Progress report

Haemostatic problems in liver disease

The liver plays a major role in the control of coagulation and as a result haemostatic problems are detected in approximately 75% of patients with liver disease.¹ The coagulation abnormalities are both complex and multifactorial and depend on the balance between hepatic synthesis and clearance of activated coagulation proteins and their inhibitors; the presence or absence of dysfibrinogenaemia; thrombocytopenia, abnormal platelet function, and disseminated intravascular coagulation.

Some patients will present with petechiae, ecchymosis or epistaxis, but most patients are asymptomatic or only bleed after venepuncture or liver biopsy. Alternatively haemorrhage may be life threatening and patients may die from variceal bleeding or from disseminated intravascular coagulation. The reasons for this disparity are not yet clear, but after the introduction of newer techniques, in particular the development of immunological assays for the antigens of coagulation proteins, our understanding of these problems has improved. The normal coagulation and fibrinolytic systems are depicted in Figures 1 and 2 while the major

Fig. 1 The coagulation cascade. HMWK=high molecular weight Kinogen, PK=Pre-Kallikrein, AT III=antithrombin III, PL=platelets, Ca^{++} =Calcium, TF=tissue factor, $\rightarrow$ =proteolytic activation, $\rightarrow$ (with a)=conversion of coagulation protein, $---$ =inhibition by plasma inhibitors, $||||$ =crosslinking, a=activated coagulation enzyme. Coagulation is initiated when blood contacts subendothelium (intrinsic pathway) or damaged tissue (extrinsic pathway). Inactive circulating precursors are activated and each activated enzyme activates the next protein in the sequence sometimes requiring cofactors (Ca^{++} , V, VIII etc). Subsequently the process is limited by plasma inhibitors (Protein C, AT III). Prothrombin time (PT) measures extrinsic and common pathways; partial thromboplastin time (PTTK) measures intrinsic and common pathways; thrombin time measures conversion of fibrinogen to fibrin.

Fig. 2 Fibrinolytic system. TPA=Tissue plasminogen activator, FDP=Fibrin Degradation products, $\curvearrowright$ =proteolytic activation, $\longrightarrow$ =conversion of coagulation protein, $-\cdot-\cdot-$ =inhibition by plasma inhibitors. Fibrinolysis is initiated when plasminogen and TPA bind to fibrin as clot forms. Active plasmin dissolves fibrin *in situ* but is rapidly neutralised by α_1 -anti-plasmin as it leaves the clot preventing systemic fibrinolysis.

coagulation defects are summarised in the Table.

Coagulation proteins may be estimated by measuring either their functional activity – that is, individual biological assays – or by an immunoassay directed towards the specific antigen. In normal circumstances functional and immunoassays have a close correlation.

Hepatic synthesis of coagulation proteins

It is generally accepted that all coagulation proteins (except for Von Willebrand factor) and inhibitors are synthesised in the liver. The evidence for this assumption was obtained from a variety of different experimental techniques. In the first instance, coagulation factors I, II, VII, IX, X, XII, XI were noted to fall after chemically induced hepatic necrosis² while factors VIII and IX were detected in isolated liver perfusates.³

More recently, secretion of fibrinogen,⁴ prothrombin⁵ and factor VII⁶ have been detected in hepatocyte culture and hepatocyte cell line cultures,⁷ while fibrinogen⁸ and prothrombin⁹ have been demonstrated in parenchymal cells by immunohistological techniques and their site of synthesis confirmed by demonstration of mRNA.¹⁰ Von Willebrand factor (vWF, formerly known as VIII R:antigen) is synthesised by vascular endothelial cells in culture¹¹ while factor VIII (antihaemophilic factor) is now known to be synthesised mainly by the hepatocyte and the spleen^{12 13} although low level synthesis takes place in a variety of other tissues¹³ in both human and guinea pig.^{12 13}

