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REVIEW ARTICLE

Autoimmune heparin-induced thrombocytopenia

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Summary. Autoimmune heparin-induced thrombocytopenia (aHIT) indicates the presence in patients of anti-platelet factor 4 (PF4)–polyanion antibodies that are able to activate platelets strongly even in the absence of heparin (heparin-independent platelet activation). Nevertheless, as seen with serum obtained from patients with otherwise typical heparin-induced thrombocytopenia (HIT), serum-induced platelet activation is inhibited at high heparin concentrations (10–100 IU mL^{-1} heparin). Furthermore, upon serial dilution, aHIT serum will usually show heparin-dependent platelet activation. Clinical syndromes associated with aHIT include: delayed-onset HIT, persisting HIT, spontaneous HIT syndrome, fondaparinux-associated HIT, heparin 'flush'-induced HIT, and severe HIT (platelet count of $< 20 \times 10^9$ L^{-1}) with associated disseminated intravascular coagulation (DIC). Recent studies have implicated anti-PF4 antibodies that are able to bridge two PF4 tetramers even in the absence of heparin, probably facilitated by non-heparin platelet-associated polyanions (chondroitin sulfate and polyphosphates); nascent PF4–aHIT-IgG complexes recruit additional heparin-dependent HIT antibodies, leading to the formation of large multimolecular immune complexes and marked platelet activation. aHIT can persist for several weeks, and serial fibrin, D-dimer, and fibrinogen levels, rather than the platelet count, may be helpful for monitoring treatment response. Although standard anticoagulant therapy for HIT ought to be effective, published experience indicates frequent failure of activated partial thromboplastin time (APTT)-adjusted anticoagulants (argatroban, bivalirudin), probably because of underdosing in the setting of HIT-associated DIC, known as 'APTT confounding'. Thus, non-APTT-adjusted therapies with drugs such as danaparoid and fondaparinux, or even direct oral anticoagulants, such as rivaroxaban or apixaban, are suggested therapies, especially for long-term management of persisting HIT. In addition, emerging data indicate that high-dose intravenous immunoglobulin can interrupt HIT antibody-induced platelet activation, leading to rapid platelet count recovery.

Keywords: autoimmunity; heparin-induced thrombocytopenia; high-dose intravenous immunoglobulin; platelet-activating antibodies; platelet factor 4 (PF4).

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Introduction

Heparin-induced thrombocytopenia (HIT), a prothrombotic adverse drug reaction, is caused by the transient production of platelet-activating antibodies of IgG class that recognize multimolecular complexes of (cationic) platelet factor 4 (PF4) bound to (polyanionic) heparin. During the past decade, it has become recognized that some patients present with clinical symptoms and laboratory features of HIT despite not having previously received heparin, either in the recent past or at all (spontaneous HIT syndrome). Sera from these patients contain antibodies that activate platelets strongly even in the absence of heparin. However, such 'heparin-independent' platelet-activating properties are not unique to spontaneous HIT syndrome, but are also found in sera of a minority of (heparin-dependent) typical HIT patients. Moreover, patients who show this *in vitro* reactivity profile are more likely to have unusual HIT syndromes such as delayed-onset HIT, persisting HIT, fondaparinux-associated HIT, and HIT induced by exposure to heparin 'flushes' (Table 1). Such patients often show unusual clinical features, such as severe thrombocytopenia (platelet count of $< 20 \times 10^9$ L^{-1}) that can persist for weeks, often accompanied by disseminated intravascular coagulation (DIC) and microvascular thrombosis. In this article, we refer to these clinical and serologic features of HIT as autoimmune HIT (aHIT).

Table 1 Autoimmune heparin-induced thrombocytopenia (aHIT) syndromes

Clinical entity	Description
Delayed-onset HIT	HIT that begins or worsens after stopping of heparin
Persisting HIT	HIT that persists for > 1 week despite stopping of heparin
Spontaneous HIT syndrome	HIT without proximate heparin exposure
Flush heparin HIT	HIT induced by exposure to heparin flushes
Fondaparinux-associated HIT	HIT that is believed to be triggered by exposure to fondaparinux
Severe HIT (e.g. platelet count of $< 20 \times 10^9$ L^{-1}) with overt DIC	Overt HIT-associated DIC defined as proven HIT with one or more of the following: relative/absolute hypofibrinogenemia, elevated INR (without another explanation), and normoblastemia (circulating nucleated red blood cells)

DIC, disseminated intravascular coagulation; INR, International Normalized Ratio.

The characteristic profile of typical HIT antibodies is that they activate platelets in a heparin-dependent fashion, which can be shown in two ways. First, patient serum activates platelets more strongly at pharmacologic unfractionated heparin (UFH) concentrations (0.1–0.3 IU mL^{-1}) than in the absence of heparin, i.e. 0 IU mL^{-1} UFH or 'buffer control'). Second, suprapharmacologic concentrations of heparin (e.g. 10–100 IU mL^{-1}) inhibit serum-induced platelet activation, an effect caused by disruption of PF4-containing multimolecular complexes at very high heparin concentrations. However, in the case of aHIT syndromes, there is substantial platelet activation at buffer control. Although our laboratories (Greifswald and McMaster) routinely perform platelet activation testing for HIT at buffer control as well as at pharmacologic/suprapharmacologic heparin concentrations, many reference laboratories only perform platelet activation assays at 0.1–0.5 IU mL^{-1} UFH and at 100 IU mL^{-1} heparin. Thus, the phenomenon of heparin-independent platelet activation underlying aHIT is underrecognized.

Molecular mechanisms of aHIT: a new concept for understanding autoimmunity

PF4 as a danger signal for the presence of pathogens

Many prokaryotic cells (bacteria) have a strongly negative surface charge. In Gram-negative and Gram-positive bacteria, lipopolysaccharides (especially lipid A) and teichoic acids, respectively, carry negative charges [1]. A surface negative charge helps bacteria to adhere to certain proteins, keeps bacteria apart, and protects bacteria from phagocytosis caused by zeta potential-mediated repulsive forces (zeta potential is the degree of electrostatic repulsion between similarly charged molecules or particles). For eukaryotic cells within multicellular organisms, however, a strong negative cell surface charge is incompatible with a united cell structure. It would seem that a fundamental prerequisite for the generation of eukaryotes is a relative paucity of surface negative charge; hence, a strong negative charge represents a warning pattern indicating the presence of prokaryotic invaders. Consistent with this hypothesis, several components of the innate immune system are activated by negative charge, e.g. classic and alternative complement systems [2], the intrinsic clotting system [2], the kininogen–bradykinin pathway [3], and even some toll-like receptors [4].

The adaptive immune system (T-cell receptors and antibodies), however, recognizes structure rather than charge [5]. These systems require 'adapter molecules' that translate charge into structure. At least two molecules, β_2-glycoprotein I and PF4, change their conformation upon binding to surfaces with strongly negative charges. β_2-Glycoprotein I opens its ring structure after binding to negatively charged molecules on bacteria, thereby exposing domain 1, the binding site for anti-β_2-glycoprotein I antibodies [6]. The association of these antibodies with bacterial infections and their causal role in the antiphospholipid syndrome are well established [7]. We summarize the current knowledge on PF4 structural change after binding to polyanions.

Conformational changes in PF4 structure

The chemokine PF4 (CXCL4) consists of four monomers that expose a ring of positive charges primarily attributable to lysines (we refer to PF4 in its tetrameric form). Each monomer contains three-stranded antiparallel β-sheets on which an aperiodic N-terminal N-domain and an amphipathic C-terminal α-helix are folded [8]. Fundamentally, close proximity of two (or more) PF4 tetramers (through charge neutralization) is required to form the HIT antigens [9]. Crystal structure analyses show how, when PF4 is complexed with the pentasaccharide fondaparinux, the latter stabilizes PF4 tetramers in an asymmetric way, with two monomers being closer together than the other two monomers, and binding of Fab fragments of the mAb KKO (recognizing PF4–heparin [PF4/H] complexes) to the stabilized open part of the tetramer [10]. These structural changes of PF4 have been quantified by circular dichroism spectroscopy, which indicates that the epitope for binding of anti-PF4/H antibodies is exposed when the amount of antiparallel β-sheets in PF4 tetramers exceeds 30% [11] (for a review of the different biophysical approaches used to characterize PF4 and PF4/H complexes, see [12]).

