Rapid Communication

Resistance to activated protein C is a risk factor for fibrostenosis in Crohn’s disease

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Abstract

Aim: To evaluate the effect of resistance to activated protein C (aPCR), the most common known inherited thrombophilic disorder, on the risk of intestinal operation of fibrostenosis in patients with Crohn's disease (CD).

Methods: In a previous study, we assessed the prevalence of aPCR in CD. In a retrospective case-controlled study, 8 of these CD patients with aPCR were now compared with 24 CD patients without aPCR, matched by gender, age at diagnosis and duration of disease in a 1:3 fashion. The primary end point was the occurrence of an intestinal CD-related operation with evidence of fibrostenosis in the bowel resection specimen.

Results: The Kaplan-Meier analysis revealed that patients with aPCR had a lower probability of remaining free of operation with fibrostenosis than patients without aPCR (P = 0.0372; exact log-rank test) resulting in a significantly shorter median time interval from diagnosis of CD to the first operation with fibrostenosis (32 vs 160 mo). At 10 years, the likelihood of remaining free of operation with fibrostenosis was 25% for patients with aPCR and 57.8% for patients without aPCR.

Conclusion: CD patients with aPCR are at higher risk to undergo intestinal operation of fibrostenosis than those without aPCR. This supports our hypothesis of aPCR being a possible risk factor for fibrostenosis in CD.

Key words: Fibrostenosis; Resistance to activated protein C; Factor V Leiden; Intestinal surgery; Crohn’s disease

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Introduction

Fibrostenotic lesions of the bowel are frequent and serious complications in Crohn’s disease (CD). They often require surgery, which may lead to short bowel syndrome in case of repeated bowel resections[1-4]. Intestinal fibrosis in CD as a consequence of chronic transmural inflammation is characterized by increased production of extracellular matrix, including fibrillar collagen, by activated mesenchymal cells, such as fibroblasts and smooth muscle cells[5-8]. However, the exact pathophysiological mechanism explaining variable disposition of CD patients to develop fibrostenotic lesions is unknown. But some risk factors have been discovered. For example, NOD2/CARD15 variants[9-11] and smoking have been associated with fibrostenosis[12,13]. Moreover, CD patients with antibody responses against *Saccharomyces cerevisiae* (ASCA), CD-related bacterial sequence (12), *E. coli* outer membrane porin C (anti-OmpC), and neutrophil nuclear antigen (pANCA) have also been reported as being more likely to develop fibrostenotic lesions[12-19].

Resistance to activated protein C (aPCR)[14], representing the most common known inherited thrombophilic disorder[17], has been shown to increase the rate of fibrosis in other inflammatory diseases, such as chronic viral hepatitis[18]. Activated protein C serves as a natural anticoagulant by cleavage and inactivation of factor Va. Factor V plays a central regulatory role in hemostasis, since it contributes to the conversion of prothrombin to active thrombin, which, on the other hand, transforms fibrinogen into fibrin. A single-point mutation in the gene encoding factor V, also known as factor V Leiden, results in a form of factor Va that is resistant to degradation by activated protein C, leading to a relative hypercoagulation state[19].

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The increase of the rate of fibrosis in chronic viral hepatitis caused by aPCR was explained by thrombotic obliterations of small portal and hepatic veins resulting in local hepatocyte death and increased development of fibrosis and by the effect of thrombin through activation of stellate cells, which are the mediators of fibrosis in the liver. However, the role of aPCR in the development of fibrostenotic lesions in CD has not been elucidated till now. The potential role of the clotting system in CD has already been shown. Granulomatous vasculitis, intravascular fibrin deposition and capillary microthrombi have been reported in CD lesions. Additionally, CD has been observed less frequently in patients with inherited haemophilic disorders. On the other hand, aPCR as a thrombophilic disorder may favor intravascular fibrin deposition and thrombotic obliteration of the small vessels of intestinal lesions in CD, resulting in increased local cell death and fibrosis.

We, therefore, hypothesized that aPCR increases the rate of fibrosis in CD patients. We expected that CD patients with aPCR are more likely to have fibrostenotic lesions of the bowel than patients without aPCR, resulting in a lower probability of remaining free of operation with fibrostenosis.

