

Plasma fibronectin in Crohn's disease

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SUMMARY The hypothesis that abnormal fibronectin metabolism in Crohn's disease could be an important mechanism leading to stricture formation or postoperative infection was tested in three related studies. (1) Lower concentrations of plasma fibronectin ($p < 0.05$) were found in 20 patients with small and large bowel Crohn's disease (mean 0.24 g/l) compared with 13 patients with more limited disease confined to only small or only large bowel (mean 0.27 g/l) or 20 healthy controls (mean 0.29 g/l). (2) In 25 patients followed for 10 days after operation for Crohn's disease, there was a significant fall in fibronectin concentrations of 43% ($p < 0.01$, Wilcoxon's rank-sum test). This fall was maximal on the second postoperative day and was more marked in patients undergoing more major operative procedures. (3) The predictive value of plasma fibronectin for subsequent stricture formation or progression was studied for one year; during which 10 patients developed strictures requiring operative treatment. Higher plasma fibronectin concentrations were related to stricture formation, although there was not a complete, predictive relationship. In this study we found that plasma fibronectin concentrations were low in patients with extensive or severe Crohn's disease, fall after operation and may be related to the risk of stricture formation. This relationship is unlikely to be of clinical value, although it shows the potential significance of fibronectin in the pathogenesis of strictures.

Fibronectin is a large glycoprotein of plasma, tissue fluids, and tissues. It is involved in several biochemical processes in chronic inflammatory disorders, and is produced by variety of mesenchymal cells.¹⁻³ Fibronectin binds to fibroblasts and macrophages;⁴ and it interacts with other connective tissue components such as collagen, hyaluronic acid, and fibrin.⁵ These biochemical activities are important in chronic inflammation as well as in the genesis of fibrous tissue in disorders such as Crohn's disease. Alterations of these functions may be important in the production of enteric fibrous strictures in Crohn's disease. Another role of fibronectin is opsonisation of particulate material. Most information has been gained on its opsonic role from studies of gelatin coated particles. But it can also have a

similar influence on certain bacteria and enhances their adherence of bacteria to mononuclear phagocytic cells.⁶ After operations in patients with Crohn's disease, falls in circulating fibronectin may increase the risk of sepsis because of impaired phagocytosis by mononuclear cells.

There have been several studies of plasma fibronectin concentrations before and after operation⁷⁻¹² but the findings are conflicting. Some report a fall in plasma fibronectin immediately after operation^{10,11} while others do not.¹² So far there have been no studies of the influence of operation on plasma fibronectin concentrations in patients with Crohn's disease in three related studies to examine: (1) The relationship between the extent and severity of the disease and fibronectin activity. (2) The influence of an operation on fibronectin concentrations. (3) The relationship between plasma fibronectin concentrations and the risk of enteric stricture or postoperative infection.

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Methods

PATIENTS

STUDY 1: FIBRONECTIN CONCENTRATIONS IN PATIENTS WITH CROHN'S DISEASE

Fresh blood specimens were collected at the same time of day (mid-morning) from 55 patients with Crohn's disease, seven with ulcerative colitis and 20 healthy controls. Plasma was separated by centrifugation. The details of age and sex in the patients as well as the distribution of the site of Crohn's disease was assessed as shown in Table 1. The inflammatory activity of the Crohn's disease was assessed biochemically using serum alpha-1-glycoprotein measured by radial immunodiffusion. Alpha-1-glycoprotein is known to correlate with the clinical activity of the disease using regression analysis.¹³

A record was kept of the patients receiving corticosteroids at the time blood was taken for fibronectin estimations. No patients in any of the studies received immunosuppressive or dietary manipulation which might independently effect plasma fibro-