Charge-related binding of polyanions to PF4 is an exothermic reaction, and thus induces energy release

along with conformational changes in PF4. A certain threshold of energy seems to be required to force PF4 into its new conformation [13]. It has been empirically observed in immunologic and functional studies that only heparin molecules with a chain length of at least 10–11 monomers are able to induce the antigens on PF4/H complexes recognized by anti-PF4/H antibodies [14,15]. As binding of polyanions to PF4 is purely charge-related, this raised the puzzling question of why only longer polyanions are able to induce the antigens for PF4/H antibodies, as smaller polyanions in sufficient quantities should also be able to neutralize charge on PF4. The explanation for this has been provided by single-molecule assessment of the interaction of long and short heparin chains with PF4 [16].

Short polyanions bind only to a single PF4 tetramer, whereas longer polyanions bridge two PF4 tetramers. If the polyanion contains enough negative charges, this overcomes the natural repulsive forces between the two PF4 molecules, bringing them into close proximity and thereby merging the two clouds of negative charge into one single charge cloud. This charge cloud fusion allows the hydrophobic parts of the PF4 molecules to come into close proximity, which creates sufficient energy to produce the conformational changes in PF4 necessary to expose the HIT antigen(s). This process only occurs with intact PF4 tetramers. In the presence of small molecules designed to interfere with PF4 tetramer formation, the anti-PF4/H antibody-binding sites on PF4/H complexes are completely blocked [17]. This complex process is biologically meaningful, as it ensures that only intact PF4 tetramers are able to undergo these polyanion-induced conformational changes. This prevents senescent protein degradation inadvertently triggering the 'danger signal' for the immune system.

Molecules inducing PF4 structural change

Besides heparin, other polyanions, such as hypersulfated chondroitin sulfate [18,19], DNA and RNA (including DNA/RNA-based aptamers [20]), polyphosphates [21,22], and bacterial wall components (e.g. lipid A) [1], can induce the conformational changes in PF4 required to expose the HIT antigen(s). Indeed, a large population study showed a close correlation between chronic periodontal infection and the presence of anti-PF4/H antibodies within a (non-heparin-exposed) general population [23]. Furthermore, binding of high concentrations of PF4 to platelets may also induce exposure of HIT antigen(s) in the absence of added polyanions [24–26]. In this case, polyanions on the platelet surface probably augment the close proximity of PF4 [22,24]. For the phenomenon of aHIT, this clearly shows that the antigens can be exposed by factors other than heparin. These might be bacterial wall components or trauma-induced nucleic acid release (possible triggers of spontaneous HIT syndrome), or platelet-derived polyanions such as chondroitin sulfate or polyphosphates (delayed-onset HIT, persisting HIT, and fondaparinux-associated HIT). Interestingly, as discussed later, in most patients with spontaneous HIT syndrome a careful history reveals a proximate proinflammatory triggering event. However, although these triggers can induce the formation of antibodies explaining aHIT, they are usually not present in sufficient concentrations and are not present for long enough (at least 5 days) to explain the clinical manifestation of aHIT, and definitely not the long persistence of aHIT over weeks or even months.

Antibody binding to PF4 and PF4/H complexes in aHIT

Litvinov *et al*. showed that the HIT-mimicking mAb KKO has higher avidity and affinity for PF4/H complexes than does RTO, a non-platelet-activating antibody that also binds to PF4 [27]. These workers had also shown that KKO but not RTO is able to cluster PF4 in the absence of heparin [28]. These findings led us to investigate the characteristics of antibodies isolated from patients with heparin-dependent HIT and those with aHIT [29]. As noted earlier, the fundamental characteristic of aHIT is that patient sera contain antibodies that activate platelets in the absence of heparin. Whereas isolation of heparin-dependent antibodies requires a column of PF4/H complexes as the solid phase, aHIT antibodies can be isolated with a PF4 column. These PF4 column-isolated antibodies do indeed activate platelets in the absence of heparin, and bind to PF4 in both a PF4 and a PF4/H enzyme immunossay (EIA), indicating that these antibodies also recognize PF4 alone. In a PF4/H EIA, these aHIT antibodies bound much more strongly to PF4/H complexes than did wholly heparin-dependent HIT antibodies when both were used in the same concentrations (Fig. 1) [29]. This indicates qualitative differences between typical heparin-dependent HIT antibodies and heparin-independent aHIT antibodies.

By the use of single-molecule force spectroscopy, which allows measurement of the binding strength of a single antibody to its antigen, it became clear that sera of patients with aHIT always contain antibodies with different binding affinities for PF4 and PF4/H complexes. The majority of antibodies in the serum bound weakly (binding force of < 60 pN) or with intermediate strength (binding force of 60–100 pN) to PF4/H complexes, but a few antibodies bound very strongly (binding force of > 100 pN) to PF4/H complexes (Fig. 2) [29]. Only the antibodies with binding forces of > 100 pN bound strongly to PF4 alone, and those with weaker binding forces bound only to PF4/H complexes.

Binding of aHIT antibodies to PF4 recruits heparin-dependent antibodies to form large immune complexes

Further studies investigated the energy release that results when purified antibodies from patients with aHIT are incubated with PF4. The thermal energy of the binding

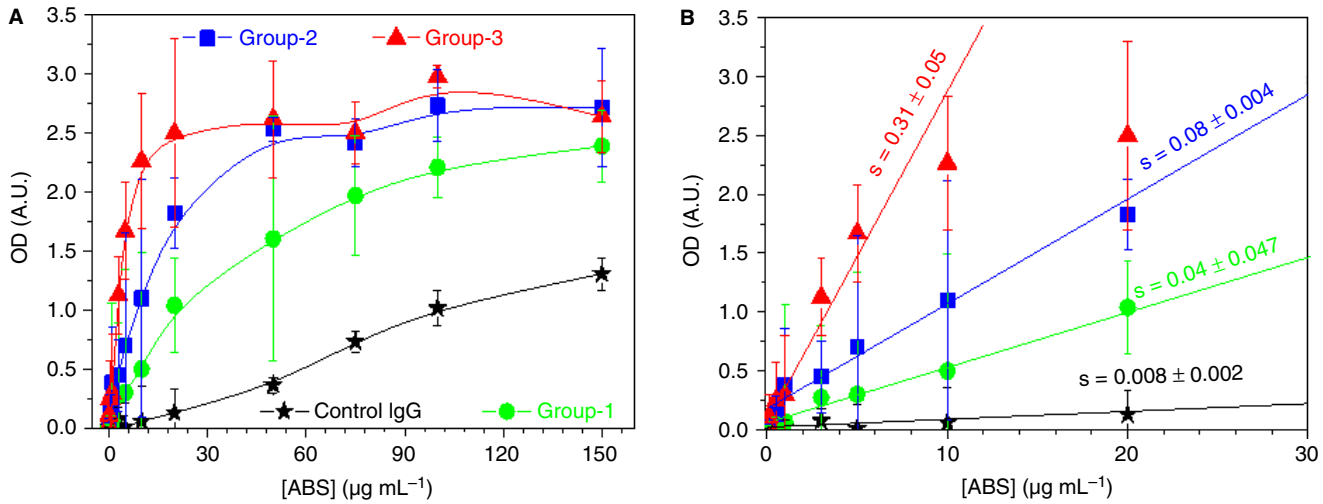

Fig. 1. Dose-dependent binding of affinity-purified anti-platelet factor 4–heparin (PF4/H) antibodies to PF4/H complexes in an enzyme immunossay (EIA). PF4/H complexes were coated on a microtiter plate, and binding of affinity-purified heparin-induced thrombocytopenia antibodies to PF4/H complexes was measured by EIA. Mean OD values ± standard deviations were averaged from five sera for each group and two sera for control IgG. (A) The curve of control IgG (black stars) provides the background reaction. The specific reactions were: lowest for anti-PF4/H antibodies that were positive by EIA but negative by functional assay (Group-1; green circles); higher for anti-PF4/H antibodies that were positive by EIA and positive by functional assay in the presence of heparin (Group-2; blue squares); and highest for anti-PF4 autoantibodies positive by EIA and by functional assay in the absence of heparin (Group-3; red triangles). (B) Slopes (s) of the curves shown in (A) for concentrations up to 20 μg mL^{-1} (at which the background for control IgG was an OD up to 0.32). [ABS], concentration of purified antibodies. AU, absorbance unit. Reprinted from: Nguyen TH, Medvedev N, Delcea M, Greinacher A. Anti-platelet factor 4/polyanion antibodies mediate a new mechanism of autoimmunity. *Nat Commun* 2017; **8:** 14945. [Color figure can be viewed at wileyonlinelibrary.com]

