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Investigating treatment approaches for immunothrombosis through an examination of its intricate mechanisms

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Abstract

Immunothrombosis refers to the processes by which the coagulation and immune systems are coactivated in the form of an aberrant and dangerous positive feedback loop. This paper highlights the bidirectional relationship between both systems in order to underline the concepts behind imbalances that may appear and result in complications. Because of its many triggers and effects throughout the body, designing treatments that do not compromise the wrong pathway - or the whole system - proves to be challenging. Through an examination of current treatment methods and the general physiology of immunothrombosis, we investigate its bidirectional nature during the process of diagnosing and treating patients. On this ground, we illustrate a possible flowchart for diagnosis and a general checklist to aid in testing the safety and efficacy of new approaches.

Keywords

Immunothrombosis, Coagulation, Immune system, Homeostasis, Hemostasis, Neutrophil extracellular trap, Cytokines, Complement cascade, C-Reactive protein, Inflammatory response

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Introduction

In many situations, the crosstalk between the coagulation and immune systems is significantly effective at controlling the spread of an infection. This crosstalk normally provides a healthy balance between immune and hemostatic systems but can, under certain circumstances, lead to unchecked rogue feedback loops that may result in thrombotic or immunological dysfunction.

The coagulation system - involving blood cells and platelets; among others - functions to regulate blood clotting. The process of stopping the flow of blood outside a damaged vessel is known as hemostasis, which involves coagulation through mechanisms that recruit multiple factors; such as platelets, fibrin, and other coagulation factors. Damage to the endothelium (the walls of a blood vessel - such as when a cut or bruise occurs) exposes platelets to the extracellular matrix and extravascular space. As the closed-circuit integrity of the blood vessels is compromised , coagulation begins in order to lessen the bleeding and leakage. It induces the extrinsic pathway that activates tissue factor (TF), a coagulation factor expressed in many cells, to bind with its ligand (such as neutrophils). Normally situated in the blood on the other side of the endothelium is Factor VII/VIIa, which binds to TF when they come in contact. The TF FVIIa complex then activates Factor X, which converts inactive prothrombin into its active form; thrombin, which is an enzyme that functions in converting fibrinogen into insoluble polymeric fibrin, which is then used to form and strengthen a clot. Simultaneously, other proteins, such as the von Willebrand factor (vWF), aid in allowing platelets to bind to the damaged vessel wall, which strengthens the forming blood clot. However, this extrinsic pathway is only effective in treating minor tissue injury.

The intrinsic pathway is designed to be activated for amplification of the coagulation cascade (1). This pathway generates a greater burst of thrombin through Factors IX, X, and XI. The products of both the extrinsic and intrinsic pathways form the common pathway as the activated platelet clump increases in size, developing into a thrombus - a blood clot formed as the end results of hemostatic events (1).

Likewise, the immune system maintains homeostasis by utilizing its innate and adaptive components to fight pathogens and other harmful agents before they spread into major organ systems. When exposed to most forms of threat or damage to tissue, inflammation occurs. Because of its many purposes, almost every cell in the body interacts with it in some manner, and it can be triggered in numerous ways. If an immune cell (primarily dendritic cells or macrophages; although granulocytes also play a significant role) detects there is an active threat - through methods like the toll-like receptors on phagocytes, the remains of unnaturally killed cells, or direct engagement with antigens - it releases cytokines that result in an inflammatory response. The primary component of this inflammatory response is increased blood vessel and capillary permeability. Endothelial cells receiving cytokines will shrink away from each other, creating small gaps in the vascular system wide enough for plasma – concurrently with the immune cells - to seep out.

Once inflammation starts, it needs constant stimulation in order to be self-sustaining. As soon as enough immune cells stop fighting or otherwise stop releasing cytokines, the effects subside (partly through mechanisms like increased regulatory T cell activation and controlled apoptosis of immune cells). This is critical to the survival of the organism because inflammation can become very dangerous if left unchecked (it is estimated that almost half of human deaths can in some way be linked to an exaggerated or unchecked inflammatory response) (2). The innate immune system has evolved to be skilled at stalling or reducing pathogen multiplication in order to buy time for the adaptive immune system to activate. However, in the interim, this inflammatory response; that can last for weeks; can become counterproductive to the detriment (or failure) of the cells, tissues or organs that it is seeking to protect. An exaggerated immune response can kill the host in as little as a few minutes (for example, as in acute anaphylactic shock). A particular example can be seen in COVID-19, where the sudden increase of inflammatory cytokines released in the lungs result in the buildup of fluid and difficulty absorbing oxygen, leading to decreased blood oxygenation and possible asphyxiation.

Following inflammation, the innate immune system also triggers the complement cascade, a collection of around 50 proteins that act as one of the most important connections within the body's immune defenses. Complement proteins saturate many fluids throughout the body, although they are most prevalent in the blood plasma (3). This is important largely because, when inflammation occurs, complement proteins flow into the tissue with blood, allowing them to respond almost instantaneously to danger anywhere in the body. Among its important functions, the cascade promotes adaptive immune activation, opsonizes and causes lysis in pathogens, and releases anaphylatoxins (which promote inflammation through the release of proteins like histamine, aggravating the phagocyte response, and signaling for reinforcements from other nearby immune cells) (4-6).

The crosstalk between inflammation and coagulation functions through a positive feedback system, activating clotting mechanisms and the complement cascade (7). Infectious agents may then be trapped by coagulation in the bloodstream, preventing them from spreading. However, upregulation in the balance of crosstalk may result in the dysregulation of this relationship, increasing the production of inflammatory cytokines and, in turn, unnecessarily enhancing dangerous coagulatory events.