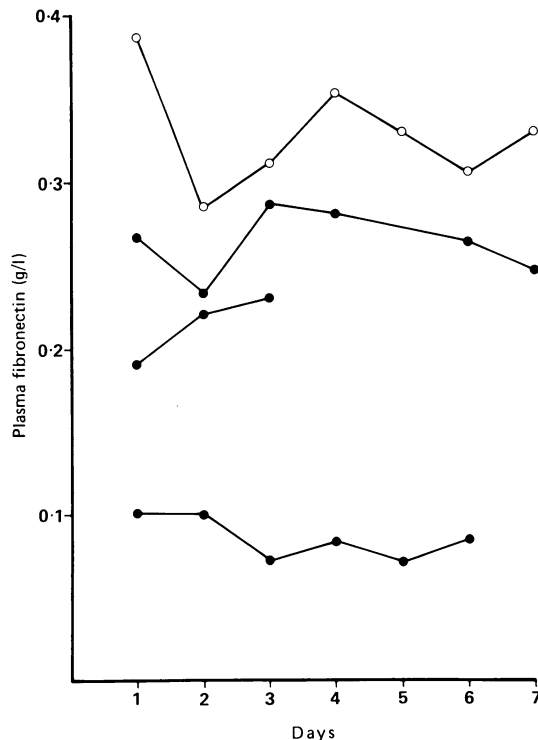


Fig. 1 Day to day variation in plasma fibronectin concentrations in three patients with Crohn's disease and a healthy control (Study 1). ○=healthy control, ●=patient with Crohn's disease.

Table 1 Details of patients in study 1: patients in study and the effect of disease site on plasma fibronectin concentrations

Group: site of disease	n	Age (yr)	Mean (range)	M:F	Mean plasma fibronectin g/l	SD
Controls	20	41	(28-82)	13/7	0.29	0.09
Crohn's disease patients	55	38	(18-78)	17/38		
Small bowel only	22				0.27	0.11
Colonic only	13				0.29	0.13
Small and large bowel	20				0.24	0.05*
Ulcerative colitis patients	7	47	(31-60)	2/5	0.31	0.09

*p<0.05 difference when compared with control values.

nectin concentrations. To determine the extent of day to day variation in plasma fibronectin, mid-morning specimens were collected daily for six days from three patients with Crohn's disease and one healthy control (Fig. 1).

The diagnosis of Crohn's disease and the extent of bowel involved with the disease was measured using standard clinical histological, radiological, and endoscopic parameters.

In those patients with Crohn's disease who were studied from day to day, the simple index of Crohn's disease activity described by Harvey and Bradshaw¹⁴ was used to assess changes in the activity of the disease. This index was filled in by the patient after explanation from one of the authors AA.

STUDY 2: THE INFLUENCE OF OPERATION ON PLASMA FIBRONECTIN CONCENTRATIONS

Specimens were collected (mid-morning) from 25 patients before and for up to nine consecutive days after operation for Crohn's disease. Their mean age was 39 years (range 12-63), there were 19 women. Surgery was subdivided into minor, intermediate, or

Table 2 Details of operations performed on 25 patients with Crohn's disease

Magnitude of procedure	n
Major (6)	
Panproctocolectomy	1
Proctectomy	2
Colectomy and ileo-rectal anastomosis	3
Intermediate (16)	
Small bowel resection	11
Freeing of adhesions	2
Strictureplasty	3
Minor (3)	
Closure of loop ileostomy	1
Dilatation of rectal stricture	1
Drainage of perianal sepsis	1

major procedures. The details of the operations are given in Table 2.

In 15 patients pre-operative nutritional assessments were made, including weight, height, triceps skin fold thickness, arm muscle circumference, grip strength and serum albumin.

STUDY 3: PROSPECTIVE EVALUATION OF PLASMA FIBRONECTIN CONCENTRATIONS AND SUBSEQUENT INTESTINAL STRICTURE FORMATION

Blood was collected (mid-morning) from 52 patients with Crohn's disease who were followed up for one year to evaluate any possible progression or formation of an enteric stricture. The patients had a mean age of 36 years (range 17–73) and there were 36 women. The presence of strictures was evaluated by a combination of either a small bowel enema with colonic barium enema or colonoscopy; except in 10 patients who underwent laparotomy.

The end point of follow up was taken as a laparotomy for obstructive symptoms due to stricturing, or else normal investigations of the small bowel and colon, at least one year after entry into the study. Two patients died.

The median follow up was 19 months (12–28) for patients remaining asymptomatic and undergoing repeat gastrointestinal radiology (n=40); nine months (range 2–14) for patients requiring laparotomy (n=10); the two deaths were at four and 14 months.

Two patients who died had no clinical or autopsy evidence of a stricture. The 10 patients who required surgery for stricture had either developed a new stricture (in four cases) or had progressive tightening of an existing stricture (in six cases).

FIBRONECTIN ASSAY

Blood specimens were collected into 2% EDTA and immediately spun to separate cells from plasma, which was collected and frozen in aliquot at -20°C until analysis. Plasma fibronectin concentrations were measured by 'rocket' immunoelectrophoresis as previously described.¹⁵ Monospecific antiserum to human fibronectin was obtained from the Immuno-Diagnostic Research Laboratory, Department of Immunology, University of Birmingham. A plasma pool from 30 healthy controls was used to calibrate the rocket assay; this was standardised using freshly purified human plasma fibronectin¹⁶ the coefficient of variation for the fibronectin assay was 5%.

