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Pathogenesis of Heparin-Induced Thrombocytopenia

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Abstract

There are currently no effective substitutes for high intensity therapy with unfractionated heparin (UFH) for cardiovascular procedures based on its rapid onset of action, ease of monitoring and reversibility. The continued use of UFH in these and other settings requires vigilance for its most serious non-hemorrhagic complication, heparin induced thrombocytopenia (HIT). HIT is an immune prothrombotic disorder caused by antibodies that recognize complexes between platelet factor 4 (PF4) and polyanions such as heparin (H). The pathogenicity of anti-PF4/H antibodies is likely due to the formation of immune complexes that initiate intense procoagulant responses by vascular and hematopoietic cells that lead to the generation of platelet microparticles, monocyte and endothelial cell procoagulant activity, and neutrophil extracellular traps (NETs), among other outcomes. The development of anti-PF4/H antibodies after exposure to UFH greatly exceeds the incidence of clinical disease, but the biochemical features that distinguish pathogenic from non-pathogenic antibodies have not been identified. Diagnosis relies on pretest clinical probability, screening for anti-PF4/H antibodies and documentation of their platelet activating capacity. However, both clinical algorithms and test modalities have limited predictive values making diagnosis and management challenging. Given the unacceptable rates of recurrent thromboembolism and bleeding associated with current therapies, there is an unmet need for novel rational non-anticoagulant therapeutics based on the pathogenesis of HIT. We will review recent developments in our understanding of the pathogenesis of HIT and its implications for future approaches to diagnosis and management.

Keywords

Heparin induced thrombocytopenia; Platelet factor 4; Heparin; Thrombocytopenia; hit

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Conflict of Interest

GMA receives royalties from Biokit manufacturer of a HIT diagnostic assay. GMA and DBC have pending intellectual property applications.

Introduction

The last two decades have witnessed remarkable progress in the development of novel anticoagulant therapies, first with the introduction of parenteral anticoagulants such as direct thrombin inhibitors (DTIs) and more recently, direct oral anticoagulants (DOACs). These agents have dramatically altered approaches to therapeutic and prophylactic anticoagulation for many clinical indications. However, no new agent comes close to replicating the clinical utility of unfractionated heparin (UFH) in settings with respect to cost, rapid onset of action, ease of monitoring, and reversibility, such as in cardiopulmonary bypass, extracorporeal membrane oxygenator (ECMO) circuits, mechanical valve prosthesis, for dialysis, and in critically ill patients at concurrent risk for thrombosis and bleeding.

However, these advantages are partially offset by the most common non-bleeding complication of UFH exposure, heparin induced thrombocytopenia (HIT). HIT is a severe and potentially fatal immunothrombotic disorder that remains challenging to diagnose and manage. Given the absence of alternatives to UFH for some common and recurrent settings, the disease burden has not abated. The prevalence of HIT is estimated to be ~20,000 cases per year (1 in 1500 hospital admissions) in the U.S.[1] with the greatest risk being in patients undergoing cardiac surgery (0.6%) and dialysis for acute kidney injury (0.5%). In-hospital mortality of patients with HIT is four-fold higher than for patients who are diagnosed with other causes of thrombocytopenia, the median length of stay is three times longer and the cost of hospitalization is four times higher.[2]

Current management strategies for patients suspected or diagnosed with HIT rely on treating the symptoms and using alternative anticoagulants, rather than addressing the underlying cause (immune complex initiated thrombosis). Emerging data suggest that current approaches may not only be incompletely effective, but also problematic in their own right. In a prospective study of 310 patients suspected of having HIT, new thromboembolic complications were diagnosed in 36% of patients with HIT despite treatment with non-heparin anticoagulants, and major bleeding events occurred in 38–44% of patients treated with non-heparin anticoagulation, irrespective of a HIT diagnosis (confirmed or suspected), two of which (13%) were fatal.[3] These findings confirm and extend results from prior studies that document a high incidence of bleeding complications (~1%/day) among patients with a suspected or confirmed diagnosis of HIT who are treated with non-heparin anticoagulants.[4–6] These data also clearly demonstrate the need for targeted therapies based on the pathophysiology of HIT. We will review recent developments in understanding the pathogenesis of HIT and highlight those findings that have diagnostic significance and/or therapeutic potential.

