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Clinical significance of "circulating fibrin monomers"

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SUMMARY During the decade 1970-9 we investigated circulating fibrin monomers in 3293 patients. Fibrinaemia was determined by means of the ethanol gelation test (EGT). This was positive in 149 patients (4·5%) and was highly correlated with fibrogenal fibrin products. In many diseases the test was only transiently positive (1 or 2 days). However in patients with circulating fibrin monomers demonstrable for more than 5 days (chronic fibrinaemia) malignant disease was associated in 63% Chronic fibrinaemia occasionally preceded overt malignancy by a long period. Overall, only 10·8% of patients with malignant disease showed chronic fibrinaemia. The clinical symptoms most often associated with chronic fibrinaemia were those of venous thrombosis (42·8%) and abnormal bleeding (10·7%). Thromboembolism in the absence of malignant disease only occasionally showed short-term positive EGT and chronic fibrinaemia was never seen. Almost half (46·5%) of patients with chronic fibrinaemia had neither thromboembolic disease nor a haemorrhagic diathesis.

The presence of thrombin-induced fibrinogen derivatives in the blood is considered by some authors to be a sign of hypercoagulability of the plasma.[1-6] Fibrin monomers are known to form complexes with fibrinogen and fibrinogen degradation products (FDP/fdp), giving rise to high molecular weight complexes which are initially soluble.[2 7] When such complex formation occurs in the blood, a condition tentatively termed fibrinaemia is present.[8] These complexes may be cleared by the reticuloendothelial system (RES) or deposited either diffusely (diffuse intravascular coagulation) or locally (local thrombosis).[9-11] Fibrin monomers can be detected by several laboratory methods which vary in laboriousness and sensitivity.[1 4 12-15]

The clinical usefulness of a laboratory test in large numbers of patients is improved when, while remaining reliable, it is easy to perform. The ethanol gelation test (EGT) for the detection of circulating fibrin monomers, introduced by Godal and Abildgard,[1] fulfills these two criteria; however its sensitivity is limited because it only becomes positive when 2·4-3·8% plasma fibrinogen is circulating in the form of fibrin monomers.[16] Using this test, fibrin monomer complexes have been detected in several diseases known to be associated with intravascular fibrin formation.[3 8 12] Although one study has evaluated this test in a large number of hospital patients on admission,[17] the clinical significance of prolonged circulation of fibrin monomers remains obscure.

In order to define the clinical value of the EGT we have performed this test in about one third of all patients admitted to our Department of Internal Medicine between 1970 and 1979. In a substantial number of these patients the test was frequently repeated to establish the effect of treatment and the course of the disease on the outcome of the test. The presence of a positive EGT was defined as fibrinaemia. We were especially interested in the significance of "chronic fibrinaemia" in its relation to thrombotic and haemorrhagic conditions and in the effect of antithrombotic drugs on the presence of fibrin monomers in vivo. In addition we have correlated the outcome of the EGT with fibrinogen levels and the level of FDP/fdp.

Materials and methods

PATIENTS

In the initial phase of the study, we performed the EGT in patients with all kinds of diseases admitted to the Department of Internal Medicine. In the last part of the study we were especially interested in patients with malignancy or suspected malignancy, thromboembolism, haemorrhagic diathesis, septicaemia, and liver disease.

The diagnosis of malignancy was in most cases confirmed by histological examination. Septicaemia was confirmed by positive blood cultures. The

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clinical diagnosis of liver cirrhosis was confirmed by liver biopsy. Venous thromboembolism was usually diagnosed by clinical examination but included in the study was a group of 37 patients with established venous thromboembolism, which has been described previously together with diagnostic criteria.[18] We performed the EGT in 3293 patients, 37·3% of those admitted to our Department.

BLOOD SAMPLING

Blood was collected by venipuncture: nine volumes of venous blood were mixed as soon as possible with one volume of 3·8% trisodium citrate solution. The plasma was separated from the cells by centrifugation for 10 min at 1260 *g* at room temperature.

