

Plasma fibronectin in normal subjects and in various disease states

NE STATHAKIS, A FOUNTAS, E TSIANOS

From the Professorial Medical Unit, Evangelismos Hospital, Athens, Greece

SUMMARY Plasma fibronectin was determined in 121 normal adults and in 149 patients. Fibronectin levels in normals were strongly influenced by sex and age. The mean value of the protein in cancer patients did not differ from that in normal controls; however, patients with cryofibrinogenaemia or extensive liver metastases had lower values whereas those with obstructive jaundice due to pancreatic carcinoma had higher values than normal controls. Fibronectin levels were greatly increased in patients with primary biliary cirrhosis and moderately elevated in nephrotic syndrome. In patients with severe infection or sepsis, plasma fibronectin did not show a consistent pattern. Patients with overt disseminated intravascular coagulation, irrespective of its cause, had the lowest plasma fibronectin concentrations.

Fibronectin (also known as cold-insoluble globulin) is a glycoprotein with a molecular weight of 450 000. It is composed of two apparently identical subunits, which are held together with disulphide bonds.^{1,2} Fibronectin, which is a normal constituent of plasma, is also widely deposited in connective tissue, blood vessel walls, and basement membranes, being a major non-collagenous component of all organ stroma. Among its several properties of special interest are the following: it crosslinks with fibrin when blood clots through the action of fibrin stabilising factor;³ it has an affinity for fibrin at both room and low temperatures⁴⁻⁶ while its affinity for fibrinogen is weak and evident only in the cold;⁶ it binds to heparin and is the only protein precipitated by heparin at +4°;⁷ it has a strong affinity and binds to denatured and, less actively, to native collagen;⁸ it mediates the adhesion of fibroblasts to collagen;⁹ and it is a mediator of the attachment of denatured collagen to macrophages.¹⁰

The concentrations of plasma fibronectin in normal subjects and in various diseases have been determined in several laboratories.¹¹⁻¹⁵ However, some of the data reported are contradictory and inadequate for final conclusions to be drawn. In the present study, we report the results of fibronectin measurements in the plasma of 121 normal subjects and of 149 patients with various disease states.

Material and methods

The clinical material studied included the following groups: (1) 121 normal subjects (61 men and 60 women), mostly blood donors; (2) 94 patients (60 men and 34 women) with advanced cancer. This group included patients with cancer of the lung (14), stomach (12), pancreas (16), colon (10), liver (8), ovaries (6), and miscellaneous other sites; (3) 24 patients with infection (pneumonia, pyelonephritis, cellulitis) or sepsis (with both Gram-positive and Gram-negative bacteraemia) (14 men and 10 women); (4) five women with primary biliary cirrhosis. This diagnosis was confirmed in all cases by liver biopsy; (5) 10 patients with nephrotic syndrome (5 men and 5 women). Histological examination of kidney biopsy material revealed membranous glomerulonephritis (6 cases), membranoproliferative glomerulonephritis (2 cases), focal glomerulonephritis (1 case), and amyloidosis (1 case); (6) 14 patients with overt disseminated intravascular coagulation (DIC) secondary to carcinoma (5), acute leukaemia (4), and septicaemia (5). All these cases had decreased fibrinogen concentration, thrombocytopenia, prolonged prothrombin and partial thromboplastin times, and increased fibrin degradation products.

PLASMA FIBRONECTIN DETERMINATIONS

Fasting venous blood, collected by venepuncture

with siliconised needles and plastic syringes, was added to a 1/10 volume of 3.8% sodium citrate in silicone-coated tubes. Plasma was prepared by centrifugation at 2400 *g* for 20 minutes at room temperature; it was frozen at -30° and thawed just before assay.

Fibronectin concentration was determined by Laurell's electroimmunoassay¹⁶ in 1% agarose containing monospecific antiserum (a generous gift from Dr Mosesson, Downstate Medical Center, State University of New York, Brooklyn, USA). Electrophoresis was performed overnight at 90 volts using barbital buffer (pH 8.6, $I = 0.05$). Plates were washed for 8-10 hours in tris-buffered saline and fixed in 1% tannic acid or squashed, dried, and stained with Coomassie brilliant blue.