The HIT Immune Response

HIT is caused by IgG antibodies that bind to PF4 complexed with polyanions such as heparin. The rate of seroconversion varies with extent of preceding platelet activation, duration of heparin exposure and drug composition (e.g. chain length). The highest rates of seroconversion occur in patients undergoing cardiac surgery, on ECMO, or after insertion of ventricular assist devices (~27–73%)[7–11], likely due to the combined effects of underlying

vascular disease,[12] persistent platelet activation leading to increased PF4 levels,[13] and exposure to high doses of UFH (~1–4 U/mL).[14] Seroconversion among medical and surgical patients varies from 4–17%[15, 16] and is lowest among pediatric patients (0–2%),[17] which has been attributed to a lower burden of vascular disease, i.e., less chronic platelet activation leading to lower circulating levels of PF4.[17]

The observation that the rate of seroconversion is markedly higher following treatment with UFH than low-molecular weight heparin (LMWH)[16,18,19] highlights the critical role antigen structure plays in this disease. PF4, an abundant cationic protein stored in form of tetramers within platelet alpha granules that is released upon platelet activation, interacts with heparin through electrostatic interactions. The crystal structure of the complex between PF4 and the heparin-derived pentamer fondaparinux provides insights[20] into how PF4 (as opposed to other cationic proteins) and how heparin, especially UFH (as opposed to other anionic compounds) form immune complexes that eventuate in the development of HIT. Binding of PF4 to “heparin” may stabilize the dominant antigenic site on the “open” surface of the asymmetric tetramer and stabilize the linearity of the oligosaccharide, which binds to the opposing surface. Heparin molecules of sufficient length can “share” PF4 tetramers and PF4 tetramers can bridge more than one heparin molecule, culminating in the formation of PF4/H oligomers.[21, 22] These results help explain the greater immunogenicity of UFH over LMWH[23] and are consistent with prior findings these complexes form at lower concentrations of UFH than LMWH[21, 24] Oligomeric complexes form over a narrow molar ratio[21, 22] and are disrupted if this ratio is perturbed, the basis for “heparin-dependent” binding seen in immunoassays and functional assays.

The requirements for PF4/H to assemble into ultralarge antigenic complexes (ULCs) are likely important for initiating the immune responses *in vivo*. Mice injected with PF4 alone or with heparin alone do not develop anti-PF4/H antibodies (<10%), but when injected with PF4 and heparin combined at ratios shown to optimize assembly of ULCs, there’s a marked increase in the rate of seroconversion (90–100%).[22] Such heparin-dependent immune responses are not limited to PF4. Other heparin-binding proteins, such as protamine and lysozyme also form ULCs *in vitro*,[25] and show similar propensity for anti-protamine (PRT)/heparin or anti-lysozyme/heparin formation in mice,[25] or high-titer anti-PRT/heparin antibodies in humans.[26] Likewise, binding of PF4 to other polyanions, such as cell-surface glycosaminoglycans (GAGs), platelet polyphosphates,[27, 28], DNA[29, 30] or multimeric von Willebrand Factor (vWF) from endothelial cells[31, 32] forms antigenic complexes that have been implicated by some in the rare reported cases of “spontaneous” HIT in patients with no prior heparin exposure or “autoimmune” anti-PF4/heparin antibodies that perpetuate the risk of thrombosis after heparin has been cleared from the circulation and metabolized.[33, 34]

