

# Haemostasis Impairment in Patients with Obstructive Jaundice

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## Abstract

As part of the multifactorial role of liver in protein synthesis, many coagulation factors, natural anticoagulants, and compounds of the fibrinolytic system are produced in the liver. A prolonged liver disease, either biliary obstruction or parenchymal liver disease, is consecutively accompanied by abnormal clotting.

In the present paper we review the haemostasis impairment in obstructive jaundice with special reference to the hepatic cirrhosis and failure, to systemic inflammation and sepsis that develops in cholestatic diseases, and finally in some other benign or malignant diseases including pancreatic adenocarcinoma, acute pancreatitis, cholangiocarcinoma, and hepatocellular carcinoma. Finally, a special reference to the possible therapeutic interventions has been made. The aim of the present review is to collect the current concepts concerning the haemostasis impairment in obstructive jaundice and provide practical guidelines for the diagnostic and therapeutic strategies. Understanding the pathophysiology of haemostatic changes in patients with cholestasis, and, more generally, liver disease, is the hallmark of accurate diagnosis and treatment.

## Key words

Obstructive jaundice - hepatocellular carcinoma - pancreatic adenocarcinoma - acute pancreatitis - haemostasis impairment - coagulation - vitamin K

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## The pathophysiology of haemostasis impairment in obstructive jaundice

As part of the multifactorial role of liver in protein synthesis, many coagulation factors (fibrinogen, prothrombin, V, VII, VIII, IX, X, XI, XII, XIII, prekallikrein,

HMWK), natural anticoagulants (antithrombin-III, heparin cofactor-II, Protein C, protein S, TFPI-1, TFPI-2), and compounds of the fibrinolytic system (plasminogen,  $\alpha_2$ -antiplasmin, TAFI) are produced in the liver (1-4). A prolonged liver disease, either biliary obstruction or parenchymal liver disease, is thus accompanied by abnormal clotting, as most usually measured by the prothrombin time and the International Normalized Ratio (INR).

The impaired production of coagulation factors by the damaged hepatocytes is superimposed by the poor absorption of vitamin K due to the absence of bile in the gut. Vitamin K is an essential cofactor for a microsomal enzyme that catalyzes the post-translational carboxylation of multiple, specific, peptide-bound glutamic acid residues in inactive hepatic precursors of factors II, VII, IX, and X. The resulting gamma-carboxyglutamic acid residues convert the precursors into active coagulation factors that are subsequently secreted by liver cells into the blood.

Despite oral (along with bile acids) or parenteral vitamin K administration in patients with obstructive jaundice, the surgeon might still face difficulty in overcoming haemostasis impairment in these patients (5). Bleeding episodes or thrombotic events may further complicate a jaundiced patient. These manifestations need careful clinical and laboratory approach for an accurate diagnosis to be established and an effective treatment to be offered (6).

Bacterial translocation plays a key role in the pathophysiology of haemostasis impairment in patients with obstructive jaundice. Numerous studies have demonstrated that obstructive jaundice significantly promotes bacterial translocation in animal models as well as in humans (7-8). In these cases, gut derived bacteria and endotoxins can cross the mucosal barrier and reach mesenteric lymph nodes or other distant tissues, thus causing a systemic inflammatory response. As a consequence, septic complications and multiple organ failure evolve in a considerably high percentage of these patients. The triggering of coagulation cascade, mainly via tissue factor (TF) pathway, is a key parameter for the final outcome; the extreme and unbalanced production of TF (mainly by TF pathway inhibitor) and the subsequent uncontrolled extrinsic tenase complex activation

may lead even to clinically evident thrombotic events and/or disseminated intravascular coagulation.

Systemic inflammation is also present in two chronic liver diseases accompanied by cholestasis: primary biliary cirrhosis and primary sclerosing cholangitis, in which a hypercoagulable state has been documented (9).

Apart from septic/inflammatory complications, which result in hypercoagulability, the underlying pathology is crucial in determining additional pathophysiological pathways of haemostasis impairment in obstructive jaundice. It is well known that malignant diseases, which cause obstructive jaundice, and especially adenocarcinomas of the pancreas, may affect coagulation in various ways. Additionally, acute pancreatitis (which may be due to choledocholithiasis) has been demonstrated to be accompanied by a prethrombotic state, mainly due to platelet stimulation (10-11).