Purified plasma fibronectin was prepared as previously described¹⁷ and served as a primary standard. The protein concentration of the purified protein was determined by absorbance at 280 nm by assuming absorption coefficient ($A_{1\text{cm}}^{1\%}$) 12.8.¹ The primary standard was used to determine the concentration of fibronectin in pooled citrated plasma from 20 normal adults. Portions of 1 ml of the pooled plasma were stored at -30° and served as secondary standards. On each plate single applications of four dilutions of the secondary standard, together with double applications of one dilution of unknown plasma, were made.

OTHER STUDIES

For the detection of cryofibrinogenaemia, 2 ml of fresh citrated plasma were incubated in a silicone-coated tube for 24 hours at $+4^{\circ}$. Positive cases formed a cryoprecipitate, which was not present in the serum of the same patient refrigerated under the same conditions.

Fibrinogen was determined by the method of Ratnoff and Menzie.¹⁸ The remainder of the coagulation tests were performed using standard techniques.¹⁹

Student's *t* test was applied to calculate the significance of differences.

Results

PLASMA FIBRONECTIN IN NORMAL SUBJECTS

The mean fibronectin concentration in the citrated plasma of 121 normal adults was $325 \pm 76 \mu\text{g/ml}$. The men had higher fibronectin plasma levels than the women ($P = 0.05$) (Table 1). The pattern of distribution of plasma fibronectin levels according to sex and age permitted the separation of the whole material into three age groups: 21-30 years, 31-50 years, and over 51 years. The mean value of each subgroup is shown in Table 2. In both men and women, fibronectin was significantly lower in the 21-30 year group than in the older age groups. The fibronectin concentrations in men aged 31-50 and over 51 were similar, while the concentration of protein was lower in women aged 31-50 years than in women over 51 years, but this difference did not reach statistical significance.

Table 1 *Fibronectin levels in normal subjects*

	Number	Age (years)	Fibronectin ($\mu\text{g/ml}$)
All normal subjects	121	40.5 ± 17.1	325.0 ± 76.0
Men	61	40.8 ± 18.3	$339.4 \pm 70.2^*$
Women	60	40.2 ± 16.2	$311.8 \pm 81.5^*$

*Men v women: $t = 1.99$ ($P = 0.05$).

Analysis of the data in women according to menopausal state showed that premenopausal women had less fibronectin ($284.6 \pm 71.9 \mu\text{g/ml}$) than postmenopausal women ($347.8 \pm 84.1 \mu\text{g/ml}$) ($P < 0.01$). When plasma fibronectin levels of the three age groups were related to sex it was found that men aged 31-50 years had significantly higher levels than women of the same age ($P < 0.05$). In the

Table 2 *Fibronectin concentrations in normal subjects: distribution according to age and sex*

	Age groups (years)					
	21-30		31-50		51-80	
	No	Fibronectin ($\mu\text{g/ml}$)	No	Fibronectin ($\mu\text{g/ml}$)	No	Fibronectin ($\mu\text{g/ml}$)
All normal subjects	40	284.4 ± 61.1	51	340.1 ± 79.2	30	354.8 ± 75.3
Men	21	300.1 ± 61.9	25	366.5 ± 70.2	15	349.6 ± 58.6
Women	19	267.1 ± 67.6	26	316.8 ± 80.4	15	360.0 ± 91.0

Statistically significant *t*-test values:

All subjects 21-30 yr v all subjects 31-50 yr: $t = 3.79$ ($P < 0.01$)
 Men 21-30 yr v men 31-50 yr: $t = 3.40$ ($P < 0.01$)
 Women 21-30 yr v women 31-50 yr: $t = 2.24$ ($P < 0.05$)
 Men 31-50 yr v women 31-50 yr: $t = 2.34$ ($P < 0.05$)

other age groups there were no significant differences in fibronectin levels between the two sexes.

