

**Title:** Shedding Light on Hemostasis in Patients With Inflammatory Bowel Diseases

Jeremy Lagrange, PhD<sup>1,2,3</sup>, Patrick Lacolley, MD<sup>1,2,4</sup>, Denis Wahl<sup>1,2,5</sup>, Laurent Peyrin-Biroulet, MD<sup>2,6,7</sup>, Véronique Regnault, PhD<sup>1,2,4</sup>

<sup>1</sup> INSERM U1116, Faculté de Médecine, Vandœuvre-lès-Nancy, France

<sup>2</sup> Université de Lorraine, Nancy, France

<sup>3</sup> Center for Thrombosis and Hemostasis, University Medical Center Mainz, Germany

<sup>4</sup> CHRU Nancy, Vandœuvre-lès-Nancy, France

<sup>5</sup> Division of Vascular Medicine, CHRU Nancy, Vandœuvre-lès-Nancy, France

<sup>6</sup> INSERM U1256, Faculté de Médecine, Vandœuvre-lès-Nancy, France

<sup>7</sup> Department of Gastroenterology, CHRU Nancy, Vandœuvre-lès-Nancy, France

**Correspondance:**

Jeremy Lagrange PhD, INSERM 1116 DCAC 9 avenue de la forêt de Haye 54 505  
Vandœuvre-lès-Nancy, France  
jeremy.lagrange@inserm.fr

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**Abstract:** Patients with inflammatory bowel diseases (IBD) have an increased risk of thrombosis, possibly due to changes in blood cells and molecules involved in hemostasis. They have increased platelet counts and reactivity as well as increased platelet-derived large extracellular vesicles. Coagulation is continuously activated in patients with IBD, based on measured markers of thrombin generation, and the anticoagulant functions of endothelial cells are damaged. Furthermore, fibrinogen is increased and fibrin clots are denser. However, pathogenesis of thrombosis in patients with IBD appears to differ from that of patients without IBD. Patients with IBD also take drugs that might contribute to risk of thrombosis, complicating the picture. We review the features of homeostasis that are altered in patients with IBD and possible mechanisms of this relationship.

## **Introduction**

Venous thromboembolism (VTE) affects between 0.5 and 2 persons per 1000 in the general population and is a major cause of death in hospitalized patients.<sup>1</sup> Independent risk factors for VTE include age, sex, genetic factors (such as mutated factor V Leiden and prothrombin G20210A, or deficiency in antithrombin, or protein C or S), surgery or trauma. Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is also an independent risk factor for first and recurrent VTE and large population-based studies have shown that the risks of both pulmonary embolism (PE) and VTE are several-fold higher in patients with IBD compared with the general population.<sup>2</sup> Meta-analyses based on large populations have revealed that the risk for VTE was increased more than two times in IBD patients.<sup>3,4</sup> VTE is a life threatening complication of IBD and hospitalized patients display higher risk of VTE<sup>5</sup>. Indeed, 80% of VTE occurred in patients with active IBD and hospitalized IBD patients with VTE had increased risk of mortality compared to hospitalized IBD patients without VTE (OR = 2.5; 95% CI: 1.83-3.43) and even in comparison to non-IBD patients with VTE (OR= 2.1; 95% CI: 1.6-2.9)<sup>6</sup>. IBD leads to an increased risk of recurrent VTE compared to patients with no IBD. After a first VTE event the probability of recurrence 5 years after discontinuation of anticoagulation therapy was higher among patients with IBD than patients without IBD.<sup>7</sup> Disease activity in IBD is also an independent risk factor for VTE. Venous events are not the only thrombotic complications in IBD patients: arterial thrombotic events (myocardial infarction, splanchnic ischemia, stroke) are also increased in IBD patients and in particular in hospitalized patients.<sup>8</sup> Active disease amplifies the arterial thrombotic risk in IBD as well as for VTE. In addition, the VTE risk directly related to the disease, recent reports suggest that newly developed drugs could also participate in the increased risk of VTE<sup>9,10</sup>. Contrary to the general population a large group of IBD patients develop VTE for no identifiable reason<sup>11</sup>. Herein we provide an extensive review of data collected in IBD patients about hemostasis alterations that we will separated between (1) platelets, (2) coagulation, and (3) fibrinolysis, in order to summarize the relevant parameters implicated in increased VTE risk.

