Estimation of prothrombin in liver disease

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SYNOPSIS Prothrombin, both by a one- and a two-stage method, and progressive antithrombin were determined on the same plasma samples from 47 patients with cirrhosis and 27 patients with acute hepatitis. In the first group prothrombin estimated by the two-stage method was significantly higher than by the one-stage method. The values were often within the normal range and were not correlated with the indices of protein synthesis. Since progressive antithrombin was also depressed, the unduly high levels measured by the two-stage method are probably related to the influence exerted by antithrombin on the two-stage method.

The opposite pattern was observed in patients with acute hepatitis, ie, the one-stage assay gave higher values than the two-stage assay. The one-stage method correlated better with the indices of protein synthesis. It is suggested that a one-stage assay should be used in patients with liver failure to evaluate the haemostatic balance before liver biopsy or surgery.

Experience gained with congenital hypoprothrombinaemia suggests that severe spontaneous bleeding may occur in patients with about 10% of prothrombin and that levels as high as 40% are necessary to avoid excessive postoperative bleeding (Biggs and MacFarlane, 1966). Abnormally low values for prothrombin have been found in liver disease by Mindrum and Glueck (1959), Biggs and MacFarlane (1962), and others. A possible relationship between prothrombin levels and haemorrhage in these patients has been suggested by Donald, Hunter, Tudhope, Walker, and Whitton (1954).

A specific assay of prothrombin was therefore carried out in patients with liver disease undergoing such diagnostic procedures as liver biopsy or such operations as portacaval shunts. In fact the prothrombin time, which is widely used as a coagulation screening test before surgery in these patients, is known to be somewhat insensitive to a reduction in true prothrombin levels, and might therefore fail to detect any tendency to bleed due to prothrombin deficiency.

Relatively high levels of prothrombin were

unexpectedly found in a number of patients with liver cirrhosis who were investigated with a two-stage assay of prothrombin (Oxford 'area' method of Biggs and Douglas, 1953). Since these values were scarcely compatible with the marked impairment of liver function as expressed by other indices of protein synthesis we felt that the problem of assaying prothrombin in liver disease should be reconsidered. Prothrombin, estimated both by one- and two-stage methods, and progressive antithrombin were therefore determined on the same plasma samples from normal subjects and from patients with liver cirrhosis and acute hepatitis.

Material

Case material included 24 normal subjects, 47 patients with decompensated cirrhosis, and 27 patients with acute hepatitis, none of whom had been previously treated with vitamin K. A diagnosis was made according to the commonly accepted clinical and laboratory criteria.

Methods

Blood was collected and citrated plasma separated as described by Hardisty and Ingram (1965). Prothrombin was estimated on plasma within five hours of collection and antithrombin on plasma samples kept frozen at -20°C for not more than 30 days. Values were expressed as percentages of average normal plasma. (Five pooled plasmas were collected and examined under the same conditions as the test samples.)

Prothrombin was determined by the two-stage method of Biggs and Douglas (Oxford 'area' method) as described by Biggs and MacFarlane (1966), with an additional subsampling from the incubation mixture after one minute and 30 seconds. The one-stage method was that of Jobin and Esnouf (1966) employing tiger snake (*Notechis scutatus scutatus*) venom. In this method 1/10 (100%) and 1/80 (12.5%) dilutions of pooled normal plasma usually gave clotting times around 14 and 33 seconds respectively, the blank clotting time being between 100 and 110 seconds.

Progressive antithrombin was determined by the method of Quick (1938), modified by defibrination at 56° for five minutes. The results were expressed as percentages of the thrombin-fibrinogen clotting time calculated after subsampling from the incubation mixture at 20 minutes to that of a pooled normal plasma in the same experimental conditions. This method is influenced by both progressive antithrombin (antithrombin III) and heparin cofactor (antithrombin II).

Results

NORMAL SUBJECTS

Mean values for prothrombin (one-stage and two-stage methods) and antithrombin in 24 normal subjects were respectively 100.4, 100, and 95%. The range, calculated from 2 standard deviations, was 79-123 for one-stage prothrombin, 70-130 for two-stage prothrombin, and 73-117 for antithrombin. These two methods of prothrombin estimation showed a significant correlation ($r = 0.54$, $P < 0.01$). No correlation was observed between either of the prothrombin assays and antithrombin ($r = 0.22$, $r = 0.07$).