Vitamin K dependent proteins

PROTHROMBIN (II), VII, IX, X

These vitamin K dependent proteins are synthesised by the liver in a precursor form. Vitamin K carboxylates the glutamic acid residues of the precursors before secretion, thus enabling them to bind to phospholipid and function in the coagulation process.¹⁴ The exact role of vitamin K in this reaction is not certain but it is thought to cleave the carbon-hydrogen bond on the glutamyl residues and that carboxylation takes place by a carbanion mechanism.¹⁵ Thus, in vitamin K deficiency (from any cause) immunologically identical but non-functional precursor forms are detected in the plasma.¹⁶

Likewise, inactive precursor forms can be detected in patients with liver disease (without vitamin K deficiency) suggesting an acquired defect of

Table Principal coagulation abnormalities in liver disease

<i>I Coagulation proteins and inhibitors</i>	<i>Clinical observation</i>	<i>Proposed mechanism</i>
Fibrinogen (I)	(i) Reduced in acute liver disease (ii) Abnormal fibrin polymerisation in cirrhosis, hepatoma (iii) Increased concentrations in cholestasis	Reduced hepatic synthesis ^{2 4} Increased sialation of fibrinogen chains B β and γ ^{28 29} ? Reduced hepatic clearance
Prothrombin (II) Factors VII, IX, X	(i) Reduced concentrations in parenchymal liver disease (ii) Discrepancy between antigen and activity assays	Reduced hepatic synthesis ^{2 5} $\pm$ reduced carboxylation in associated Vit K deficiency ¹⁶ ? Production of abnormal protein
Factor V	(i) Reduced in parenchymal liver disease (ii) Normal/raised in cholestasis	Reduced synthesis ? Reduced clearance
Factor VIII and VWF	(i) Normal activity with increased antigen concentrations in both parenchymal and cholestatic liver disease	? Production of abnormal protein ²⁵ ? Reduced clearance of antigens ²⁵
Factor XI, XII Pre-Kallikrein High molecular weight Kininogen, Factor XIII Antithrombin III	Reduced concentrations in severe disease only	Reduced hepatic synthesis ²
Plasminogen	Reduced concentrations in parenchymal liver disease	(i) Reduced synthesis ³⁴ (ii) Consumption in DIC ³⁴
α_2 antiplasmin	Reduced concentrations in parenchymal liver disease	(i) Increased turnover and catabolism (ii) Reduced synthesis ⁴⁰ Reduced synthesis
<i>II Platelet abnormalities</i>		
Quantitative	Reduced numbers	(i) Decreased survival ^{40 45} — Hypersplenism — DIC — Platelet associated antibodies ^{46 48} (ii) Decreased production — alcohol — folate — viral hepatitis
Qualitative	Reduced platelet aggregation	(i) Decreased arachidonic acid in platelets ⁵⁰ (ii) Reduced volume of platelets ⁴⁹
<i>III Disseminated intravascular coagulation</i>		
	Reduced concentrations of all coagulation proteins, reduced platelets, increased FPDs, Fibrin monomer	Activation of coagulation cascade caused by reduced clearance of activated factors leading to secondary fibrinolysis ³⁸

carboxylation.¹⁷ Vitamin K does not have any effect on the synthesis of these proteins as there was no increase in the low levels of prothrombin antigen (immunoassay) or activity (functional assay) in cirrhotics after vitamin K therapy.^{18 19} It is interesting to note that in this group of cirrhotic patients there was a relative excess of non-functional antigen

which is unexplained but may represent release of the protein from damaged hepatocytes or perhaps, production of abnormal proteins.