The prevalence of anti-PF4/H serconversions and the early onset isotype-switched response (IgG antibodies appearing within 5–10 days of heparin) in HIT has been attributed to a recall response to a prior sensitizing event, such as bacterial infection. This premise is based on observations of PF4 binding to bacterial surfaces[35–37] and heightened anti-PF4/H seroconversion in patients with acute[38] or chronic bacterial infection.[39] Other studies implicate innate immune mechanisms more directly. For example, PF4/H ULCs bind to “natural IgM” in the blood[40], activate the classical pathway of complement, and the

complement-coated antigens/complexes bind to B-cells via the complement receptor CD21.[41]

The presence of high-titer isotype antigen-specific IgG responses also strongly denote involvement of adaptive immunity in HIT. Other supporting data come from findings of a restricted T cell repertoire in patients with HIT[42, 43] and the requirement for T cells in the murine HIT immune response.[44–46] Recent genome wide association (GWAS) studies, though involving small numbers of patients, identified several candidate genes,[47, 48] including an HLA-DRB3*01:01 allele associated with a greater risk of developing HIT[49]; these findings will require validation in larger cohorts.

The Clinical Syndrome of HIT

Thrombocytopenia with or without thrombosis is the salient clinical feature of HIT. An unexplained fall in the platelet count of >30–50% in the proper temporal relationship to heparin exposure is evident in ~95% of patients 5–14 days after their initial exposure to heparin.[50] Thrombocytopenia may occur more rapidly (<24 hours) in patients with circulating anti-PF4/H antibodies.[51] Platelet counts <20K may presage an increased thrombotic risk in patients with high anti-PF4/heparin antibody levels (odds ratio, OR >8 for thrombosis)48 or, more often, point to an alternative diagnosis in seronegative patients. In a time-course study of 12 heparin-naïve patients who developed HIT amidst a clinical trial, the platelet count began to fall ~ 2 days (range 1–5 days) after seroconversion, but did not meet diagnostic criteria (>50% decline) for another 2 days of drug exposure. Patients with thrombocytopenia alone, i.e. “isolated HIT” are high risk for developing subsequent thrombotic complications (30–50%) over the ensuing week making timely diagnosis and intervention imperative.[52] Thrombocytopenia may be absent in the rare cases of localized thrombosis leading to heparin-induced skin necrosis,[53] while others may have delayed manifestations up to three weeks after heparin exposure (“delayed-onset HIT”).[54, 55]

HIT is an aggressive thrombotic disorder. Retrospective, prospective and epidemiologic studies document new or progressive thromboembolic complications (TECs) in ~20–50% of patients who develop thrombocytopenia,[1, 3, 6, 52, 56–60] with an estimated event rate of ~5%/day for new thrombosis, amputation or death.[61] TECs may first be identified after, concurrent with, or, less commonly, before thrombocytopenia develops.[23] Thromboses in large vessels are more evident clinically but microvascular thrombosis leading to ischemic extremities and tissue injury in the presence of detectable pulses is not unusual.[50] Venous thrombosis was more common when UFH was used more widely.[62] Arterial thrombi involving atherosclerotic vessels[63] and at sites with indwelling catheters[1, 50, 59] are increasingly common due to continued requirements for UFH for cardiovascular surgery. The prothrombotic stimulus of HIT is so intense that risk factors such as deficiencies of protein C, S or anti-thrombin III or the presence of factor V Leiden make little impact on prevalence.[63, 64] The only consistent laboratory parameter shown to correlate with the risk of TEC is antibody burden indicated by OD in ELISAs[65–67] or strong activation in functional assays.[68, 69]

The stimulus for thrombosis begins with formation of ULICs. As discussed above, binding of heparin or similar polyanions in solution stabilizes the conformation of the PF4 tetramer and nucleates incorporation of additional molecules of heparin and PF4 into a larger antigenic complex (Figure 1).[20] This permits incorporation of multiple IgG anti-PF4 antibodies in each complex forming soluble “ultralarge immune complexes” (ULICs) that reach dimensions exceeding a micron in size.[21] Monoclonal antibodies that stabilize PF4 in its monomeric configuration prevent assembly of ULICs and interfere with HIT antibody-mediated platelet activation *in vitro* and thrombus formation *in vivo*.[20]