PLASMA FIBRONECTIN IN CANCER PATIENTS

The mean fibronectin concentration in cancer patients did not differ from that of matched normal controls (Table 3). However, of the patients, 19 had fibronectin concentrations above (7 patients) or below (12 patients) the normal limits.

Table 3 *Plasma fibronectin levels in patients with advanced cancer*

Group	No	Age (years)	Fibronectin ($\mu\text{g/ml}$)
All patients with cancer	94	60.3 \pm 11.4	348.0 \pm 121.0
All controls	48	57.4 \pm 13.9	350.0 \pm 74.7
Male patients	60	61.2 \pm 10.6	344.1 \pm 125.3
Matched controls	31	58.7 \pm 16.3	353.5 \pm 64.8
Female patients	34	58.3 \pm 12.1	356.7 \pm 111.2
Matched controls	17	55.0 \pm 11.3	343.6 \pm 84.3

The origin of the cancer (evaluated in carcinoma of the lung, stomach, pancreas, colon, ovaries, and liver) did not influence the fibronectin concentration. In contrast, fibronectin levels were influenced by some complications of the disease. As shown in Table 4, patients with cryofibrinogenaemia had a small but significant decrease in fibronectin levels. In addition, patients with extensive neoplastic infiltration of the liver had low plasma fibronectin whereas the protein was significantly elevated in patients with obstructive jaundice due to carcinoma of the pancreas (Table 5).

Table 4 *Plasma fibronectin in cancer patients with or without cryofibrinogenaemia*

Cryofibrinogenaemia	No	Age (yr)	Fibronectin ($\mu\text{g/ml}$)
(+)	11	55.8 \pm 11.5	282.2 \pm 110.2
(-)	83	60.7 \pm 11.4	357.5 \pm 115.4*

*Patients (+) v patients (-): $t = 2.12$ ($P < 0.05$).

Table 5 *Plasma fibronectin in cancer patients with extensive liver metastases or cholestasis*

Group	No	Age (yr)	Fibronectin ($\mu\text{g/ml}$)
Cancer patients with extensive liver metastases	10	62.6 \pm 10.0	259.8 \pm 79.9*
Patients with obstructive jaundice secondary to pancreatic carcinoma	9	58.7 \pm 11.7	444.6 \pm 81.6†
Controls	48	57.4 \pm 13.9	350.0 \pm 74.7

*Patients v controls: $t = 3.28$ ($P < 0.01$).

†Patients v controls: $t = 3.98$ ($P < 0.001$).

FIBRONECTIN IN SEVERE INFECTIONS AND SEPSIS, PRIMARY BILIARY CIRRHOSIS, AND NEPHROTIC SYNDROME (Table 6)

The mean fibronectin concentration of patients with severe infections and sepsis did not differ from that of matched normal controls. However, among the patients nine had decreased and one increased values.

In patients with primary biliary cirrhosis fibronectin levels were significantly elevated ($P < 0.001$).

A small but significant elevation of fibronectin was also found in patients with nephrotic syndrome ($P < 0.05$).

Table 6 *Plasma fibronectin in patients with severe infection or sepsis, primary biliary cirrhosis, and nephrotic syndrome*

Group	No	Age (years)	Fibronectin ($\mu\text{g/ml}$)
Severe infections and sepsis	24	52.7 \pm 12.6	284.4 \pm 183.6*
Matched controls	15	50.4 \pm 11.6	346.4 \pm 83.2
Primary biliary cirrhosis	5	45.4 \pm 7.9	630.8 \pm 136.0†
Matched controls	8	45.3 \pm 7.4	332.5 \pm 49.7
Nephrotic syndrome	10	42.4 \pm 13.6	443.0 \pm 127.0‡
Matched controls	12	44.1 \pm 12.2	332.0 \pm 64.2

* $t = 1.4$ ($P > 0.05$).

† $t = 4.7$ ($P < 0.001$).

‡ $t = 2.5$ ($P < 0.05$).