STATISTICAL ANALYSIS

Differences between plasma fibronectin concentrations and the various patient groups were analysed using Student's unpaired *t* test with a two tail *p* value.

Table 3 Fibronectin concentrations in Crohn's disease patients and controls. The effect of steroids

	Group (total n)	Subdivided by disease site			Mean plasma fibronectin g/l	SD
		Small bowel	Colon	Both		
Controls	(20)	–	–	–	0.29	0.09
Crohn's disease patients currently given steroids	(22)	6	–	10	0.24*	0.04
Crohn's patients not currently given steroids	(33)	16	7	10	0.28	0.12

*Difference is $p < 0.05$ when compared with control values.

Changes in plasma fibronectin concentrations before and after operation were assessed using the Wilcoxon's rank-sum test.

Results

STUDY 1: ANALYSIS OF PLASMA FIBRONECTIN CONCENTRATIONS

Plasma fibronectin concentrations show considerable daily variation in three patients with Crohn's disease, though no more so than in a normal control (Fig. 1). In this longitudinal study there was no correlation between plasma fibronectin concentrations and either Hb, ESR, orosomucoid concentrations, serum albumin concentrations, platelets count or a clinical index of disease activity.¹⁴

Despite such day to day variation, mean plasma fibronectin concentrations are significantly reduced in patients with Crohn's disease, both the small and large bowel (Table 1), compared with normal controls ($p < 0.05$) (Student's unpaired *t* test). Patients with Crohn's disease in either the small bowel or the colon alone did not have reduced plasma fibronectin concentrations compared with controls ($p < 0.10$, Student's unpaired *t* test).

Patients with Crohn's disease receiving corticosteroids (Table 3) had significantly reduced plasma fibronectin levels compared with the normal controls ($p < 0.05$ Student's unpaired *t* test) regardless of the site of their disease. There was no evidence of a direct or indirect relationship between plasma fibronectin and the serum concentrations of alpha-1-glycoprotein, an indicator of inflammatory activity in Crohn's disease (Fig. 2). Plasma fibronectin concentrations were measured in seven patients with ulcerative colitis. Five of these had pancolitis and two had distal colitis reaching the splenic flexure on barium enema examination. Histologically all cases had

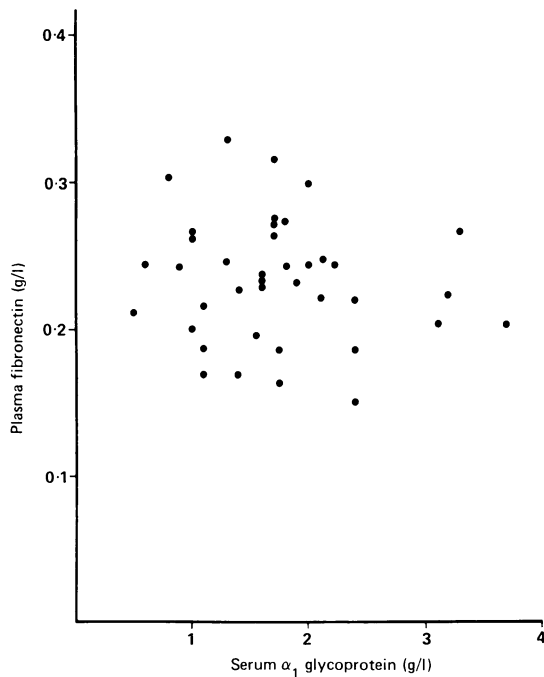


Fig. 2 Variation of plasma fibronectin concentration with serum glycoprotein in 39 patients with Crohn's disease.

active disease at the time of the study (classified as Truelove and Richards class II).¹⁷

Plasma fibronectin concentrations in patients with ulcerative colitis (n=7) were not dissimilar to those in control individuals: the mean plasma fibronectin concentration was 0.31 g/l (SD 0.09) (Table 1).