Thus, the above mentioned mechanisms that affect haemostasis in obstructive jaundice are discussed in the following four paragraphs: the first one refers to vitamin K insufficiency in obstructive jaundice, the second describes the effect of ongoing liver fibrosis and cirrhosis on haemostasis, the third analyzes the interlinkage of sepsis and haemostasis and its clinical significance in patients with obstructive jaundice and the last one focuses on certain entities that manifest with obstructive jaundice and may by themselves interfere with the haemostatic mechanism.

### Vitamin K deficiency in obstructive jaundice

Vitamin K is an essential co-factor for the synthesis of factors II, VII, IX and X, as well as of proteins C, S and Z, as it catalyzes gamma-carboxylation of the glutamic acid in their amino-terminal region. The reduction of these proteins in plasma may reflect vitamin K deficiency. This is not caused by liver injury *per se*, but it is frequently associated with liver disease. In fact, the molecular result of severe vitamin K deficiency is the production of decarboxylated precursors, named PIVKA (precursors induced by vitamin K absence), which have diminished activity (12).

Vitamin K is a fat-soluble vitamin requiring bile salts for its absorption from the gut. The intestinal bacterial flora is involved as well, either participating in the bile salt metabolism or producing small amounts of vitamin K. Thus, reduced intestinal absorption of vitamin K occurs during intra- or extrahepatic cholestasis resulting in vitamin K deficiency in patients with obstructive jaundice. These patients often present haemorrhagic diathesis, despite the presence of a merely normal coagulation profile as estimated by the prothrombin time (13-14).

Apart from the prothrombin time, measurement of PIVKA levels (and especially PIVKA-II, or des-gamma-carboxylated prothrombin) has been used for the assessment of the severity of vitamin K deficiency, but this procedure is prone to errors if hepatocellular carcinoma has not been carefully excluded. The parenteral administration of 10 mg vitamin K

replenishes serum levels, normalizes prothrombin time and prevents from bleeding episodes.

### Progressive hepatic failure and cirrhosis

As liver fibrosis evolves and hepatic failure is settled in a cirrhotic environment, a generalized derangement of haemostasis becomes evident through laboratory tests and, later on, through clinical signs and symptoms. The ongoing evolution toward cirrhosis in a patient with cholestasis is rare in malignant diseases, as the time is too short in most cases. Nevertheless, in other benign models of obstructive jaundice, such as primary sclerosing cholangitis, cirrhosis is the inevitable result. The possibility of cirrhosis should be evaluated in every patient who has a medical record of chronic cholestatic syndrome of undetermined etiology.

Thrombocytopenia in cirrhosis is profound and is mainly explained by the increased platelet sequestration in the enlarged spleen (congestive splenomegaly) (15). Moreover, a reduction in thrombopoietin levels has been suggested to contribute to this anomaly, as liver transplantation increases thrombopoietin levels and reverses thrombocytopenia independently of the size of spleen (16). Nevertheless, conflicting results regarding plasma thrombopoietin levels in chronic and acute liver failure have been published (17-19). Other causes for thrombocytopenia as reduced platelet half-life, presence of autoantibodies, especially in patients with primary biliary cirrhosis or sclerosing cholangitis, folic acid deficiency and ethanol toxicity on megacaryopoiesis, especially in alcohol abusers have been proposed (20-23). Finally, the presence of disseminated intravascular coagulation, even low-grade, is still debatable (24).

Platelet function defects are often encountered in patients with chronic or acute liver disease. In vitro platelet aggregation in response to ADP, arachidonic acid, collagen, and thrombin has been shown to be defective (25,26). Also, platelet-vessel wall interaction studied under flow conditions has been shown to be impaired (27). Impaired aggregation might be caused by defective platelet signal transduction mechanisms, an acquired storage pool deficiency, and decreased levels of arachidonic acid (required for thromboxane A<sub>2</sub> production) in the platelet membrane (28-30). Furthermore, increased production of prostacyclin and nitric oxide (both powerful platelet inhibitors) by the endothelial cells may contribute to impaired platelet function in vivo (31,32). Finally, platelet vessel wall interaction may be defective in patients with liver disease due to proteolysis of platelet receptors by plasmin, or due to the presence of a reduced haematocrit (33-35).

Apart from platelet defects, decreased synthesis of coagulation factors is observed in patients with liver impairment. Merely all proteins that constitute the coagulation cascade are synthesized in the liver and for many of them, the liver is the exclusive site of production. The degree of the reduction of the procoagulants is related to the severity of liver damage, bleeding diathesis and, finally,

prognosis. Usual coagulation tests are not affected until plasma levels of the relevant factors fall below 30-40% of normal and the specific tests for each factor, although available, are not very informative in the routine clinical practice. As factor V and especially VII have the shorter half-lives (12 and 4-6 hours, respectively), their assessment might be helpful in acute liver failure. As factor VII and fibrinogen are acute phase proteins, they are initially increased and their substantial decrease might underline the presence of disseminated intravascular coagulation.