PROTEIN C

Protein C is a recently discovered vitamin K dependent protease^{20 21} which is a glycoprotein with a molecular weight of 62 000 daltons. It is activated by thrombin and thrombomodulin (produced by vascular endothelial cells) and has been shown to increase the levels of plasminogen activator and to inactivate factors VIII and V *in vitro*²² suggesting that it is a natural inhibitor of blood coagulation. There is controversy about the effect of protein C on circulating concentrations of VIII and V *in vivo*, but low levels of functional activity²³ and of protein C antigen have been detected in patients with chronic liver disease²⁴ and with vitamin K deficiency.²⁵ Raised concentrations of factor VIII activity and of VIII:antigen fell after vitamin K in 13 vitamin K deficient patients with obstructive jaundice, but not in 23 patients with vitamin K replete chronic liver disease. There was no change in protein C antigen or factor V levels in either group after vitamin K. Although these results need to be correlated with a functional assay for protein C, these data suggest that protein C may not have a significant effect on factors VIII and V in liver disease.²⁵

QUALITATIVE ABNORMALITIES OF COAGULATION PROTEINS

Qualitatively abnormal proteins such as transcobalamin, thyroxine binding globulin and fibrinogen are known to be synthesised by primary liver tumours³¹ but it is now apparent that structurally abnormal non-functional proteins may also be synthesised in hepatocellular disease further interfering with coagulation.

Fibrinogen

Acquired dysfibrinogenaemia should be suspected in any patient with a prolongation of the plasma thrombin time in the absence of abnormal concentrations of fibrinogen or raised fibrinogen degradation products (FDPs). It may develop in 50–78% of patients with chronic liver disease.^{26 27} The prolonged thrombin time is because of abnormal fibrin polymerisation (Fig. 1) caused by an increase in the sialic acid content of the β and α chains of fibrinogen.^{28 29} Reduction of the sialic acid content by neuraminidase returns the fibrin polymerisation and the thrombin time to normal.^{27 30} The increase in sialic acid content may be because of excess production of sialic acid transferase²⁹ or reversion to fetal fibrinogen or another acquired defect in processing oligosaccharide chains.³¹

Factor VIII

Raised concentrations of both factor VIII (antihaemophilic factor) and Von Willebrand factor (vWF) have been noted in liver disease³² but poorly explained. A study of 85 patients with liver disease showed a disproportionate increase in the non-functional antigens VIII:antigen and vWF:antigen compared with the biological activities of these proteins. Concentrations were highest in patients with alcoholic liver disease or cholestasis. Although this may represent decreased hepatic clearance of these antigens

by the hepatic Kupffer cells it is also possible that there may be production of an abnormal hypoactive factor VIII in liver disease.²⁵

The plasma concentration of factor VIII may help to differentiate coagulation abnormalities secondary to fulminant hepatitis or disseminated intravascular coagulation (DIC). Factor VIII concentrations are low in DIC, as it is consumed, but often very high in fulminant hepatitis, presumably because of the release of factor VIII although this is not proven.³³ It may also represent continued or increased extrahepatic synthesis of factor VIII.¹³

The development of immunological assays for the antigens of coagulation proteins has led to the demonstration of other discrepancies between antigen level and functional activity in liver disease such as prothrombin¹⁹ and antithrombin III^{34 35} and it is possible that synthesis of other non-functional coagulation proteins will also be demonstrated in liver disease.

Protein inhibitors of blood coagulation

The coagulation reaction is controlled by various physiological mechanisms which include the clearance of activated coagulation factors by the liver and the synthesis of plasma inhibitors such as antithrombin III (AT III), α_2 macroglobulin, α_1 -antitrypsin, C₁ inhibitor and protein C.

