

CONCISE REPORT

Serum human cartilage glycoprotein 39 as a marker of arthritis associated with inflammatory bowel disease

L Punzi, M Podswiadek, R D'Inca, M Zaninotto, D Bernardi, M Plebani, G C Sturniolo

Ann Rheum Dis 2003;62:1224–1226. doi: 10.1136/ard.2002.004408

Background: Common blood markers of arthritis are difficult to interpret in arthritis associated with inflammatory bowel disease (IBD) owing to the coexistence of two inflammatory events. No specific serological disease marker is available for IBD.

Objective: To determine a value of serum human cartilage glycoprotein 39 (HC gp39) as a marker of arthritis associated with IBD.

Methods: Serum levels of HC gp39 and ultrasensitive C reactive protein (CRP) were determined in 121 patients with IBD: 58 without arthritis (IBD-nonA) and 63 with arthritis (IBD-A), and in 20 healthy controls. IBD was classified as active (aIBD) and non-active (naIBD), and patients with IBD-A were classified as type I, II, and III arthritis by clinical activity indices.

Results: HC gp39 was higher in IBD-A than in IBD-nonA ($p < 0.001$) and controls ($p < 0.01$), while no difference was found between IBD-nonA and controls. CRP was increased in both IBD-A and IBD-nonA compared with the controls ($p < 0.01$ and < 0.05 , respectively) and in aIBD-nonA v naIBD-nonA ($p < 0.05$), but no difference in CRP was found between aIBD-A and naIBD-A. Finally, a correlation was found between the number of affected joints (NAJ) and HC gp39 ($r = 0.6$, $p < 0.001$).

Discussion: Increased serum levels of HC gp39, which were higher in IBD-A than in IBD-nonA, suggest that this substance might be a marker of arthropathy in IBD. HC gp39, because of its relationship with NAJ in IBD-A, may also be proposed as a disease activity marker in arthritis associated with IBD.

Joint involvement is a common feature in patients with inflammatory bowel disease (IBD), in particular, ulcerative colitis (UC) and Crohn's disease (CD).¹ Although these arthropathies are usually included in the group of seronegative spondylarthritides, their clinical spectrum may vary greatly, being found as arthralgia, peripheral and axial disease, or isolated enthesopathy.² Blood markers generally considered useful in the assessment of joint diseases, such as erythrocyte sedimentation rate or C reactive protein (CRP), are difficult to interpret in arthropathies associated with IBD as an increase in their value might be attributed to the joint disease or to the IBD. Thus it would be useful to have a substance specifically acting as a marker of just one of the two diseases. Because no such specific serological disease markers have yet been identified for IBD,³ we performed this study to determine whether the serum level of human cartilage glycoprotein 39 (HC gp39), a substance produced from activated articular chondrocytes and synoviocytes,⁴ might be a marker of arthritis associated with IBD.

PATIENTS AND METHODS

Patients

A hundred and twenty one patients with IBD (49 women and 72 men; mean (SD) age 42.9 (12.1) years, range 21–69), 58 without articular manifestation (IBD-nonA; mean age 44.3 (11.6) years; 32 with CD and 26 with UC) and 63 with an inflammatory arthropathy (IBD-A; mean age 38.8 (12.8) years; 35 with CD and 28 with UC) entered the study. Serum determinations were also carried out in 20 healthy controls (mean age 30.8 (5.9) years). IBD was diagnosed by clinical, radiological, endoscopic, and histological criteria.

We divided the patients into those with active and non-active IBD (respectively aIBD and naIBD) according to international criteria for the remission of IBD (for CD, CDAI < 150 and for UC, Powell-Tuck < 2).^{5, 6}

The diagnosis of inflammatory arthropathy was based on a physical examination performed by two rheumatologists and was made according to European Spondylarthropathy Study Group (ESSG) criteria for spondyloarthropathies,⁷ and the patients were classified according to three clinical patterns: peripheral oligoarticular (type I), peripheral polyarticular (type II), and axial (type III), as suggested by Orchard.^{2, 8} The joint count was performed in all three subtypes and, subsequently, the number of affected joints (NAJ) was established.

We excluded patients with fever, infectious diseases, liver affections, history of malignancies, and those who were over 70, because of a possible increase in HC gp39 in these conditions. The patients with IBD were not treated with corticosteroids for at least six months before the study. All patients were taking mesalazine (2400 mg daily) or sulfasalazine (3000–4000 mg daily) for at least six months. Oral paracetamol and topical treatment were permitted.

Laboratory investigations

Serum from all patients was tested for HC gp39 by a third generation enzyme linked immunoadsorbent assay (ELISA-3; Metra Biosystems, USA; analytical sensitivity 20 ng/ml). CRP was detected by a high sensitivity nephelometric method (analytical sensitivity 0.04 mg/l).