## Platelets

### *Quantitative, qualitative and functional platelet alterations*

Increased platelet count in IBD was first reported in 1968<sup>12</sup> and described since in many other studies (**Figure 1**)<sup>13-18</sup> and was regarded as reactive thrombocytosis. Platelet count was considered as a marker to distinguish IBD from infective diarrhea.<sup>19</sup> In the dextran sulfate sodium (DSS)-induced mouse model of colitis, platelet count and platelet turnover were increased.<sup>20</sup> Both circulating activated platelets and platelets aggregated with neutrophils, monocytes, or lymphocytes were elevated following DSS treatment.

Mean platelet volume (MPV) is also affected by reactive thrombocytosis. MPV decrease is associated with an increased thrombopoiesis. There are two possible explanations for this MPV decrease in IBD: elevated inflammation activates megakaryopoiesis leading to an accelerated production and decreased half-life of platelets<sup>21</sup>, or, larger platelets that are known to be more active could infiltrate sites of inflammation leading to a lower MPV of blood platelets. MPV is related both to inflammatory and thrombotic disorders.<sup>22</sup> MPV was often reported as being decreased and associated with disease activity in IBD.<sup>23-25</sup> However, a sex difference might exist with females being less affected by MPV variations.<sup>17</sup> Interestingly MPV was found to be a good predictor in CD patients for sustained response to anti-tumor necrosis factor (TNF)- $\alpha$  therapy (Infliximab).<sup>26</sup>

Concerning platelet functions, activation and aggregation were found to be increased in IBD with an effect of diseased activity.<sup>27</sup>

Others found an increased aggregation response even in inactive-phases.<sup>18</sup> Markers of platelet activation such as platelet factor 4 (PF4) were found to be increased and even correlated with activity of the disease.<sup>27,28</sup> It was shown that without any stimulation, platelets from IBD patients presented spontaneous aggregation and hypersensitivity.<sup>29</sup> More recently protease-activated receptors (PAR)-1 and -4 that mediate thrombin signaling were found to increase platelet responsiveness in whole blood platelet aggregometry in CD.<sup>30</sup> Another factor that could influence platelet reactivity is von Willebrand Factor (VWF). Endothelial cells produce 80% of total VWF, the remaining 20% being synthesized by megakaryocytes and stored in platelets. VWF is important for thrombus formation via binding and transport of factor VIII (FVIII) and by mediating platelet activation and adhesion via the platelet receptor glycoprotein (GP) Iba.<sup>31</sup> IBD are associated with increased circulating levels of VWF and a decrease in the level and activity of a disintegrin and metalloprotease with thrombospondin repeats, member 13 (ADAMTS13)<sup>32</sup>, which cleaves large VWF multimers to smaller, less-active multimers, thereby determining VWF hemostatic and proinflammatory potential. VWF plasma levels are also increased with disease activity in IBD.<sup>33</sup> The complex formed between VWF and ADAMTS13 was increased in active and in inactive disease states.<sup>32</sup> In UC patients an increased VWF in active disease was considered as a marker of endothelial dysfunction triggered by systemic inflammation.<sup>34</sup> Importantly a lower incidence of IBD in patients with inherited bleeding disorders, such as hemophilia A (FVIII deficiency) and B (FIX deficiency), and von Willebrand's disease has been reported.<sup>35</sup> Finally, in mice lacking ADAMTS13 DSS-induced colitis was worsened while administration of ADAMTS13 decreased colitis severity indicating a direct role of VWF in IBD pathogenesis.<sup>36</sup> Platelet contribution to inflammation as well as platelet-derived large extracellular vesicles (P-LEV) in IBD are described in the supplement.