Soluble ULICs may initiate prothrombotic responses, but it is likely that the key event that sustains the risk of thrombosis is the development of large oligomeric immune complexes on the surface of platelets, monocytes and neutrophils leading to activation of FcγIIA receptors (FcγRIIA). The requirement for platelet FcγRIIA to develop thrombocytopenia and thrombosis in response to HIT antibodies was demonstrated in a murine model comparing response in receptor null (wild type) and receptor-expressing transgenic mice.[70] A histidine (H)/arginine (R) polymorphism at amino acid 131 in the extracellular domain of FcγRIIA modulates *in vitro* platelet activation by immune complexes. Individuals with the RR allotype may be more prone to thrombosis because endogenous IgG_2 binds and competes less well for platelet activation by ULICs.[71] However, studies looking at the importance of this polymorphisms as a risk factor for TEC have been inconclusive.[72]

Cell surface ULICs engage FcγRIIA’s leading to receptor clustering, phosphorylation of the tyrosine residues on its immunoreceptor tyrosine-based activating motif (ITAM) that provide a docking site for Syk kinase. Receptor binding initiates downstream signaling involving Bruton tyrosine kinase (BTK) and Tec.[73] Inhibiting Syk kinases prevents HIT antibody-mediated platelet aggregation *in vitro* and thrombocytopenia and thrombosis in murine models.[74, 75] Inhibition of platelet aggregation by HIT antibodies by platelet BTK inhibitors has also recently been reported[76] but the clinical utility of this approach in such a rapidly developing disease has not been assessed.

FcγRIIA-bearing monocytes, platelets, and neutrophils differ in their binding and response to HIT ULICs that may be relevant to disease expression and mitigation. For example, HIT antibodies bind more efficiently to monocytes than to platelets due to a greater abundance and differences in the composition of GAGs that increase binding of PF4.[77] Activation of monocytes leads to expression of cell surface tissue factor[78, 79] and generation of thrombin.[75] Depletion of monocytes from whole blood and blocking of tissue factor reduced platelet accumulation and fibrin generation in a microfluidic injury model.[75] Monocyte depletion *in vivo* markedly attenuates clot formation, but exacerbates thrombocytopenia, likely due to redistribution of ULICs to platelet FcγRIIAs.[77] Thrombin generated by activated monocytes augments platelet FcγRIIA signaling through protease activated receptor 1 to generate highly procoagulant “coated” platelets.[75] Together, these studies suggest that co-activation of platelets by thrombin and through direct activation by ULICs contribute to thrombocytopenia and thrombosis.

Neutrophils are also subject to FcγRIIA-dependent activation by HIT antibodies.[80, 81] HIT ULICs stimulate neutrophil adhesion to endothelial cells downstream of thrombi, promote

their retrograde migration into venous thrombi and generate NETs stabilized by HIT ULICs that develop resistance to degradation by DNAse.[30] NETosis can be induced indirectly through expression of P-selectin on activated platelets and directly through platelet-independent mechanisms.[29] Recent studies suggest that the ability of PF4 and the murine monoclonal HIT-like antibody KKO ULICs to stabilize NETs could be therapeutically exploited for treatment of sepsis.[82] In these studies, deglycosylated KKO, modified to minimize FcγR activation and/or complement activation, promoted NET compaction, reduced net degradation and decreased mortality in a murine model of sepsis.[82]

Endothelial cells lacking FcγRIIA are also activated in HIT through a unique mechanism. *In vitro*, binding of HIT antibodies initiates complement-dependent deposition of platelets and expression of tissue factor.[83] In a mouse model of thrombosis, platelets release PF4, which binds to heparan sulfate and other GAGs expressed by endothelial cells and propagates ULIC formation downstream[32] In turn, injured endothelium release large multimers of von Willebrand factor (vWF) that bind PF4 and HIT ULICs and propagate thrombosis.[31]