Statistical analysis

We used the Kruskal-Wallis test for analysis of the variance, Student's *t* test for the differences, and Pearson's test for correlations. A *p* value of < 0.05 was considered significant. To determine significant differences between group means in the analysis of variance we applied a post-test analysis (Dunn's multiple comparison test).

Abbreviations: aIBD, active IBD; CD, Crohn's disease; CRP, C reactive protein; HC gp39, human cartilage glycoprotein 39; IBD, inflammatory bowel disease; IBD-A, IBD with arthritis; IBD-nonA, IBD without arthritis; naIBD, non-active IBD; RA, rheumatoid arthritis; NAJ, number of affected joints; OA, osteoarthritis; UC ulcerative colitis,

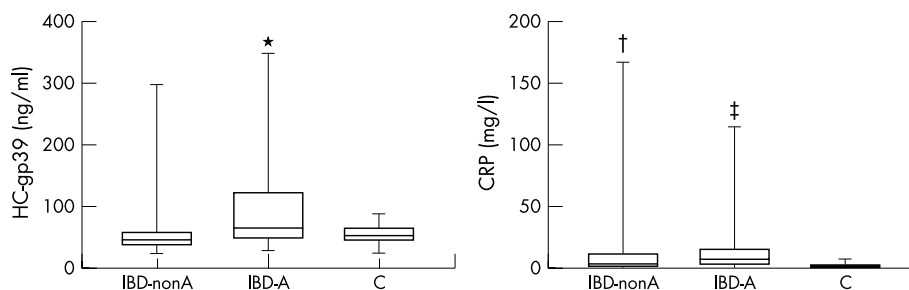


Figure 1 Serum levels of HC gp39 and CRP in patients with IBD-A, IBD-nonA, and controls (C). Significant differences (Student's *t* test): * $p < 0.001$ IBD-A *v* IBD-nonA; † $p < 0.05$ IBD-nonA *v* controls; ‡ $p < 0.01$ IBD-A *v* controls.

RESULTS

Figure 1 shows the data for HC gp39 and CRP in the two IBD subgroups: 58 IBD-nonA and 63 IBD-A, and in the 20 healthy controls. As shown, the levels of HC gp39 in IBD-A were significantly raised in comparison with both IBD-nonA ($p < 0.001$) and controls ($p < 0.01$), while no differences were found between IBD-nonA and controls. CRP levels in IBD-A and IBD-nonA were higher than in controls ($p < 0.01$ and < 0.05 , respectively); however, no difference was observed between IBD-A and IBD-nonA. For the subgroups (table 1, fig 2) the highest levels of HC gp39 were found in aIBD-A (122.6 (78.6) ng/ml), higher than in naIBD-A (105.8 (97.6) ng/ml, $p = \text{NS}$), in aIBD-nonA (72.6 (49) ng/ml, $p = \text{NS}$), in naIBD-nonA (58.4 (49.4) ng/ml, $p < 0.001$), and in controls (51.9 (14.1)). Also naIBD-A was higher than naIBD-nonA (105.8 (97.6) *v* 58.4 (49.4), $p < 0.01$). The only differences for CRP were seen between aIBD-nonA (22.4 (18.5)) and naIBD-nonA (5.1 (6.1), $p < 0.05$), and in all the groups *v* controls. No significant correlation was found between HC gp39 and CRP in all the patients considered together except for the IBD-A ($r = 0.49$; $p < 0.05$).

Five (8%) patients had type I arthropathy, 12/63 (19%) type II, and 46/63 (73%) type III. There were no differences in HC gp39 and CRP levels among these subgroups. The number of active joints was higher in patients with type II and III arthropathy (3.3 (2.3) and 2.4 (1.8), respectively) than in type I (1.3 (0.6)), though not significant. Finally a strong correlation was observed between HC gp39 and the NAJ ($r = 0.6$, $p < 0.001$) in all the patients with IBD-A. No correlation between CRP and NAJ was found.

No differences in the levels of CRP and HC gp39 were found between UC and CD.

DISCUSSION

This study shows that serum levels of HC gp39 in patients with IBD-A are higher than in those with IBD-nonA; the latter have levels similar to those found in normal subjects.