FIBRONECTIN IN OVERT DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

The mean value of fibronectin was much lower in patients with DIC (107 \pm 66.6 $\mu\text{g/ml}$) than in corresponding normal controls (336 \pm 71.0 $\mu\text{g/ml}$) ($P < 0.001$). In 14 out of 16 patients studied, fibronectin levels were lower than the lower limits of normal.

Discussion

The results of the present study indicate that plasma fibronectin concentrations in normal adults are strongly influenced by sex and age. Men had higher fibronectin concentrations than women. This finding, which confirms previous observations,^{11,12} was significant only for the age group 31-50 years. Women under 30 tended to have lower values whereas those over 50 years did not differ from the men. Both men and women showed a significant increase in fibronectin concentrations from the third to the fourth decade of life. In addition, premenopausal women had lower fibronectin levels than postmenopausal women. Although these complex relations of fibronectin levels to sex and age imply hormonal influences, it is very likely that other factors may play an important role. In any event,

these variations in plasma fibronectin must be taken into consideration in any study on the behaviour of this protein in various disease states.

There is little information concerning plasma fibronectin in patients with cancer. Bruhn and Heimburger¹³ reported normal levels in an unstated number of patients with malignancies. In the present study, although the mean value of plasma fibronectin in patients with advanced cancer did not differ from that of the matched controls, 20% of the patients had fibronectin concentrations above or below the normal limits. This may indicate that among cancer patients there are subgroups with abnormally high or low plasma fibronectin levels. Interesting in this respect are the results of Mosher and Williams,¹² who found that although fibronectin was elevated in a small number of patients with metastatic breast carcinoma, it was decreased in severely ill patients with cancer. Analysis of our data revealed that the site of origin did not influence plasma fibronectin, at least for the carcinomas of the lung, stomach, liver, pancreas, and ovaries, for which an adequate number of patients was studied. In contrast, fibronectin levels were related to some complications of the disease, namely, obstructive jaundice, cryofibrinogenaemia, and extensive liver metastases. The low values found in the last group of patients corroborate the results of Mosher and Williams¹² that this protein is decreased in severely ill patients.

The relatively low fibronectin levels in patients with cryofibrinogenaemia may be due to enhanced catabolism of the protein secondary to intravascular fibrin formation. Fibronectin complexes with fibrin both *in vitro*⁶ and *in vivo*.²⁰ These complexes, which precipitate in the cold *in vitro*, are rapidly cleared from the blood *in vivo*. As a result, fibronectin levels are usually very low in overt DIC,²¹ a finding confirmed in the present study. In cryofibrinogenaemic cancer patients without overt DIC, fibronectin levels are intermediate between normal and those in overt DIC.

Patients with obstructive jaundice due to pancreatic carcinoma had slightly elevated plasma fibronectin. Forkman *et al.*¹⁴ reported very high levels in patients with recurrent cholestasis of pregnancy. Similarly, we found that the protein was elevated in patients with primary biliary cirrhosis. It appears, therefore, that an increased plasma fibronectin concentration is a general characteristic of cholestasis irrespective of its aetiology. A slight but significant elevation of fibronectin levels also seems to characterise nephrotic syndrome.

Plasma fibronectin did not follow a consistent pattern in patients with severe infection or sepsis. Mosher²² found a significant decrease in plasma

fibronectin in severely ill monkeys with experimental Rocky Mountain spotted fever which he attributed to fibronectin and fibrin deposition and lysis in infected blood vessels. However, he did not find any change in experimentally induced *Salmonella typhi* and *Streptococcus pneumoniae* bacteraemia. These observations in animals may explain our results in clinical material where a great range of values was obtained.