reaction between anti-PF4 antibodies from patients with aHIT and PF4 ($\Delta H = -3.5 \pm 0.86 \times 10^7$ cal mol^{-1}) [29] was even stronger than the thermal energy of the interaction of a 16-mer heparin with PF4 ($\Delta H = -7.26 \pm 1.36 \times 10^3$ cal mol^{-1} [13]), which is capable of inducing the conformational changes allowing binding of heparin-dependent antibodies to PF4/H. This indicates that aHIT antibodies can induce the critical conformational changes within PF4. In other words, these antibodies bind with each of their two Fab arms to two different PF4 tetramers, thus forcing them together, resulting in their charge clouds being merged (Fig. 3). This provides the energy needed to induce the conformational changes in PF4 leading to the formation of > 30% antiparallel β-sheets and thus to closely-approximated clusters of PF4, expressing the antigen(s) recognized by heparin-dependent anti-PF4/H antibodies. In the presence of platelet surface polyanions, the formation of PF4 complexes by aHIT antibodies is probably facilitated, and can even be achieved, by antibodies with slightly lower binding forces.

This phenomenon allows typical heparin-dependent anti-PF4/H antibodies to bind to these nascent PF4–autoantibody IgG complexes with the same binding forces as PF4/H complexes. By these mechanisms, the relatively few high-avidity antibodies recruit the large proportion of lower-avidity antibodies into the growing immune complexes. This explains why a small subgroup of high-avidity antibodies are able to induce the formation of large immune complexes, which can readily activate platelets via the platelet Fc receptors.

Serial dilution of serum from patients with aHIT typically results in eventual loss of buffer reactivity; that is, the reactivity of the diluted serum resembles the reactivity seen with typical (heparin-dependent) HIT sera. This can be explained by 'out-dilution' of the much lower concentration of the high-avidity autoantibodies relative to the heparin-dependent antibodies.

As discussed later in this review, understanding the pathogenesis of aHIT provides a rationale for therapeutic interventions to interfere with aHIT antibody-mediated platelet activation, such as: (i) detaching PF4 from platelet surfaces (e.g. danaparoid or partially desulfated heparin); (ii) blocking platelet Fc receptors with high-dose intravenous immunoglobulin (IVIg); and (iii) removal of antibodies by plasma exchange (same effect as *in vitro* dilution of plasma).

Clinical autoimmune HIT syndromes

Delayed-onset HIT

Twelve patients with delayed-onset HIT, six presenting as outpatients and six as inpatients, were reported in 2001 [30]. A 5-day gap between the last heparin exposure and the onset of HIT-related thrombocytopenia was used as a definition. However, the term 'delayed-onset' is a misnomer, as the timing of the onset of thrombocytopenia is not necessarily greater than seen in typical-onset HIT (i.e. 5–10 days after immunizing heparin exposure [31]). Nevertheless, this name was intended to help the clinician

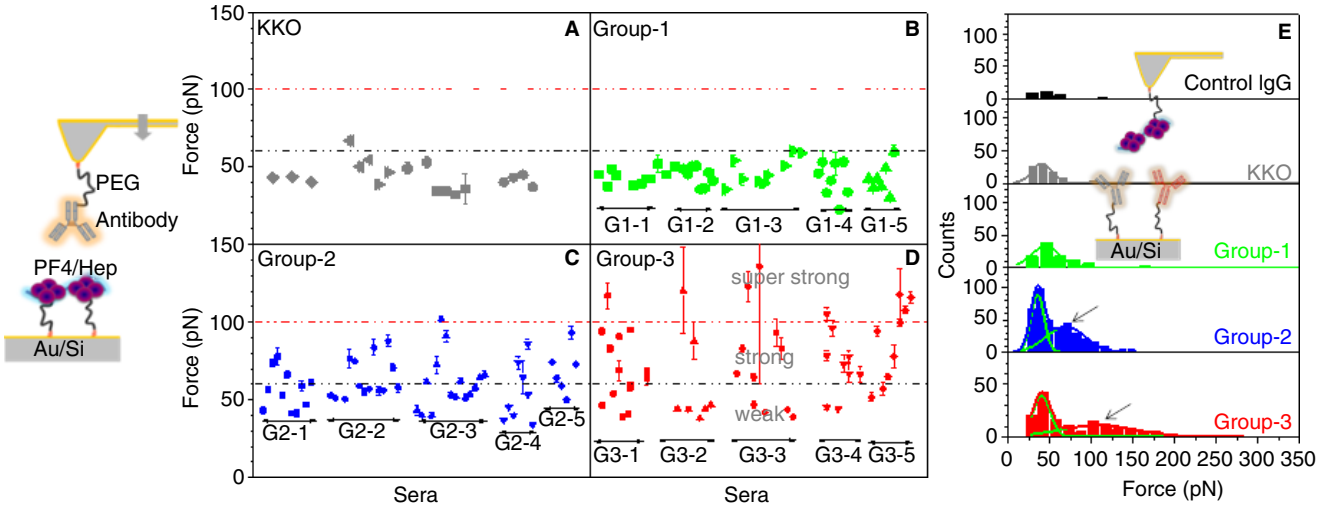

Fig. 2. Differences in binding characteristics of single anti-platelet factor 4 (PF4)–heparin (PF4/H) (typical heparin-induced thrombocytopenia [HIT]) antibodies and anti-PF4 autoantibodies (autoimmune HIT [aHIT] antibodies). An individual antibody was attached to each cantilever of an atomic force microscope (via polyethylene glycol [PEG] bound to antibody Fc) for determination of the forces (expressed in pN) involved in binding to PF4/H complexes (see leftmost cartoon). (A) Experiments conducted with KKO (a mAb that mimics human heparin-dependent platelet-activating HIT antibodies). (B–D) Experiments performed with individual antibodies isolated from the same patient serum (6–15 different isolated antibodies per patient serum, and five different patient sera per HIT antibody group, for each of three defined anti-PF4/H antibody groups, as follows: (B) Group-1 (green), anti-PF4/H antibodies without platelet-activating properties (enzyme immunossay [EIA]-positive, heparin-induced platelet activation [HIPA]-negative); (C) Group-2 (blue), anti-PF4/H antibodies with wholly heparin-dependent platelet-activating properties (EIA-positive and HIPA-positive at 0.2 U mL^{-1} heparin, but HIPA-negative at buffer control); and (D) Group-3 (red), anti-PF4/H antibodies with heparin-independent and heparin-dependent platelet-activating antibodies (EIA-positive and HIPA-positive at 0.2 U mL^{-1} heparin and buffer control. Each dot in (A)–(D) shows the mean and standard error of the rupture forces for each respective antibody (n = 1000 measurements per antibody). Whereas Group-1 antibodies bind to PF4/H complexes with a binding strength generally < 60 pN (dotted/dashed black line), a subset of Group-2 and Group-3 antibodies bind with forces > 60 pN ('strong'), with a subset of Group-3 antibodies binding to PF4/H complexes with rupture forces > 100 pN ('superstrong'; red dotted/dashed line). (E) Reverse experimental design (rightmost cartoon): a PF4/H complex was immobilized on the tip of the atomic force microscope cantilever, and antibodies were immobilized on the substrate. The force histogram for control IgG (black) was used to determine the background. KKO (gray) and Group-1 antibodies (green) showed only one force distribution, whereas two distributions were observed for Group-2 (blue) and Group-3 (red) antibodies. Group-3 antibodies can be regarded as aHIT antibodies. Au/Si; Hep, heparin. Reprinted, with modifications, from: Nguyen TH, Medvedev N, Delcea M, Greinacher A. Anti-platelet factor 4/polyanion antibodies mediate a new mechanism of autoimmunity. *Nat Commun* 2017; **8:** 14945. Au/Si, gold and silicium coating. [Color figure can be viewed at wileyonlinelibrary.com]

remember that HIT can begin several days after discontinuation of heparin. This first article also reported a high frequency of thrombosis (100%) and overt DIC (25% with hypofibrinogenemia) in the 12 patients with delayed-onset HIT. Moreover, the authors pointed out that the magnitude of heparin-independent platelet activation (i.e. serotonin release at 0 U mL^{-1} heparin) was higher in sera from these patients than in randomly selected HIT sera. Remarkably, delayed-onset HIT can occur after a single injection of heparin [32].