Table 1 HC gp39 and CRP serum levels in: IBD-A, IBD-nonA, aIBD, and naIBD subgroups with and without arthritis

Subgroups	Patients (n)	HC gp39 (ng/ml)	CRP (mg/l)
aIBD-A	20	122.6 (78.6)‡§	24.5 (35)*
naIBD-A	43	105.8 (97.6)†	10.4 (14)*
aIBD-nonA	16	72.6 (49)	22.4 (18.5)*
naIBD-nonA	42	58.4 (49.4)	5.1 (6.1)§
Controls	20	51.9 (14.1)	1.3 (1.5)

Serum levels of HC gp39 and CRP in aIBD-A, naIBD-A, aIBD-nonA, naIBD-nonA, and controls. The Kruskal-Wallis analysis: *v* naIBD-nonA * $p < 0.05$, † $p < 0.01$, ‡ $p < 0.001$; *v* controls § $p < 0.05$, ¶ $p < 0.001$.

However, no differences were found between aIBD-A and naIBD-A or between aIBD-nonA and naIBD-nonA. This strongly suggests that HC gp39 is a marker of arthropathy in patients with IBD.

HC gp39, also described as YKL-40, is a protein with an apparent molecular weight of 42 kDa, mainly produced by human chondrocytes and synovial cells, but also by macrophages and neutrophils.⁹⁻¹⁰ It is one of the most abundant proteins secreted in vitro by chondrocytes and synoviocytes in rheumatoid arthritis (RA).⁴ Furthermore, HC gp39 messenger RNA is expressed in the cartilage from patients with RA or osteoarthritis (OA) but not in healthy adult cartilage.¹¹ Although the physiological function of HC gp39 is unknown, it has been noted that this protein contains several DR4 peptide binding motifs and may be a target of the immune response in RA.¹² Furthermore, it has been shown that the presence of HC gp39+ cells in RA synovium correlates with the degree of joint destruction.¹³ Although the serum levels of HC gp39 in RA and severe OA were higher than in controls and correlated with disease severity,¹⁴⁻¹⁵ HC gp39 may not be considered specific for joint diseases as it is found in other non-articular inflammatory or neoplastic disorders, probably as a result of neutrophil or macrophage production.¹⁴ The slight increase of HC gp39 in aIBD compared with naIBD may confirm its extra-articular synthesis.

However, its increased serum levels in our patients with IBD-A in comparison with IBD-nonA, and the absence of influence by bowel inflammation, strongly suggest that HC gp39 is a marker of arthritis associated with IBD. To our knowledge, this is the first substance until now which shows such properties.

The results of this study also indicate that HC gp39 can be proposed as a disease activity marker of arthritis associated with IBD. This is not surprising because similar properties were also seen in RA, in which HC gp39 was correlated with clinical activity¹⁶ and with CRP,¹⁷ a largely accepted serological marker of disease activity in RA. In our study the correlation between HC gp39 and CRP was found only in IBD-A, probably because of the influence of the bowel inflammation on CRP. This latter observation seems in keeping with a specific role for HC gp39 in IBD-A.

Recently, HC gp39 was determined in the serum of patients with ankylosing spondylitis treated with infliximab.¹⁸ Although HC gp39 changes were not statistically significant, they did correlate with the Bath indices including BASDAI, BASFI, and BASGI. This property of HC gp39 and the results obtained in our study led us to suggest that HC gp39 is a marker of articular damage and not an acute response index. In conclusion, HC gp39, a protein mainly produced by chondrocytes and synoviocytes, may be a useful disease marker in IBD, able to detect the presence, and perhaps the degree, of joint disease in these patients.

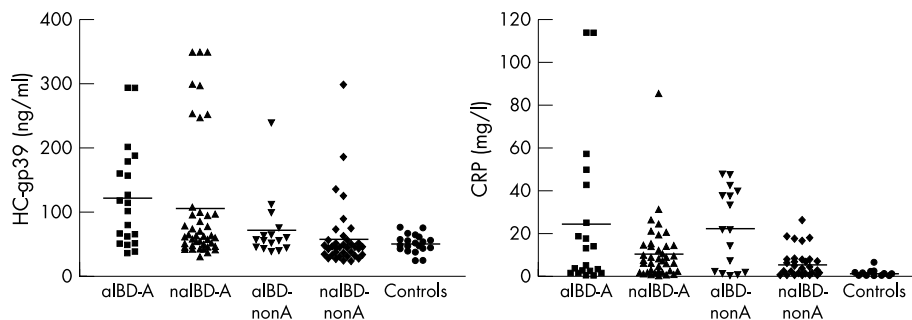


Figure 2 The Kruskal-Wallis analysis for HC gp39 and CRP in alBD-A, naIBD-A, alBD-nonA, naIBD-nonA, and controls.