*In summary, IBD patients present clear increased platelet count and decreased MPV with higher reactivity which could participate in the increased thrombotic risk. Inflammation increases platelet responsiveness through CD40 and PARs pathways and P-LEV having an important procoagulant activity are highly increased. Moreover, given the involvement of platelets in coagulation, inflammation and immune responses, antiplatelet drugs could positively affect the clinical course of IBD by reducing both the procoagulant and inflammatory states. Interestingly, less frequent rectal bleeding was reported in IBD patients taking antiplatelet therapy which highlights the possible role of platelets as modulators independent of their hemostasis function.<sup>37</sup> In the general population platelet count was found to be associated with arterial thrombosis but not with venous thrombosis<sup>38</sup> while in IBD patients platelet count was associated with the overall risk of thrombosis.<sup>39</sup>*

## **Coagulation**

Inhibition of the coagulation cascade has been taken into account for a long time by physicians dealing with IBD. The use of low-molecular weight heparin (LMWH) is part of the common prophylaxis administered to all patients hospitalized following disease flare. The benefit of LMWH is still discussed but several studies reported a use in active but not in moderately active UC.<sup>40,41</sup> A summary of the primary and secondary hemostasis is visible in the supplement.

### *In vivo coagulation activation and thrombin formation*

*In vivo* markers of thrombin generation, prothrombin fragments 1 and 2 (F1+2) generated during the conversion of prothrombin to thrombin and thrombin-antithrombin complexes (TAT) have been measured in some publications (**Table 1**). All publications reported an

elevation of TAT and F1+2 both in active CD and UC while changes were not clear in inactive diseases.<sup>15-17,42-44</sup> This implies that there is upregulation of the coagulation system and thus increased thrombin formation during the active disease state, though this is less clear in periods of disease inactivity. Prothrombin concentration was indifferent in IBD patients<sup>45,46</sup>. Concerning the measurements of antithrombin (AT); they showed high variations and were contradictory depending on the study.<sup>13,15,46,47</sup> The thrombin-generating capacity assessed *in vitro* by CAT was not significantly different compared to controls but significantly higher after addition of thrombomodulin (TM), to evaluate inhibition through the APC pathway, and adjustment to CRP levels.<sup>45</sup> A second report described also a markedly increased thrombin generation only in acute-phase IBD.<sup>48</sup> In pediatric patients, thrombin generation was increased but with a prolonged lag time and correlated with disease activity.<sup>49</sup> Therefore, it remains unclear whether the thrombin-generating capacity is increased among IBD patients. Other diseases such as antiphospholipid antibodies and common genetic mutations of coagulation factors (FV Leiden, prothrombin G20210A) affecting thrombin formation and risk of VTE are discussed in the supplement. All together, the current findings on thrombin generation changes are not conclusive to explain the increased risk of VTE and arterial thrombosis in IBD patients despite the increased *in vivo* activation of coagulation highlighted by increased *in vivo* markers.

### *Extrinsic pathway*

In IBD where the thrombotic risk is increased, modifications of an important number of coagulation factors have been reported.<sup>13,14,47</sup> The literature for the coagulation factor changes are presented in the **supplementary table**. TF, the trigger for the extrinsic pathway of coagulation, is present in the sub-endothelial layers of the vascular wall (**Figure 2**). Alterations of the anticoagulant properties of the vascular endothelium are discussed in the supplement.

In blood, the so-called blood-borne TF can be expressed by activated monocytes and by LEV.<sup>50</sup> In IBD, TF activity related to monocytes as well as LEV exposing TF were found to be increased.<sup>51,52</sup> However, LEV exposing TF were not associated with *in vivo* coagulation activation (indirectly accessed by plasma levels of D-dimer, a fibrinolysis product). TF plasma activity was correlated to the level of TAT, which is another marker for *in vivo* coagulation activation, but not with inflammatory markers or clinical activity in IBD.<sup>53</sup>

Interestingly TF was found to be detrimental in experimental colitis.<sup>54,55</sup> The use of an antibody against TF in the DSS model of colitis or genetic ablation of TF with 1% remaining TF activity to avoid embryonic lethality has been reported to limit the immune cell influx into the colon and to lower inflammation and organ damage.