In general, immunothrombosis, when initiated by inflammation, induces the coagulation cascade by activating key factors involved, such as tissue factor and thrombin. Cytokines produced as a result of inflammation activate the platelet-activating factor, causing platelets to start adhering to each other. Additionally, as a result of stimuli such as microorganisms or pro-inflammatory cytokines, neutrophil extracellular traps (NETs) are triggered. NET decondensed nuclear content is first released into the cytoplasm of a stimulated neutrophil, after which a rupture of the plasma membrane occurs, releasing the chromatin as well as granular proteins into the extracellular space. This pool traps pathogens in its sticky genetic material, histones, and antimicrobial peptide substance, and then kills them using neutrophil

elastase (NE), peptidyl-arginine deiminase 4 (PAD4), and cathepsin G (CG) (8, 9). The process of immune function attracts inflammatory cytokines to the site, further escalating the total response. The increased number of cytokines triggers the endothelium to release Weibel Palade bodies (vWF and P-selectin): binding molecules that aid platelets and other substances in sticking to the endothelial surface. The release of both vWF and P-selectin facilitates platelet aggregation, eventually progressing into the coagulation cascade. Platelet activation can also result in platelet-monocyte aggregates, which induce tissue factor and coagulation as well (10).

On the other hand, akin to the immune system, coagulation presents a significant route to developing immunothrombosis through coagulatory aspects that induce inflammation. One such example involves thrombin, which causes an increase in inflammation. Most directly, thrombin cleaves complement proteins, resulting in the release of pro-inflammatory anaphylatoxins (6). Additionally, in the intrinsic pathway, Factor Xa converts prothrombin into thrombin. Expressed by cells including leukocytes, platelets, and endothelial cells, protease-activated receptors (PARs) mediate Factor Xa and thrombin's pro-inflammatory functions. Once activated, Factor Xa activates PAR1 and PAR2, while thrombin activates PAR3. These concurrent activations result in inflammatory cytokine release by PAR-expressing cells. Furthermore, in the case of an injury, thrombus formation in a blood clot involves the release of platelets. P-selectin in platelets' alpha granules promotes recruitment of leukocytes by activating β2 integrin, an integrin/receptor that allows for leukocyte adhesion to the endothelium (10). Figure 1 shows the intricate and intertwined pathways of immune activation and coagulation.

Understanding the mechanisms behind these networks allows for a deeper comprehension and greater focus on the crosstalk of inflammation and coagulation. Considering the existence of the bidirectional relationship between both systems, there remain many different modes in which immunothrombosis can be activated. Therefore, with the varied therapeutic approaches for immunothrombosis, it is important to explore ways to improve upon current patient treatment strategies. We propose two such possible routes through the introduction of a treatment checklist and a treatment algorithm flowchart.

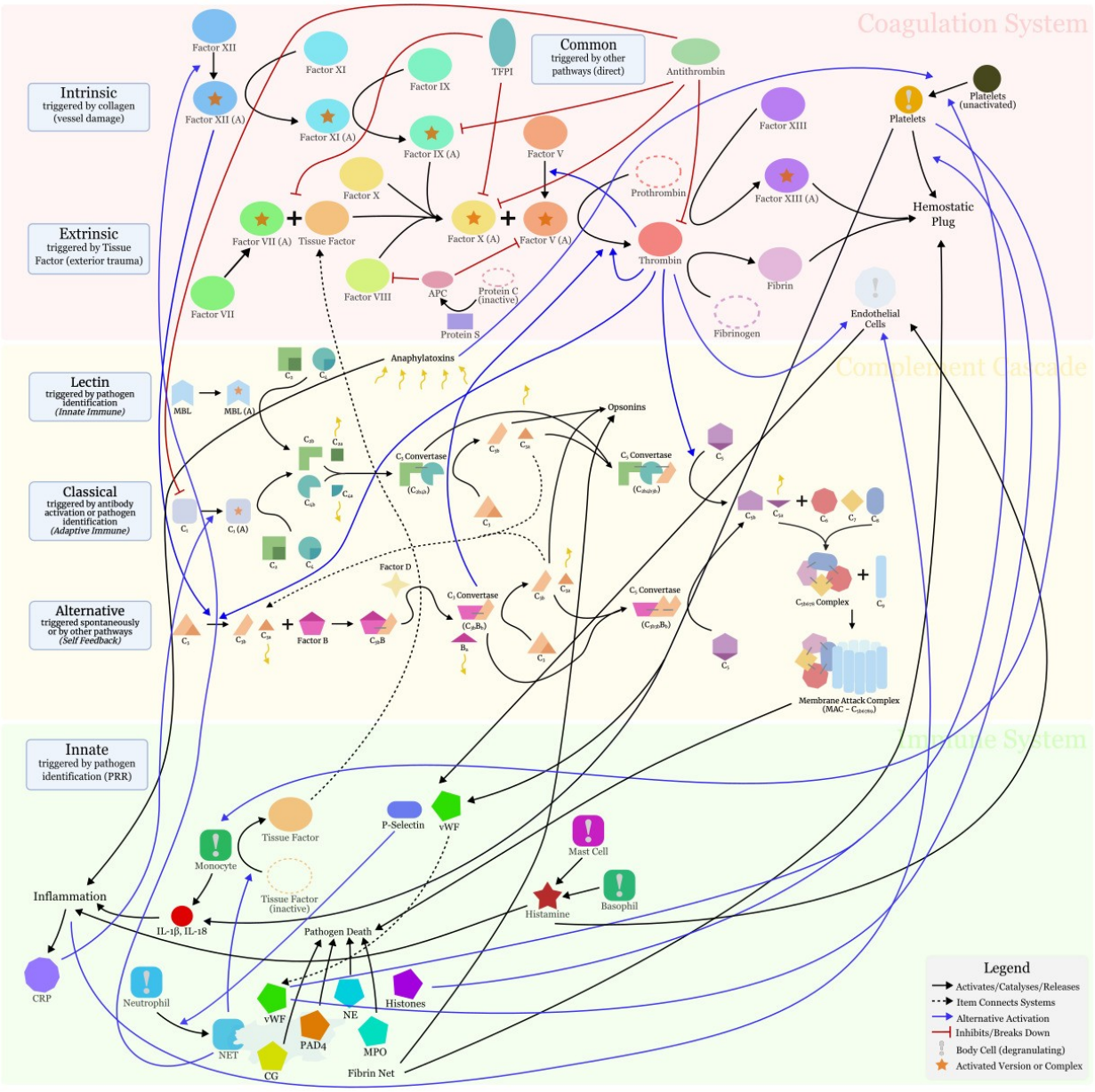

Figure 1. Connections between the Coagulation and the Immune systems (including complement proteins). The shown interactions are not exhaustive (1,3,6,7,10,14). This diagram was partially made using the BioRender.com software.

