JF. Failure of early heparin cessation as treatment for heparin-induced thrombocytopenia. *Am J Med*. 1999;106(6):629-635.
  94. Selleng K, Schutt A, Selleng S, Warkentin TE, Greinacher A. Studies of the anti-platelet factor 4/heparin immune response: adapting



- the enzyme-linked immunosorbent spot assay for detection of memory B cells against complex antigens. *Transfusion*. 2010;50(1):32-39.
95. Pötzsch B, Klövekorn WP, Madlener K. Use of heparin during cardiopulmonary bypass in patients with a history of heparin-induced thrombocytopenia. *N Engl J Med*. 2000;343(7):515.
  96. Lubenow N, Kempf R, Eichner A, Eichler P, Carlsson LE, Greinacher A. Heparin-induced thrombocytopenia: temporal pattern of thrombocytopenia in relation to initial use or reexposure to heparin. *Chest*. 2002;122(1):37-42.
  97. Warkentin TE, Sheppard J-AI. Serological investigation of patients with a previous history of heparin-induced thrombocytopenia who are re-exposed to heparin. *Blood*. 2014;123(16):2485-2493.
  98. Lo GK, Juhl D, Warkentin TE, Sigouin CS, Eichler P, Greinacher A. Evaluation of pretest clinical score (4 Ts) for the diagnosis of heparin-induced thrombocytopenia in two clinical settings. *J Thromb Haemost*. 2006;4(4):759-765.
  99. Cuker A, Gimotty PA, Crowther MA, Warkentin TE. Predictive value of a 4Ts scoring system for heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood*. 2012;120(20):4160-4167.
  100. Crowther M, Cook D, Guyatt G, et al. Heparin-induced thrombocytopenia in the critically ill: interpreting the 4Ts test in a randomized trial. *J Crit Care*. 2014;29(3):470.e7-470.e15.
  101. Linkins LA, Bates SM, Lee AY, Heddle NM, Wang G, Warkentin TE. Combination of 4Ts score and PF4/H-PaGIA for diagnosis and management of heparin-induced thrombocytopenia: prospective cohort study. *Blood*. 2015;126(5):597-603.
  102. Cuker A, Arepally G, Crowther MA, et al. The HIT Expert Probability (HEP) Score: a novel pre-test probability model for heparin-induced thrombocytopenia based on broad expert opinion. *J Thromb Haemost*. 2010;8(12):2642-2650.
  103. Chong BH, Burgess J, Ismail F. The clinical usefulness of the platelet aggregation test for the diagnosis of heparin-induced thrombocytopenia. *Thromb Haemost*. 1993;69(4):344-350.
  104. Tomer A. A sensitive and specific functional flow cytometric assay for the diagnosis of heparin-induced thrombocytopenia. *Br J Haematol*. 1997;98(3):648-656.
  105. Padmanabhan A, Jones CG, Curtis BR, et al. A novel PF4-dependent platelet activation assay identifies patients likely to have heparin-induced thrombocytopenia/thrombosis (HIT). *Chest*. 2016;150(3):506-515.
  106. Pouplard C, Amiral J, Borg JY, Laporte-Simitsidis S, Delahousse B, Gruel Y. 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*. 1999;111(5):700-706.
  107. Sun L, Gimotty PA, Lakshmanan S, Cuker A. Diagnostic accuracy of rapid immunoassays for heparin-induced thrombocytopenia. A systematic review and meta-analysis. *Thromb Haemost*. 2016;115(5):1044-1055.
  108. Nagler M, Bachmann LM, Ten Cate H, Ten Cate-Hoek A. Diagnostic value of immunoassays for heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood*. 2016;127(5):546-557.
  109. Cuker A, Cines DB. How I treat heparin-induced thrombocytopenia. *Blood*. 2012;119(10):2209-2218.
  110. Warkentin TE, Greinacher A, Gruel Y, et al. Laboratory testing for heparin-induced thrombocytopenia: a conceptual framework and implications for diagnosis. *J Thromb Haemost*. 2011;9(12):2498-2500.
  111. Nellen V, Sulzer I, Barizzi G, Lammle B, Alberio L. Rapid exclusion or confirmation of heparin-induced thrombocytopenia: a single-center experience with 1,291 patients. *Haematologica*. 2012;97(1):89-97.
  112. Leroux D, Hezard N, Lebreton A, et al. Prospective evaluation of a rapid nanoparticle-based lateral flow immunoassay (STic Expert® HIT) for the diagnosis of heparin-induced thrombocytopenia. *Br J Haematol*. 2014;166(5):774-782.