Authors' affiliations

L Punzi, M Podswiadek, Division of Rheumatology, University of Padova, Italy

R D'Inca, G Sturniolo, Division of Gastroenterology, University of Padova, Italy

M Zaninotto, D Bernardi, M Plebani, Department of Laboratory Medicine, University of Padova, Italy

Correspondence to: Professor L Punzi, Division of Rheumatology, Department of Medical and Surgical Sciences, University of Padova, Via Giustiniani 2, 35128 Padova, Italy; punzireu@unipd.it

Accepted 23 March 2003

REFERENCES

- Greenstein AJ, Janowitz HD, Sachar DB. The extraintestinal complications of Crohn's disease and ulcerative colitis: a study of 700 patients. *Medicine (Baltimore)* 1976;**55**:401-12.
- Orchard TR, Wordsworth BP, Jewell DP. Peripheral arthropathies in inflammatory bowel diseases: their articular distribution and natural history. *Gut* 1998;**42**:387-91.
- Nielsen OH, Vainer B, Madsen MS, Seidelin JB, Heegard NHH. Established and emerging biological activity markers of inflammatory bowel disease. *Am J Gastroenterol* 2000;**95**:359-67.
- Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem* 1993;**268**:25803-10.
- Best WR, Beckett JM, Singleton JW. Rederived values of the eight coefficients of the Crohn's disease activity index (CDAI). *Gastroenterology* 1979;**77**:843-6.
- Powell-Tuck J, Day DW, Buckell NA, Wadsworth J, Lennard-Jones JE. Correlations between defined sigmoidoscopic appearances and other measures of disease activity in ulcerative colitis. *Dig Dis Sci* 1982 Jun;**27**:533-7.
- Amor B, Dougados M, Listrat V, Menkes CJ, Dubost JJ, Roux H, et al. Evaluation of the Amor criteria for spondylarthropathies and European Spondylarthropathy Study Group (ESSG). A cross-sectional analysis of 2,228 patients. *Ann Med Interne (Paris)* 1991;**142**:85-9.
- Smale S, Natt RS, Orchard TR, Russel AS, Bjarnason I. Inflammatory bowel disease and spondylarthropathy. *Arthritis Rheum* 2001;**44**:2728-36.
- Kirkpatrick RB, Emery JG, Connor JR, Dodds R, Lysko PG, Rosenberg M. Induction and expression of human cartilage glycoprotein 39 in rheumatoid inflammatory and peripheral blood monocyte-derived macrophages. *Exp Cell Res* 1997;**237**:46-54.
- Volck B, Ostergaard K, Johansen JS, Garbarsch C, Price PA. The distribution of YKL-40 in osteoarthritic and normal human articular cartilage. *Scand J Rheumatol* 1999;**28**:171-9.
- Volck B, Johansen JS, Stollenberg M, Garbarsch C, Price PA, Østergaard M, et al. Studies on YKL-40 in knee joints of patients with rheumatoid arthritis and osteoarthritis. Involvement of YKL-40 in the joint pathology. *Osteoarthritis Cartilage* 2001;**9**:203-14.
- Verheijden GFM, Rijnders AWM, Bos E, Coenen-de-Roo CJJ, van Staveren CJ, Miltenburg AMM, et al. Human cartilage glycoprotein-39 as a candidate autoantigen in rheumatoid arthritis. *Arthritis Rheum* 1997;**40**:1115-25.
- Baeten D, Boots AM, Steenbakkers PG, Elewaut D, Bos E, Verheijden GF, et al. Human cartilage gp-39+, CD16+ monocytes in peripheral blood and synovium: correlation with joint destruction in rheumatoid arthritis. *Arthritis Rheum* 2000;**43**:1233-43.
- Vos K, Steenbakkers P, Miltenburg AMM, Bos E, van den Heuvel MW, van Hogezaand RA, et al. Raised human cartilage glycoprotein-39 plasma levels in patients with rheumatoid arthritis and inflammatory conditions. *Ann Rheum Dis* 2000;**59**:544-8.
- Johansen JS, Williamson MK, Rice JS, Price PA. The distribution of YKL-40 in osteoarthritic and normal human cartilage. *Scand J Rheumatol* 1999;**28**:171-9.
- Johansen JS, Stollenberg M, Hansen M, Florescu A, Horslev-Petersen K, Lorenzen I, et al. Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity. *Rheumatology (Oxford)* 1999;**38**:618-26.
- Harvey S, Whaley J, Eberhardt K. The relationship between serum levels of YKL-40 and disease progression in patients with early rheumatoid arthritis. *Scand J Rheumatol* 2000;**29**:391-3.
- Maksymowych WP, Jhangri GS, Lambert RG, Mallon C, Buenviaje H, Pedrycz E, et al. Infliximab in ankylosing spondylitis: a prospective observational inception cohort analysis of efficacy and safety. *J Rheumatol* 2002;**29**:959-65.