THE ETHANOL GELATION TEST

The EGT was performed according to Godal and Abildgard.[1] In brief: 0·5 ml plasma was mixed with 0·5 ml 50% ethanol and was left undisturbed at room temperature for exactly 10 minutes. It was then examined for gel formation by gently tilting the tube. Only when clear gel formation occurred, was the test considered positive. When only precipitation or change of opacity occurred in the absence of gel formation, the test was considered to be negative.

Although the results of a single determination are included in the results, the final analysis only refers to those patients for whom the test was positive on at least two occasions, necessitated as a check against false positive tests arising from a technically poor venipuncture.

FIBRINOGEN

Fibrinogen was determined as described by Strengers and Asberg.[19] Normal value: 200-400 mg/100 ml.

FIBRINOGEN/FIBRIN DEGRADATION PRODUCTS (FDP/fdp)

FDP/fdp were determined by the method described by Laurell.[20] Four ml blood were collected into 0·04 ml aprotinine (Transylol: 100 000 KIU/ml) and kept at 37°C for 2 hours, after which it was centrifuged for 10 min at 1260 *g*. One ml of the supernatant serum was incubated with 20 U thrombin at 37°C for 15 min and then centrifuged for 15 min at 1260 *g*. The samples were stored at −20°C until testing. All normal subjects had a level below 1·5 mg/100 ml serum.

THROMBIN

Thrombin (Topostasin, Roche, Basle) was dissolved in saline (0·9% NaCl) to a final concentration of 500 U/ml. Freshly prepared thrombin solution was used.

Results

Between 1970 and 1979 we performed the EGT on 6782 occasions in a total of 3293 patients with various diseases. In 149 of these (4·5%) the test was positive at least once. Since the detection of a positive EGT prompted further investigations, it was possible to determine the duration of this abnormality. As is shown in Table 1 the test was positive for only one day in 83 patients, for two to five days in 21 patients and for more than five days in 45 patients. The longest positive EGT was followed for five years.

Table 1 *Duration of the positive ethanol gelation test in 149 patients*

Duration	*Ethanol gelation test*			*Total*
	Positive once	*Positive twice*	*Positive more than twice*	
One day	57	16	10	83
Two days		7	5	
Three days			3	21
Four days			3	
Five days		1	2	
Six days		1	3	45
> six days			41	
Total	57	25	67	

CLINICAL DIAGNOSIS

The data in Table 2 demonstrate that a large variety of disorders may be associated with a positive EGT. It appears that short-term fibrinaemia is found mostly in patients with acute diseases including septicaemia, bacterial meningitis, abruptio placentae and dissecting aortic aneurysm. In 10 out of 60 patients, malignant disease was associated with short-term fibrinaemia. Of the 44 patients with chronic fibrinaemia, 28 (63·6%) had a malignancy and 12 of these presented with venous thromboembolism. In one patient the EGT was positive for four years before a tumour became apparent. The malignancies most often associated with chronic fibrinaemia were carcinomas of the pancreas, gall bladder, prostate, and bronchus (Table 3).

THROMBOHAEMORRHAGIC CONDITIONS, FDP/fdp AND FIBRINAEMIA

Thirty-seven patients had established venous thromboembolism, but without malignancy, fibrinaemia was detected in only two of these and this was short-term and never a chronic phenomenon. The most prominent clinical symptom of chronic fibrinaemia was venous thromboembolism (Tables 2 and 4). In patients with malignancy and chronic fibrinaemia, a haemorrhagic diathesis was present only occasionally and in about half of such patients thrombohaemorrhagic symptoms were absent (Table

Table 2 *Associated diseases in patients showing a positive ethanol gelation test*