MATERIALS AND METHODS

Patients and study design
This is a retrospective case-control study on the influence of aPCR on the presence of fibrostenotic lesions found at intestinal CD-related operations in CD patients. In a previous study on risk factors for thromboembolism in inflammatory bowel disease (IBD), we assessed the prevalence of aPCR in patients with IBD, including 77 patients with CD. We had detected 8 CD patients with aPCR who were now compared with CD patients without aPCR selected from the same database. CD patients with aPCR (n = 8) and without aPCR (n = 24) were matched by gender, age at diagnosis (± 10 years) and duration of disease (± 3 years) in a 1:3 fashion. A colleague blinded for the clinical course of the patients performed the matching. All patients included in the study were Caucasians and had been in routine care at our institution (Medical University of Vienna, Department of Internal Medicine IV, Division of Gastroenterology and Hepatology, Vienna). The diagnosis of CD was based on established criteria of clinical, radiological, endoscopic, or histological findings. The location of CD was determined according to the Vienna classification. The information on the clinical course, smoking habits, and the medical and surgical management was reviewed from the charts of the patients. A “smoker” was defined as a patient who had smoked at least seven cigarettes weekly for at least one year. Immunosuppressants, such as azathioprine, were considered for analysis if the duration of treatment had been at least 3 mo.

The primary end point of the study was the presence of fibrostenosis found at intestinal CD-related operations documented by the pathological and/or surgical report, respectively. The secondary end point was the occurrence of intestinal CD-related operations regardless of fibrostenosis. Only intestinal operations, such as bowel resections, strictureplasties and gastrointestinal bypass-surgery, were included in the analysis. Creations of a stoma, intestinal reconstructions, exploratory laparatomies, and surgery of complicated perianal CD were excluded from the analysis. Two observers blinded for the aPCR status of the patients (W.R. and J.P.) reviewed the pathological and surgical reports of the patients for the presence of fibrostenosis. The Ethics Committee of the Medical University of Vienna approved this study.

Definition of fibrostenotic lesions
Stricturing disease is defined by the Vienna classification as the occurrence of constant luminal narrowing demonstrated by radiologic, endoscopic, or surgical examination combined with prestenotic dilatation and/or obstructive signs or symptoms but without evidence of penetrating disease. In our retrospective analysis, for the purpose of defining fibrostenotic lesions at the time of surgery, we modified this definition for four reasons. First, strictures of the bowel in CD may result not only from fibrostenotic lesions but also from inflammatory obstruction without fibrosis. Second, internal fistulas are often associated with strictures and such mechanical factors may even favor the development of fistula, which might explain the coexistence of fibrostenotic lesions and perforation. Third, radiologic, endoscopic, and surgical examination may report contradictory results. The most reliable data on complicated disease are available after resection from surgical and pathological reports. In these reports, fibrostenotic lesions were defined as luminal narrowing and bowel wall thickening on naked-eye examination of the surgical resection specimen. Histologically, strictures were recognized by thickening of the muscularis mucosa and by fibrosis of the submucosa. And fourth, we excluded “obstructive symptoms” from the definition, since it may be difficult to differentiate clinically from other disease-related complications (e.g. inflammation, fistula and abscess).

We, therefore, defined fibrostenotic lesions as the occurrence of bowel wall thickening and luminal narrowing on naked eye examination in the pathological and surgical report found at intestinal CD-related surgery as well as the occurrence of thickening of the muscularis mucosa and of fibrosis of the submucosa in the histological part of the pathological report. In case of discrepancies between pathological and surgical examination, the pathological report of the surgical resection specimen was rated with higher priority than the surgical report. In case of strictureplasties and gastrointestinal bypass surgery, if a surgical resection specimen was not available, the assessment of fibrostenosis was based solely on the surgical report. The diagnosis of a fibrostenotic lesion was irrespective of evidence of penetration.

Assay system
aPCR was determined as described previously using the assay Coatest® aPC™ Resistance (Chromogenix, Mölndal, Sweden) according to the manufacturer’s instructions. The normal range of the aPC ratio was > 1.9 and aPCR, therefore, was diagnosed if the aPC ratio was ≤ 1.9. This
test with predilution of samples with factor V-deficient plasma has been shown to have a very high sensitivity and specificity of nearly 100% for the diagnosis of factor V Leiden, including a discrimination between heterozygous and homozygous subjects. The location of disease was classified according to the Vienna classification.