The main qualitative disorder that may accompany liver failure is dysfibrinogenemia, which is characterized by abnormal polymerization of fibrin monomers as a consequence of hypersialylation of the fibrinogen molecule.

Advanced liver disease is also characterized by the presence of hyperfibrinolysis, which is revealed by the shortened euglobin clot lysis time and elevated levels of D-dimers, FDPs and fibrin and attributed mainly to the reduced clearance of fibrinolytic agents, mainly tPA. Additionally, low levels of  $\alpha_2$ -antiplasmin and thrombin activatable fibrinolysis inhibitor (due to the impaired protein production from hepatocytes) may contribute to the progressive enhancement of this phenomenon (36). Whether hyperfibrinolysis is totally a primary procedure or is partly an effect of the continuous triggering of coagulation has not been answered yet (37-39).

In a recent study, an exhaustive analysis of primary and secondary haemostatic mechanism in 32 cirrhotic patients showed that all variables except fibrinogen, factor XIII, plasminogen inhibitor and TFPI were impaired. PFA-100 after ADP stimulation, PT activity, factor X, factor V, fibrin and plasminogen were independently correlated with the severity of cirrhosis and declined from normal mean in the early stages of the disease, suggesting that haemostasis impairment is present even in subclinical cirrhosis (40).

In spite of the fact that the net outcome of the alteration in the haemostatic system in cirrhotic patients is a bleeding diathesis, thrombosis of the portal vein has been frequently observed in these patients. Thus, in case of a sudden deterioration of a cirrhotic patient, portal vein thrombosis should be carefully excluded from the differential diagnosis (41). However, the development of thrombosis might be attributed to local circulation parameters and mainly to the reduced blood flow in the portal vein. This aspect is enhanced by the findings of a recent study, which suggest that the feared coagulopathy in cirrhotics is more a myth than a reality, as these patients generate adequate thrombin when endogenous thrombin potential assay is performed (42,43).

### **Systemic inflammation/sepsis and haemostasis impairment in patients with obstructive jaundice. The central role of TF**

The occurrence of disseminated intravascular coagulation in obstructive jaundice and its relation to biliary tract

infection has been early recognized (44). Elevated plasma levels of endotoxin, cytokines and C-reactive protein in patients with obstructive jaundice and positive bile cultures were temporarily improved after drainage (45). Infection enhances the production of the cytokines interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF) that are able to activate clotting and fibrinolysis via stimulation of the extrinsic pathway (46). Endotoxins, produced by bacteria, stimulate TF expression on macrophages and clotting activation via an oxidative process (47,48). A relationship has been demonstrated between TF levels and markers of lipid peroxidation, clotting activation, and fibrinolysis in cirrhotic patients (49). Hyperfibrinolysis delays clotting activation through clotting factor consumption and inhibition of fibrin polymerization, and reduces platelet adhesion and aggregation as well (50). Platelet functions are further impaired by increased prostacyclin levels, which are induced by endotoxin and endothelin via nitric oxide formation (51). How these phenomena induced by sepsis can trigger bleeding remains speculative and requires further study.

The relationship between coagulation and inflammation is still poorly understood. Blood clotting, apart from leading to fibrin deposition and platelet activation, results in vascular cell activation, thus contributing to leukocyte activation (52). On the other hand, sepsis and septic shock is known to trigger activation of the extrinsic coagulation pathway, as has been clinically shown by ELISA measurements of the TF in septic patients (53). TF over-expression is normally compromised by the TF pathway inhibitor (TFPI) (54). Nevertheless, septic patients who present insufficient TFPI balancing mechanism, have poor prognosis as the over-production of TF can not be outweighed (55). Other anticoagulants, as antithrombin and activated protein C, have been found to exert antiinflammatory properties (52). In fact, recombinant activated protein C (drotrecogin-alfa) has demonstrated direct activity in blocking thrombin formation, enhancing fibrinolysis and diminishing the expression of inflammatory molecules; under this profile, the drug is now indicated in severe sepsis (with APACHE II 25 or more or with two or more organs having impaired function).