Together, these studies begin to unravel some of the diverse shared and cell-specific prothrombotic pathways initiated by HIT ULICs that may help to explain why inhibition of FXa and thrombin may not suffice to prevent recurrent TECs. Mechanisms involved in ULIC formation, mediators of FcγR cellular activation (e.g., cellular FcγR's, signaling proteins, or NETs), and/or endothelial cells (e.g., vWF multimers, complement) serve as checkpoints for novel interventions.

Laboratory Testing in HIT

Current approaches to diagnosis involve use of clinical algorithms, such as the 4Ts[84] to assess pre-test probability and confirmation by laboratory testing. As a stand-alone test, a low 4Ts score has excellent sensitivity and a negative predictive value that exceeds 98% outside of the ICU or post-cardiopulmonary surgical settings.[85] However, an intermediate (4Ts ≥4) or high 4T's score (≥6) has a much more limited positive predictive value (14% and 64%, respectively) due to low specificity (33–64%).[86,87] Due to the high sensitivity of immunoassays, HIT can be excluded with ~99% certainty when anti-PF4/H antibodies are not detected.[85, 88]

The challenge with HIT laboratory testing is the poor specificity of commonly available immunoassays. As stated earlier, development of anti-PF4/H antibodies is many times more common than HIT, with seroconversions occurring in ~50% of patients exposed to heparin after cardiovascular surgery.[7–9] IgG isotype[89] and antibody titer correlate with clinical disease but cannot be totally relied upon for individual cases due in part to the reported inter- and intra-assay variability in reported OD values among commercial assays.[90] The proportion of anti-PF4/H antibodies detected by ELISA that cause platelet activation *in vitro* depends on the pretest probability of disease, ranging from <1% in those with low 4T scores to 40–50% in those at greatest risk.[58, 69, 91, 92] Increased utilization of rapid immunoassays, [93, 94] or tandem immunoassays combined with Bayesian analysis,[95] may improve the turnaround time and diagnostic accuracy, but has not been demonstrated to have comparable value in critically ill patients on ventricular assist devices (VADs) or extracorporeal

membrane oxygenators (ECMOs).[96] Given the reported differences in sensitivity/specificity as well as occurrence of discordant classifications when results of different commercial immunoassays have been compared,[90] is important for each laboratory to validate the immmunoassays they choose with known positive and negative controls.

Diagnostic specificity is increased with detection of anti-PF4/H antibodies that activate platelets *in vitro* by engaging FcγRIIA receptors[97] at heparin concentrations[98, 99] that favor formation of ULICs. Binding of HIT IgG to platelets is accompanied by complement C3 deposition,[98] which may enhance platelet clearance, activation, and release of procoagulant platelet microparticles that contribute to thrombosis (as discussed above).[100, 101]

The presence of heparin-dependent platelet activating antibodies tracks closely with clinical disease.[68] A test is considered positive if addition of therapeutic concentrations of heparin (0.1–1.0 units/ml) to a source of patient plasma or serum is required to activate normal platelets. Confirmation may be enhanced if platelet activation is prevented by supra-therapeutic heparin doses (typically 5–100U/ml) associated with disruption of antigenic complexes. It may be necessary to adsorb or digest heparin in the test plasma to affirm drug-dependence. Rare cases of heparin-independent, so called “autoimmune HIT” have been reported.[33, 34, 102]

