Coagulation and Fibrinolysis in Inflammatory Bowel Disease and in Giant Cell Arteritis

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Key Words

Coagulation · Fibrinolysis · Crohn's disease · Ulcerative colitis · Giant cell arteritis

Abstract

Background: In inflammatory bowel disease (IBD), gut microvascular thrombosis as well as thromboembolic complications have repeatedly been observed. We examined the long-term course of markers of coagulation and fibrinolysis in relation to clinical disease activity.

Materials and Methods: In a prospective study, prothrombin fragment 1 and 2 (F1.2), thrombin-antithrombin complex (TAT), antithrombin, D-dimer, plasmin- α_2 -antiplasmin complex (PAP) and plasminogen activator inhibitor-1 (PAI-1) were measured in 20 patients with Crohn's disease (CD), 18 with ulcerative colitis (UC), and 19 with giant cell arteritis during active and inactive disease, as well as in 51 controls without inflammation.

Results: Levels of F1.2, TAT, D-dimer, PAP and PAI-1 were significantly higher in active versus inactive CD and UC. However, even after 12 months of follow-up, in CD and UC the mean levels of F1.2, D-dimer and PAP were significantly higher than the levels of the controls. **Conclusions:** Levels of F1.2, D-dimer and PAP were markedly raised for a long time in clinically inactive IBD, underlining a chronic state of hypercoagulation and enhanced fibrinolysis.

Introduction

In patients with inflammatory bowel disease (IBD), clinical assessment of disease activity as well as the response to treatment may be difficult, and biochemical markers reflecting these processes would be helpful [1]. Most widely used are acute phase proteins, like C-reactive protein (CRP), synthesized in the liver as a response to mediators such as interleukin-6, probably liberated from the inflammation in the gut. In general, a close link exists between coagulation and inflammation, and fibrin degradation products are capable of inducing an inflammatory response [2]. The observation of intestinal microvascular damage in Crohn's disease (CD) [3–6] as well as fibrinoid microvascular thrombi in the gut mucosa of patients with ulcerative colitis (UC) [7] could be of etiologic importance.

Recurrent thromboembolic complications have been reported in 10–13% of patients with IBD [8, 9], with a 3-fold increased risk of developing deep venous thrombosis and pulmonary embolism in an age- and sex-matched population-based study [10]. Also, inherited disorders of hypocoagulation appear to protect against IBD [11]. However, no single inherited procoagulant disorder, like e.g. factor V Leiden [12–15], was found to account for the risk of venous thrombosis in IBD [16]. Although alterations in blood markers of coagulation and fibrinolysis have been found in patients with IBD [16–26], the duration of follow-up was relatively short.

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In a prospective manner and during a longer time of follow-up than was studied previously, we investigated the course of markers for coagulation activation [prothrombin fragment 1 and 2 (F1.2), thrombin-antithrombin complex (TAT)] and coagulation inhibition (anti-thrombin, AT), as well as fibrinolysis activation (D-dimer) and fibrinolysis inhibition [plasmin- α_2 -antiplasmin complex (PAP), plasminogen activator inhibitor-1 (PAI-1)]. These protein complexes or end products with relatively long plasma half-lives were chosen to reliably characterize both coagulation and fibrinolysis processes, as well as the relationship with disease activity over a longer time span. The markers were determined in patients with IBD, as well as in patients with a well-known vasculitis (giant cell arteritis, GCA), to demarcate the specificity of our findings. GCA has characteristics of a granulomatous disease similar to CD, although in GCA, medium to large arteries are generally involved [27]. However, GCA is well diagnosed with a temporal artery biopsy, while in patients with a small-vessel vasculitis like in Henoch-Schönlein purpura or (microscopic) polyangiitis, a uniform histology diagnosis is more difficult to obtain.

Our hypothesis was that in active but also in inactive IBD and GCA, levels of coagulation and fibrinolysis were raised. Furthermore, to assess the possible role of coagulation and fibrinolysis factors as markers of disease activity, a correlation with indices of clinical activity as well as biochemical activity like the erythrocyte sedimentation rate (ESR) or CRP was investigated.