Discussion

Therapeutic approaches to immunothrombosis

The relevance of immunothrombosis as evidenced in the COVID-19 pandemic has increased the focus on therapeutic approaches to aid patients (11). Treatments center on blocking or inhibiting the key effectors of immunothrombosis, including specific factors in inflammation - such as NETs - in order to mitigate any negative impact on the host. Due to the bidirectional relationship between inflammation and coagulation, immunothrombosis can be caused by a large number of unique triggers. Often, atypical triggers - such as cytokines activating protease receptors – have not yet been sufficiently researched in order for effective treatments to emerge.

In the following two sections, we examine the current treatment approaches targeting specific factors of immunothrombosis, specifically through NETs and coagulatory mechanisms. By taking into account these existing immunothrombosis treatments, we then consider how treatments can be best utilized in patients.

Targeting inflammation: NETs

In the context of the many immune related triggers of immunothrombosis, NETs are among a crucial trigger to target, due to their ability to significantly promote the positive feedback loop between coagulation and inflammation, particularly as occurs during NETosis. A current NETosis inhibitor is DNase: a nuclease, made by the pancreas and salivary glands, that degrades the DNA phosphodiester backbone of NETs through hydrolysis, in a concentration-dependent manner (12).

Recently, recombinant human DNases have been developed, and are commonly used in patients who have a mutation in the DNase sequence - resulting in abnormally low amounts of production and therefore an increased amount of NET production (12). When inhibition of NETs and PAD enzymes successfully occurs, this treatment is effective in fighting against inflammation and NETs in disorders such as colon cancer, multiple sclerosis, and rheumatoid arthritis (13). In tandem with the recombinant human DNases used for the listed disorders and diseases, these DNases are also currently being researched to determine their safety and efficiency in lowering dangerously high NET levels in COVID-19 patients, as well as patients undergoing an exacerbated inflammatory response, as occurs after thrombectomy.

The success in other diseases has supported DNases as an effective treatment option. However, concerns remain about DNase's NET degradation demonstrating potential. NET releasing neutrophils cannot distinguish between therapeutic or pathogen mediated attempts at abrogating the NET response. Hence, they release cytokines, as a defense mechanism, which are equally effective in abrogating therapeutic or pathogen mediated attempts to decrease NET. This could result in further inflammation and autoimmune dysfunction, which could be extensively detrimental (especially in older and/or immunocompromised individuals). There is also a risk of an increased susceptibility to infection, since the PAD enzymes that function in microbial resistance are inhibited.

Targeting Coagulation

Treatments of immunothrombosis, depending on the experienced route of damage, can involve targeting coagulation directly. Warfarin, an anticoagulant drug, is effective at reducing procoagulant factors (prothrombin and Factors VII, IX, and XI), though the exact pathway of action remains unclear. Whether warfarin's inhibition of thrombin production leads to decreased activation of thrombin activatable fibrinolysis inhibitor (TAFI), which then subsequently promotes fibrinolysis (the degradation of clots), is unknown. Regardless, warfarin often showcases effectiveness as a treatment option able to combat immunothrombosis.

However, it is significant to note that only low doses may be given to the patient. This is because warfarin functions in degrading both procoagulant factors as well as in inhibiting anticoagulant mechanisms such as Protein C. Since Protein C has a short half life of only 8 hours, its levels will often decrease faster than the decrease in procoagulant factors and, as a result, in some cases increase coagulation (14). Therefore, since the level of dosage needs to be taken into account, warfarin's status as the most effective option in decreasing coagulation (particularly in COVID-19 patients), is debatable among the scientific community (14).

Another approach in this context includes the use of heparin (another anticoagulant), which induces antithrombin (as well as other anticoagulant factors) to increasingly progress the neutralization of active procoagulants. Histones are also affected, which is noteworthy due to their ability to induce thrombin and coagulation characteristics (NETs) (12).

Heparin takes a different pathway of action than warfarin by regulating a varied range of pro-coagulant factors that may put a patient at risk for extensive blood clotting. This is achieved through inactivation of any proteins and mechanisms that are typically responsible for coagulation. On the other hand, warfarin is effective at decreasing the coagulant mechanisms once coagulation levels are high. If the individual experiencing immunothrombosis has begun to exhibit inflammation and has a high possibility of developing increased coagulation as well, heparin may be a better option; but if clotting rates have already been initiated, warfarin is the more applicable option. As a result, immunothrombosis' variability in the appearance of coagulation makes both drugs equally crucial, depending on the state of the patient. However, neither may be equally effective as a general application for all scenarios.

Treatment checklist

In order to ease the complexity of a medical professional's job, treatments should preferably have been extensively tested and contain as much relevant information on their function before being brought into practice. We have constructed a theoretical model that could be used by physicians and entities involved in drug testing to collect such important information in a consistent manner, in the form of a checklist. Primarily, this checklist serves as a rating system for treatments already in existence in order to test their theoretical viability as a treatment against immunothrombosis. However, the checklist is designed as a proof-of-concept and is not intended to be used in actual medical scenarios in its current state.

The checklist is divided into three specific categories. The first, titled "Immune System Interactions," includes items relevant to the immune system. Likewise, the second, titled "Coagulation System Interactions," includes items related to the coagulation system-side of immunothrombosis. The last section, titled "General Physiology," is included as a reminder that immunothrombosis is often a full-body event that takes into account many more systems than what treatments typically focus on. For example, a treatment designed for an otherwise healthy individual with minor clotting may not take into account a compromised immune system that can be associated with immunothrombosis (similar to a cytokine storms or cell exhaustion) and therefore can possibly cause further difficulties for the patient.

When using the checklist, the medical professional, pharmaceutical company, or other group interested in checking, designing or repurposing a treatment will go down the list, checking existing documentation or conducting new research for each item. If the statement in that item is decided to be true, that box is checked. If the user determines the statement to be false, that box is to be crossed. If there is no convincing available information on the topic and the user is unable to create the suggested scenario, that box is left blank.