Platelet activation can be assessed based on aggregation detected by a change in light transmission, release of radiolabeled serotonin (^{14}C-Serotonin release assay, SRA), flow cytometry to detect microparticles, or by flow cytometry to detect expression of P-selectin (P-selectin Expression Assay, PEA), among other endpoints. The SRA using washed platelets is reported to have diagnostic sensitivity of ~80–90% and specificity ranging from ~85–95% depending on the clinical population.[7, 103–105] The sensitivity of functional assays might be enhanced by adding exogenous PF4 to donor platelets[91, 104, 106] However, the lack of availability of functional assays at most medical centers, leads to slow turn-around times and limits their clinical utility in practice. The reader is referred to recent comprehensive reviews of these functional assays and their clinical utility.[107–109]

Management of HIT

Current guidelines recommend immediate discontinuation of heparin therapy and institution of non-heparin alternative therapies when HIT is suspected by an intermediate or high 4Ts score; in patients at high risk for bleeding, confirmation by ELISA may be warranted.[110] To date, there have been no randomized prospective treatment studies. Parenteral therapies include the DTIs (argatroban and bivalirudin), factor Xa inhibitors such as danaparoid (not available in US) and the synthetic pentasaccharide fondaparinux. The choice of therapeutic agent is guided by drug half-life, patient co-morbidities (hepatic or renal disease), and availability. The reader is referred to comprehensive reviews[111, 112] and recent guidelines[110] for additional information on the use of non-heparin anticoagulants in HIT.

A main concern with use of non-heparin parenteral anticoagulants is the high rates of recurrent thrombotic and bleeding complications. Recent prospective and retrospective studies indicate mortality rates of ~20%[1, 3, 6, 60] in part due to progressive or recurrent

TECs. Rates of amputations are unaffected by treatment and are ~5-fold higher in patients with HIT than among control cohorts.[1, 6]. As noted earlier, there is a high rate of major bleeding, not only in patients diagnosed with HIT, but also those suspected of HIT while awaiting confirmatory testing.[3] Morbidity from bleeding is heightened by lack of reversal agents for the DTIs and risk of rebound TECs due to anticoagulant discontinuation or use of newer anti-Xa reversal agents.[113]

DOACs are being increasingly used as first line therapy for patients who can tolerate anticoagulation,[114] but sufficient clinical data are unavailable to meaningfully assess their safety and efficacy. A recent multi-center, prospective study of rivaroxaban for HIT was terminated early due to poor recruitment.[115] Of the 12 patients who completed this study, one had a recurrent TEC while on therapy and one had an amputation.[115] Few patients at the highest risk of TEC and bleeding who are at greatest need for new forms of treatment, i.e. post-surgery, VADs, ECMO, etc., many of whom also have impaired renal or heparin function, have been subject to study.

Warfarin remains a safe alternative oral anticoagulant choice once patients have been bridged with parenteral anticoagulants until platelet counts recover.[110] Warfarin therapy without bridging is associated with complications of warfarin skin necrosis and venous limb gangrene[116, 117] due to reduced synthesis of protein C and impaired generation of activated protein C (aPC); i.e. PF4 binds to chondroitin sulfate residues on thrombomodulin and enhances aPC activation,[118, 119] an effect reversed by HIT antibodies.[120]

Anti-platelet drug are ineffective as stand-alone approaches (with the exception of prostacyclin which requires circulatory support), as they neither totally inhibit the intense activation of platelets through multiple pathways in HIT nor interfere with other cell activating effects of HIT antibodies.[121] Intravenous immunoglobulin (IVIG) and therapeutic plasma exchange (TPE) are the only approaches in use with disease modifying potential. High dose IVIG, which interferes with FcγRIIA dependent cell activation,[122] is increasingly being used for refractory disease or in patients with high risk of bleeding. Although epidemiologic studies point to an increased thromboembolic adverse events with the IVIG due to FXIa contamination,[123] this has not been observed to date in the highly prothrombotic background in HIT.[124] TPE removes IgG antibodies from the circulation, reducing antibody burden.[125] Case series show TPE may have an adjunctive role prior to re-exposure to heparin for emergent cardiac surgery.[125–127]