Statistical analysis

All computations were performed with the use of SAS software, version 9 (SAS Institute Inc., Cary, NC, USA). Data were presented as frequencies, percentages and median values with range, respectively. Differences between groups were analyzed using the fisher’s exact test for categorical data and the Mann-Whitney U-test for continuous data. Survival time methods were used to analyze the time from diagnosis of CD to the first intestinal CD-related operation with fibrostenosis and from diagnosis of CD to the first intestinal CD-related operation, respectively, (uncensored observations) or the duration of follow-up among patients without intestinal CD-related operation with fibrostenotic lesions and without intestinal CD-related operation, respectively (censored observations). The probability of survival free of intestinal CD-related operation with fibrostenosis and survival free of intestinal CD-related operation regardless of the presence of fibrostenosis, respectively, was estimated according to the Kaplan-Meier method. Differences between Kaplan-Meier curves of both groups were analyzed using the exact log-rank test for unequal survival time.

RESULTS

Study population

The clinical data of both patient groups are summarized in Table 1. Eight patients had an aPC ratio below 1.9 and were diagnosed as having aPCR: one patient had an aPC ratio of 1.19 consistent with homozygous for factor V Leiden and the other 7 patients had aPC ratios ranging from 1.46 to 1.64 consistent with heterozygous for factor V Leiden. Twenty-four patients had values of the aPC ratio within the normal range. Neither patient group differed in terms of location of disease, and percentage of smokers and patients who had been treated with azathioprine (Table 1). Furthermore, the number of cigarettes per day, the duration of smoking, and the dosage and duration of treatment with azathioprine did not differ between either patient group (data not shown). No immunosuppressants other than azathioprine had been administered. None of the patients had received infliximab or any other biological agent. Patients with aPCR had a history of venous thromboembolic complications diagnosed by imaging procedures significantly more often than the patients without aPCR (P < 0.01). Most of the thromboembolic events had been deep venous thromboses of the legs and pulmonary emboli.

Intestinal CD-related surgery

Six of 8 patients with aPCR and 16 of 24 patients without aPCR underwent intestinal surgery. Altogether, 44 intestinal CD-related operations (13 in patients with aPCR and 31 in patients without aPCR) had been performed. All operations had been intestinal resections, except for one case of gastroentero-anastomosis, which was necessary to circumvent the obstructed duodenum in a patient with CD of the upper gastrointestinal tract. A strictureplasty and a resection of another bowel segment performed at the same surgical procedure in a patient with aPCR were counted as one operation. The location of the bowel resections did not differ between both patient groups and was most likely of the terminal ileum (Table 2). In addition to bowel resections, three balloon dilations of fibrostenotic

| Table 1 Characteristics of 32 patients with Crohn’s disease (frequencies, %, median values with range) |
|---|---|---|
| With aPCR | Without aPCR |
| (n = 8) | (n = 24) |
| Sex ratio (M/F) | 3/5 | 9/15 |
| Age at diagnosis (yr) | 30 (13-53) | 31 (10-46) |
| Duration of disease (mo) | 140 (86-257) | 145 (65-272) |
| aPC ratio | 1.52 (1.19-1.64) | 2.31 (2.08-2.66) |
| Patients with VTE in the history | 5 (63%) | 2 (8%) |
| Smokers | 4 (50%) | 19 (79%) |
| Patients under azathioprine | 4 (50%) | 15 (63%) |
| Location of CD | Terminal ileum | 2 (25%) | 4 (17%) |
| | Colon | 1 (12.5%) | 5 (21%) |
| | Ileocolon | 4 (50%) | 12 (50%) |
| | Upper gastrointestinal tract | 1 (12.5%) | 3 (12%) |
| | Patients having undergone an operation | 10/16 (62.5%) | 6 (25%) |
| | Patients having undergone an operation with fibrostenosis | 6 (75%) | 16 (67%) |
| | Patients with fibrostenosis at 1st operation | 6/6 (100%) | 10/16 (62.5%) |
| | Location of fibrostenosis at 1st operation | Terminal ileum | 4 | 8 |
| | Colon | 2 | 0 |
| | Upper gastrointestinal tract | 0 | 2 |
| | Median time from diagnosis of CD to 1st operation (mo) | 32 | 80.5 |
| | Median time from diagnosis of CD to 1st operation with fibrostenosis (mo) | 32 | 160 |

aPCR: resistance to activated protein C; CD: Crohn’s disease; VTE: venous thromboembolism. The patients were divided into the two groups according to the value of the aPC ratio: < 1.9 (diagnosis of aPCR) and > 1.9 (normal range), respectively.