Based on the completed checklist, the user can examine the results of each item within their respective categories to consider the theoretical effectiveness of the treatment. High scores within a section (indicated by a high number of checked boxes) may suggest that the treatment could be effective against those certain immunothrombosis scenarios. Conversely, lower scores within that area (indicated by a high number of crossed out boxes) may suggest that the treatment would likely not be effective in fighting that particular immunothrombosis presentation. Lastly, an empty score (indicated by a high number of open boxes) implies the need for further relevant research in order to determine the treatment's possible effects in this area. There is no definite cutoff for any of these conclusions, as the checklist is intended to serve as an analysis tool, not as a definitive decision maker. Instead, a reviewer can use the provided information when considering whether to move forward with embarking on a particular course of treatment in specific immunothrombosis settings, interpreting it through their own judgment.

The appendix presents two sample checklists (tables 3 and 4): one for a commonly used immunothrombosis drug and one that has minimal documented relevance. Note that we do not have the capability to run actual tests and are not trained medical professionals, so the information available in these samples is based purely off of public documentation and is not to be taken as fact, but instead shows how a user might go about employing the checklist.

Table 1. Immunothrombosis treatment development checklist

Immunothrombosis Treatment Development Checklist Guidelines: Check the boxes below if the suggested treatment for immunothrombosis encompasses any of the listed characteristics. Checking a box is designed to present a gap or aspect of the treatment to possibly consider before presenting it to investors, committees, or patients. ** - If treatment uses a drug, consider these* *† - If treatment uses a surgical procedure, consider these* *‡ - If treatment uses living (biotic) matter, consider these* *§ - Applies to all treatments* This tool is a proof of concept, it is not to be used for actual medical practices.		
Immune System Interactions	**‡ A mixture of isolated macrophages and the treatment contains elevated levels of cytokines (primarily IL-1, IL-6, TNF-α, and IFN-α)* [1]	☐
	**‡ A mixture of isolated neutrophils and the treatment contains elevated levels of TNF-α, PADs, or cytotoxins (e.g. granzymes and perforin)* [2]	☐
	**‡ A mixture of neutrophils and treatment contains elevated levels of NET condensed chromatin (or some other indicator of increased NETosis)* [3]	☐
	**‡ A mixture containing the treatment, MBL, C_1, C_2, and C_4 contains elevated levels of C_3 Convertase or anaphylatoxins (C_{2a} and C_{4a})* [4]	☐
	**‡ Treatment results in overactive adaptive immune activity, such as that expressed during an allergic reaction* [5]	☐
	**‡ A solution with the treatment and blood (or isolated C_3) contains substantial quantities of C_{3a} and C_{3b}* [6]	☐
Coagulation System Interactions	**‡ Blood treated with the treatment scores abnormally on a thrombin time blood test* [7]	☐
	**‡ Blood treated with the treatment scores abnormally on a coagulation factor test* [8]	☐
	**‡ A mixture with a blood sample and the treatment continues to contain elevated levels of coagulation factors (Protein C, Factor X, etc) for an extended time (more than 8 hours)* [9]	☐
	**† A mixture with a blood sample and the treatment contains abnormal levels of platelet function/concentration* [10]	☐
General Physiology	*§ Negative side effects are known to worsen following treatment (such as infection)* [11]	☐
	§ Treatment requires additional medications or procedures in addition to the original (at the same time, e.g. antibiotics) [12]	☐

Table 2. Reasoning behind sections on the checklist in Table 1.

Reasoning Behind Sections on Checklist Below is the reasoning behind the descriptions chosen in each section of the checklist. The number on the left corresponds to the superscript found in the relevant box above.	
1.	*Although many cells in the body produce cytokines, macrophages are one of the prime producers of such responses, and so are appropriate places to start a search. Interleukins, interferons, and tumor necrosis factors are common examples of indicators that an immune response has been triggered.* (15)
2.	*Although often deactivated, during an infection (or some other kind of immune distress) neutrophils are a key component of the immune defenses, and will be found in most tissues and fluids. As such, moderation of neutrophil-related responses (such as through general cytokine production) is important to consider when developing treatments.*
3.	*Although often deactivated, during an infection (or some other kind of immune distress) neutrophils are a key component of the immune defenses, and will be found in most tissues and fluids. As such, moderation of neutrophil-related responses (primarily the triggering of NETs due to their close connection to immunothrombosis) is important to consider when developing treatments.*
4.	*Activation of the complement cascade will result in both coagulative and immune activities, and therefore should be considered with caution. C_1, C_2, and C_4 are the primary instigators of the classical pathway, and therefore should be monitored for this reason. Since naturally the pathway can be activated by certain carbohydrates, this test will check for similar compounds. MBL, C_2, and C_4 are the primary instigators of the lectin pathway, and therefore should be monitored for this reason. Since naturally the pathway can also be activated by certain antibodies, this test will also check for overactive antibodies.*
5.	*Although difficult to directly predict or classify, allergic reactions (and other activity from granulocytes and lymphocytes) can be very dangerous in a patient.*
6.	*Activation of the complement cascade will result in both coagulative and immune activities, and therefore should be considered with caution. C_3 is a critical component at the center of all pathways of the complement cascade, and can also be activated directly by coagulation factors (thrombin).*
7.	*Thrombin time is a test done on a blood sample that tests the activity of fibrinogen after a standardized addition of thrombin. Abnormal results may indicate coagulatory disruption caused by the treatment in question, with normal results falling around 12-19 seconds. Other tests can be done for a similar effect, like prothrombin time (PT) and activated partial thromboplastin time (PTT).* (16)
8.	*There are many types of coagulation factor tests, including INR time, complete blood count (CBC), and Factor V assay. Improper concentrations of blood cells or coagulation factors may indicate coagulatory disruption caused by the treatment, and therefore a worth taking into account when analyzing the effects of the treatment.* (17, 18)
9.	*Several relevant proteins have half lives of 8 or less hours, and therefore this may be an effective initial measurement period. However, others have much longer periods. Immunothrombosis involves the coagulation and inflammation crosstalk at high levels of clotting and inflammatory responses, due to a positive feedback loop. Both systems continuously depend on the other to sustain the ability to induce responses. It's significant to consider that an anticoagulant treatment that fails to lower thrombin level or coagulation factor level may not have accounted for both sides of the crosstalk. This is because high levels of inflammation may continue to activate coagulation.* (19)
10.	*Treatments such as anti-platelet drugs function in decreasing platelet numbers. This remains effective in reducing extensive platelet action that had resulted in high levels of clotting, often seen in*

	immunothrombosis. However, if an incorrect treatment dosage is given, it may overly eliminate platelet level. This will result in dangerously high levels of bleeding if the coagulation and inflammation crosstalk remains.
11.	*Some treatments are associated in some way with certain negative side effects. For example, surgery puts individuals into a situation where they are more vulnerable to infection. When considering a treatment for immunothrombosis, the possibility of these side effects being more dangerous than previously thought should be seriously considered.*
12.	*The more complicated a solution gets, the more things can go wrong. The ideal solution would be a single effector and straight-forward, which allows for more complete testing (for example, one of the secondary treatments may react with the original, along with one of the body's components, to present challenges). Additionally, drugs like antibiotics should be used sparingly in general.*