Regarding coagulation FVII which forms a complex with TF to activate FX, contradictory results have been published. Yazici *et al* did not find a significant difference for FVII activity in IBD patients while Hudson *et al* found FVII activity significant increased.<sup>13,56</sup> In both studies a difference appeared between CD and UC patients with a higher procoagulant activity of FVII in CD patients. FX was found to be increased in active UC and increased FV was described for both CD and UC.<sup>14,15,47</sup> Activated FX (FXa) in concert with FVa can convert prothrombin to thrombin, the key enzyme of the coagulation cascade. Activated FX was not correlated to clinical activity of IBD.<sup>53</sup>

### *Intrinsic pathway*

Again the variation of circulating FXII concentration varies in IBD.<sup>15,16</sup> FXI is one of the markedly increased coagulation factors in active and inactive disease (**Figure 2 and supplementary table**).<sup>15,16</sup> FVIII procoagulant activity was reported to be increased in most

publications.<sup>13–15,45,47</sup> The effect of this coagulation factor on the occurrence of deep-vein thrombosis is well documented and could have a pivotal role in the thrombotic risk associated with IBD.<sup>57</sup> However elevated FVIII was not found to be an independent predictor of the risk of recurrent VTE.<sup>7</sup>

TF was not the only coagulation factor capable of limiting DSS-induced colitis in mice. Indeed FIX deficient mice presented a decreased colonic neutrophil infiltration.<sup>58</sup> Moreover, the intestinal epithelium was described to be able to synthesize FIX. Local synthesis of coagulation factors could increase PARs activation and induce detrimental cellular effects. Intriguingly, mice lacking FXII were not protected against DSS-induced colitis.<sup>59</sup> This dichotomy in the intrinsic pathway may be the result of thrombin-driven FXI activation which was described to trigger and amplify vascular inflammation independently of FXII.<sup>60</sup> Indeed, mice lacking FXI were protected against angiotensin II-induced vascular inflammation while mice lacking FXII display normal vascular inflammation. Further studies should focus on the role of ectopic synthesis of coagulation factors triggered by active IBD.

*In summary, no strong definitive data have been published on most coagulation factors and most of the conclusions were based on a limited number of patients. Large-scale studies with higher numbers of patients and systematic measurement of coagulation factors in relation with age and disease activity will be necessary to conclude on the possible causal effect of some coagulation factors in increasing the thrombotic risk in IBD.*

*The relevance of thrombin generation was established in the prediction of a first VTE event but until now no longitudinal studies have been conducted to evaluate the usefulness of this tool to stratify thrombotic risk in IBD patients.<sup>61</sup> Moreover, some coagulation factors (TF, FXa, thrombin) are now well described to have direct or indirect cellular effects through the PARs and could play a role on microvascular alterations present in IBD patients.<sup>62</sup> Finally, other autoimmune diseases, such as the antiphospholipid syndrome, could also have an increased prevalence in IBD and have a role in the pathogenesis of thrombosis. Very few data are available concerning the direct relation between coagulation proteins and VTE risk in IBD patients. Protein S deficiency is associated with a very high VTE risk while other factors as FVIII were not statistically different<sup>39</sup>*

## **Fibrin clot formation and fibrinolysis alteration**

### *Fibrin clot formation*

Discrepant results have been published regarding the circulating concentrations of fibrinogen, some studies reporting increased fibrinogen in CD and UC<sup>13,16,46,49</sup> and others only in CD<sup>13,56</sup> or UC.<sup>17</sup> These differences might result from the fact that fibrinogen concentration is well known to be correlated with inflammation which is highly variable depending on the disease activity state. In order to stabilize the fibrin clot, FXIII forms cross-links with fibrin polymers. FXIII was decreased in active IBD patients and correlated with disease activity.<sup>44,63,64</sup> Decreased circulating levels of FXIII may result from an increased consumption due to the formation of microthrombi.

As for *in vivo* markers of fibrin formation, results from laboratory tests for plasma clotting (APTT: activated partial thromboplastin time, PT: prothrombin time) were not consistent (**Table 1**). Some reported no changes in APTT or PT in CD or UC patients while others presented slightly or significant delayed clotting times.<sup>13,15–17,46,47</sup> No study has presented faster clotting times.