References

- Mosesson MW, Umfleet RA. The cold insoluble globulin of human plasma. I. Purification, primary characterization and relationship to fibrinogen and other cold-insoluble fraction components. *J Biol Chem* 1970; **245**:5728-36.
- Mosesson MW, Chen AB, Huseby RM. The cold insoluble globulin of human plasma: studies of its essential structural features. *Biochim Biophys Acta* 1975; **386**: 509-24.
- Mosher DF. Cross-linking of cold-insoluble globulin by fibrin stabilizing factor. *J Biol Chem* 1975; **250**:6614-21.
- Ruoslahti E, Vahei A. Interaction of soluble fibroblast surface antigen with fibrinogen and fibrin. Identity with cold-insoluble globulin of human plasma. *J Exp Med* 1975; **141**:497-501.
- Stemberger A, Hörmann H. Affinity chromatography on immobilized fibrinogen and fibrin monomer. II. The behaviour of cold-insoluble globulin. *Hoppe-Seyler's Z Physiol Chem* 1976; **357**:1003-5.
- Stathakis NE, Mosesson MW, Chen AB, Galanakis DK. Cryoprecipitation of fibrin-fibrinogen complexes induced by the cold-insoluble globulin of plasma. *Blood* 1978; **51**:1211-22.
- Stathakis NE, Mosesson MW. Interactions among heparin, cold-insoluble globulin and fibrinogen in formation of the heparin precipitable fraction of plasma. *J Clin Invest* 1977; **60**:855-65.
- Ruoslahti E, Engvall E. Immunochemical and collagen-binding properties of fibronectin. *Ann NY Acad Sci* 1978; **312**:178-91.
- Gold LI, Pearlstein E. Fibronectin-collagen binding and requirements during cellular adhesion. *Biochem J* 1980; **186**:551-9.
- Hörmann H, Jelinic V. Fibronectin. VII. Binding of cold-insoluble globulin and denatured collagen by macrophages. *Hoppe-Seyler's Z Physiol Chem* 1980; **361**: 379-87.
- Fyrand D, Solum NO. Studies on cold-insoluble globulin in dermatological patients. I. Immunochemical quantitation in citrated plasma from patients with increased amounts of heparin precipitable fraction (HPF). *Thromb Res* 1976; **9**:447-55.
- Mosher DF, Williams EM. Fibronectin concentration is decreased in plasma of severely ill patients with disseminated intravascular coagulation. *J Lab Clin Med* 1978; **91**:729-35.
- Bruhn HD, Heimburger N. Factor-VIII-related antigen and cold-insoluble globulin in leukaemias and carcinomas. *Haemostasis* 1976; **5**:189-92.
- Forkman B, Ganrot PO, Gennser G, Rannevik G. Plasma protein pattern in recurrent cholestasis of pregnancy. *Scand J Clin Lab Invest* 1972; **29** (Suppl 124):89-96.
- Ganrot PO. Variation of the concentrations of some plasma proteins in normal adults, in pregnant women and in newborns. *Scand J Clin Lab Invest* 1972; **29** (Suppl

- 124):83-93.
- ¹⁶ Laurell C-B. Electro-immunoassay. *Scand J Clin Lab Invest* 1972;**29** (Suppl 124):21-37.
- ¹⁷ Chen AB, Mosesson MW. An improved method for purification of the cold insoluble globulin of human plasma (CIG). *Anal Bioch* 1977;**79**:144-9.
- ¹⁸ Ratnoff OD, Menzie C. New method for determination of fibrinogen in small samples of plasma. *J Lab Clin Med* 1951;**37**:316-20.
- ¹⁹ Dacie JV, Lewis SM. *Practical haematology*, 5th ed. Edinburgh, London, and New York: Churchill Livingstone, 1975.
- ²⁰ Sherman LA, Lee J. Fibronectin: its blood clearance and interaction with fibrin in vivo. *Thromb Haemost* 1979;**42**:121.
- ²¹ Matsuda M, Yoshida N, Aoki N, Watabayashi K. Distribution of cold-insoluble globulin in plasma and tissues. *Ann NY Acad Sci* 1978;**312**:74-92.
- ²² Mosher DF. Changes in plasma cold-insoluble globulin concentration during experimental Rocky Mountain spotted fever infection in rhesus monkeys. *Thromb Res* 1976;**9**:37-45.

Requests for reprints to: Dr NE Stathakis, Department of Medical Oncology, St Bartholomew's Hospital, London EC1A 7BE, England.