Treatment flowchart

Due to the large number of therapeutic options already available to treat immunothrombosis, it can be difficult to find the right treatment for each patient and for the right scenario or presentation. We hence propose the following treatment algorithm flowcharts with the purpose of showing medical professionals the specific characteristics of coagulation and inflammation that may be fueling the crosstalk in a patient (considering that immunothrombosis has many triggers), as well as suggesting relevant treatments for the patient's state and presentation.

Figures 2, 3, and 4 are sub-parts of one large flowchart meant to encompass a large number of possible tests. Based on whether a patient has been considered for treatment due to symptoms of abnormal coagulation or abnormal inflammation, a medical professional will run the listed tests. The result of the test, whether abnormal (high or low) or normal, will dictate the next steps to take (such as the corresponding treatment or running another test). Figure 5 provides an alternative flowchart that takes advantage of several known pathways in order to streamline the diagnostic process. In either chart, as a treatment provider travels further down the boxes, they will ideally be able to identify the specific trigger in the patient's body that is likely causing the imbalance and identify the relevant treatment routes that can be taken to address it. However, if the flowchart's results indicate no major correlation to immunothrombosis, the treatment provider can also refer to the bottom of the chart, where diseases and disorders with similar symptoms are listed.

A box listed as *Other Possible Tests* is also included for each section, with more tests for other, less common immunothrombotic characteristics. Nevertheless, with the understanding that there are numerous triggers to immunothrombosis, it should be considered that a patient may experience a characteristic that is not addressed in the flowchart.

A major drawback of these models is the impracticality of running multiple tests one after another, considering the availability, cost, and time required. Not all health providers have these resources, especially in underfunded or overcrowded locations. Additionally, patients undergoing immunothrombosis-related stress are likely to be in immediate danger and require action very quickly, so they cannot afford the time it takes for test results to return. As such, a symptomatic approach to treatment

is more advised in most scenarios. Considering these substantial flaws, the charts found below are intended as proofs of concept meant to show the possibility of addressing immunothrombosis with more precise treatments, not as actual guidelines.