Antithrombin III is the most important natural inhibitor. It is a single chain glycoprotein synthesised by the liver.³⁴ Its main action is to neutralise thrombin and this reaction is accelerated by heparin.^{36 37} Low concentrations of AT III in cirrhosis and acute hepatitis may be caused by reduced synthesis of the protein or to its consumption in disseminated intravascular coagulation.³⁴ There is some evidence that a qualitatively abnormal protein may be synthesised in liver disease as some workers have noted a discrepancy between the immunoassay and the functional assay.^{34 35}

Disseminated intravascular coagulation (DIC)

Disseminated intravascular coagulation is a pathologic stimulation of the coagulation cascade resulting in intravascular coagulation, consumption of coagulation proteins (including factor VIII) and platelets leading to a haemorrhagic state. A compensatory fibrinolysis develops.³⁸

The pathogenesis of disseminated intravascular coagulation in liver disease is multifactorial and includes the release of tissue thromboplastin from liver cells; reduced clearance of activated coagulation proteins; reduced synthesis of inhibitors – for example, antithrombin III and protein C; and circulating endotoxin.³⁹ Any of these mechanisms may activate the coagulation system and subsequently stimulate fibrinolysis leading to disseminated intravascular coagulation. There is ample evidence that while the catabolism and turnover of fibrinogen is increased in liver disease^{39 40} normal concentrations are maintained by increased synthesis.⁴⁰ The catabolism of plasminogen is also increased, but synthesis is reduced leading to reduced turnover.⁴⁰ Treatment with heparin to inhibit the coagulation cascade reduced the catabolism of both fibrinogen and plasminogen implying that activation of the coagulation reaction is the

primary abnormality in disseminated intravascular coagulation.^{39 40} Likewise transfusion of AT III in patients with cirrhosis and deficiency of AT III reduced the fractional catabolic rate of fibrinogen suggesting that decreased AT III concentration may have contributed to continued activation of the coagulation cascade.⁴¹

Opinions vary about the incidence and severity of disseminated intravascular coagulation in liver disease – in general there is low grade disseminated intravascular coagulation in most cirrhotic patients but this may not be clinically significant.^{39 40 42} Disseminated intravascular coagulation occurs after insertion of the Le Vein shunt but regresses if the shunt becomes blocked or reversed.⁴³ The mechanism of postshunt disseminated intravascular coagulation is unclear but may be because of the release of tissue thromboplastin in the recirculated ascites⁴³ as disseminated intravascular coagulation does not occur when the ascitic fluid is filtered.⁴⁴

Abnormalities of platelet dynamics and function

Abnormalities of platelets may be qualitative or quantitative (Table). Thrombocytopenia may be caused by decreased survival because of splenic sequestration;^{40 45} to platelet associated IgG in chronic active hepatitis;^{46 47} or to platelet antibodies in alcoholic cirrhosis.⁴⁸ Impaired production of platelets may be an associated factor in alcoholic liver disease or in viral hepatitis.

Platelet function as measured by adherence to glass beads or ADP induced aggregation may also be abnormal in liver disease either because there is a reduction in the numbers of the larger, more haemostatically active platelets⁴⁹ or because of altered cholesterol/phospholipid ratios leading to reduced availability of arachidonic acid for prostaglandin production.⁵⁰

Evaluation of the risk of bleeding

As outlined above, the risk of bleeding in any individual patient depends on the balance between the synthesis and clearance of the coagulation proteins and their inhibitors (Table, Figs. 1 and 2). This is of particular importance when assessing the risk of bleeding in patients undergoing liver biopsy or surgery, in assessing prognosis, or in estimating the reasons for variceal bleeding.

It is clear that the risk of bleeding cannot be easily evaluated from routine haemostatic tests. Ewe⁵¹ in a unique study of 200 patients undergoing laparoscopic liver biopsies found no correlation between standard tests such as prothrombin time and platelet count and the 'liver bleeding time'. Although this study cannot be directly compared with closed liver biopsies it does suggest that peripheral blood coagulation values do not reflect *in vivo* tissue clotting. Liver biopsies carried out on patients with less than 60 000 platelets caused bleeding in three of 13 patients⁵² and it is therefore prudent to assess platelet function in patients with platelet counts less than 60 000 and to protect with platelet transfusions if function is impaired.