Rice *et al.* [33] also reported patients with delayed-onset HIT. Although some of the cases resembled those reported by Warkentin *et al.* [30], others were patients who presented with thrombosis and normal platelet counts several weeks after having received heparin, but who then experienced abrupt platelet count falls when heparin was readministered. This latter group of patients do not necessarily have aHIT, but could simply be rapid-onset HIT patients in whom wholly heparin-dependent platelet-activating antibodies continued to circulate in the plasma at the time of heparin re-exposure [31,34]. On the other hand, such cases could also represent patients who had aHIT with subsequent platelet count recovery, but with late development of thrombosis leading to rapid-onset HIT when heparin was administered [35].

More recently, the concept of delayed-onset HIT has been expanded to also include those patients whose platelet counts continue to fall despite heparin being stopped [36,37]. In other words, there is no requirement for a minimum gap in time between heparin cessation and onset of aHIT; rather, the underlying concept is that the progression and evolution of HIT is occurring independently of whether heparin is being given. In this regard, aHIT is a more encompassing term than delayed-onset HIT.

Persisting HIT

HIT is an unusually transient adverse drug reaction, with HIT antibodies becoming undetectable (by serotonin release assay [SRA]) at a median of 40 days following HIT [31]. However, antibodies in aHIT patients may persist for much longer. This means that aHIT patients can

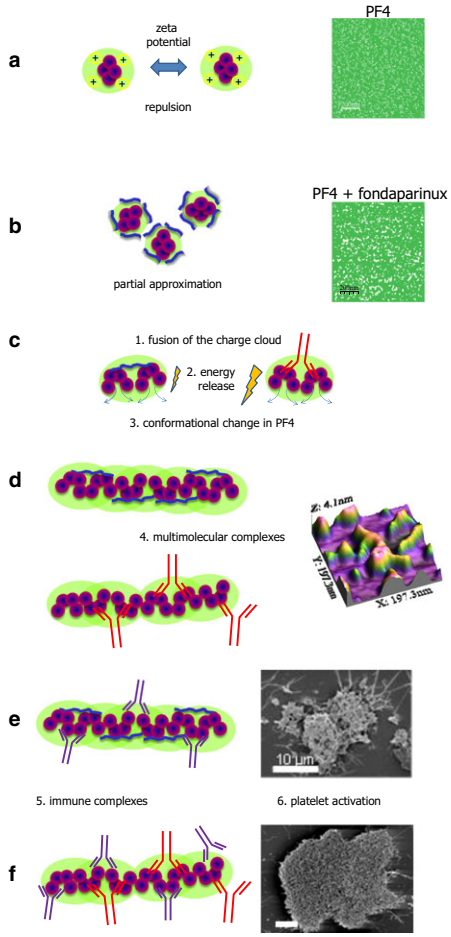

Fig. 3. Generation of the heparin-induced thrombocytopenia (HIT) antigen(s) by polyanions and anti-platelet factor 4 (PF4) autoantibodies. (A) PF4 is strongly positively charged. The resulting zeta potential causes repulsion of single PF4 molecules. This is shown by atomic force microscopy in the right panel, where the white spots represent single PF4 molecules. (B) In the presence of short polyanion chains, the positive charge of PF4 is partially neutralized, and the PF4 molecules can approach each other, as shown in the right panel by atomic force microscopy. (C) Longer polyanion chains can bridge two PF4 molecules, resulting in fusion of the individual charge clouds of a single PF4 molecule; this is an exothermic reaction, and the released energy is high enough to induce a conformational change in PF4 whereby two monomers on one side come closer together and the other two monomers open up [10]. This process can also be induced by some high-avidity anti-PF4 autoimmune HIT (aHIT) autoantibodies (red IgG molecules) [29]. (D) Large complexes can be formed between PF4 and polyanions, and between PF4 and aHIT autoantibodies (shown in red). The right panel shows the ridge-like PF4-containing structures in complex with longer polyanions as demonstrated by atomic force microscopy [9]. (E) Anti-PF4–heparin (PF4/H) antibodies (blue IgG antibodies) bind to PF4–polyanion complexes, and activate platelets via the platelet Fc receptors (not shown). The right panel shows an electron photomicrograph of the resulting platelet aggregates. (F) The same anti-PF4/H antibodies bind to the PF4-containing complexes formed by anti-PF4 aHIT autoantibodies (shown in red). These complexes contain more IgG, because both the heparin-dependent (blue IgG) antibodies and the aHIT autoantibodies (red IgG) bind. This is probably the reason why aHIT autoantibodies cause stronger platelet activation and much larger and denser platelet aggregates, as shown by the electron photomicrograph. The white scale bar represents 10 µm. The electron photomicrographs shown in (E) and (F) were taken from the supplementary material published with reference [29]. [Color figure can be viewed at wileyonlinelibrary.com]

have thrombocytopenia that lasts for several weeks or even a few months following the recognition of HIT and the cessation of heparin. The definition of persisting HIT has included patients whose platelet count takes > 1 week to recover (this review article) to those whose platelet count recovery requires > 1 month [38]. Recovery time beyond a week probably suffices to define persisting HIT, as ≈ 90% of patients with HIT show platelet count recovery within this time period [35], and the short half-life of heparin (< 1 h) means that heparin is no longer present after this time period. Demonstrating heparin-independent platelet activation by patient serum in the absence of heparin (buffer reactivity) supports a diagnosis of persisting HIT versus another (non-HIT) explanation for ongoing thrombocytopenia. Platelet activation by these sera, however, is still inhibited (or strongly delayed) in the presence of high heparin concentrations (100 IU mL^{-1}). Inhibition by high concentrations of heparin usually distinguishes HIT antibody reactivity, whether typical or autoimmune, from non-HIT-related platelet activation (e.g. attributable to circulating immune complexes). Table 2 contrasts the laboratory profile of aHIT with that of typical HIT, and discusses issues of potential cross-reactivity with danaparoid and fondaparinux (discussed later in this review). As a general rule, aHIT sera test strongly positive in PF4-dependent EIAs (> 2.0 OD units), although no single cutoff reliably distinguishes aHIT antibodies from typical HIT antibodies.

Some reports [39,40] have shown a striking inverse correlation between platelet counts and heparin-independent serum-induced serotonin release, supporting a role for autoreactive HIT antibodies in the pathogenesis of this disorder (Fig. 4). The same inverse correlation is seen in the heparin-induced platelet activation (HIPA) test, in which the buffer reaction occurs very quickly (within 5 min), and at the same time as the reaction in the presence of low concentrations of heparin. Also, an association between HIT serum reactivity at buffer control and prolonged recovery from thrombocytopenia has been reported [41].