### *Fibrinolysis*

Fibrinolysis is the pathway counteracting coagulation and degrading the fibrin clot. The key molecule of fibrinolysis is plasmin. Its inactive zymogen (plasminogen) is activated mainly by tissue plasminogen activator (t-PA) or urokinase plasminogen activator (uPA). Plasmin in return can be inhibited by plasminogen activator inhibitor-1 and 2 (PAI-1, -2) via inhibition of tPA and uPA. Hypofibrinolysis and alterations in thrombus resolution constitute a risk factor for VTE.<sup>65</sup> As for changes in coagulation, a reduction in the fibrinolytic capacity of IBD patients is not clear but some findings suggest an imbalance of fibrinolysis (**Table 2**). Plasminogen is unchanged<sup>14-16,46</sup> while variations for tPA are inconsistent since some studies show an increase<sup>16,44</sup> and others a decrease for both antigen and activity<sup>46,66</sup> or no variations at all.<sup>67,68</sup> PAI-1 concentrations were measured in several studies and discrepant findings have been reported. However, PAI-1 is most likely increased in IBD.<sup>16,44,46,67-70</sup> Recently Kaiko *et al.* showed that PAI-1 expression was highly elevated in IBD patients with active disease who did not respond to anti-TNF therapy.<sup>71</sup> PAI-1 exacerbated mucosal damage by blocking tPA and inhibition of PAI-1 reduced both mucosal damage and inflammation. PAI-1 can interact with specific matrix components such as vitronectin or low-density lipoprotein-1 and uPA, with its receptor, play a role in cell migration.<sup>72</sup> PAI-1 inhibition was found to decrease vascular smooth muscle cell migration and neointima formation.<sup>73</sup> These cellular effects may have a role in IBD but have not yet been studied. Very recently PAI-1 was discovered to be able to directly bind FXIa on the surface of endothelial cells, thereby inducing its clearance and degradation.<sup>74</sup> This new mechanism emphasizing a crosstalk between coagulation, fibrinolysis and endothelial function may be impaired in IBD patients as they exhibit alterations in all these processes. In line with the PAI-1 increase suggesting a decrease of fibrinolysis, thrombin-activatable fibrinolysis inhibitor (TAFI) was found to be decreased in IBD patients.<sup>69</sup>

Focusing on clot lysis is a more integrative way to evaluate fibrinolysis compared with separate measurements of fibrinolysis-related molecules. Indeed, IBD patients display a denser fibrin network with prolonged clot lysis time (**Figure 3**).<sup>75</sup> Moreover, in IBD patients with a history of thromboembolic events the area under the fibrinolytic curve, which reflects the overall coagulation/fibrinolysis profile, was increased.<sup>75</sup> Fibrinolysis alteration is very likely related to the thrombotic risk associated with IBD and could be used as a parameter for risk assessment.

D-dimer results from fibrin degradation and are fibrinolysis markers. Most studies in IBD patients presented increased D-dimers<sup>13,15,16,44,67,70,76,77</sup> but some did not find any differences.<sup>14,34,47</sup> (**Table 2**). However, in all publications focusing on D-dimers and diseased activity a positive correlation was reported which is also in favor of a continuous activation of the coagulation during disease flares.

*In summary, fibrinogen is increased in active IBD and clot formation is affected with an elevated consumption of FXIII while the coagulation times are not significantly impaired. However, fibrinolysis is decreased as shown with PAI-1 elevation. The balance between coagulation activation and fibrinolysis conterregulation is dysregulated towards a prothrombotic phenotype. In the study of Andrade et al, as for FVIII, fibrinogen concentration was not found to be significantly associated with the occurrence of VTE in IBD patients. Concerning fibrinolysis itself no data are available.*

## Conclusion

In IBD patients VTE may be considered unprovoked in the absence of recent surgery with general/spinal/epidural anesthesia for longer than 30 min, hospital admission and confinement to bed (only bathroom privileges) for acute illness for at least three days, or active malignancy (i.e., no curative treatment received, ongoing curative treatment, or recurrence/progression

despite curative treatment), and patients with an age < 65. In this situation minor clinical risk factors should be investigated together with laboratory workup, including proteins C and S and antithrombin deficiencies, factor V Leiden and 20210 prothrombin gene mutations as well as assays for antiphospholipid antibodies. Unprovoked proximal deep vein thrombosis or pulmonary embolism, recurrent VTE and major thrombophilia such as antithrombin deficiencies or antiphospholipid syndrome may require long term anticoagulant treatment.