LIVER CIRRHOSIS

In patients with severe cirrhosis of the liver the prothrombin, assayed by one- and two-stage methods, and the antithrombin were significantly decreased. Mean values were respectively 40, 66, and 41%; variations from the normal were highly significant ($P < 0.001$), (Fig. 1). Prothrombin values assayed by the two-stage method were significantly higher than

by the one-stage method ($P < 0.001$). Several samples had unexpectedly high potencies which were within the normal range in contrast to the marked impairment of liver function as expressed by other biochemical indices, determination of prothrombin time, and assays of other clotting factors. No correlation between the one- and two-stage methods was observed ($r = 0.20$; Fig. 2). The two-stage method showed no correlation with indices of protein synthesis such as serum albumin and pseudocholinesterase, which showed on the contrary a positive correlation with the one-stage assay ($r = 0.37$, $P < 0.01$; $r = 0.30$, $0.02 > P > 0.01$). The ratios between two-stage and one-stage estimations of prothrombin in each plasma sample, taken as an index of the discrepancy between the two assays, were inversely correlated with progressive antithrombin ($r = 0.29$) though the correlation was weak ($0.05 > P > 0.02$).

ACUTE HEPATITIS

Prothrombin was shown to decrease in patients with acute hepatitis when assayed by both one-

Fig. 1 Prothrombin (one- and two-stage method) and progressive antithrombin in 48 patients with decompensated cirrhosis of the liver. The shaded areas correspond to the normal limits, the broken lines to the mean values obtained in the group of cirrhotics, and triangles indicate the patients who bled spontaneously.

Fig. 2 Correlation between results of the two-stage and the one-stage assays of prothrombin.

and two-stage methods. Mean values (73% and 58% respectively) were significantly different from normals ($P < 0.001$). Prothrombin assayed by the one-stage method was higher than with the two stage method in these patients ($0.02 > P > 0.01$). Antithrombin gave a mean value of 96%, which is not significantly different from normal. However, a larger variability was evident, both very high and very low values being observed (Fig. 3). No correlation was observed between prothrombin (both one- and two-stage methods) and antithrombin. Serum bilirubin and transaminase were not correlated with either prothrombin or antithrombin. On the contrary, a significant correlation could be established between one-stage prothrombin, serum albumin ($r = 0.48$, $P < 0.01$), and pseudocholinesterase ($r = 0.51$, $P < 0.01$). No correlation was seen between the latter two parameters and two-stage prothrombin.

SPONTANEOUS BLEEDING

Fourteen patients with liver cirrhosis showed spontaneous bleeding (gastrointestinal bleeding, epistaxis, large bruises) when admitted or during their period in hospital. Prothrombin estimated with the one-stage assay was lower than 40%, with one exception (42%). The two-stage assay gave higher values, which were within the normal range in four cases and Quick's prothrombin

Fig. 3 Prothrombin (one- and two- stage methods) and progressive antithrombin in 27 patients with acute hepatitis. The key is the same as in Figure 1.

time was normal in four of these patients (Table).

POSTOPERATIVE BLEEDING

Patients were not submitted to liver biopsy or portacaval shunts unless prothrombin was higher than 40% with the venom assay. No abnormal bleeding was observed in these cases.

Discussion

Since it was realized that the classical prothrombin time is somewhat insensitive to the concentration in plasma, various methods have been proposed to measure specifically this clotting factor. The one-stage method consists of modifications of the original one-stage test of Quick (1938) whose specificity is increased by the addition of serum (containing factors VII and X in excess) together with adsorbed plasma as a source of factor V and fibrinogen. Tiger snake venom has been shown by Jobin and Esnouf

Case No.	Prothrombin (%)		Prothrombin Time (%)
	One-stage Method	Two-stage Method	
1	32	64	45
2	16	42	34
3	26	73	60
4	20	107	53
5	33	65	50
6	26	69	57
7	26	89	29
8	37	61	74
9	14	106	47
10	22	52	67
11	19	44	61
12	42	41	40
13	20	45	68
14	30	60	69
Normal range 79-123			66-123

Table Values for prothrombin and for Quick prothrombin times in 14 patients with bleeding

(1966) to have properties similar to those of activated factor X (Xa) and is therefore independent in variations of factors VII and X in the plasma sample tested. There is a good correlation in normal subjects between the Tiger snake-venom assay and the two-stage assay of prothrombin, so that the venom method can be considered a reliable assay of prothrombin.