| Table 2 Intestinal CD-related surgery in 32 patients with Crohn’s disease (frequencies, %, median values with range) |
|---|---|---|
| With aPCR | Without aPCR |
| (n = 8) | (n = 24) |
| Patients having undergone an operation | 6 (75%) | 16 (67%) |
| Patients having undergone an operation with fibrostenosis | 6 (75%) | 13 (54%) |
| Patients with fibrostenosis at 1st operation | 6/6 (100%) | 10/16 (62.5%) |
| Location of fibrostenosis at 1st operation | Terminal ileum | 4 | 8 |
| | Colon | 2 | 0 |
| | Upper gastrointestinal tract | 0 | 2 |
| | Median time from diagnosis of CD to 1st operation (mo) | 32 | 80.5 |
| | Median time from diagnosis of CD to 1st operation with fibrostenosis (mo) | 32 | 160 |

aPCR: resistance to activated protein C; CD: Crohn’s disease. Operations: intestinal CD-related operations, Operations with fibrostenosis: intestinal CD-related operations with fibrostenosis. The location of fibrostenosis was classified according to the Vienna classification for location of Crohn’s disease. P < 0.05 vs patients without aPCR.
lesions had been performed in two patients with aPCR but not in patients without aPCR. These interventions were not included in the analysis.

**Operations with fibrostenosis: primary end point**

There was a clear tendency for fibrostenosis to be found more often in the presence of aPCR. At the first intestinal CD-related operation, fibrostenotic lesions were found in all 6 (100%) patients with aPCR, but only in 10 out of 16 (62.5%) patients without aPCR ($P = 0.079$). The Kaplan-Meier analysis revealed that patients with aPCR had a significantly lower probability of remaining free of operation with fibrostenosis compared to patients without aPCR ($P = 0.0372$; exact log-rank test). This was also represented by the median time from diagnosis to the first operation with fibrostenosis being significantly shorter in patients with aPCR than in patients without aPCR (32 vs 160 mo; Table 2). In a further step, the calculation was limited to 120 mo, since this time interval was just below the median observation time of the patients (140 and 145 mo, respectively) and, therefore, more than half of the patients were under follow-up during the first 10 years after diagnosis of CD ($P = 0.0216$; exact log-rank test) (Figure 1). At 10 years, the likelihood of remaining free of operation with fibrostenosis was 25% for patients with aPCR and 57.8% for patients without aPCR.

Both observers who reviewed the pathological and surgical reports of the patients for fibrostenotic lesions agreed on the presence or absence of fibrostenosis in all operations. In 4 out of 44 operations, a fibrostenosis was described in the pathological report but was not mentioned in the surgical report. In all these four cases (1 patient with aPCR and 3 patients without aPCR), the surgeons described inflammatory masses that made it difficult to diagnose fibrostenosis in the surgical situation.

**Overall operations: secondary end point**

Concerning any intestinal CD-related operation without regard to whether it revealed fibrostenosis or not, patients with aPCR did not undergo operations more often than patients without aPCR. Six of 8 (75%) patients with aPCR and 16 of 24 (67%) patients without aPCR had undergone intestinal surgery ($P = 0.32$) (Table 2). Furthermore, the median time interval from diagnosis of CD to the first operation (Table 2), and the probability of remaining free of intestinal surgery (Kaplan-Meier survival curve not shown) did not significantly differ between the two patient groups ($P = 0.24$).

**DISCUSSION**

To our best of knowledge, this is the first report on an association between resistance to activated protein C and fibrostenosis in Crohn’s disease. In a case-controlled study setting, aPCR increased the risk of fibrostenotic lesions found at intestinal surgery as described by surgical and/or pathological reports. CD patients with aPCR had a significantly lower probability of remaining free of intestinal operations with fibrostenosis. These data support our hypothesis that aPCR accelerates the development of fibrosis in intestinal lesions in CD patients.