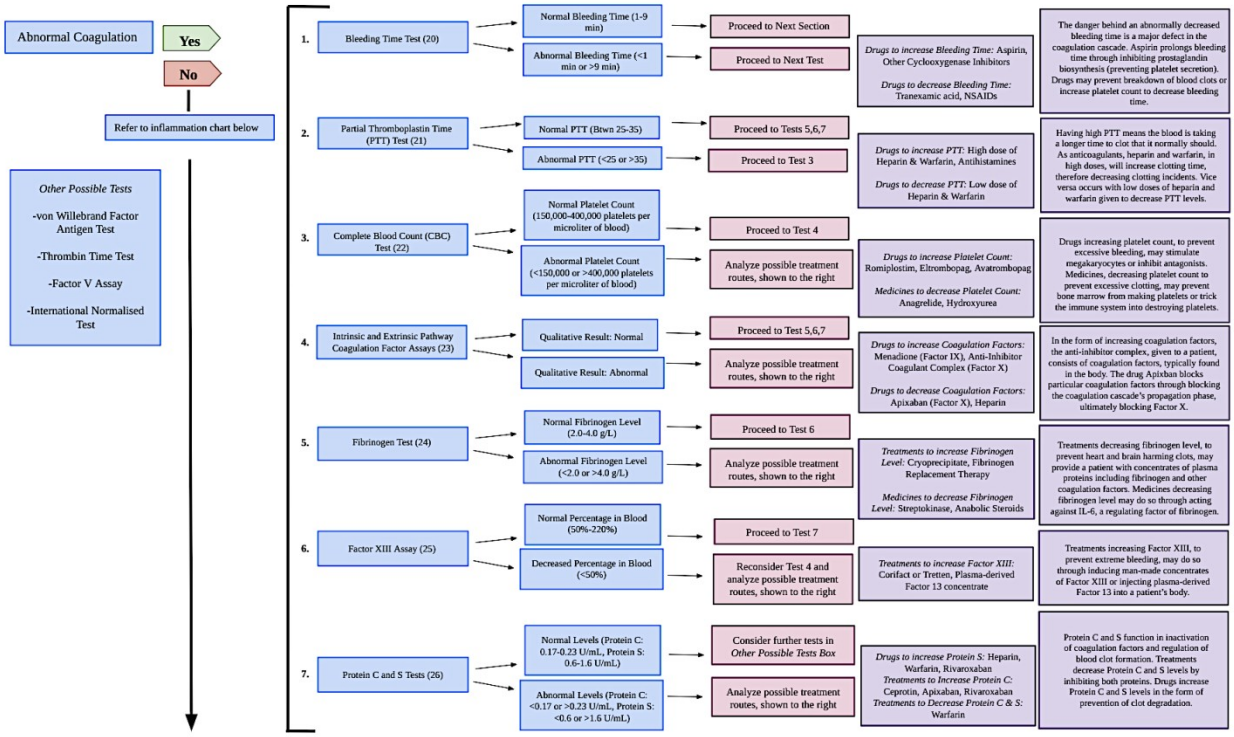

Figure 2. Coagulatory aspect of immunothrombosis

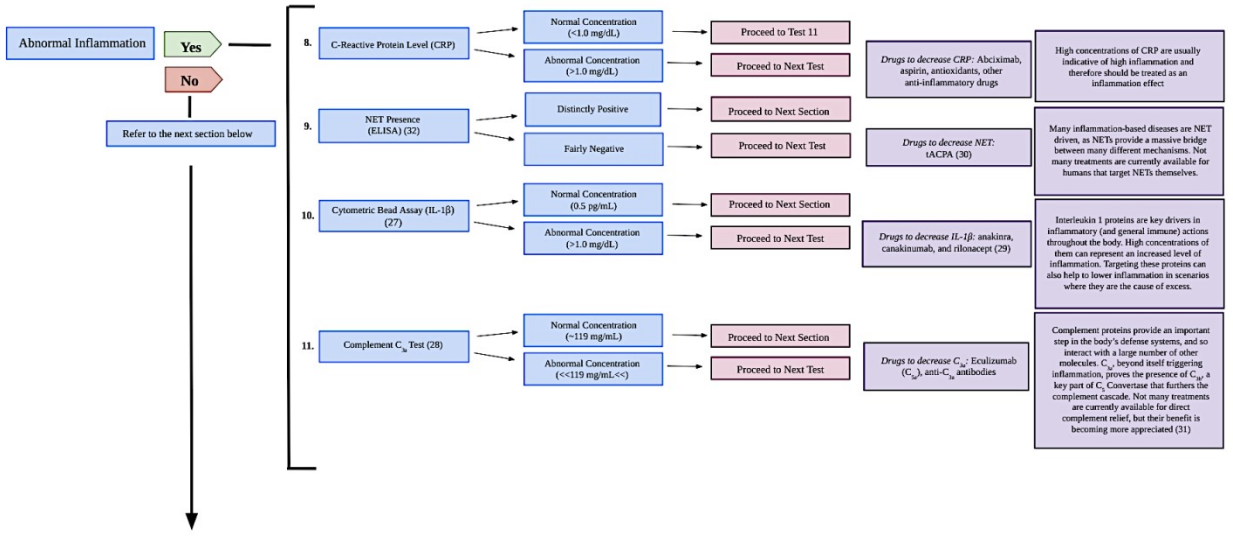

Figure 3. Inflammatory aspect of immunothrombosis (Continuation of Figure 2)

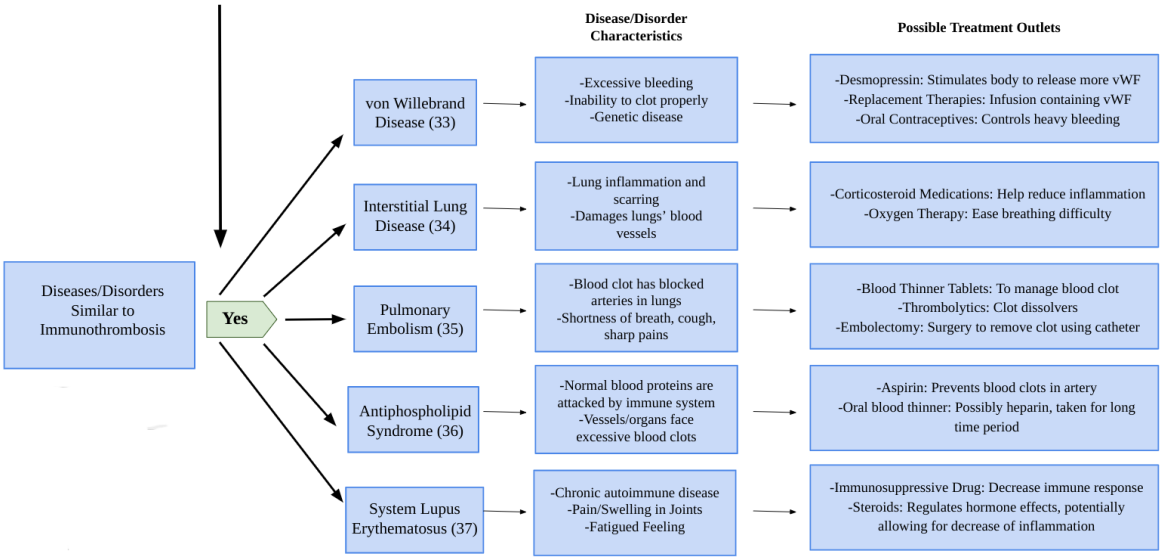

Figure 4. Treatment routes of diseases or disorders similar to immunothrombosis (Continuation of Figure 3)