Spontaneous HIT syndrome

To date, ≈ 20 cases of spontaneous HIT syndrome have been reported [42–54]. Affected patients resemble those with HIT both clinically (thrombocytopenia and thrombosis – often including adrenal hemorrhagic infarction) and serologically (strong positive results in PF4-dependent EIA and SRA/HIPA), despite the absence of proximate (closely preceding) heparin exposure. The presence

Table 2 Laboratory features of heparin-induced thrombocytopenia (HIT) antibodies

Clinical presentation of HIT	PF4-dependent EIA	Functional (platelet activation) test using washed platelets (e.g. serotonin release assay or heparin-induced platelet activation test)				
		Buffer (0 IU mL^{-1} heparin)	UFH 0.1–0.3 IU mL^{-1}	Fondaparinux/ danaparoid	UFH 100 IU mL^{-1}	Heparin 0.1–0.3 IU mL^{-1} + IV.3
No HIT	Negative or (usually weakly) positive	Negative	Negative	Negative	Negative	Negative
Non-HIT platelet activation	Negative or (usually weakly) positive	Positive	Positive	Positive	Positive	Positive/negative
Typical (or rapid-onset) heparin-dependent HIT	Positive (usually strongly positive)	Negative	Positive	Negative	Negative	Negative
aHIT	Positive (usually very strong reactivity, e.g. OD > 2.0 units)	Positive*	Positive	Positive	Negative	Negative
	Serial diluted sera (amount of dilution required differs among sera)					
aHIT antibodies not cross-reacting with either fondaparinux or danaparoid	Positive (usually very strong reactivity, e.g. OD > 2.0 units)	Negative	Positive	Negative	Negative	Negative
	Diluted serum (required dilution grade differs between sera)					
aHIT antibodies cross-reacting with either fondaparinux or danaparoid	Positive (usually very strong reactivity, e.g. OD > 2.0 units)	Negative	Positive	Positive	Negative	Negative

aHIT, autoimmune heparin-induced thrombocytopenia; EIA, enzyme immunoassay; IV.3, Fc receptor blocking antibody; PF4, platelet factor 4; UFH, unfractionated heparin.

*Serum/plasma is diluted 1 : 5 for washed platelet activation assays, so residual heparin is an infrequent explanation for buffer reactivity; nevertheless, this is a potential explanation for serum/plasma-induced platelet activation at buffer control, particularly if the sample is obtained while the patient is receiving therapeutic-dose heparin. One approach is to repeat the platelet activation test with a sample obtained on the day after discontinuation of heparin; true heparin-independent serum/plasma-induced platelet activation will still be evident 1 day after discontinuation of heparin.

of aHIT antibodies is characteristic, and should be considered to be a serologic criterion [45].

Some patients with normal platelet counts who experience an unexpected abrupt platelet count fall upon receiving heparin although no previous (or recent) heparin had been given have also been reported [42,47,48]. Such patients have been shown to have HIT antibodies but without heparin-independent serum-induced platelet-activating properties. Although the absence of thrombocytopenia on presentation and the lack of aHIT antibodies argue against classifying these patients as having aHIT, it is possible that some might have had a recent episode of (unrecognized) aHIT, especially if they present with a thrombotic episode that leads to administration of heparin with (unmasking of) HIT.

Most reported spontaneous HIT patients had preceding orthopedic surgery (usually, knee replacement surgery), suggesting that one or more factors (e.g. release of knee cartilage glycosaminoglycans or RNA, owing to tourniquet-related cell damage) linked to this type of surgery is able to, rarely, trigger an HIT-like immune response. Other patients appear to have had preceding infection, suggesting that exposure to microorganisms might have helped to trigger an HIT-like immune response. However, some patients have had no apparent preceding illness.

Fondaparinux-associated HIT

Fondaparinux is an antithrombin-dependent, sulfated pentasaccharide with selective activated factor X (FXa) inhibition. HIT antibodies do not usually show cross-reactivity with fondaparinux, either *in vitro* [55] or *in vivo* [56]. Nevertheless, there are rare examples of *de novo* HIT in which the timing of the HIT immune response coincides with preceding fondaparinux post-surgery thromboprophylaxis [57–62]. In some of these patients, *in vitro* cross-reactivity of the HIT antibodies with fondaparinux was shown; that is, patient serum showed greater serotonin release (over buffer control) in the presence of pharmacologic concentrations of fondaparinux [61,62].

Although fondaparinux is now widely used as an (off-label) treatment for HIT [63], a few reports have implicated fondaparinux failure, including persisting or worsening thrombocytopenia with uncontrolled DIC. In these report, *in vitro* cross-reactivity for fondaparinux was demonstrated [38,54,64].

Sometimes, however, fondaparinux is an 'innocent bystander', whereby aHIT triggered by preceding heparin occurs during subsequent thromboprophylaxis with fondaparinux, and no fondaparinux cross-reactivity is shown

[39,65]. In such patients, switching from prophylactic-dose to therapeutic-dose fondaparinux, to treat HIT, was successful [39]. On the other hand, thrombocytopenia occurring during fondaparinux administration in a patient who recently received heparin can also be associated with *in vitro* (and presumably *in vivo*) cross-reactivity for fondaparinux [66] (Table 2; see also 'Treatment of aHIT').

Flush heparin HIT

Rarely, HIT can occur in association with exclusive exposure to heparin 'flushes' used to manage intravascular catheters [67–69]. The sera of such patients also contain aHIT antibodies that activate platelets independently of heparin, with platelet count recovery being associated with waning of serum-induced heparin-independent platelet activation [69]. The strong association between aHIT and flush heparin HIT helps to explain how HIT can occur with such small quantities of heparin [69].

HIT-associated DIC

Most patients with HIT have moderate degree of thrombocytopenia (median platelet count, 50–60 $\times$ 10^9 L^{-1}); however, for $\approx$ 15% of patients with HIT, the platelet count nadir is $< 20 \times 10^9$ L^{-1} [70]. In our experience, many of these patients have severe HIT-related complications, including overt DIC and microvascular thrombosis, and HIT sera that show heparin-independent platelet-activating properties [36,41,71]. These patients can also develop bleeding [38,64], although the role of hemorrhagic infarction (HIT-associated thrombosis) versus complication of anticoagulation versus the role of severe thrombocytopenia as such remains unclear.

Treatment of aHIT

Table 3 summarizes the approaches used for the treatment of aHIT. Several of the strategies listed are not licensed, but are based on theoretical considerations and favorable anecdotal experience.

Heparin cessation and avoidance/reversal of vitamin K antagonists (VKAs)

Although heparin cessation is a tenet of HIT management, many patients with aHIT are no longer receiving heparin (delayed-onset HIT and persisting HIT) or have never received heparin (spontaneous HIT syndrome). Clearly, heparin cessation does not interrupt the pathogenesis of aHIT (and, indeed, stopping heparin could even increase hypercoagulability). Nonetheless, in some patients, giving heparin could have adverse consequences through the recruitment of coexisting heparin-dependent HIT antibodies (Fig. 3).

VKAs are especially dangerous in patients with aHIT, owing to the risk of protein C depletion during ongoing hypercoagulability [72] and greater potential for activated partial thromboplastin time (APTT) confounding (discussed subsequently) [37,73]. If aHIT is recognized in a patient already receiving a VKA, vitamin K should be promptly given [37,74–76].

Alternative anticoagulation: principles

Patients with aHIT require aggressive therapeutic-dose anticoagulation with an alternative anticoagulant pending platelet count recovery (and usually for longer to manage associated thrombosis). Although patients with aHIT often have severe thrombocytopenia ($< 20 \times 10^9$ L^{-1}), this is not a contraindication for anticoagulation, and, indeed, patients usually require high therapeutic levels of anticoagulation to control the hypercoagulable state. An important principle is that prolonged thrombocytopenia in aHIT patients despite the use of an alternative anticoagulant is not necessarily an indicator of ineffective anticoagulation or drug cross-reactivity; rather, it usually represents the inherent platelet-activating effects of aHIT antibodies (which can last for several weeks or months). Nevertheless, thrombocytopenia can sometimes also indicate inadequate anticoagulation; thus, it is our practice to perform serial measurements of fibrinogen and fibrin D-dimer levels, as well as direct measurements of anticoagulant level (when available), to judge the adequacy of anticoagulation [40]. Worsening of thrombocytopenia or of DIC parameters during treatment with an alternative anticoagulant should generally prompt a dose increase, but, in the case of treatment with danaparoid or fondaparinux, should also prompt testing for drug cross-reactivity (discussed subsequently) [38].