Complete conversion of prothrombin into thrombin in the presence of tissue extracts with measurement of the yield of thrombin is the principle underlying the two-stage assays, which are, however, influenced by antithrombins and require the addition of fractions supplying in excess the other components of the extrinsic system. Therefore Biggs and Douglas (1953) devised a modification of the two-stage assay of prothrombin where the area enclosed under the curve of thrombin formation is measured and compared with that from a reference plasma containing a known amount of prothrombin. It was concluded that the observed areas provide a specific measurement of prothrombin level in a given sample, independent of the concentration of clotting factors governing the rate of prothrombin conversion. However the assay is influenced by the level of antithrombin and is thus reliable only when it is present in normal amounts.

Since this test was devised, it has become clear that abnormal levels of progressive antithrombin may be encountered in liver disease. It is commonly decreased in liver cirrhosis (Jurgens, 1953; Hensen and Loeliger, 1963; von Kaulla and von Kaulla, 1967; and other workers). Results are contradictory in acute hepatitis, where high as well as low levels have been reported by different workers (Koller and Fritschy, 1943; Innerfield, Angrist, and Boyd, 1952; Forell and Koller, 1953, and others).

In the patients with cirrhosis, who had consistently low levels of progressive antithrombin,

estimation of prothrombin with the two-stage method gave higher results than the one-stage assay in 37 of 47 patients. The values were often within the normal range, in agreement with the results of Cowling (1956) and of Donaldson, Davies, Darg, and Richmond (1969) who found relatively high prothrombin levels with the same method in patients with chronic liver disease. They were scarcely compatible with the occurrence of spontaneous bleeding and with the severity of liver failure as judged by the impairment of other test of protein synthesis, whereas a good correlation was obtained between albumin, cholinesterase, and the one-stage assay of prothrombin. Quick's prothrombin time was also shown to be often normal in these series of patients with bleeding (Table). The decreased capacity of inactivating thrombin showed by cirrhosis plasmas might possibly account for this discrepancy observed between the one- and two-stage assays of prothrombin. This view is supported by the existence of a negative correlation between progressive antithrombin and the index of discrepancy of the two assays. When progressive antithrombin is decreased, larger amounts of thrombin formed by prothrombin conversion are detectable at any time in the incubation mixtures, causing overestimation of prothrombin when measured with the two-stage 'area' method. An additional factor possibly contributing to overestimation of prothrombin in the two-stage assay is hypofibrinogenaemia, which is often encountered in these patients. Fibrin possess the capacity of binding a proportion of the thrombin formed in a clotting mixture, a property designated 'antithrombin I' (Hardisty and Pinniger, 1956). The opposite pattern was observed in acute hepatitis, ie, the two-stage assay of prothrombin gave lower values than the one-stage assay. It might be expected that this discrepancy could be accounted for by high levels of thrombin inhibitors, but in fact we were unable to find consistently high levels of progressive antithrombin in our patients and no correlation was seen between either assay and antithrombin. However, in this group of patients also the one-stage assay was the better correlated with the indices of protein synthesis.

From the present study it appears, therefore, that the one-stage method employing tiger snake venom, which is not influenced by antithrombin (Jobin, 1965), is more reliable than the two-stage 'area' method for measuring prothrombin in liver disease. Estimation of prothrombin with this method in patients with liver cirrhosis shows that values under 40%, which are considered necessary for optimal haemostasis, are often attained. A high proportion of these patients with low levels of prothrombin were seen to bleed spontaneously. Therefore, although depletion of clotting factors is possibly only one of the causes of abnormal bleeding

in chronic liver failure, it might be advisable to add this test to the prothrombin time for the evaluation of the haemostatic balance before surgery or during bleeding episodes.

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