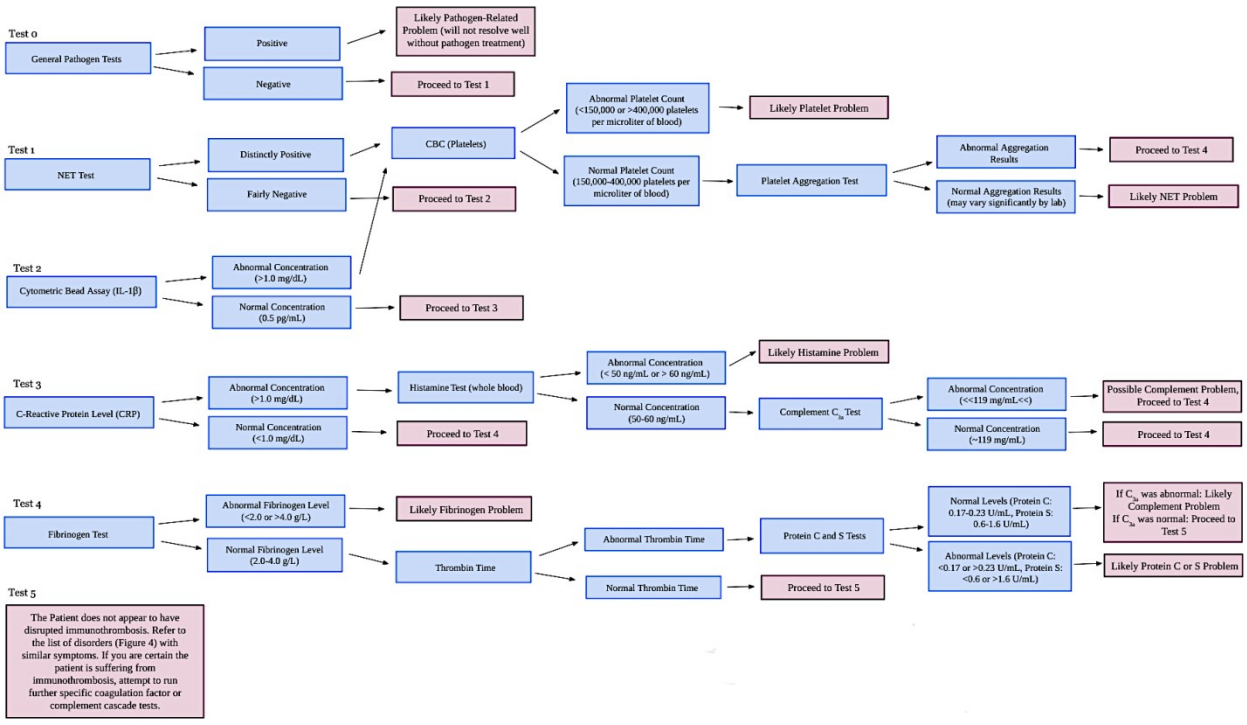

Figure 5. A different format to organize the treatment algorithm flowchart

Conclusion

The immune and coagulation systems are crucial to the everyday survival of organisms. Composed of many intricate processes and pathways, together they are extremely effective at maintaining homeostasis even in the presence of immense stress. The many modes in which they can be activated include mechanisms like thrombin, tissue factor, and platelets working to induce inflammatory cytokines, as NETs and vWFs activate platelets as well as other coagulation factors. These examples of interactions only account for a few of the many other forms in which the crosstalk may be triggered. However, this relationship can become dangerous in the form of immunothrombosis: when the systems become dysregulated. Therefore, considering the varied modes of activation, the treatment approaches that already exist can fail to consider less common triggers of immunothombosis, such as the involvement of thrombin and protease receptors. Beyond not strengthening our understanding of these systems, this shortfall could potentially sabotage the effectiveness of the administered treatments in a patient due to a lack of identification of the fundamental problem.

Since it is often superimposed on other diseases and/or conditions, immunothrombosis may be overlooked or misdiagnosed. Retrospective analysis indicates that immunothrombosis continues to appear in many places, such as in cancerous cells that release inflammatory cytokines that induce tissue factor (38). With future experimentation, it is important to have an increasing focus on inspecting the entangled systems of segments that make up the complex structure of the human body and the gaps in research that continue to exist within the intricate relationship between coagulation and inflammation. To aid in this search, this article outlines a general guide to providing treatments based on immunothrombosis pathways through a flowchart and a checklist for testing proposed solutions. A greater understanding of the complex relationships between the immune and coagulation systems may bring to light a more rounded and nuanced picture of the workings of immunothrombosis and provide better opportunities for the development of safe and efficient treatments.

Abbreviations

APC: “Activated Protein C” is a coagulation regulator protein (natural anticoagulant), CBC: “Complete Blood Count” is a wide-range general blood health blood test, CG: “Cathepsin G” is a protease similar to NE that is released in NETs, COVID-19: “Coronavirus Disease 2019” is the disease caused by the SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) Virus and was responsible for the 2020 pandemic, CRP: “C-Reactive Protein” is a blood plasma protein whose concentration is positively connected to the extent of inflammation, $C_{1\text{-}9}$: “Complement Proteins 1 through 9” make up the majority of the complement cascade, ELISA: “Enzyme Linked Immunosorbent Assay” detects and counts certain antibodies, antigens, proteins and hormones, FVII-XIII: “Factors 7 through 13” are important coagulation factors, IFN-α: “Interferon Alpha” is an innate antiviral cytokine, IL-1β/18: “Interleukins 1β and 18” are common proinflammatory cytokines. See Figure 1 in (14) for a more intricate model, IL-1/6: “interleukins 1 and 6” are families of proinflammatory cytokines, INR: “International

Normalized Ratio" is a blood clotting time test based off of PT results, MBL: "Mannan-Binding Lectin" is a key complement trigger that detects common pathogen-related molecules, MPO: "Myeloperoxidase" is a cytotoxic enzyme released in NETs, NE: "Neutrophil Elastase" is a protease released in NETs, NET: "Neutrophil Extracellular Traps" are networks of extracellular fibers, primarily composed of DNA from neutrophils, which bind pathogens, NSAIDs: "Nonsteroidal anti-Inflammatory Drugs" are a common family of diverse drugs, PAD-4: "Peptidyl-Arginine Deiminase 4" is a key enzyme involved with NETs, PAR: "Protease-Activated Receptors" are a family of receptors important in inflammation pathways, PT: "Prothrombin Time" is a blood clotting time test, PTT: "Partial Thromboplastin Time" is a blood clotting time test, tACPA: "Therapeutic anti-Citrullinated Protein Antibody" is an experimental NET-related drug (see (36)), TF: "Tissue Factor" is an important coagulation factor, TFPI: "Tissue Factor Pathway Inhibitor" is a coagulation regulator protein (natural anticoagulant), TNF-α: "Tumor Necrosis Factor Alpha" is an innate and inflammation cytokines, vWF: "von Willebrand Factor" is an important coagulation factor