Materials and Methods

Patients

In a prospective study, performed at the Department of Internal Medicine of the Atrium Medical Center Heerlen, The Netherlands, 38 newly diagnosed in- or outpatients with IBD were enrolled from December 1991 to December 1994 and followed in an active and inactive phase of disease. The Ethics Committee of the Atrium Medical Center Heerlen approved the study and patients provided written informed consent before entry. Twenty patients with CD and 18 patients with UC were included with a median duration of follow-up of 12 (range 6–21) months. In CD and UC, the diagnosis was documented according to the criteria of Lennard-Jones [28]. Although it was not an exclusion criterion in this study, no patients were categorized as having indeterminate colitis.

In CD, the severity of disease was classified according to the Crohn's Disease Activity Index (CDAI) [29]. Active disease was defined as a CDAI score >150 . At entry, 6 patients had a CDAI >450 , 11 patients had a CDAI score between 300 and 450 and in 3 patients, the CDAI ranged from 150 to 299.

In patients with UC, severity of disease was classified according to the Truelove-Witts criteria [30]. For statistical purposes, the classification was quantitatively modified [31]. The score in severe disease

was between 15 and 18, in moderate disease between 9 and 14, in mild disease between 4 and 8 and in inactive disease it was 3 or less. At entry, disease activity was severe in 12 patients, moderate in 6 patients and none had mild or inactive disease.

In 19 patients with a histologically proven and newly diagnosed GCA, activity was assessed according to the criteria of the ARA [27], as well as by the ESR and CRP levels. Since the GCA group was considerably older than the group of IBD patients, separate groups of controls were included.

Twenty-five age- and sex-matched orthopedic patients, seen before elective minor surgery, served as a control group for patients with IBD. They had no signs of vasculitis or gastrointestinal disease. Also, 26 age- and sex-matched controls, consisting of 19 healthy females of an older age and 7 male urology patients seen in the outpatient clinic because of benign prostate hypertrophy, were all without signs of inflammation and served as a control group for patients with GCA.

None of the patients was on oral anticoagulants or aspirin. Patients with preexisting liver disease, with coexisting malignancy, or who were pregnant and patients who refused consent were excluded from the study.

Depending on the severity of disease, therapy in IBD consisted of oral 5-aminosalicylic acid (5ASA) and/or prednisone (starting dose varying between 0.5 and 1 mg/kg/day), azathioprine (2.5 mg/kg), and was sometimes combined with enema therapy. Prednisone was started in case of insufficient clinical response to 5ASA treatment in active CD (CDAI >150) and moderate to severe UC (Ulcerative Colitis Activity Index, UCAI >8). In active GCA, oral prednisone (absolute dose 40 mg/day) was started, and according to the clinical and acute phase response, dosage was gradually lowered [32]. None of the patients received plasma derivatives other than packed cells, and none of the patients with IBD had surgery during follow-up.

Plasma samples were collected during active UC, CD and GCA before conventional therapy, and – after intervals of 3 months – in inactive disease while on low-dose prednisone (less than 15 mg/day), azathioprine and/or mesalazine (oral or enema) in the IBD group. In the noninflammatory controls, blood samples were taken twice within a month before surgery. All patients with UC underwent a sigmoidoscopy and/or colonoscopy at entry, and in CD (an additional) selective small bowel enteroclysis was performed. However, unlike the clinical activity and laboratory investigations, follow-up endoscopies were not performed in a standardized manner.

The characteristics of patients and controls are shown in table 1.