STUDY 2: EFFECT OF OPERATION ON PLASMA FIBRONECTIN CONCENTRATION

There was a significant 24% reduction in mean plasma fibronectin concentrations in the first day after operation ($p < 0.05$) Wilcoxon's rank-sum test). A further fall occurred the following day (Fig. 3). Thereafter there was a gradual return towards pre-operative levels. When patients undergoing operation were subdivided into those undergoing intermediate (n=16) or major procedures (n=6) it was noted that the fall in plasma fibronectin after operation was more marked in those undergoing major operations (Table 2) (Fig. 4). In those patients who had undergone major procedures the fall for plasma fibronectin was 47% two days later. The similar fall for those undergoing intermediate surgery was 30%.

Nutritional assessment of 15 patients before operation showed that the levels of fibronectin were unrelated to patients weight for height, triceps skin

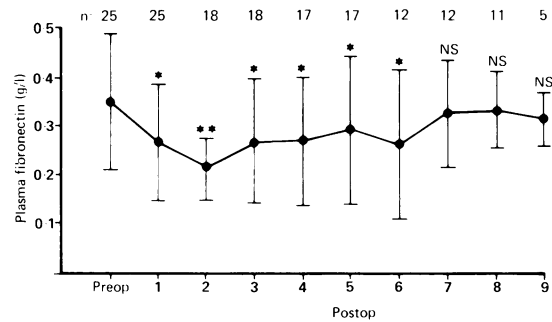


Fig. 3 Mean changes in plasma fibronectin concentrations in 25 patients with Crohn's disease following their immediate post-operative course. The numbers of patients studied on each day are shown. Error bars indicate SD. Significance of fall from pre-operative levels: * $p < 0.01$; NS= $p > 0.10$ (Study 2).

fold thickness, arm muscle circumference, grip strength or serum albumin.

Three patients developed septic complications after operation (one wound abscess, two perianal abscesses). The plasma fibronectin concentration was reduced, compared with preoperative levels, in only one of them. In contrast, 18 patients had a low fibronectin concentration postoperatively with no clinically apparent septic complication. Four patients received transfusions of blood (1-3 units), mainly during the operation, and in none was there an increase in concentration of fibronectin 24 hours later.

STUDY 3: PLASMA FIBRONECTIN CONCENTRATIONS AND SUBSEQUENT STRICTURE FORMATION OR STRICTURE PROGRESSION

The 10 patients who needed an operation for stricture formation or progression during the year of observation originally had a mean plasma fibronectin concentration of 0.27 g/l (SD 0.08). The 42 patients who did not had a mean concentration of 0.24 g/l (SD 0.08). The patients without stricture formation or progression had a lower mean fibronectin concentration but

Table 4 Relationship of plasma fibronectin levels to the development of a subsequent stricture in patients with Crohn's disease

	Initial plasma fibronectin >0.22 g/l	At or below 0.22 g/l
Patients subsequently developing stricture (n=10)	90% (9)	10% (1)
Patients who did not develop stricture (n=42)	60% (25)	40% (17)

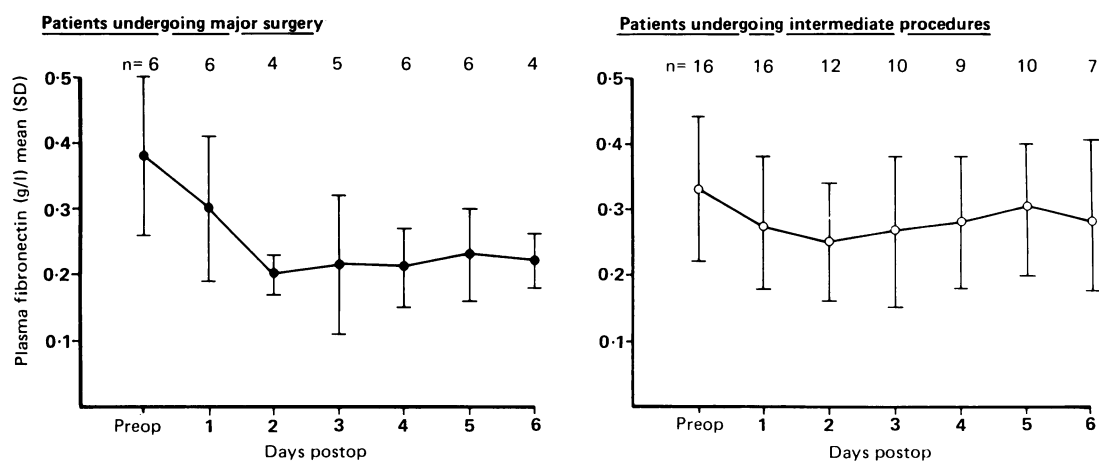


Fig. 4 Mean changes in plasma fibronectin concentrations in 25 patients with Crohn's disease after their immediate postoperative course related to nature of operative procedure. ○=intermediate procedure, ●=major procedure. The numbers in each group are indicated (Study 2).

there was a considerable range of results and the absolute difference did not reach a conventional level of significance ($p > 0.10$ by Student's unpaired *t* test). Many of the patients without strictures had low concentrations of plasma fibronectin, which suggests that it may be a negative risk factor for stricture development, and this is shown in Table 4.