	Patients				
	EGT Positive once		EGT Positive twice		EGT Positive more than five days
	Tested once	Tested more than once[1]	Tested twice	Tested more than twice	
Sepsis (gram negative)	1	3	1		2
Malignancy without thromboembolism	3[2]	5		3	13
Malignancy with thromboembolism		2			12
Malignancy with haemorrhagic diathesis	1				3
Venous thromboembolism without malignancy	2	5		3	4[3]
Heroine addict with diffuse intravascular coagulation (DIC)		1			
Meningitis (bacterial)		4		3	1
Abruptio placentae				1	
Liver cirrhosis	1	4		3	3
Aneurysm cardiac				1	
aortic dissecting	2[2]	1	1	3	
Nephrotic syndrome					1
Leukaemia	1				3
Autoimmunohaemolytic anaemia and DIC					1
Unknown	5	5	2		2
Miscellaneous	2[4]	10[5]	1[6]	3[7]	

[1]The test was initially positive but negative when repeated.
[2]One patient had a dissecting aortic aneurysm and lung cancer.
[3]One patient had a venous thromboembolism with sepsis.
[4]Intoxication with analgesic drugs, bleeding ulcer with shock.
[5]Leptospirosis, vasculitis, diabetic coma, Guillain-Barré syndrome, haemolytic anaemia, pneumonia, malaria, Marfan's syndrome, emphysema, infected delivery.
[6]Myocardial infarction.
[7]Vasculitis, ulcerative colitis, tuberculosis.

Table 3 *Patients with chronic fibrinaemia and associated malignancy*

Carcinoma of the pancreas or gallbladder	12
Prostatic carcinoma	6
Bronchial carcinoma	5
Carcinoma of the stomach	1
Seminoma	1
Carcinoma of the breast	2
Carcinoma of indeterminate site	1
Total	28

4). ^{125}I-fibrinogen was injected in 3 of these patients to find out if subclinical thrombosis was present. Leg scanning in these patients was negative, but the in vivo half-life of the injected ^{125}I-fibrinogen was shortened (mean value: 28 h, normal: 4-5 days). Arterial thrombi were not seen in patients with chronic fibrinaemia.

Thromboembolism was found in malignancy without chronic fibrinaemia in 11 of 231 patients (4·7%). The serum FDP/fdp level was determined in 115 patients with malignancy and a negative EGT. The FDP/fdp level was less than 2 mg/100 ml in 57 of these patients. Three of these showed clinical thromboembolism (5·2%). In the residual 58 patients the serum FDP/fdp level was 2 mg/100 ml or more and eight of these showed clinical thromboembolism (13·7%). Thirty-seven patients had established venous thromboembolism, but without malignancy, fibrinaemia was detected in only two of these and this was short-term and never a chronic phenomenon.

Table 4 *Thrombohaemorrhagic disease associated with malignancy and chronic fibrinaemia in 28 patients*

Thromboembolism	12
Haemorrhagic diathesis	3
Neither of these conditions	13

RELATION BETWEEN FIBRINOGEN, FDP/fdp AND FIBRINAEMIA

The distribution of fibrinaemia at various fibrinogen levels is shown in Table 5. The majority of patients with fibrinaemia had a fibrinogen level between 50 and 500 mg/100 ml; at fibrinogen concentrations of more than 500 mg/100 ml the incidence of a positive

Table 5 *Fibrinogen levels correlated with a positive ethanol gelation test*

Fibrinogen level (mg/100 ml)	Patients		%
	Total number	Positive EGT	
50	3	1	33·3
50-150	186	58	31·0
150-500	3488	362	10·4
500-600	421	20	4·8
600	381	20	5·3

EGT dropped. The level of FDP/fdp in serum exceeded 1·5 mg/100 ml and was more than 2 mg/100 ml in 97·3% and 93% respectively of all patients with a positive EGT (Table 6). The level of FDP/fdp in serum exceeded 1·5 mg/100 ml and was more than 2 mg/100 ml in 29% and 21% respectively of all patients with a negative EGT (Table 6). High fibrinogen levels do not exclude the possibility of a negative ethanol gelation test.