Methods

All blood samples were drawn fasting between 8 and 10 a.m. to avoid diurnal variation [33]. After a resting period of 20 min, non-traumatic venipuncture was performed in a standardized manner by trained operators. After discarding the first 2 ml of blood, 4.5 ml of blood was drawn into test tubes with 0.5 ml citrate and immediately kept on ice. Within 1 h from sample collection, all test tubes were centrifuged for 30 min (3,000 g) at 4°C. Platelet poor plasma was aspirated from the central part of the plasma, without disturbing either the top layer or the buffy coat. The plasma was snap-frozen in aliquots, stored at -70°C in plastic tubes and thawed in a water bath at 37°C for 5 min, immediately prior to serial analysis. Plasma concentrations of F1.2, TAT, D-dimer, PAP and PAI-1 were determined by sandwich-type enzyme immunoassays, and the activity of AT was measured by a chromogenic substrate (Boehringer Mannheim). The

Table 1. Characteristics of the patients and control groups (C1 for CD and UC and C2 for GCA)

	CD	UC	GCA	C1	C2
Patients	20	18	19	25	26
Female	14	11	14	16	19
Male	6	7	5	9	7
Median age, years	34 (20–71)	47 (17–86)	72 (65–88)	41 (22–74)	71 (67–84)
Median follow-up, months	12 (6–21)	12 (6–18)	10 (6–22)	1 (0.5–1)	1 (0.5–1)
Smoking	8	4	6	6	7
Oral contraceptive use	5	4	0	8	0

Figures in parentheses represent range.

intra-assay coefficient of variation was between 5 and 10% for the assays.

CRP was measured by an enzyme immunoassay (Behring, Germany; normal values 0–5 mg/l), and the ESR was measured according to the Westergren method (normal values 5–15 mm/h).

Statistical Analysis

All patients were included in the follow-up data. In UC, CD and GCA, median values with the minimum-maximum range (table 1) or mean values with the 95% confidence intervals (CI) for the mean (table 2) were calculated in case of a skewed or even distribution of data, respectively. To test differences for significance, the Mann-Whitney signed-rank test was performed for the longitudinal patient follow-up as well as the Mann-Whitney U test to compare the three patient groups with the age- and sex-matched control groups (table 2). Furthermore, the Spearman rank correlation coefficient and the two-tailed significance of the correlation were calculated between coagulation, fibrinolysis, and other biochemical markers and disease activity indices. A correction for multiple comparisons was performed using the Bonferroni-adjusted level of significance. Calculations were done with CD and UC as separate groups, not with IBD as a whole. For statistical analysis, SPSS 10.0 software was used (SPSS, USA).

Results

Markers of Coagulation Activation and Inhibition in Active and Inactive Disease (table 2)

F1.2 was significantly higher in active and inactive CD, UC and GCA compared to the controls ($p < 0.0001$). Also, F1.2 values were higher in active than in inactive CD or UC ($p = 0.005$) unlike in active versus inactive GCA ($p = 0.297$). TAT was higher in active CD, UC and GCA compared to the controls ($p < 0.0001$), and higher in active versus inactive CD or UC ($p < 0.001$) unlike GCA ($p = 0.402$). AT activity was normal in active and inactive IBD, but raised in inactive compared to active GCA ($p < 0.0001$).

In the noninflammatory control groups, laboratory values were within normal limits.

Markers of Fibrinolysis Activation and Inhibition in Active and Inactive Disease (table 2)

D-dimer was significantly higher in active and inactive CD, UC and GCA compared to the controls ($p < 0.0001$), and higher in active versus inactive CD, UC or GCA ($p < 0.0001$). PAP was higher in active CD, UC and GCA compared to the controls ($p < 0.0001$), as well as in active versus inactive CD ($p = 0.002$), UC ($p < 0.0001$) or GCA ($p < 0.0001$). PAI-1 was higher in active UC and GCA, but not in active CD compared to the controls ($p = 0.003$, $p = 0.001$, $p = 0.582$, respectively). PAI-1 levels were reduced in inactive compared to active CD ($p = 0.011$), UC ($p < 0.0001$) and GCA ($p = 0.006$).

PAP and PAI-1 values were normal in the controls, but D-dimer was higher in the elderly (GCA) controls ($p = 0.001$).