In principle, the alternative anticoagulants used to treat typical HIT, such as argatroban, bivalirudin, danaparoid, and fondaparinux [74,75], and probably also direct oral anticoagulants (DOACs) [40], can be used to treat aHIT. Ironically, the most established agents for treating HIT are associated with specific problems in managing aHIT, including, commonly, APTT confounding (argatroban and bivalirudin) and, less often, *in vitro*/*in vivo* cross-reactivity (danaparoid and fondaparinux). Thus, recent favorable experience with the use of DOACs for treating aHIT [40] is of considerable interest.

Alternative anticoagulation: direct thrombin inhibitors (DTIs)

The DTIs argatroban and bivalirudin are routinely monitored with a global coagulation test, the APTT. However, this bears the risk of APTT confounding because of associated DIC, with the potential for 'therapeutic' APTT values (reflecting low levels of prothrombin, FXI and FXII rather than DTI drug levels) to be

Table 3 Management of autoimmune HIT

Treatment measure	Effects	Risk of thrombosis	Should be done	Considerations
Stop heparin	Contradictory: heparin can both anticoagulate and promote platelet activation	+/−	☑	-aHIT is often recognized after heparin has already been stopped -Most complications of aHIT occur after heparin is stopped
IVC filters or central venous catheters	HIT promotes artificial surface thrombosis	↑	☒	-Artificial surfaces promote thrombus formation; alternative anticoagulants used in HIT do not inhibit contact factor activation
VKAs	Fail to inhibit HIT hypercoagulability; lower protein C levels	↑	☒	-Reverse VKAs (if given) with vitamin K -Avoid VKAs prior to resolution of aHIT (do not give VKAs until a platelet count of at least 150×10^9 L^{-1} is achieved)
Alternative (non-heparin) anticoagulation	Reduces thrombin generation	↓	☑	-Requires anticoagulation in the upper therapeutic range -Duration of anticoagulation depends on: • platelet count recovery • thrombotic event(s) (3–6 months) • whether platelet count decreases again after anticoagulant dose reduction, in which case resume higher dosing
Intravenous argatroban/ bivalirudin	Reduce thrombin generation	↓		-APTT confounding (risk of underdosing) -Monitor by the use of dTT or ECA (if available) -Follow with serial D-dimer and fibrinogen levels
Intravenous danaparoid	Reduces thrombin generation; disrupts HIT antigens	↓		-Reduce dose by 30% in cases of severe renal insufficiency -Monitoring aFXa (0.6–1.2 aFXa U mL^{-1}); higher doses might be needed in severe aHIT -Long half-life poses difficulty with bleeding -Potential for danaparoid cross-reactivity
Subcutaneous fondaparinux	Reduces thrombin generation	↓		-Renal function dependency -Monitoring aFXa (see text) -Potential for fondaparinux cross-reactivity (diagnosis requires serial dilutions with platelet activation assay)
Oral rivaroxaban Oral apixaban Oral edoxaban	Reduce thrombin generation	↓		-Anecdotal reports exist of successful outcomes of aHIT treated with rivaroxaban
Oral dabigatran	Reduces thrombin generation	↓		-Anecdotal reports do not comment on whether patients had aHIT or not
High-dose IVIg	Inhibits platelet Fc receptors; potentially, downregulation of B cells	↓	? Second-line therapy	-1 g kg^{-1} on two consecutive days -Additional anticoagulation is required -Recurrence of thrombocytopenia can occur
Therapeutic plasma exchange	Reduces HIT antibody levels	?	? Third-line therapy	-Additional anticoagulation is required -aHIT antibodies redistribute from interstitial compartment, so more than one treatment is usually needed -Does not inhibit ongoing antibody synthesis

aFXa, anti-activated factor X; aHIT, autoimmune heparin-induced thrombocytopenia; APTT, activated partial thromboplastin time; dTT, diluted thrombin time; ECA, chromogenic ecarin assay; IVC, inferior vena cava; IVIg, intravenous immunoglobulin; VKA, vitamin K antagonist. Several treatment approaches listed (e.g. fondaparinux, direct oral anticoagulants [e.g. rivaroxaban and apixaban], high-dose IVIg, and therapeutic plasma exchange) are not licensed for the treatment of HIT, and are included because of theoretical considerations and because of favorable anecdotal experience. ?, uncertain.

nonetheless associated with DTI underdosing [41,73]. Typical clinical situations for APTT confounding are: severe HIT-associated DIC, concurrent VKA therapy, or hepatic insufficiency. The plasma-diluted thrombin time assay [77] or chromogenic ecarin clotting assay [78] are much less affected by coexisting coagulopathies, but the dose–response curve for each DTI needs to be established in each laboratory. If, despite therapeutic APTT values, further clotting occurs during DTI therapy, APTT confounding is the most likely explanation. A recent review of DTI therapy for cases of spontaneous HIT syndrome post-knee replacement surgery found failure of DTI therapy in six of seven (86%) patients [54].