On the fundamental side, until now clear changes in the coagulation system which were reported by several groups for active CD and UC include increased TAT, F1+2 and D-dimer for the *in vivo* markers of coagulation and fibrinolysis and elevated circulating FV, FVIII, VWF, fibrinogen and PAI-1. However, coagulation changes are hardly related to the procoagulant state of IBD patients since clotting times are unchanged or even delayed despite an important increase in fibrinogen concentration. Substantial increases in terms of platelet reactivity and the clear increased platelet count in IBD patients argues strongly for a crucial role of platelets in the increased thrombotic risk. P-LEV are also good candidates as contributors to this increased risk since they can act at different vascular beds far from the ones where platelets are aggregated. Fibrinolysis was also identified as strongly altered in IBD. Additionally, it is clear that inconsistent data have been reported in different studies performed on the hemostasis system in IBD. Some of the discrepancies could originate from pre-analytical abnormalities in the preparation of blood and plasma samples. Therefore, further studies should follow the guidelines from the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH)<sup>78-80</sup>. Finally, the endothelial barrier was impaired with decreased surface expression of anticoagulant proteins. So far, no extensive review has been completed on the effect of IBD treatments in active and inactive diseases and on the thrombotic risk. A recent meta-analysis on the VTE risk in IBD after steroid or anti-TNF- $\alpha$  medications reported a higher risk after steroid treatment but a protection with anti-TNF- $\alpha$ .<sup>81</sup> Important open questions are summarized below. This review provides a framework for better understanding of how IBD affects hemostasis and in particular platelet and fibrinolytic function. In the upcoming years, studies should be designed in a way that integrate all blood cells and components in IBD risk stratification since the use of individual plasma components or platelet activation are not sufficient to give a complete overview of the blood-dependent procoagulant state.

### Open research questions

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Could antiplatelet therapy improve disease activity?

Is there an indication for anticoagulant treatment in the active phase of IBD?

How do cellular effects of coagulation factors (tissue factor, activated factor X, thrombin) affect IBD pathogenesis?

Are other autoimmune diseases augmented in IBD and worsening the risk of thrombosis?

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**Search strategy and selection criteria:** Each protein and cell of hemostasis was searched in PubMed with the terms inflammatory bowel disease, Crohn's disease or ulcerative colitis and also with the abbreviation of each term. Only papers published in English were reviewed. The final reference list was generated on the basis of relevance to the broad scope of this Review.

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**Table 1: Clotting times, thrombin generation and markers of *in vivo* thrombin generation in IBD.**

Markers	Population	Observed modification	Ref.
Activated partial thromboplastin time (APTT)	IBD patients	=	13
		=	46
		↗ in active CD and UC females	17
	UC patients	= with UC activity	15
Prothrombin time (PT)	IBD patients	=	13,47
		↗ PT	16
		↗ PT	46
		=	17
Calibrated automated thrombinography (CAT)	IBD pediatric patients	Endogenous thrombin potential ↗ in active and inactive IBD compared to controls	82
	IBD patients	= Endogenous thrombin potential	45
Thrombin-antithrombin complex (TAT)	IBD patients	↗ in active IBD	42
		↗ in active compared to inactive	70
	UC patients	↗ in active and ischemic UC	15
		= in actives UC (high variability)	34
Fragment 1+2 (F1+2)	CD and UC patients	Highly ↗ in active and inactive IBD	16
		↗ in active IBD	42
		↗ in active IBD and with a tendency in inactive IBD	44
		↗ both in CD and UC patients	76
	UC patients	↗ in active compared to inactive IBD	70
		↗ in active and ischemic UC	15
	IBD Young patients	↗ in active but not in inactive IBD	43
		=	67
IBD pediatric patients	correlated with the disease activity	49	