Correlations between Coagulation and Fibrinolysis Markers

In CD and UC, F1.2 correlated with TAT, D-dimer, PAP (all $p < 0.0001$), and PAI-1 ($p = 0.012$). TAT correlated with D-dimer, PAP and PAI-1 ($p < 0.0001$). AT did not correlate with the other coagulation and fibrinolysis markers in CD and UC, except for TAT in UC ($p = 0.009$). D-dimer correlated with PAP ($p < 0.0001$) in CD and UC, as well as PAI-1 in CD ($p = 0.002$), but not in UC. PAP correlated with PAI-1 in UC ($p = 0.007$) unlike in CD.

In GCA, the markers F1.2 and TAT did not correlate with the other coagulation and fibrinolysis markers. D-dimer correlated with PAP and PAI-1 ($p < 0.0001$ and $p = 0.006$), and PAP correlated with AT ($p = 0.002$) and PAI-1 ($p = 0.003$).

Table 2. F1.2, TAT, AT, D-dimer, PAP, PAI-1, ESR and CRP in active and inactive CD (n = 20), UC (n = 18) and GCA (n = 19)

Laboratory tests	CD, CDAI		UC, UCAI		GCA, ARA		C1	C2
	>150	<150	>4	<4	active	inactive		
F1.2 (0.26–1.14 mmol/l)								
Mean	1.70	0.87	1.61	1.04	2.37	1.52	0.56	0.70
95% CI	0.99–2.41	0.73–1.01	1.24–1.97	0.74–1.34	1.11–3.64	1.04–1.99	0.46–0.67	0.56–0.84
Significance	***	**	***	**	***	***		
TAT (0.7–4.2 µg/l)								
Mean	5.55	2.34	5.35	2.85	5.08	3.77	2.34	2.24
95% CI	3.61–7.49	1.50–3.18	3.93–6.77	1.85–3.85	3.14–7.02	3.09–4.43	1.94–2.75	1.81–2.68
Significance	***	n.s.	***	n.s.	***	**		
AT (80–120%)								
Mean	96.5	98.8	105.4	102.8	97.0	116.3	98.9	100.0
95% CI	89.7–103.2	93.6–103.9	95.7–115.1	99.3–106.2	89.5–104.5	109.9–122.6	94.8–103.0	95.1–105.0
Significance	n.s.	n.s.	n.s.	n.s.	n.s.	***		
D-dimer (0–400 µg/l)								
Mean	1,469	583	1,509	566	1,780	853	283	414
95% CI	1,139–1,799	373–794	1,126–1,891	389–744	1,151–2,410	188–1,518	244–321	352–476
Significance	***	***	***	***	***	***		
PAP (80–470 µg/l)								
Mean	948	550	1,059	540	1,162	735	282	259
95% CI	745–1,151	373–727	844–1,274	456–624	1,017–1,306	617–853	242–322	215–302
Significance	***	***	***	***	***	***		
PAI-1 (4.0–43.0 µg/l)								
Mean	31.0	16.3	47.6	16.4	49.2	25.4	26.8	30.0
95% CI	21.5–40.5	12.5–20.2	35.7–59.5	13.0–19.7	37.1–61.3	14.6–36.3	21.9–31.7	25.7–34.2
Significance	n.s.	**	**	*	**	*		
ESR (5–15 mm/h)								
Mean	51	10	42	11	82	14	15	17
95% CI	34–68	6–13	32–51	9–13	69–95	11–17	7–18	10–20
Significance	***	*	***	n.s.	***	n.s.		
CRP (0–5 mg/l)								
Mean	122	6	78	7	108	6	7	8
95% CI	83–161	5–7	54–102	6–9	72–143	5–7	5–9	6–10
Significance	***	n.s.	***	n.s.	***	n.s.		

Mean and 95% CI of the mean (lower to upper bounds) are shown. The significance of the differences between the means of the separate groups of activity in IBD and GCA, and their respective group of controls (C1 for IBD, C2 for GCA) was calculated by means of the Mann-Whitney U test.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$. n.s. = Not significant.