The primary end-point in the present study was the occurrence of an intestinal CD-related operation with fibrostenosis. Fibrostenotic lesions were referred to bowel wall thickening and luminal narrowing on naked-eye examination and to patho-histological features. This definition of fibrostenosis was modified from the definition of disease behavior used in the Vienna classification and was irrespective of evidence of penetration. The most important reasons for modification were the association of internal fistula and fibrostenosis in multiple bowel resection specimens as well as the possible occurrence of inflammatory bowel obstruction without fibrostenosis. It is difficult or sometimes impossible to distinguish inflammatory from fibrostenotic strictures by current diagnostic techniques, including imaging procedures and endoscopy, leaving the question of what is the gold standard for the differential diagnosis? Is there any method, which is superior to direct visualization by the surgeon and the pathologist as well as to patho-histological investigation? From the literature, good evidence cannot be retrieved. Thus, we based our evaluation of fibrostenosis on the assumption that surgical and pathological reports deliver the most reliable data on the presence of fibrostenosis and are, therefore, superior to imaging procedures in the diagnosis of fibrostenotic strictures on expert opinion.

aPCR is the most common known inherited thrombophilic disorder known until now. The genetic basis of aPCR is a single-point mutation in the gene encoding for coagulation factor V, also known as factor V Leiden. Factor V Leiden has a 2%-7% prevalence in most Caucasian populations and is identified in 20%-50% of patients with venous thromboembolism.

In addition to the increased risk of venous thromboembolism, aPCR and other thrombotic risk factors have also been shown to accelerate the rate of fibrosis in inflammatory diseases, such as chronic viral hepatitis. Thrombotic obliterations of small portal and hepatic veins and activation of the stellate cells by
thrombin were assumed to cause increased fibrosis in the liver[38]. The effect of thrombin is mediated through activation of thrombin receptors on the stellate cells, which are up-regulated during chronic liver injury[39]. Furthermore, aPCR significantly increased pulmonary fibrosis in bleomycin-induced inflammatory lung injury in mice[40]. Additionally, an association between aPCR and fibrosis has been described in a transgenic factor V Leiden mouse model with enhanced fibrin deposition in multiple tissues[41]. In CD, an influence of the clotting system on the pathomechanism has been assumed based on the finding of intravascular fibrin deposition and capillary microthrombi[21,22] and a reduced frequency of CD in patients with inherited haemophilic disorders[23].

The association between aPCR or any other thrombotic risk factor and fibrosis in CD has not been previously investigated. An increased rate of fibrosis may lead to fibrostenotic lesions of the bowel, which often require surgery. Intestinal fibrosis and fibrostenosis are caused by uncontrolled proliferation of mesenchymal cells and excessive production of extracellular matrix proteins, including fibrillar collagen[5,6]. Fibroblasts cultured from fibrostenotic lesions in CD patients, for instance, have been shown to synthesize an increased amount of collagen type III[32]. A precondition for the development of fibrosis is a chronic transmural inflammation, which is a typical feature of CD. Infiltrating immune cells are considered to secrete cytokines and growth factors, such as transforming growth factor beta (TGF-β), in the mesenchymal layers, which might lead to activation of fibroblasts[6,45].

Some risk factors predispose to fibrostenosis in CD. Several studies reported that CD-associated NOD2/CARD15 variants are associated with fibrostenotic lesions[21-23] and, furthermore, that CD patients who are positive for ASCA, I2, anti-OmpC, or pANCA are also more likely to develop fibrostenosis[25,27]. Additionally, smoking has been shown to predispose to ileal localization of CD and to fibrostenotic lesions[10-11]. But for all the itemized risk factors, it remains to be determined whether the association with fibrostenosis is independent or secondary to association with ileal involvement.