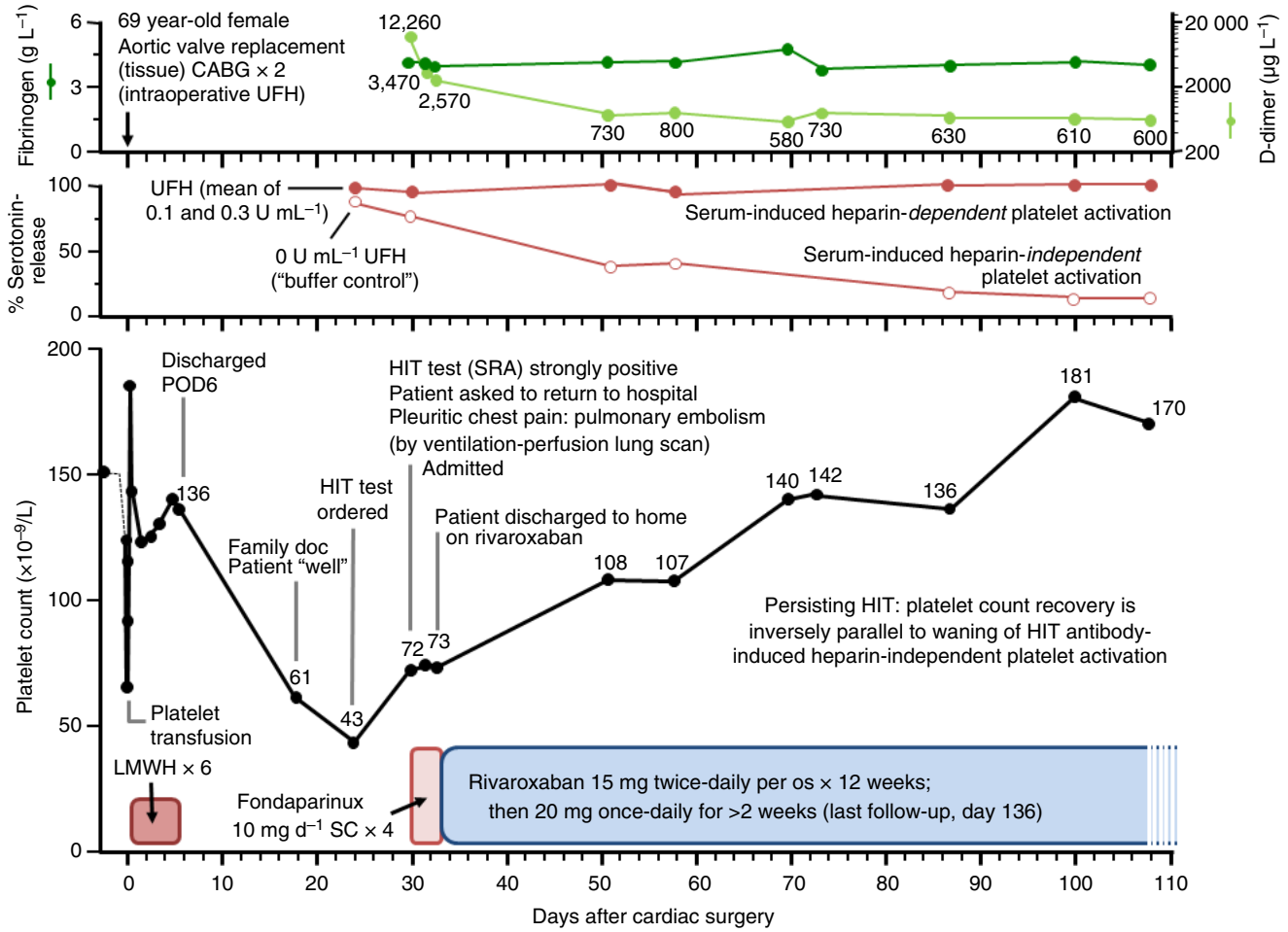

Fig. 4. Patient with autoimmune heparin-induced thrombocytopenia (aHIT) (delayed-onset and persisting heparin-induced thrombocytopenia [HIT]) who was switched from fondaparinux to rivaroxaban during acute thrombocytopenia. The gradual recovery in platelet count paralleled the gradual decline in serum-induced percent serotonin release at 0 U mL^{-1} heparin (see open circles). CABG, coronary artery bypass grafting; LMWH, low molecular weight heparin; POD, postoperative day; SC, subcutaneous; SRA, serotonin release assay; UFH, unfractionated heparin. Reprinted from: Warkentin TE, Pai M, Linkins LA. Direct oral anticoagulants for treatment of HIT: update of Hamilton experience and literature review. *Blood* 2017; **130**: 1104–13. [Color figure can be viewed at wileyonlinelibrary.com]

Alternative anticoagulation: danaparoid and fondaparinux

An advantage of danaparoid and fondaparinux is that dosing is not based on global coagulation assays such as the APTT. Danaparoid sodium has a further advantage: it is the only commercially available anticoagulant that, at therapeutic concentrations, can directly interfere with HIT pathogenesis by detaching PF4 from the platelet surface, and disrupting the PF4-containing antigen complexes [79,80]. Thus, danaparoid has a two-fold action in HIT, by inhibiting thrombin generation (anticoagulation) [81] and interfering with platelet activation by immune complexes. In addition, danaparoid's long half-life facilitates stable anticoagulation (although this poses problems if bleeding occurs). Danaparoid's pharmacokinetics are dependent on renal function, and 30% dose reduction and anti-FXa (aFXa) monitoring should be performed in cases with severe renal insufficiency. The high plasma levels of danaparoid required for the treatment of aHIT (0.8–1.0 aFXa U mL^{-1} versus 0.5–0.8 U mL^{-1}) can readily be achieved through intravenous administration.

Fondaparinux appears to be effective for the treatment of HIT [82–87]. Like danaparoid, it can be monitored by aFXa activity to avoid overdosing [82,87]. Fondaparinux clearance is strongly dependent on renal function, with dose reduction and monitoring being required in cases with renal insufficiency. In our experience, subcutaneous fondaparinux is well suited for prolonged anticoagulation in patients with aHIT (requiring much lower volume injections then subcutaneous danaparoid). However, sera from some patients with aHIT show *in vitro* cross-reactivity with fondaparinux, particularly those from patients with fondaparinux-associated HIT [61,62]. This phenomenon has also been observed in some patients with other presentations of aHIT, including spontaneous HIT syndrome [54] or delayed-onset and persisting HIT

[38,64]. It is challenging to demonstrate fondaparinux cross-reactivity, as it is crucial to demonstrate platelet activation in the presence of fondaparinux that exceeds that seen in buffer control; this usually requires serial dilution of patient serum to achieve the appropriate *in vitro* conditions (Table 2) [61,62]. In cases of fondaparinux cross-reactivity, the diluted samples will show a negative result with buffer, but strong platelet activation in the presence of fondaparinux, whereas, in cases with non-cross-reacting antibodies, no increase in platelet activation over buffer control will be seen in the presence of fondaparinux. In our experience, a more common problem than fondaparinux cross-reactivity is underdosing: thus, especially in cases of persisting thrombocytopenia and/or further thromboses, the dose of fondaparinux should be increased (target peak levels, > 1.0 aFXa U mL^{-1}), but trough levels can also be monitored (and maintained at < 1.0 aFXa U mL^{-1} to avoid accumulation). Furthermore, as noted earlier, monitoring of serial levels of fibrinogen and fibrin D-dimer levels is helpful to identify patients in whom aHIT-induced thrombin generation is not sufficiently controlled by the anticoagulant dose used.

Alternative anticoagulation: DOACs

Data are sparse regarding the use of DOACs for the treatment of aHIT. However, case reports do suggest that rivaroxaban can be effective in controlling hypercoagulability in aHIT patients [39,40] (Fig. 4), even in cases with initial failure of fondaparinux [64] and argatroban [54] (Fig. 5). A theoretical disadvantage of DOACs in the treatment of severe aHIT could be the fluctuating drug levels over a period of 24 h. We suggest the use of higher and more frequent doses of DOAC (e.g. rivaroxaban, 15 mg twice daily rather than 20 mg once daily) until the platelet count has recovered. DOAC therapy also facilitates outpatient management of persisting HIT.

Duration of anticoagulation in aHIT

As a general rule, patients with aHIT require aggressive anticoagulation until the platelet count has stabilized in the range of the pre-heparin platelet count. Even when the platelet count appears to stabilize at mildly reduced levels (e.g. 100–120 $\times 10^9$ L^{-1}), this indicates ongoing HIT antibody-induced platelet and coagulation activation. Patients with aHIT may require anticoagulation for several weeks or even months. In parallel, monitoring of buffer reactivity strength helps to identify the persistence of heparin-independent platelet-activating antibodies. Each time that the anticoagulation dose is reduced (e.g. from 15 mg of rivaroxaban twice daily to 20 mg once daily), the platelet count, fibrin D-dimer level and fibrinogen level should be monitored over the subsequent days. A new decrease in platelet counts accompanied by an increase in D-dimer level and a reduction in fibrinogen level indicates that the higher dose should be further maintained. Once aHIT autoantibody reactivity is greatly diminished, the indication for further therapeutic-dose anticoagulation depends on existing levels of thrombosis and comorbidities. Ultimately, aHIT is a transient disorder, with no patients having been reported with spontaneous relapse, so indefinite anticoagulation is not warranted.

IVIg

The use of high-dose IVIg to treat HIT was described > 25 years ago [88]. *In vitro* studies have shown that the Fc domain of IgG inhibits, in a dose-dependent fashion, activation of platelets by HIT antibodies [89,90]. Until recently, IVIg use had attracted little attention for the treatment of HIT, in part because of approval of alternative (non-heparin) anticoagulants. However, the recognition of aHIT is changing this view: several recent reports [64,90–92] have indicated a high probability of abrupt platelet count recovery following treatment with high-dose IVIg.

Dosing is typically 1 g kg^{-1} given twice over two consecutive days. Tvito *et al*. summarized 12 cases of aHIT treated with IVIg [64]. Features included very low platelet count nadirs (median, 15 $\times 10^9$ L^{-1}) and persistence of thrombocytopenia (prior to administration of IVIg); in all cases, platelet counts abruptly recovered after admistration of IVIg. One patient, who received only 1 g kg^{-1} IVIg, developed recurrence of thrombocytopenia, which resolved with a second dose of IVIg [90]. Although IVIg use does not supplant the need for aggressive anticoagulation, it interrupts aHIT antibody-induced platelet activation, which could otherwise last for weeks or months, increasing the risk of breakthrough thrombosis.

Plasma exchange

Plasma exchange has been used successfully to reduce HIT antibody levels prior to cardiac surgery and planned heparin re-exposure [90,93]. Theoretically plasma exchange should also reduce aHIT antibodies and improve outcomes in aHIT. A few anecdotal reports of refractory HIT with improvement after plasma exchange are consistent with this hypothesis [94,95].