A potent pathway, which explains sepsis and coagulation pathways interference, is the stimulation of *F3* expression in peripheral blood cells and endothelium, which normally lack this mechanism, directly by lipopolysaccharides (LPS) and peptidoglycans or indirectly by TNF- $\alpha$ , VEGF, IL-1 $\beta$ , IFN-1 $\gamma$  and many other inflammation mediators (56,57).

*F3* is the gene coding for TF. TF is a protein having a large extracellular domain (219 residues), a small transmembrane domain and a small cytoplasmic tail. Its role is to form a trimolecular complex with FVIIa and FX (activating FX) and thus initiating coagulation (58-59). *F3* is expressed in normal conditions mainly in the brain, lung, placenta and kidney and, after stimulation, in the peripheral blood cells and endothelium. Trace amounts are detected in plasma (60).

The physiological importance of TF was demonstrated in experiments on transgenic mice, to which *F3* knocking-out had been lethal (61).

*F3* is also expressed with another splice variant, which includes *F3* exons 1, 2, 3, 4 and 6 and leads to the production of the alternatively spliced Human TF (as-HTF). As-HTF is a protein, which lacks the transmembrane and cytoplasmic tail of TF and has a unique termination sequence due to the exons 4/6 fusion. Both TF and as-HTF share the same active catalytic domain and the same pro-coagulant properties, acting as propagators of the coagulation process in the borders of newly synthesized thrombi. Tissue factor is membrane-bound whereas as-HTF circulates freely (62).

The role of the TF in the systemic inflammatory response accompanying cholestasis has been investigated in the elegant study of Semeraro *et al* (63). These investigators studied the procoagulant activity of peripheral blood monocytes in 41 patients with severe obstructive jaundice and in 27 non-jaundiced control patients using a one-stage clotting assay. Mononuclear cells from jaundiced patients, tested immediately after isolation, expressed low levels of procoagulant activity, which were, however, significantly higher than in cells from controls ( $p < 0.01$ ). In addition, after incubation in short-term cultures with and without endotoxin, these cells generated more procoagulant activity than did the control ones ( $p < 0.001$ ). No significant difference in the procoagulant activity was found between patients with and without malignancy in either group. The relief of biliary obstruction resulted in the reduction of both serum bilirubin levels and monocyte procoagulant activity. Endotoxin-induced monocyte procoagulant activity was about threefold higher in the jaundiced patients who died than in the survivors ( $p < 0.001$ ). In rabbits made icteric by bile duct ligation and separation (15 days), the endotoxin-induced monocyte procoagulant activity was markedly increased as compared with sham-operated animals ( $p < 0.005$ ). In all instances, procoagulant activity was identified as TF. The increased capacity of the mononuclear phagocytes to produce procoagulant activity might explain the activation of blood coagulation in severe obstructive jaundice.

A well determined paradigm of how systemic inflammation, apart from true sepsis, can be interlinked with coagulation in clinical practice is chronic cholestatic liver disease due to primary biliary cirrhosis or primary sclerosing cholangitis. These entities are characterized by a better outcome of variceal bleeding and less blood loss in liver transplantation, suggesting the presence of a hypercoagulable state. During their course, levels of factors VIII and vW are increased, while proteins C, S, Z, antithrombin III,  $\alpha_2$  macroglobulin and heparin cofactor II are all reduced. This imbalance along with the presence of antiphospholipid, anticardiolipin and antineutrophil cytosolic autoantibodies in many patients favour hypercoagulability.(9) In a recent study, hypercoagulability in non-cirrhotic patients with primary biliary cirrhosis and primary sclerosing cholangitis has been attributed to the elevated fibrinogen and the hyperactivity of platelets, which

is not observed in non-cholestatic liver disease (chronic hepatitis C and alcoholic cirrhosis). These changes are believed to be the result of a marked systemic inflammatory activity. Whether this phenomenon involves platelets directly or indirectly (through TF expression) has not been clarified yet (52).

## Underlying pathology in patients with obstructive jaundice as an additional mechanism of haemostasis impairment

**a. Pancreatic adenocarcinoma.** Data from *in vitro* and *in vivo* studies show that the coagulation cascade is activated in human pancreatic carcinoma. As a result of the intrinsic hypercoagulable state, pancreatic cancer is associated with a high risk of developing thromboembolic disease. Moreover, proteins that are part of the coagulation cascade have been proved to be important for angiogenesis; thus, induction of coagulation cascade leads also to the induction of angiogenetic signalling pathways. More specifically, TF, apart from being the key molecule in the triggering of the extrinsic pathway of the coagulation cascade, leads to the upregulation of vascular endothelial growth factor (VEGF) and downregulation of thrombospondin, which serves as angiogenesis inhibitor. The role of TF in the angiogenetic control can explain why expression of TF is associated with a poor prognosis.