Correlations of Coagulation and Fibrinolysis Markers with Clinical and Biochemical Indices of Disease Activity (table 3)

In CD, the CDAI as well as the ESR and CRP correlated with F1.2, TAT, D-dimer, and PAP, but not with AT. Also, PAI-1 correlated with the CDAI and ESR, but not with CRP. In UC, the UCAI as well as the ESR and CRP correlated with F1.2, TAT, D-dimer, PAP and PAI-1, but not AT. In GCA, the ESR did not correlate with F1.2 or TAT, but inversely correlated with AT ($r = -0.428$; $p = 0.005$). The ESR however correlated with D-dimer ($r = 0.726$; $p < 0.0001$), PAP ($r = 0.710$; $p < 0.0001$), and PAI-1 levels ($r = 0.425$; $p = 0.005$).

Correlation of Coagulation and Fibrinolysis Markers with the Extent of Bowel Involvement at Entry

The endoscopic extension of bowel involvement in patients with active CD or UC was investigated as well as its relationship to the coagulation and fibrinolysis markers.

Patients with CD involving only the small bowel ($n = 10$) or only the colon ($n = 4$), and CD of both small and large bowel segments ($n = 6$) were distinguished. Levels of F1.2, TAT and AT, as well as D-dimer, PAP and PAI-1 were not significantly different according to disease localization and extension. However, the number of patients does not allow us to make definite statements in this respect.

Table 3. Spearman-rank correlation coefficient values and the two-tailed significance of the correlation are shown between the coagulation and fibrinolysis laboratory tests, and clinical (CDAI, UCAI) and biochemical (ESR, CRP) indices of activity in CD and UC

Laboratory tests	CD			UC		
	CDAI	ESR	CRP	UCAI	ESR	CRP
F1.2	0.376	0.453	0.382	0.383	0.483	0.390
Significance	***	***	***	***	***	***
TAT	0.442	0.369	0.450	0.508	0.541	0.448
Significance	***	***	***	***	***	***
AT	0.051	0.037	−0.042	0.085	0.244	0.047
Significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
D-dimer	0.514	0.502	0.475	0.433	0.522	0.462
Significance	***	***	***	***	***	***
PAP	0.412	0.265	0.269	0.502	0.553	0.449
Significance	***	**	**	***	***	***
PAI-1	0.265	0.317	0.213	0.455	0.465	0.354
Significance	**	**	n.s.	***	***	**
ESR	0.633			0.703		
Significance	***			***		
CRP	0.806			0.736		
Significance	***			***		

** $p < 0.015$ (Bonferroni-adjusted significance); *** $p < 0.0001$. n.s. = Not significant.

Patients with UC involving only the rectum and/or sigmoid colon ($n = 9$), and UC extended to the more proximal colon segments (left-sided colitis: $n = 3$; pancolitis: $n = 6$) were distinguished. Levels of TAT ($p = 0.022$) as well as D-dimer ($p = 0.031$), PAP ($p = 0.002$) and PAI-1 ($p = 0.049$) were significantly higher in more extensive UC, and a correlation with the extent of bowel involvement at entry was found with TAT ($r = 0.534$; $p < 0.0001$), D-dimer ($r = 0.477$; $p = 0.002$), PAP ($r = 0.446$; $p = 0.004$), PAI-1 ($r = 0.463$; $p = 0.003$), as well as CRP ($r = 0.618$; $p < 0.0001$).

The Course of Coagulation and Fibrinolysis Markers over Time in CD and UC

In CD, after 12 months of follow-up, the mean F1.2 was higher by a factor of 1.5 than the control value (0.86 and 0.56 mmol/l, respectively; $p < 0.0001$), and in 3 of 18 patients tested it was above the upper reference value. As a contrast, TAT equalized the mean control value between 3 ($p = 0.048$) and 6 months ($p = 0.892$). The AT activity in CD was stable and not different from the controls during 12 months of follow-up (fig. 1).

The mean D-dimer level in CD was higher by a factor of 1.9 than in the controls (524 and 283 $\mu\text{g/l}$; $p < 0.0001$;

above upper reference in 11 of 18 patients), and the mean PAP level was higher by a factor of 1.7 after 12 months (486 and 282 $\mu\text{g/l}$; $p < 0.0001$; above upper reference in 8 of 18 patients). The initially higher mean values of PAI-1 became lower by a factor of 1.7 as compared to the controls (16.0 and 26.8 $\mu\text{g/l}$; $p = 0.005$; in 1 of 18 patients below the lower reference value) (fig. 2).