Perspective

HIT is not only a serious medical disease, but also serves as a model of an immune complex-mediated disorder with a structurally defined antigen, tests to measure clinically relevant antibodies and murine models to investigate pathogenesis and treatment. The last two decades of research have expanded our understanding of the role of “ultralarge immune complexes” that assemble on the surfaces of vascular and intravascular cells and initiate prothrombotic FcγR-dependent and independent mechanisms, some of which are not affected by the anticoagulants that we rely upon for management. A more refined understanding of the key antigenic epitopes within PF4, means to identify the subset of anti-

PF4 antibodies that promote ULIC formation, use of complement inhibitors to block antibody production and cell damage, and possibly novel signaling inhibitors may point to better predictive models to forestall the development of HIT and rational disease specific non-anticoagulant strategies to mitigate its most severe sequelae.

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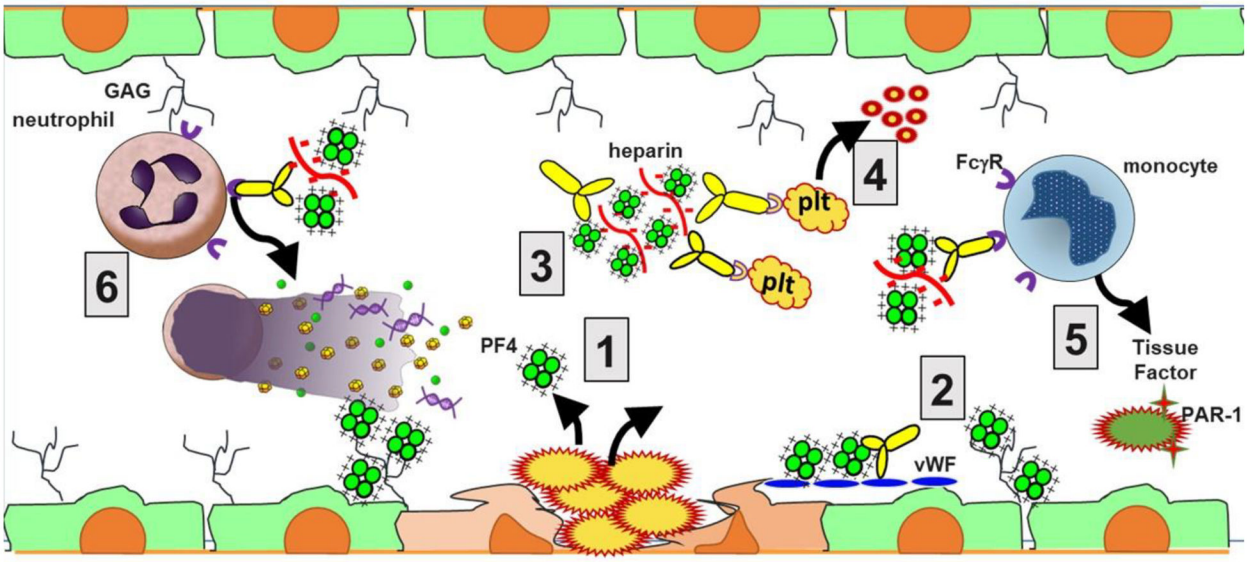

Figure. Thrombosis in HIT is a multicellular event.

1) PF4 is released from activated platelets especially at sites of endothelial injury, e.g. (atherosclerosis, thrombosis or catheterization). 2) PF4 forms antigenic complexes with cell surface GAGs or vWF extruded from activated endothelium. 3) Also forms large antigenic complexes in the circulation after exposure to heparin. 4) ULCs and cell-associated antigenic complexes bind HIT antibodies forming ULICs that engage FcgRIIA on platelets, leading to release of procoagulant microparticles. 5) monocytes, leading to expression of tissue factor and generation of thrombin which transactivates platelets via PAR-1 to form coated platelets and 6) neutrophils leading to degranulation and formation of NETS.