Hypercoagulability accompanying pancreatic cancer is created by three distinct mechanisms: 1) tumour cells stimulate platelet adhesiveness and aggregation *in situ*, a process which has been evaluated as essential for the development and metastasis of the tumor, as activation of the coagulation pathway is interlinked with activation of angiogenesis pathway; 2) circulating carcinoma mucins (e.g. Ca 19-9) induce the formation of circulating microthrombi (without the participation of thrombin in that process) and thus contribute to the occlusive/ischaemic microangiopathy often observed in pancreatic cancer, and 3) tumour cells produce various procoagulant factors, especially TF and prothrombin.

In an interesting study, the expression of TF was observed immunohistochemically in about one half of pancreatic tumors (29 out of 55 samples), but never in healthy pancreatic tissue (0 out of 18 samples). Moreover, TF expression by tumor cells was found to correlate significantly with histological grade: while 77% of poorly differentiated tumours produced TF, only 20% of well-differentiated tumours presented the same pattern (64). In another study, the *in situ* formation of TF, prothrombin and fibrinogen, has been demonstrated in pancreatic tumours. Interestingly, both TF pathway inhibitor and plasminogen activators had been found in trace quantities. These data can explain the prothrombotic potential that is generated *in situ* in cases of pancreatic tumours (65).

Platelet aggregation induced by tumour cells is an important process for hematogenous metastasis, apart from



contributing to the prothrombotic state. An *in vitro* study has demonstrated that malignant pancreatic cells can induce platelet aggregation with a thrombin-dependent mechanism (66). A possible association between the cell-surface sialylation of tumour cells and their ability to aggregate platelets and induce thrombosis has been referred (67). This observation is in keeping with newer data suggesting that the circulating carcinoma mucins, such as Ca 19-9, interact with platelet P-selectin and leukocyte L-selectin and that these interactions generate platelet-rich microthrombi without thrombin production. This process may well be inhibited by heparin but not by warfarin; thus, unfractionated or low-molecular-weight heparin is much more effective than oral anticoagulants in treating malignant disease associated with thrombosis (68).

Thrombotic events in portal vein may additionally compromise haemostasis through lowering platelet number as a result of pooling in the enlarging spleen. Portal vein thrombosis is a major complication of pancreatic carcinoma and in some cases it remains subclinical or asymptomatic. In case of acute or increased abdominal pain, jaundice and progressive ascites in a patient with a medical record of pancreatic cancer, an abdominal CT would be useful to assess the diagnosis of portal vein thrombosis (69).

**b. Cholangiocarcinoma.** Cholangiocarcinoma progresses slowly, infiltrates the duct walls and leads to biliary tract obstruction. It has been reported that prothrombin time and activated partial thromboplastin time are important determinants for survival after surgery for cholangiocarcinoma (70). Hui *et al* found no significant difference in the prothrombin time and activated partial thromboplastin time between cholangiocarcinoma patients with and without cirrhosis (71). In a recent study, a relation between prolongation of the activated partial thromboplastin time and aminotransferase (AST and ALT) levels was observed. As there was no significant correlation of the APTT anomaly with alkaline phosphatase or serum bilirubin, the investigators hypothesized that this bleeding tendency might be attributed to the excessive liver parenchymal involvement seen in the majority of patients and be treated as a form of hepatocellular failure (72).

**c. Hepatocellular carcinoma.** Although hepatocellular carcinoma seldom results in true obstructive jaundice, cholestasis observed during its course is mainly attributed to damaged hepatocytes and progressive hepatic failure. Nevertheless, as this tumour is accompanied by various haemostatic derangements, it is included in this chapter mainly for differential diagnosis purposes.

Hepatocellular carcinoma results in dysfibrinogenemia, an acquired disorder of haemostasis that is characterized by the presence of excessive number of sialic acid residues on the molecule of fibrinogen which interact with the enzymatic activity of thrombin and is caused by an increased activity of the enzyme sialyl-transferase (73). This enzyme is fetal and can be reexpressed in tumour cells (74). The clinical result of dysfibrinogenemia is the production of abnormally polymerized fibrin, which leads to the

disproportionally severe prolongation of the thrombin time comparing with the mild prolongation of PT and PTT and the normal amounts of fibrinogen.