Immunosuppressive drugs do not seem to prevent the formation of fibrostenosis. Azathioprine has been shown to improve mucosal healing but not the development of fibrostenosis in CD, especially in the ileocecal region[44-46]. A recent retrospective study on the use of immunosuppressive drugs and the need for intestinal surgery described only a tendency of reduced probability of formation of fibrostenosis over the time, despite a significant increase of the use of azathioprine in the same time period[46]. However, the only immunosuppressive drug given to the patients in the present study was azathioprine, which was equally frequently administered to the patients of both groups. We can, therefore, exclude any influence of medical treatment on the results of our study.

Furthermore, there was no difference in smoking habits between the two patient groups. But all the other risk factors for fibrostenosis, such as NOD2/CARD15 variants, ASCA, I2, anti-OmpC, and pANCA, were not fully available and, therefore, not included in the analysis. It was thus not possible to investigate the influence of these risk factors on our results. A further limitation might be the small number of patients included in the study. Since the effect of aPCR on fibrostenosis in CD was unknown prior to the present investigation, a calculation of the sample size was not possible. Our aim was to perform a pilot study on an association between aPCR and fibrostenosis in CD and by this way to generate the basis for a working hypothesis.

aPCR did not significantly influence the number of overall intestinal CD-related operations regardless of fibrostenosis. Therefore, we have no evidence that other indications for surgical treatment in CD except fibrostenotic strictures are influenced by aPCR.

In summary, our results give rise to this hypothesis that aPCR may be a risk factor for fibrostenosis in CD. The effect of aPCR might not be specific and coagulation might generally be involved in the development of fibrosis in CD, disposing patients with any thrombophilic disorder also to have an increased tendency to fibrostenosis. We are aware that our data should be verified by further larger trials, including data about other known risk factors for fibrostenosis. However, our study contains the first reference to an association between aPCR and fibrostenosis in CD and may provide a new insight in the pathogenesis of fibrostenosis in CD.

REFERENCES

1. Greenstein AJ, Lachman P, Sachar DB, Springhorn J, Heimann T, Janowitz HD, Aufses AH Jr. Perforating and non-perforating indications for repeated operations in Crohn’s disease: evidence for two clinical forms. *Gut* 1988; 29: 588-592
2. Poggioni G, Pierangeli F, Laureti S, Ugolini F. Review article: indication and type of surgery in Crohn’s disease. *Aliment Pharmacol Ther* 2002; 16 Suppl 4: 59-64
3. Andersson P, Olaison G, Boden A, Nyström PO, Sjödahl R. Surgery for Crohn colitis over a twenty-eight-year period: fewer stomas and the replacement of total colectomy by segmental resection. *Scand J Gastroenterol* 2002; 37: 68-73
4. Cosnes J, Nion-Larmurier I, Beaugerie L, Afnan P, Tiret E, Gendre JP. Impact of the increasing use of immunosuppressants in Crohn’s disease on the need for intestinal surgery. *Gut* 2005; 54: 237-241
5. Pucilowska JB, Williams KL, Lund PK. Fibrogenesis. IV. Fibrosis and inflammatory bowel disease: cellular mediators and animal models. *Am J Physiol Gastrointest Liver Physiol* 2000; 279: G653-G659
6. Van Asche G, Geboes K, Rutgeerts P. Medical therapy for Crohn’s disease strictures. *Inflamm Bowel Dis* 2004; 10: 55-60
7. Lesage S, Zouali H, Cezeard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O’Morain C, Gasull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jarnot AS, Thomas G, Hugot JP. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; 70: 845-857
8. Abreu MT, Taylor KD, Lin YC, Hang T, Gaennerie J, Landers CJ, Vasiliaskas EA, Kam LY, Rojany M, Papadakis KA, Rotter JI, Targan SR, Yang H. Mutations in NOD2 are associated with inflammatory bowel disease: evidence for two clinical forms. *Gastroenterology* 2000; 123: 679-688
9. Brant SR, Picco MF, Achkar JP, Bayless TM, Kane SV, Brazzinskis A, Nouvet FJ, Bonen D, Karban A, Dassopoulos T, Karaliukas R, Beaty TH, Hanauer SB, Duerr RH, Cho JH. Defining complex contributions of NOD2/CARD15 gene mutations, age at onset, and tobacco use on Crohn’s disease phenotypes. *Inflamm Bowel Dis* 2003; 9: 281-289
10 Bridger S, Lee JC, Bjarnason I, Jones JE, Macpherson AJ. In siblings with similar genetic susceptibility for inflammatory bowel disease, smokers tend to develop Crohn’s disease and non-smokers develop ulcerative colitis. Gut 2002; 51: 21-25