In UC, after 12 months the mean F1.2 count was higher by a factor of 1.5 than in the controls (0.89 and 0.56 mmol/l, respectively; $p = 0.009$; above upper reference in 6 of 17 patients). However, TAT equalized the controls between 3 ($p = 0.024$) and 6 months ($p = 0.225$). As in CD, the AT activity course in UC did not differ from the controls (fig. 3).

The mean D-dimer level was higher by a factor of 2.0 in UC than in the controls (555 and 283 $\mu\text{g/l}$; $p < 0.0001$; above upper reference in all 17 patients tested), and the mean PAP level was higher by a factor of 1.6 than in the controls (461 and 282 $\mu\text{g/l}$; $p < 0.0001$; above upper reference in 11 of 17 patients) after 12 months. As in CD, initially higher mean values of PAI-1 became lower by a factor of 1.7 as compared to the controls (15.9 and 26.8 $\mu\text{g/l}$; $p = 0.005$; no scores below the lower reference value) (fig. 4).

Fig. 1. Mean levels of F1.2, TAT and AT on entry and during 12 months of follow-up in 20 patients with CD. Sample sizes of F1.2, TAT and AT at 0, 3, 6, 9 and 12 months were 20, 20, 18, 20 and 18. The interrupted line shows the course of the CDAI. AT levels were in the normal range during 12 months of follow-up. Within 6 months, the mean TAT level was equal to the control group. However, even after 12 months of follow-up, the mean F1.2 levels were 1.5 times higher than the levels of the control group.

Fig. 2. Mean levels of D-dimer, PAP and PAI-1 on entry and during 12 months of follow-up in 20 patients with CD. Sample sizes of D-dimer, PAP and PAI-1 at 0, 3, 6, 9 and 12 months were 20, 20, 18, 20 and 18. The interrupted line shows the course of the CDAI. After 12 months of follow-up, the mean D-dimer and PAP levels were 1.9 and 1.7 times higher than the levels of the control group. However, PAI-1 levels were 1.7 times lower in CD as compared to the control group after 12 months of follow-up.

Discussion

Not only in the active, but also in the inactive phase of IBD we found a significantly higher level of coagulation and fibrinolysis as compared to the age- and sex-matched controls. Second, long-term patterns of coagulation and fibrinolysis were similar in GCA and IBD. This suggests that common pathways, e.g. microvascular thrombosis or vasculitis, may play a role in both IBD and GCA. Although we did not examine coagulation activity in biopsies in the present study, we and others have shown that microthrombi are present in gut mucosa even when histology shows no inflammation [7, 34]. We therefore hypothesize that hypercoagulation and disturbed fibrinolysis might add to the chronicity of these diseases.

Unlike the study of Henegan et al. [35], but analogous to the study of van Bodegraven et al. [36], we did not find a significantly low AT level in IBD as compared to the controls. In GCA however, levels of AT were significantly lower in active versus inactive GCA. Also, TAT levels were raised both in active and inactive GCA, and this might point to a disseminated intravascular coagulation with more consumption of AT during active disease as compared to IBD [37].

In the two noninflammatory control groups, the only difference was a higher D-dimer in the older patients, also found by others [38]. Although we cannot find a clear explanation, it might be a feature of more background atherosclerosis occurring in the elderly. However, we would have expected that the other markers would be raised to some degree as well [39, 40].

In this study, a clinical remission was not documented with follow-up endoscopy and histology in IBD. Therefore, it is possible that at least part of the raised hemostasis activity was due to ongoing inflammation. At entry, levels of coagulation (TAT) as well as fibrinolysis factors (D-dimer, PAP, PAI-1) were lower in proctosigmoiditis than in more extensive UC. Although this suggests a relationship between the size of the mucosal defect and the magnitude of coagulation activation, these markers also correlated with CRP, fitting in with the concept of a more generalized systemic inflammatory and hemostatic response.