Additionally, a posttranslational defect in gamma carboxylation induced by tumour cells is considered to account for the elevated levels of decarboxylated prothrombin that characterize hepatocellular carcinoma (75-77). Decarboxylated prothrombin is antigenically identical to that produced during warfarin therapy. Moreover, elevated D-dimer levels have been detected in hepatocellular carcinoma and reflect the tumor stage and vascular invasion of the malignancy (78). The relation between haemostasis and tumour growth has been clinically evaluated in terms of heparin administration in patients with malignant diseases; similar trials are proposed for hepatocellular carcinoma (79).

Another mechanism of haemostasis impairment is the over-production of functionally intact factors of coagulation and fibrinolysis. A case report, which describes the immunohistochemically documented production of antithrombin-III by hepatocellular carcinoma cells, to which haemorrhagic diathesis was attributed, is referred as a paradigm (80).

Controversially, apoptotic hepatocellular carcinoma HepG2 cells have been demonstrated to accelerate blood coagulation through the expression of a phosphatidyl serine-dependent pro-coagulant surface in a recent study (81). These investigators took into consideration that: a) intrasinusoidal microthrombosis is considered to be a cause of massive hepatocyte death in fulminant hepatic failure, and b) generally, apoptotic cells express phosphatidyl serine outside the plasma membrane, which is also expressed on the surface of activated platelets and accelerates fibrin-thrombus formation. They thus postulated that the acceleration of blood coagulation on the surface of apoptotic hepatocytes may occur because hepatocytes are in direct contact with plasma that passes through the fenestrations of the sinusoidal endothelium. The above mentioned hypothesis was tested by investigating the coagulation activity of apoptotic hepatocytes. This mechanism may well contribute to the prothrombotic potential of acute or chronic hepatic failure and the rate of apoptosis may be a crucial parameter for this phenomenon (82).

**d. Acute pancreatitis.** The pathophysiology of acute pancreatitis has not been elucidated. Numerous mechanisms have been proposed to explain the initiation as well as the propagation of pancreatic tissue damage.

One theory is that of the «oxidative stress», according to which the overproduction of free radicals, not neutralised by scavenger molecules, plays a crucial role in setting off the initial «spark» initiating pancreatitis. Another theory is the «self-digestion» process, which is claimed to be the result of activation of pancreatic enzymes inside the gland, as a consequence of restricted damage serving as “first hit”.

What is intriguing is that, after the initiation of acute pancreatitis, the same type of histological and biochemical derangements follow. Nevertheless, the ongoing procedure

is characterized by a broad spectrum of manifestations varying from simple oedematous pancreatitis to necrotizing forms of the disease. A common denominator of these manifestations has been an increased thrombogenicity, documented as early as 20 years ago in hypothermic patients who presented intrapancreatic thrombosis.

In a recent paper, a strong prognostic value was reported for extensive coagulation activation and a poor outcome in severe necrotizing pancreatitis. Changes in protein C, antithrombin III (AT III), D-dimer and plasminogen activator inhibitor - 1 (PAI-1) levels indicating exhaustion of fibrinolysis and coagulation inhibitors, predicted a bad prognosis (83). This is in keeping with the results of another recent article, where it was claimed that the D-dimers levels in plasma reflect the expression of pancreatitis and the extension of systemic involvement (84).

Two of our recently published articles assessed platelet activation in mild forms of acute pancreatitis. In the first study, we focused on two successive end points: (i) the activation of platelets during acute pancreatitis and (ii) the alterations of platelet number and indexes between onset and remission of the disease, which reflect the bone marrow response (10). In a group of 54 patients with acute pancreatitis, activated platelet ratio (APR) was estimated using flow cytometry at onset and remission. The first end-point of the study was reached at patient 14 as APR was found elevated at the onset of acute pancreatitis ( $p=0.01$ ). The second end-point was fulfilled at patient 12 for the mean platelet volume (MPV), platelet large cell ratio (P-LCR) and platelet distribution width (PDW), which were found elevated at remission of the disease ( $p<0.01$ ) but not for platelet number, until the last patient ( $p=0.34$ ). The elevated APR at the onset in combination with the elevation of the platelet indexes in later stages of acute pancreatitis may imply a direct involvement of platelets in the systemic inflammatory process of the disease, which leads to consumption, compensated by an immediate bone marrow response (10).

In our second study, we evaluated alterations of platelet function by using a recently developed platelet function analyser (PFA-100™) (11). Sixteen patients with acute edematous pancreatitis were studied along with 32 normal controls. The hemostatic capacity of platelets was tested in citrated blood and standard cartridges containing collagen-ADP or collagen-epinephrine. A statistically significant shortening of the collagen-ADP closure time, but not of that of the collagen-epinephrine time was noted. These findings confirm an increased platelet adhesiveness and aggregation in the early stages of the inflammatory process of acute pancreatitis, which may underline the prethrombotic potential of the disease (11).