In practice, all patients for liver biopsy should have a prothrombin time, a partial thromboplastin time and a platelet count done. If either the

prothrombin time or the partial thromboplastin time are prolonged greater than three seconds then it is worthwhile giving intravenous vitamin K (10 mg) for a maximum of three days. If coagulation time does not correct on this regime (likely in severe hepatocellular disease) then the biopsy should either be deferred or done through the transvenous route.⁵³ Alternatively a guided biopsy under either ultrasound or computed tomography scanning can be plugged with gelfoam.^{54 55}

Causes of variceal bleeding

The causes of variceal bleeding are disputed and multifactorial. Attempts to correlate the incidence of bleeding with coagulation status have had limited success, but two recent studies have made the interesting suggestion that variceal bleeding is related to a hypercoagulable state⁵⁶ leading to disseminated intravascular coagulation.⁵⁷

Prognostic value of coagulation proteins

It is generally agreed that an increased prothrombin ratio unresponsive to vitamin K implies poor hepatic synthetic ability and severe hepatocellular disease. Some workers have attempted to estimate the prognostic value of individual coagulation factors. Biland and coworkers⁵⁸ found that concentrations of factor V, XIII and plasminogen were useful prognostic indicators while Ekindjiran *et al*⁵⁹ using multivariate analysis of 131 patients felt that factor V and AT III concentrations were helpful. In view of its short half-life factor VII is a good guide to severity of liver disease⁶⁰ and of prognosis.⁶¹ Despite many detailed studies, however, no particular haemostatic value has been shown to be of definite value.

Therapy of haemostatic abnormalities

The role of fresh frozen plasma, vitamin K, and supportive measures in the treatment of bleeding in liver disease are well known and these remain the standard therapy.

Prothrombin complexes were used some years ago but are now contraindicated because of the risks of hepatitis⁶² and of thrombo-embolic episodes because of the presence of activated coagulation factors.^{63 64} As heparin inhibits the coagulation cascade it has been used for treating disseminated intravascular coagulation, and although coagulation values improved,^{42 65} it has not been shown to have significant clinical value and therapy remains supportive.^{42 66}

Newer therapeutic measures include the use of AT III and Desmopressin (D-amino-8-D arginine vasopressin-DDAVP). Infusion of concentrates of AT III have been effective in patients with disseminated intravascular coagulation and fulminant hepatitis^{67 68} or acute fatty liver of pregnancy.⁶⁹ Attempts to prevent disseminated intravascular coagulation post Le Veen shunt in cirrhotic patients by infusions of AT III have not been as successful⁷⁰ but adequate controlled trials have not yet been done. Antithrombin III concentrates are expensive and large amounts are required to correct the deficit. As with other concentrates they carry a risk of hepatitis and AIDS and increased use in a clinical setting may well

depend on the development of synthetic or pasteurised AT III.

Desmopressin (DDAVP) is a vasopressin analogue which produces a two to three-fold increase in plasma concentrations of factor VIII and vWF and is a useful therapeutic agent in mild haemophilia.⁷¹ Agnelli and colleagues⁷² have shown that infusion of DDAVP in eight patients with cirrhosis unexpectedly decreased the prothrombin time and raised plasma values of factors VII, VIII, IX, and XII. A more recent study in five patients with cirrhosis confirmed increases in VIII, VIII:Ag, VIII:RiCof, vWF:Ag and IX, and XII. The bleeding time and partial thromboplastin time were shortened but there was no change in prothrombin time.⁷³ Although the associated rise of plasminogen activator in these patients may be a potential drawback further studies are required to confirm the effect on coagulation in liver disease and to evaluate its role in therapy.

In conclusion, the haemostatic defects in liver disease are complex and multifactorial, they are often unpredictable and the mechanisms elusive. Nevertheless, the development of immunological techniques has shown that both qualitative and quantitative abnormalities of coagulation proteins and protein inhibitors may interfere with function. It is hoped that future research will lead not only to a better understanding of these defects but to more effective therapy.

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