After treatment, CRP levels rapidly declined but our treatment regimen with mesalazine, prednisone and azathioprine did not alter the pattern of hypercoagulation and fibrinolysis during long-term follow-up. Although patients were clinically well, our data suggest that their dis-

Fig. 3. Mean levels of F1.2, TAT and AT on entry and during 12 months of follow-up in 18 patients with UC. Sample sizes of F1.2, TAT and AT at 0, 3, 6, 9 and 12 months were 18, 16, 18, 17 and 17. The interrupted line shows the course of the UCAI. AT levels were in the normal range during 12 months of follow-up. Within 6 months, the mean TAT level was equal to the control group. However, even after 12 months of follow-up, the mean F1.2 levels were 1.5 times higher than the levels of the control group.

Fig. 4. Mean levels of D-dimer, PAP and PAI-1 on entry and during 12 months of follow-up in 18 patients with UC. Sample sizes of D-dimer, PAP and PAI-1 at 0, 3, 6, 9 and 12 months were 18, 16, 18, 17 and 17. The interrupted line shows the course of the UCAI. After 12 months of follow-up, the mean D-dimer and PAP levels were 2.0 and 1.6 times higher than the levels of the control group. However, PAI-1 levels were 1.7 times lower in UC as compared to the control group after 12 months of follow-up.

ease was still subclinically active. In theory, the medication could have promoted this disturbed pattern of hemostasis.

Corticosteroids can induce neutrophilia [41], and apart from fibrinolysis, high levels of D-dimer could result from neutrophilia in IBD and GCA, due to enhanced elastase release by these polymorph nuclear cells [42, 43]. However, in CD and UC, the use of steroids resulted in a moderate decrease in D-dimer, F1.2 and PAP, and no significant difference in D-dimer was found between patients with or without steroid use. Corticosteroids might have reduced PAI-1 levels as in Henoch-Schönlein purpura [44], and in our study, levels of PAI-1 became low during follow-up. However, in IBD, steroids were generally withdrawn within 3–6 months, while mean PAI-1 levels did not change significantly between 3 and 12 months of follow-up.

To our knowledge, no direct effect of azathioprine on coagulation has been described. The use of azathioprine could have led to (opportunistic) infections, like e.g. cytomegalovirus reactivation, resulting in a disturbed coagulation [45]. However, no clinical signs of chronic (systemic) infections were encountered in our follow-up group. With oral 5ASA, a lower spontaneous ex vivo and

in vitro platelet activation has been described in patients with IBD [46]. However, no secondary rise in coagulation and fibrinolysis factors has been documented.

It is unknown what exactly happens early in the course of IBD. Indirect evidence has come from recent studies that the activation of hemostasis and fibrinolysis is most likely a result of stimulation by certain cytokines from the inflammatory process in the gut, such as tumor necrosis factor- α [47]. Anti-tumor necrosis factor- α antibody (Remicade®) treatment was accompanied by a clear reduction in coagulation and fibrinolysis activity as studied in 8 patients with CD [48], suggesting a secondary role for the coagulation and fibrinolysis system in CD.

Are the markers tested also clinically useful? A positive correlation was found between the CDAI as well as the UCAI with F1.2, TAT, D-dimer, PAP and PAI-1. Also, TAT, D-dimer, PAP and PAI-1 correlated with the extent of bowel involvement at entry. However, the persistently higher than normal levels of F1.2, D-dimer and PAP preclude their use as rapid diagnostics for measuring the influence of therapy, unlike e.g. an acute phase reactant like CRP [24, 49].

Nevertheless, the measurement of plasma concentrations of F1.2, TAT, D-dimer, PAP and PAI-1 could be

useful as an additional tool. They might reflect inadequate control of the inflammatory process in the gut, and possibly a systemic tendency towards thromboembolic complications [50]. Future therapeutic intervention studies with drugs which could influence both inflammation and microvascular thrombosis [51] should therefore be welcomed.

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