Evidence supports a role for thrombosis in the ongoing process of pancreatic tissue damage. What remained unclear until recently was the exact mechanism of coagulation initiation. One study evaluated coagulation activation after islet transplantation and the subsequent release of insulin. Even in the absence of signs of portal thrombosis, they

concluded that the endocrine, but not the exocrine, cells of the pancreas synthesise and secrete active TF. The clotting reaction triggered by pancreatic islets in vitro could be abrogated by blocking the active site of TF with specific antibodies or site-inactivated factor VIIa, a candidate drug for inhibition of TF activity in vivo. Thus, blockade of TF could represent a new therapeutic approach that might increase the success of islet transplantation in patients with type 1 diabetes, in terms of both the risk of intraportal thrombosis and the need for islets from more than one donor (85).

These results are supported by the demonstration that pancreatic duct cells can produce TF, possibly explaining graft rejection following islet transplantation when islet preparations are not 100% devoid of any epithelial cells (86). The destruction of epithelial pancreatic duct cells due to mechanical reasons in pancreatitis, especially of biliary etiology, might account for the enhanced primary hemostasis observed in the latter (10).

Therefore, even minimal pancreatic tissue damage, including epithelial components, may trigger TF-dependent coagulation initiation. This may not be limited to local thrombotic events, but can practically initiate a systemic inflammation process (SIRS) involving upregulation of adhesion molecule expression and chemokine production. At this point, the role of platelet activating factor (PAF), together with other proinflammatory cytokines in the pathogenesis of SIRS should not be underestimated (87). PAF increases vascular permeability, induces leukocyte infiltration, oedema and tissue injury, and has a negative inotropic effect (88). A propagation phase, through a mechanism of positive feedback loop, might result in systemic manifestations of unpredictable extent (89,90).

The question arises whether SIRS, well known to accompany acute pancreatitis, is a result of the key role of TF in interlinking between coagulation and inflammation. TF is produced in two forms: the first represents a cell-bound molecule having a transmembrane region and the second a soluble molecule lacking this transmembrane domain as a result of alternative splicing (asTF) (62).

Although TF has been shown to act as an adhesion molecule, cytokine receptor and signal transduction molecule, enhancing the inflammatory process, its splice variant asTF probably lacks these attributes, serving only as propagator of coagulation (91).

Another molecule, TFPI (TF Pathway Inhibitor) impedes TF function (92). Thus, while the ratio (TF+asTF)/TFPI indicates the thrombotic potential, the TF/asTF ratio is a measure of balance between thrombosis and inflammation. Measuring TF/asTF in early stages of acute pancreatitis would enable us to propose whether the interlinkage between thrombosis and inflammation is impaired towards one direction.

What is the real consequence of the thrombosis of the microvasculature during acute pancreatitis? As preventing further complications remains a major therapeutic goal for patients suffering from the disease, the role of anti-

thrombotic agents cannot be underestimated. Indeed, many recent articles focus on the usefulness of low molecular weight heparin during the course of acute pancreatitis (93). An anti-TF monoclonal antibody, having been clinically tested in sepsis with not much success, still remains to be evaluated in acute pancreatitis. Moreover, biosynthetic TFPI may be proven useful in controlling the rapid extra-pancreatic tissue damage that can follow an episode of acute pancreatitis.

### **Treatment of haemostatic abnormalities in patients with obstructive jaundice**

Vitamin K deficiency is likely in patients with cholestatic disease. A dose of 10 mg of vitamin K for 3 days is likely to correct prothrombin time prolongation in these patients. The intravenous administration of vitamin K has the risk of anaphylaxis. S.c. administration is characterized by an inconstant rate of absorption and i.m. injection should be avoided because of the risk of hematomas.

Low platelet count is not believed to be threatening when platelet number exceeds 50.000/ $\mu$ l. Whenever platelet number falls beyond this limit during a bleeding episode or before a surgical procedure, platelet transfusion is advisable. One unit of platelet concentrate increases the peripheral platelet count by about 10.000/ $\mu$ l. The increase in platelet count is less in patients with hypersplenism, as the majority of the transfused platelets are sequestered in the enlarged spleen. Recombinant thrombopoietin for the correction of platelet count in cirrhotics is still under investigation.