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Appendix

Table 3. Heparin as a candidate for the checklist

HEPARIN **Immunothrombosis Treatment Development Checklist** *This tool is a proof of concept, it is not to be used for actual medical practices.*			
Immune System Interactions	**‡ A mixture of isolated macrophages and the treatment contains elevated levels of cytokines (primarily IL-1, IL-6, TNF-α, and IFN-α)*		Little research is available on this topic. Macrophages created while heparin is present can show an anti-inflammatory tendency (39).
	**‡ A mixture of isolated neutrophils and the treatment contains elevated levels of TNF-α, PADs, or cytotoxins (e.g. granzymes and perforin)*		Little research is available on this topic. Heparin has been shown to decrease some neutrophil activity (40).
	**‡ A mixture of neutrophils and treatment contains elevated levels of NET condensed chromatin (or some other indicator of increased NETosis)*	☐	No experimental data on this topic could be found
	**‡ A mixture containing the treatment, MBL, C_1, C_2, and C_4 contains elevated levels of C_3 Convertase or anaphylatoxins (C_{2a} and C_{4a})*	☐	No experimental data on this topic could be found
	**‡ Treatment results in overactive adaptive immune activity, such as that expressed during an allergic reaction*		Heparin has been known to frequently cause allergic reactions in some patients (41).
	**‡ A mixture with the treatment and blood (or isolated C_3) contains substantial quantities of C_{3a} and C_{3b}*	☒	Heparin inhibits the production of thrombin, which allows for the splitting of C_3, so directly influences the complement cascade (42).
Coagulation System Interactions	**‡ Blood treated with the treatment scores abnormally on a thrombin time blood test*	☒	Heparin had increased thrombin time to the normal thrombin time range of 12 to 19 seconds. Scores were generally Normal.
	**‡ Blood treated with the treatment scores abnormally on a coagulation factor test*	☒	Heparin will typically produce normal resultson Factor X assay and prothrombin time test, both types of coagulation factor tests. However it will not impact other major coagulation factors, meaning abnormal scores may remain

			on those tests
	**‡ A mixture with a blood sample and the treatment continues to contain elevated levels of coagulation factors (Protein C, Factor X, etc) for an extended time (more than 8 hours)*	☒	Heparin typically begins *working* within 1-2 hours upon injection. Therefore, if applicable dosage is given elevated levels should lower before 8 hours
	**† A mixture with a blood sample and the treatment contains abnormal levels of platelet function/concentration*	☒	Typically as thrombin is inhibited platelet function/concentration should decrease, due to decreased coagulation *However, in the event of the patient experiencing Heparin Induced Thrombocytopenia (HIT), platelet function /concentration will increase to abnormal amounts (43).
General Physiology	*§ Negative side effects are known to worsen following treatment (such as infection)*	☐	In general heparin is a successful drug with small side effects. Not enough information could be found to conclusively label this box.
	§ Treatment requires additional medications or procedures in addition to the original (at the same time, e.g. antibiotics)	☒	Heparin does not usually require additional medications or procedures.
Conclusion Heparin demonstrates the ability to significantly decrease coagulation, which as a result can be inferred to lower inflammation. The possibility of side effects such as allergic reactions and/or the chance of leading to HIT remain. Overall, heparin appears to be a generally effective and safe drug to be used against immunothrombosis, in a coagulatory sense.			

Table 4. Acetaminophen as a candidate for the checklist

ACETAMINOPHEN **Immunothrombosis Treatment Development Checklist** *This tool is a proof of concept, it is not to be used for actual medical practices.*			
Immune System Interactions	**‡ A mixture of isolated macrophages and the treatment contains elevated levels of cytokines (primarily IL-1, IL-6, TNF-α, and IFN-α)*	☒	Elevated levels of cytokines, as a result of acetaminophen, only occur during high dosage of the drug in the form of intoxication (44)

	**‡ A mixture of isolated neutrophils and the treatment contains elevated levels of TNF-α, PADs, or cytotoxins (e.g. granzymes and perforin)*	☒	Only an overdosage typically results in a local inflammatory response, and ultimately neutrophil accumulation in the liver (45).
	**‡ A mixture of neutrophils and treatment contains elevated levels of NET condensed chromatin (or some other indicator of increased NETosis)*	☒	Generally, acetaminophen results in this state of NETosis or NET condensed chromatin, during overdosage, without a high level of relevance during normal dosage.
	**‡ A mixture containing the treatment, MBL, C_1, C_2, and C_4 contains elevated levels of C_3 Convertase or anaphylatoxins (C_{2a} and C_{4a})*	☒	Typically, reactions in relevance to anaphylatoxins and C_3 Converstase do not occur during normal acetaminophen dosage (46).
	**‡ Treatment results in overactive adaptive immune activity, such as that expressed during an allergic reaction*		Acetaminophen has been reported to having inflammatory properties, developing symptoms such as swelling of the face, throat, ankles, or lower legs
	**‡ A mixture with the treatment and blood (or isolated C_3) contains substantial quantities of C_{3a} and C_{3b}*	☐	Much experimental data on this topic could not be found.
Coagulation System Interactions	**‡ Blood treated with the treatment scores abnormally on a thrombin time blood test*	☒	Generally only in the case of overdosage, acetaminophen will have a possible result of prolonged thrombin time. This increases accounts of bleeding. Typically acetaminophen is not relevant in targeting coagulatory mechanisms.
	**‡ Blood treated with the treatment scores abnormally on a coagulation factor test*	☐	Acetaminophen is generally not relevant in targeting coagulatory mechanisms, as it is typically used for pain and fever filled situations.
	**‡ A mixture with a blood sample and the treatment continues to contain elevated levels of coagulation factors (Protein C, Factor X, etc) for an extended time (more than 8 hours)*	☐	Acetaminophen is generally not relevant in targeting coagulatory mechanisms, as it is typically used for pain and fever filled situations.

	**† A mixture with a blood sample and the treatment contains abnormal levels of platelet function/concentration*	☒	Generally only in the case of overdosage, acetaminophen will have a possible result of inhibiting platelet aggregation, resulting in high accounts of bleeding. Typically acetaminophen is not relevant in targeting coagulatory mechanisms.
General Physiology	*§ Negative side effects are known to worsen following treatment (such as infection)*	☒	Acetaminophen is typically successful at fighting pain and fever symptoms. Its effects typically are consistent at lasting for 4-6 hours, and even 8 hours depending on the dosage amount (47).
	**‡ Requires long-term repeated use of the treatment (e.g. daily drug consumption)*	☒	Apart from constant use every 4-6 hours during time of symptoms (and with consideration of the state of the patient), daily dosage should not be participated in, to avoid dangerous intoxication (48).
Conclusion Acetaminophen showcases an ability in targeting reactions of the immune system such as fever symptoms or inflammation, with little relevance to coagulation, other than indirect effects of fighting inflammation lowering coagulatory symptoms (due to immunothrombosis crosstalk). Since it is built more specifically as a pain and fever fighting treatment, acetaminophen doesn't hold a sense of effectiveness for the coagulation side of immunothrombosis and generally only highlights relevance to immunothrombosis when needing to fight particular immune system reactions in a patient, similar to those of the common cold or a fever.			