Bleeding complications

Some aHIT patients with extremely low platelet counts may be at increased bleeding risk, as observed in four of 12 patients with a platelet nadir of $< 20 \times 10^9$ L^{-1} [64]. Bleeding sites include intracranial [38,53,96] and adrenal [50–52]. In some cases (e.g. adrenal), it is likely that bleeding occurs in association with primary thrombosis (i.e. hemorrhagic infarction), although, in other types of bleeding

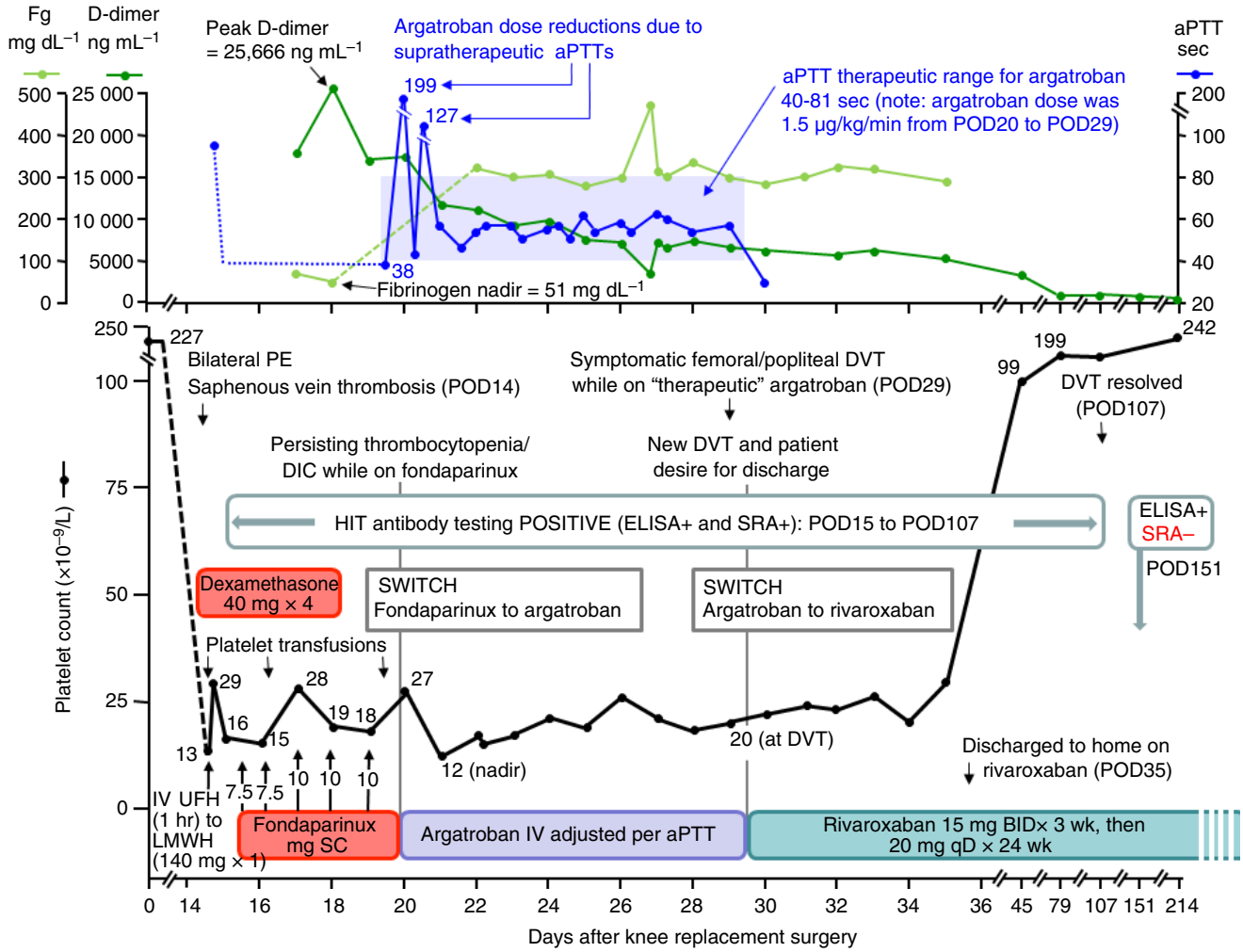

Fig. 5. Clinical course of patient with spontaneous heparin-induced thrombocytopenia (HIT) syndrome after knee replacement surgery. Upper panel: serial coagulation test results: fibrinogen (Fg), fibrin D-dimer (D-dimer), and activated partial thromboplastin time (APTT). The blue-shaded rectangle shows the target APTT therapeutic range for argatroban used in this patient. Two supratherapeutic APTT values resulted in argatroban dose reductions. Lower panel: serial platelet count values. Key clinical events, including timing of thrombotic events and changes in anticoagulant therapy, are also shown. BID, twice daily; DIC, disseminated intravascular coagulation; DVT, deep vein thrombosis; IV, intravenous; LMWH, low molecular weight heparin; PE, pulmonary embolism; POD, postoperative day; qD, once-daily; SC, subcutaneous; SRA, serotonin release assay; UFH, unfractionated heparin; US, ultrasound. Reprinted from: Poudel DR, Ghimire S, Dhital R, Forman D, Warkentin TE. Spontaneous HIT syndrome post-knee replacement surgery with delayed recovery of thrombocytopenia: a case report and literature review. *Platelets* 2017; **28**: 614–20. [Color figure can be viewed at wileyonlinelibrary.com]

(intracranial), anticoagulation and severe thrombocytopenia as such could be contributory factors. Thus, in cases of severe thrombocytopenia [97] and clinically relevant bleeding, platelet transfusions can be appropriate despite the general proscription of their use in HIT patients [74,98].

Intravascular devices and inferior vena cava (IVC) filters

aHIT is associated with extreme hypercoagulability. Although concomitant severe thrombocytopenia and venous thrombosis can prompt the use of an IVC filter in these patients, this should be avoided. This is because IVC filters strongly predispose to local thrombosis, including massive venous thrombosis and limb necrosis requiring amputations [98]. Moreover, the anticoagulants typically used in managing HIT (argatroban, bivalirudin, danaparoid, and fondaparinux) are not good at inhibiting contact factor-induced activation, contributing to failure of anticoagulation [99].

Conclusion

aHIT is an often devastating hypercoagulability state that paradoxically results in thrombotic complications even when heparin is no longer being given. It requires special laboratory diagnostics (buffer control and serial dilutions

for diagnosis and to exclude cross-reactivity of certain alternative anticoagulants) and special treatment considerations. Massive thrombin generation requires high therapeutic-dose anticoagulation, which is often not achieved with DTIs, owing to APTT confounding, which is a frequent problem in aHIT. IVIg seems to be an important additional option for interfering rapidly with aHIT pathogenesis. The recently reported mechanism of aHIT autoantibodies recruiting 'typical' (heparin-dependent) antibodies [29] may have major implications for understanding other autoimmune disorders in hemostasis.

Addendum

A. Greinacher contributed the sections on the pathogenesis of aHIT. K. Selleng contributed the sections on the treatment of HIT. T. Warkentin contributed the sections on the clinical presentation of HIT. All authors approved the final version of the manuscript.

Disclosure of Conflict of Interests

A. Greinacher reports receiving consulting fees from Aspen Germany, Chromatec, Bristol-Meyer Squibb, and Boehringer Ingelheim; research support and consulting fees from Instrumentation Laboratory; and royalties from Informa (Taylor & Francis). K. Selleng reports receiving research support and consulting fees from Johnson&Johnson; speaker fees and travel support from CSL Behring; a research contract, consulting fees and travel support from Janssen Cilag; and travel support from Bayer Vital and Novo Nordisk. T. E. Warkentin reports receiving consulting fees from Aspen Global and Octapharma; research support and consulting fees from W. L. Gore, Instrumentation Laboratory, and Medtronic Diabetes; royalties from Informa (Taylor & Francis); and consulting fees related to medical–legal testimony.

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