11 Picco MF, Bazzoli TM. Tobacco consumption and disease duration are associated with fistulizing and stricturing behaviors in the first 8 years of Crohn’s disease. Am J Gastroenterol 2003; 98: 363-368

12 Mow WS, Vasiliaslaukas EA, Lin YC, Fleschner PR, Papadakis KA, Taylor KD, Landers CJ, Abreu-Martin MT, Rotter JI, Yang H, Targan SR. Association of antibody responses to microbial antigens and complications of small bowel Crohn’s disease. Gastroenterology 2004; 126: 414-424

13 Vasiliaslaukas EA, Kam LY, Karp LC, Gaiennie J, Yang H, Targan SR. Marker antibody expression stratifies Crohn’s disease into immunologically homogeneous subgroups with distinct clinical characteristics. Gut 2000; 47: 487-496

14 Smith BR, Arnott ID, Drummond HE, Nimmo ER, Satsangi J. Disease location, anti-Saccharomyces cerevisiae antibody, and NOD2/CARD15 genotype influence the progression of disease behavior in Crohn’s disease. Inflamm Bowel Dis 2004; 10: 521-528

15 Kleb FH, Bataille F, Bertea CR, Herfarth H, Hofstädter F, Scholmerich J, Rogler G. Association of perinuclear antineutrophil cytoplasmic antibodies and anti-Saccharomyces cerevisiae antibodies with Vienna classification subtypes of Crohn’s disease. Inflamm Bowel Dis 2003; 9: 302-307

16 Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C; prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA 1993; 90: 1004-1008

17 Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet 1993; 342: 1503-1506

18 Wright M, Golden R, Heller S, Knapp S, Froshdham A, Hennig B, Hill A, Apple R, Cheng S, Thomas H, Thursz M. Factor V Leiden polymorphism and the role of fibrosis development in chronic hepatitis C virus infection. Gut 2003; 52: 1206-1210

19 Bertina RM, Koekeman BP, Koster T, Rosendaal FR, Divren RJ, de Ronde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64-67

20 Marra F, Grandallano G, Valente AJ, Abboud HE. Thrombin stimulates polimeration of liver fat-storing cells and expression of monocyte chemotactastic protein 1: potential role in liver injury. Hepatology 1995; 22: 780-787

21 Wakefield AJ, Sawyerr AM, Dhillon AP, Pittilo RM, Sawyerr AM, Pounder RE. Pathogenesis of Crohn’s disease: multifocal gastrointestinal infarction. Lancet 1989; 2: 1057-1062

22 Sankey EA, Dhillon AP, Anthony A, Wakefield AJ, Sim R, More L, Hudson M, Sawyerr AM, Pounder RE. Early mucosal changes in Crohn’s disease. Gut 1993; 34: 375-381

23 Thompson NP, Wakefield AJ, Pounder RE. Inherited disorders of coagulation appear to protect against inflammatory bowel disease. Gastroenterology 1995; 108: 1011-1015

24 Novacek G, Miehler W, Kaptios S, Katzenschlager R, Speiser W, Vogelsang H. Thromboembolism and resistance to activated protein C in patients with inflammatory bowel disease. Am J Gastroenterol 1999; 94: 685-690

25 Malchev H, Iwei K, Brandes JW, Goebell E, Hems H, Sommer H, Jesdinsky H. European Cooperative Crohn’s Disease Study (ECCDS): results of drug treatment. Gastroenterology 1984; 86: 249-266

26 Gasche C, Schölmerich J, Brynsvok J, D’haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR. A simple classification of Crohn’s disease: report of the Working Party for the World Congress of Gastroenterology, Vienna 1998. Inflamm Bowel Dis 2000; 6: 8-15

27 Oberhuber G, Stangl PC, Vogelsang H, Schober E, Herbst F, Gasche C. Significant association of strictures and internal fistula formation in Crohn’s disease. Virchows Arch 2000; 437: 295-297