Discussion

Plasma fibronectin concentrations are lower in patients with extensive Crohn's disease involving the small and large bowel than in control individuals. The extent of the Crohn's disease was assessed using contrast radiology and colonoscopy in three patients, and contrast radiology alone in 52 patients. Of these 52 patients, however, subsequent laparotomy within six months provided a further opportunity to assess the extent of the Crohn's disease. Unfortunately, we did not have access to the technique of isotopic scanning using indium labelled granulocytes.

Patients with Crohn's disease requiring treatment with corticosteroids were also found to have lower plasma fibronectin concentrations than control individuals. These findings probably reflect a correlation between plasma fibronectin and extent rather than severity of disease. In support of this, there was no correlation between plasma fibronectin and serum alpha-1-glycoprotein concentrations in a large number of patients studied. Studies in three patients included daily measurement of plasma fibronectin as well as the criteria of Harvey and Bradshaw.¹⁴ These criteria include, clinical index of disease activity, ESR, serum albumin, and platelets; no relationship

was shown between plasma fibronectin and disease activity. These findings suggest that plasma fibronectin is not of a measure of activity of the Crohn's disease.

Low serum fibronectin concentrations in patients with Crohn's disease could result from decreased production, increased metabolism, or greater deposition of fibronectin in tissues. Plasma fibronectin has a relatively rapid turnover¹⁸ and because of this patients with Crohn's disease show considerable day to day variation in plasma fibronectin concentrations. Fibronectin concentrations are low in nutritionally deprived patients.^{19,20} A combination of rapid turnover and malnutrition could rapidly reduce fibronectin concentrations. It may explain the low concentrations seen in patients with extensive Crohn's disease. Increased tissue deposition has been shown in experimental animal models in a variety of circumstances.^{21,22}

Tissue deposition would also explain the relationship between low levels of fibronectin and extensive disease. The marked postoperative fall in fibronectin concentrations we observed are similar to those reported by Chadwick *et al*¹⁰ in patients undergoing major gastrointestinal operations and by Gauperaa *et al*¹¹ after a wider range of surgical procedures. The factors which could lower fibronectin levels postoperatively include its role in opsonic removal of debris from the circulation and its deposition in the tissues together with changes in its synthesis. Not all studies have found low plasma fibronectin concentrations after operation. Hadjis *et al*¹² found that none of 16 patients having operations for obstructive jaundice had reductions in fibronectin. Part of the

explanation for this discrepancy could be caused by the differences in the measurement and methods used; Hadjis *et al*¹² preferred an affinity binding method to the immunoassay techniques which have been used in the other studies.

There is a high risk of postoperative sepsis in Crohn's disease^{23,24} and this risk is probably greater than in patients with ulcerative colitis. Patients with Crohn's disease may be predisposed to postoperative septic complications because of pre-existing sepsis, such as a fistula or abscess formation or because of an existing stoma.²³ Additional factors may be low plasma fibronectin concentrations or a rapid fall immediately after operation. These could predispose to septic complications because of loss of opsonin activity.

We observed that patients with Crohn's disease who developed or renew existing strictures do not initially have low plasma fibronectin concentrations. Andre *et al*,²⁵ showed that high concentrations were associated with subsequent stricture development. These two results are similar. Low plasma fibronectin concentrations may be protective against stricture development whereas higher concentrations are more likely to lead to tissue deposition which, in turn, will sometimes lead to fibrosis and stenosis. Indeed, there is evidence from animal models that plasma fibronectin may be deposited into tissues in pathological situations such as healing wounds.²⁶

In conclusion, there appears to be little value in measuring plasma fibronectin concentrations to monitor the clinical progress of Crohn's disease, assess its severity, or predict likelihood of developing strictures in a particular patient with Crohn's disease. For the present, fibronectin remains a research investigation that may help in the understanding of the progression of pathological changes that characterise Crohn's disease. Nevertheless, our results show the potential importance of connective tissue proteins in the pathogenesis of Crohn's disease.

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