Prolonged bleeding time, mainly attributed to low platelet count, may be reversed by the administration of 0.3  $\mu$ g/kg of 1-deamino-8-D-arginine vasopressin (DDVAP), especially when surgical procedures should be undertaken. Although DDVAP increases the plasma levels of factor VIII and vWF, the exact mechanism of DDVAP action remains unknown.

Deficiencies in coagulation factors may be corrected by fresh frozen plasma (93). Administration of 10-20 ml/kg body weight may curtail prothrombin time prolongation to less than 3 seconds. Nevertheless, the correction of coagulopathy lasts no more than 12-24 hours (as FVII has a half-life of 4-6 hours). The lack of correction after adequate fresh frozen plasma transfusion indicates the presence of dysfibrinogenemia or FDPs. Fluid overload is a frequent complication in fresh frozen plasma administration as large quantities (1-1.5 lt) may be required. Besides, the risk of infection can not be underestimated; solvent detergent-treated plasma reduces this possibility, but it is devoided of factor VIII, proteins S and C and  $\alpha$ 2-antiplasmin.

Instead of fresh frozen plasma infusion, plasma exchange has been used with similar results regarding the treatment of coagulopathy without the risk of volume overload (94).

As an alternative solution, the infusion of prothrombin concentrates, containing only the vitamin K dependent coagulation factors, may only partly correct the coagulopathy and has the risk of thromboembolic complications and disseminated intravascular coagulation.

Cryoprecipitates contain factors VIII and XIII, fibrinogen, vWF and fibronectin. One unit of cryoprecipitate (20-30 ml) is enough for every 10 kg of body weight. The administration of cryoprecipitates is indicated when plasma fibrinogen levels fall below 100 mg/dl as a consequence of disseminated intravascular coagulation or massive blood transfusion.

A new approach is the administration of recombinant activated factor VII (rFVIIa). In preliminary reports, a dose of 80  $\mu$ g/kg normalized prothrombin time for more than 12 hours in patients with cirrhosis. However, the prolongation of the prothrombin time induced by the rFVIIa does not necessarily reflect hemostatic efficacy and care must be taken in patients with subclinical disseminated intravascular coagulation (95).

In cases of hyperfibrinolysis and concomitant bleeding, the need for antifibrinolytic agents as  $\epsilon$ -aminocaproic acid, tranexamic acid or aprotinin should be estimated. Again thromboembolic events are a major threat; thus, the use of these agents must stop after the successful management of haemostasis. Aprotinin has a lower relative risk for these complications (96).

### **Conclusion**

The haemostatic derangement in a patient with obstructive jaundice is multifactorial and difficult to assess. A general rule is that a doctor has to treat the patient, not the laboratory findings. The information given by the coagulation assays should be carefully studied and interpreted through the clinical practice.

An uncomplicated but prolonged benign cholestasis will drive to haemorrhagic diathesis. Prophylactic administration of vitamin K should be performed in these cases. If septic complications and/or pancreatic involvement is superimposed, the net effect on haemostasis might be a prothrombotic state; thus, low-molecular-weight heparin might be helpful in selected patients.

Unresolved cholestasis may progressively lead to liver dysfunction and evolution of cirrhosis. In these cases, more generalized haemostatic disorders affecting practically all pathways are observed: thrombocytopenia, decreased synthesis and clearance of coagulation factors and inhibitors, dysfibrinogenemia, hyperfibrinolysis and overt disseminated intravascular coagulation along with portal vein stasis and thrombosis may converge to a single patient. The advice of a haematologist concerning the administration of platelets, fresh frozen plasma, cryoprecipitates, prothrombin complex precipitates, recombinant factor VII, DDVAP or antifibrinolytic agents is essential when treating such a patient.

When malignancy has been documented, the situation is more complicated. Mucous adenocarcinomas (e.g. of the pancreas or the colon) and hepatocellular carcinomas can induce activation of haemostasis. Thromboembolic events, especially in the former, are common and serious complicating events resulting in poor prognosis. The use of low-molecular weight heparin fractions in these patients, apart

from preventing thrombosis and embolism, may compromise tumour growth through inhibition of a TF mediated angiogenesis mechanism.

Understanding the pathophysiology of haemostatic changes in patients with cholestasis, and, more generally, liver disease, is the key of accurate diagnosis and treatment. The combination of good knowledge with close inspection of every patient could lead to the most promising result.

### Competing interests

None declared.

### Authors' contributions

All authors contributed equally to this work. All authors read and approved the final manuscript.

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