**Table 2: Fibrinolysis markers.**

Marker	Population	Observed modification	Ref.	
Fibrinogen	IBD patients	↗ in CD and UC	16,46,47	
		↗ in active but not inactive IBD	32	
		↗ in CD (and with activity) but not in UC.	13,56	
		↗ in active IBD	17	
		UC patients	↗ in active and ischemic UC	15,34
		CD patients	↗ in active CD patients	43
IBD young patients	IBD pediatric patients	↗ in active disease	67	
		↗	49	
		= in CD increased in UC	14	
Plasminogen	IBD patients	=	46	
		= in active and inactive IBD	16	
		UC patients	= with UC activity	15
Tissue plasminogen activator (tPA)	IBD patients	↗ in active and inactive IBD	16	
		↗ in active = in inactive (antigen)	44	
		↘ antigen	66	
		↘ activity	46	
		= antigen	68	
		Young IBD patients	= antigen	67
Urokinase (uPA)	IBD patients	↘ antigen	68	
TAFI	IBD patients	↘ but not associated with activity	69	
plasminogen activator inhibitor-1 (PAI-1)	IBD patients	↘ antigen	68	
		↗ in active and inactive IBD	16	
		= in active and inactive (antigen)	44	
		↗ with activity (antigen)	69	
		↗ in active compared to inactive	70	
		↗	46	
		Young IBD patients	↗ in active compared to inactive (antigen)	67
D-Dimer	IBD patients	= fibrinogen degradation products	47	
		↗ fibrinogen degradation products	76	
		↗ D-dimer in CD (and with activity)	13	
		↗ D-dimer in active disease and ↗ fibrinogen degradation products	16	
		= D-dimers	14	
		↗ in active compared to inactive	70	
		↗ in active = inactive IBD	44	
		Young IBD patients	↗ in active IBD	67
		CD patients	↗ in active CD	77
		UC patients	↗ in active and ischemic UC	15
	= in actives UC (high variability)	34		

**Figure 1: Platelet alterations in IBD patients.** IBD patients display increased platelet count related to reactive thrombocytosis. Mean platelet volume (MPV) is decreased and platelet-derived large extracellular vesicles (P-LEV) are increased and both are associated with the disease activity. P-LEV have high procoagulant activities and in vivo prothrombin activation is increased in active IBD. Increase in platelet reactivity results from (i) increased circulating concentrations of von Willebrand factor (VWF) and fibrinogen that activate platelet receptors (GPIIb/IIIa and GPIb-V-IX); (ii) activation of thrombin receptor proteases activated receptor (PAR)-1 and -4; (iii) autoactivation through the CD40 ligand (CD40L) released following PAR-1 activation and cleaved from the platelet surface; ie soluble CD40 (sCD40), can in turn activate platelets via CD40. Platelet activation leads to increased released of dense and  $\alpha$  granule contents including fibrinogen, VWF, platelet factor 4 (PF4), factor (F) V, XI, or plasminogen activator inhibitor-1 (PAI-1). VWF-cleaving protease (ADAMTS13) is increased and may limit VWF functions.

**Figure 2. Changes in pro- and anticoagulant reactions in IBD patients.** (A) IBD leads to increased large extracellular vesicles (LEV) bearing tissue factor (TF). TF can also come from subendothelial layers in case of a vascular breach. Endothelium alteration impairs its anticoagulant properties: tissue factor pathway inhibitor (TFPI) activity is decreased. Prothrombin conversion to thrombin activation is increased due to elevated levels of procoagulant factors (FVII, FV, FX). (B) Thrombin activation amplifies its own formation which is exacerbated by increased levels of procoagulant factors (FXI, FXI, FVIII) leading to increased thrombin generation as shown by elevated concentrations in prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin complex (TAT). Shedding of thrombomodulin (TM) and endothelial protein C receptor (EPCR) are increased. The thrombin-induced anticoagulant pathway, the conversion of protein C (PC) to activated PC (APC), is also altered reducing the downregulation of the coagulation cascade. Black curved arrows: “conversion to”; green arrows: “activation of” red T-bar: “inhibition of”. Little black arrows next to the factors or complexes indicate if it is predominately increased or decreased in IBD patients in the literature.

**Figure 3. Fibrinolysis modifications in IBD patients.** Fibrinogen concentration increases with inflammation. Thrombin leads to fibrin fiber polymerization. Thrombin activates also FXIII which crosslinks fibrin in order to stabilize the clot. Fibrinolysis is decreased due to increased plasminogen activator inhibitor 1 (PAI-1) that inhibits tissue plasminogen activator (tPA) and urokinase-type plasminogen activator and, plasmin activation inhibition in IBD. The fibrin network is denser and clot lysis time delayed. Fibrin degradation products (FDP and D-Dimer) hallmark continuous activation of fibrinolysis. TAFI: thrombin-activatable fibrinolysis inhibitor.