28 Kaptios S, Quehenberger P, Jilma B, Handler S, Fabinger-Fasching I, Männahler C, Speiser W. Improved characteristics of aPC-resistance assay: Coatest aPC resistance by predilution of samples with factor V deficient plasma. Am J Clin Pathol 1996; 106: 588-593

29 Tripodi A, Negri B, Bertina RM, Mannucci PM. Screening for the FV:Q506 mutation-evaluation of thirteen plasma-based methods for their diagnostic efficacy in comparison with DNA analysis. Thromb Haemost 1997; 77: 436-439

30 Hall C, Andersson NE, Andras M, Zetterberg U, Rosen S. Evaluation of a modified APPT-based method for determination of APC resistance in plasma from patients on heparin or oral anticoagulant therapy. Thromb Res 1998; 91: 203-209

31 Kalbfleisch JD, Prentice RL. The statistical analysis of failure time data. 2nd ed. Hoboka, New Jersey: John Wiley & Sons, Inc, 2002

32 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958; 53: 457-481

33 Heinzle G, Gnanat M, Schenmer M. Exact log-rank tests for unequal follow-up. Biometrics 2003; 39: 1151-1157

34 Ridker PM, Hennekens CH, Lindpaintner K, Stampler MJ, Eisenberg PR, Milketch MJ. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. N Engl J Med 1995; 332: 903-917

35 Rees DC, Cox M, Clegg JB. World distribution of factor V Leiden. Lancet 1995; 346: 1133-1134

36 Eichinger S, Pabinger I, Stümpflen A, Hirschl M, Bialoczycky C, Schneider B, Mannhalter C, Miar E, Lechner K, Kyrlle PA. The risk of recurrent venous thromboembolism in patients with and without factor V Leiden. Thromb Haemost 1997; 77: 624-628

37 Papatheodoridis GV, Papaconstantinou E, Andrioti E, Cholongitas E, Petraki K, Kontopoulou I, Hadziyannis SJJ. Thrombotic risk factors and extent of liver fibrosis in chronic viral hepatitis. Gut 2003; 52: 404-409

38 Poujol-Robert A, Rosmoroduc O, Serfaty L, Coullet F, Poupon R, Robert A. Genetic and acquired thrombotic factors in chronic hepatitis C. Am J Gastroenterol 2004; 99: 527-531

39 Marra F, DeFranco R, Crappone C, Milani S, Pinzani M, Pellegri G, Laffi G, Gentili P. Expression of the thrombin receptor in human liver: up-regulation during acute and chronic injury. Hepatology 1998; 27: 462-471

40 Xu Z, Westrick RJ, Shen YC, Eitzman DT. Pulmonary fibrosis is increased in mice carrying the factor V Leiden mutation following bleomycin injury. Thromb Haemost 2001; 85: 441-444

41 Cui J, Eitzman DT, Westrick RJ, Christie PD, Xu ZJ, Yang AY, Purkayastha AA, Yang TL, Metz AL, Gallagher KP, Tyson JA, Rosenberg RD, Ginsburg D. Spontaneous thrombosis in mice carrying the factor V Leiden mutation. Blood 2000; 96: 4222-4226

42 Stallmach A, Schuppun D, Riese HH, Matthes H, Riecken EO. Increased collagen type III synthesis by fibroblasts isolated from strictures of patients with Crohn’s disease. Gastroenterology 1992; 102: 1920-1929

43 McGaick BC, Hughes K, Tighe PJ, Mahida YR. Differential expression of TGF-beta isoforms by normal and inflammatory bowel disease intestinal myofibroblasts. Am J Physiol Cell Physiol 2002; 282: C172-C182

44 Sandborn WJ, Van O EC, Zins BJ, Tremaine WJ, Mays DC, Lipsky J. An intravenous loading dose of azathioprine decreases the time to response in patients with Crohn’s disease. Gastroenterology 1995; 109: 1808-1817

45 DeHaens G, Geboes K, Ponette E, Penninckx F, Rutgeerts P. Healing of severe recurrent ileitis with azathioprine therapy in patients with Crohn’s disease. Gastroenterology 1997; 112: 1475-1481

46 DeHaens G, Geboes K, Rutgeerts P. Endoscopic and histologic healing of Crohn’s (ileo-) colitis with azathioprine. Gastrointest Endosc 1999; 50: 667-671

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