Table 6 *Correlation between a positive or negative ethanol gelation test and an elevated level of FDP/fdp in serum*

	EGT positive	*EGT negative*
FDP/fdp ⩾ 1½ mg/100 ml	97·3%	29%
FDP/fdp ⩾ 2 mg/100 ml	93%	21%

EFFECT OF TREATMENT ON CHRONIC FIBRINAEMIA

In all patients with a positive EGT for more than 5 days, intravenous heparin administration at a dosage of 1500 U/h to 7500 U/h was followed by a negative EGT. When, instead of heparin, coumarin derivatives were administered, the EGT remained positive even when the prothrombin time was prolonged more than twice control value. In those patients whose positive EGT had become negative upon heparin administration (except in one patient), coumarin derivatives failed to maintain a negative EGT.

In some patients with prostatic carcinoma, it was possible to evaluate the effect of hormonal treatment. These patients showed a negative EGT after induction of a remission by hormonal treatment. A negative EGT became positive again when heparin treatment was discontinued, except in patients with prostatic carcinoma who responded to hormonal treatment. Two patients "escaped" hormonal treatment for prostatic carcinoma. In one there was a reappearance of the fibrinaemia after a few months. Fibrinaemia did not reappear in the second patient even after several years.

Discussion

The aim of the present study was to define the clinical significance of fibrin monomers as detected by the EGT. Short-term fibrinaemia was found to be present in a number of patients with acute disorders such as septicaemia, abruptio placentae, dissecting aortic aneurysm and venous thromboembolism (Table 2). Since anticoagulant (heparin) treatment was instituted in patients with venous thromboembolism shortly after diagnosis, it is reasonable to suppose that such treatment could have influenced fibrinaemia. One might expect, however, when the EGT is repeated after withdrawal of heparin treatment, to see the EGT become positive again when the thrombin producing process is still operative.

Long-term fibrinaemia was found most often in patients with malignancy. Chronic fibrinaemia may be present before the malignant process is diagnosed. In one of our patients with chronic fibrinaemia, repeated examinations during a 4 year follow-up period failed to detect malignancy; finally, after this period, a non-Hodgkin's lymphoma was found. Peuscher *et al.*,[18] have reported that fibrinopeptide A (a peptide cleaved from the fibrinogen molecule by thrombin) or the in vitro generation of fibrinopeptide A or both, are often increased in patients with metastatic malignancy, indicating that local or systemic thrombin is present.

Several mechanisms have been postulated to explain how activation of blood coagulation and fibrin formation in such cancer patients may proceed. Among the activating substances are: tumour-derived thromboplastin-like material,[21–23] proteolytic enzymes[24 25] and platelet factors.[26–28] In addition penetration of the malignant process in the vessel wall may be responsible for activation of blood coagulation in some patients.[29] It is intriguing that circulating monomer complexes may be present for such a long period without giving detectable organ damage, for example, in the kidney or lungs. Apparently they are completely soluble and defence systems, such as fibrinolysis, are stimulated to prevent systemic fibrin deposition. The increased level of FDP/fdp in almost all patients with fibrinaemia reflects augmented fibrinolysis. It is far from clear whether or not coagulation (formation of a fibrin net) is present in patients with chronic fibrinaemia. So chronic diffuse intravascular coagulation does not seem to be an appropriate description for all these conditions. Since 42·8% of patients with chronic fibrinaemia suffered from venous thrombosis or pulmonary embolism, the question arises as to whether fibrin monomers can also be only a result of thrombosis. Therefore we studied 37 patients with established thromboembolism, but without malignancy. None of them had chronic fibrinaemia. Besides that, we found no local signs of thrombosis in three patients with chronic fibrinaemia by ^{125}I-fibrinogen leg scanning.

It has been reported that almost 50% of the patients in which the EGT is positive display an increased fibrinogen level (> 500 mg/100 ml).[16 17] This is not in agreement with our results.

The effect of anticoagulant treatment on fibrinaemia was not uniform. In all patients the EGT became negative upon heparin treatment although

the dosage required varied widely. It appears most logical to relate the effect of heparin to its inhibitory action on activated coagulation factors (X_a) and thrombin through antithrombin III. When instead of heparin, coumarin derivatives were used, chronic fibrinaemia persisted despite adequate prolongation of the prothrombin time.

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