  113. Linkins LA, Dans AL, Moores LK, et al. Treatment and prevention of heparin-induced thrombocytopenia: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest*. 2012;141(2, suppl):e495S-e530S.
  114. Warkentin TE, Elavathil LJ, Hayward CP, Johnston MA, Russett JI, Kelton JG. The pathogenesis of venous limb gangrene associated with heparin-induced thrombocytopenia. *Ann Intern Med*. 1997;127(9):804-812.
  115. Kowalska MA, Krishnaswamy S, Rauova L, et al. Antibodies associated with heparin-induced thrombocytopenia (HIT) inhibit activated protein C generation: new insights into the prothrombotic nature of HIT. *Blood*. 2011;118(10):2882-2888.
  116. Watson H, Davidson S, Keeling D. Guidelines on the diagnosis and management of heparin-induced thrombocytopenia: second edition. *Br J Haematol*. 2012;159(5):528-40.
  117. Kelton JG, Arnold DM, Bates SM. Nonheparin anticoagulants for heparin-induced thrombocytopenia. *N Engl J Med*. 2013;368(8):737-744.
  118. Chong BH, Gallus AS, Cade JF, et al. Prospective randomised open-label comparison of danaparoid with dextran 70 in the treatment of heparin-induced thrombocytopenia with thrombosis: a clinical outcome study. *Thromb Haemost*. 2001;86(5):1170-1175.
  119. Lewis BE, Wallis DE, Berkowitz SD, et al. Argatroban anticoagulant therapy in patients with heparin-induced thrombocytopenia. *Circulation*. 2001;103(14):1838-1843.
  120. Alatri A, Armstrong AE, Greinacher A, et al. Results of a consensus meeting on the use of argatroban in patients with heparin-induced thrombocytopenia requiring antithrombotic therapy - a European Perspective. *Thromb Res*. 2012;129(4):426-433.
  121. Savi P, Chong BH, Greinacher A, et al. Effect of fondaparinux on platelet activation in the presence of heparin-dependent antibodies: a blinded comparative multicenter study with unfractionated heparin. *Blood*. 2005;105(1):139-144.
  122. Schindewolf M, Steindl J, Beyer-Westendorf J, et al. Frequent off-label use of fondaparinux in patients with suspected acute heparin-induced thrombocytopenia (HIT)—findings from the GerHIT multi-centre registry study. *Thromb Res*. 2014;134(1):29-35.
  123. Bhatt VR, Aryal MR, Shrestha R, Armitage JO. Fondaparinux-associated heparin-induced thrombocytopenia. *Eur J Haematol*. 2013;91(5):437-441.
  124. Kang M, Alahmadi M, Sawh S, Kovacs MJ, Lazo-Langner A. Fondaparinux for the treatment of suspected heparin-induced thrombocytopenia: a propensity score-matched study. *Blood*. 2015;125(6):924-929.
  125. Benken ST, Tillman N, Dajani S, Shah A, Thomas T. A retrospective evaluation of fondaparinux for confirmed or suspected heparin-induced thrombocytopenia in left-ventricular-assist device patients. *J Cardiothorac Surg*. 2014;9:55.
  126. Cegarra-Sanmartín V, González-Rodríguez R, Paniagua-Iglesias P, et al. Fondaparinux as a safe alternative for managing heparin-induced thrombocytopenia in postoperative cardiac surgery patients. *J Cardiothorac Vasc Anesth*. 2014;28(4):1008-1012.
  127. Linkins LA, Warkentin TE, Pai M, et al. Rivaroxaban for treatment of suspected or confirmed heparin-induced thrombocytopenia study. *J Thromb Haemost*. 2016;14(6):1206-1210.
  128. Sharifi M, Bay C, Vajo Z, Freeman W, Sharifi M, Schwartz F. New oral anticoagulants in the treatment of heparin-induced thrombocytopenia. *Thromb Res*. 2015;135(4):607-609.
  129. Warkentin TE, Makris M, Jay RM, Kelton JG. A spontaneous prothrombotic disorder resembling heparin-induced thrombocytopenia. *Am J Med*. 2008;121(7):632-636.
  130. Jay RM, Warkentin TE. Fatal heparin-induced thrombocytopenia (HIT) during warfarin thromboprophylaxis following orthopedic surgery: another example of 'spontaneous' HIT? *J Thromb Haemost*. 2008;6(9):1598-1600.
  131. Pruthi RK, Daniels PR, Nambudiri GS, Warkentin TE. Heparin-induced thrombocytopenia (HIT) during postoperative warfarin thromboprophylaxis: a second example of postorthopedic surgery 'spontaneous' HIT. *J Thromb Haemost*. 2009;7(3):499-501.