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Serum cartilage oligomeric matrix protein (COMP) level is a marker of disease activity in relapsing polychondritis

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ABSTRACT

Objectives. *Relapsing polychondritis (RP) is a rare and severe disease which may lead to destruction of elastic cartilages. Until now, no reliable biomarker of disease activity in RP has been available. This study was designed to measure serum levels of cartilage biomarkers during both active and inactive phases of the disease.*

Methods. *Serum levels of cartilage oligomeric matrix protein (COMP), chondroitin sulfate 846 epitope (CS846) of proteoglycan aggrecan and collagen type II collagenase cleavage neoepitope (C2C) were measured retrospectively in 21 subjects with RP. The Wilcoxon matched-pairs signed-rank test was used for statistical comparisons of biomarker levels in active and inactive phases of RP.*

Results. *Only the serum level of COMP was significantly increased during disease flares. Steroids did not alter the serum cartilage-related biomarker levels. However, during the active phase, C2C levels were significantly higher in steroid treated patients compared with non-steroid treated patients.*

Conclusion. *This study suggests that serum COMP level may be useful for monitoring disease activity of RP. Further prospective studies are required to confirm this result.*

Introduction

Relapsing polychondritis (RP) is a rare autoimmune systemic disorder of unknown origin characterized by recurrent, widespread chondritis of the auricular, nasal, and laryngotracheal cartilages (1). Inflammation of other proteoglycan-rich tissues such as the eyes, heart, blood vessels, inner ears, and kidneys may also be present (1, 2). Disease activity is commonly assessed by clinical signs, an increase of acute phase reactants such as erythrocyte sedimentation rate and C-reactive protein, and by imaging such as laryngoscopy, high-resolution laryngotracheal computed tomography or magnetic resonance imaging of the trachea (1-3).

However we lack a reliable marker that reflects ongoing cartilage damage and disease activity in RP (3). Cellular infiltration by proinflammatory cells leads to high turnover of major elastic cartilage matrix components such as collagens, matrilin-1, cartilage oligomeric matrix protein (COMP) and the proteoglycan aggrecan. As a consequence, specific autoantibodies against type II, IX, and XI collagens, matrilin-1 and COMP may develop (1, 3 ,4, 5). Disease activity may be assessed by the presence of those autoantibodies. For instance, the presence of autoantibodies against matrilin-1 is linked to respiratory involvement (4). Only a few studies have measured circulating levels of cartilage matrix molecules in RP (1, 3, 4). For example, the urinary collagen type II neoepitope has been shown to reflect the severity of RP (3). In a case report, it has also been shown that the serum COMP level decreased during a flare of RP (6). Although never previously studied in RP additional biomarkers, such as the chondroitin sulfate 846 epitope (CS846) of aggrecan (7), and collagen type II collagenase cleavage neoepitope (C2C), sometimes known as Col2-3/4$C_{Long\ mono}$ (8), have been studied in other arthritic conditions, and shown promising results (7, 8). In this retrospective study, we sought to demonstrate whether the cartilage biomarkers (COMP, CS846, and C2C) correlated with disease activity in RP.

Patients and methods

Patients

We retrospectively selected 21 patients (16 women and 5 men) with RP, defined by the Michet criteria (9). These had been followed in the Internal Medicine Department of Pitié-Salpêtrière Hospital, Paris, France. For each patient, a serum sample was collected within 3 weeks of the initial presentation for a clinical flare (active phase) and during an inactive phase of the disease. Clinical flares were defined as chondritis involving at least 2 of 3 sites (auricular, nasal, or laryngotracheal cartilage), or one of these sites and 2 others manifestations, including ocular inflammation (conjunctivitis, keratitis, episcleritis, uveitis), audiovestibular symptoms (hearing loss or vertigo), or seronegative inflammatory arthritis (10). Eleven patients were treated with corticosteroids during

Table I. Baseline characteristics of the 21 patients.*

	Median [range] or number (%)
Demographics	
Age, years	51 [42-54]
Female gender	16 (76.2%)
Clinical characteristics	
Nasal chondritis	8 (38.1%)
Auricular chondritis	14 (66.7%)
Laryngotracheal chondritis	3 (14.3%)
Arthritis	5 (23.8%)
Ocular inflammation	3 (14.3%)
Ear symptoms	3 (14.3%)
Cartilage destruction	6 (28.6%)
Disease duration, months	80 [21-122]
Erythrocyte sedimentation rate, mm/hr	21 [10-41]
C-reactive protein, mg/L	5 [4-19]

*Qualitative variables are expressed as number (%), quantitative variables as median [interquartile range].

the active phase and 5 during the inactive phase. The mean steroid dosage was 7.5 mg/d prednisone equivalent. Other treatments included methotrexate, cyclosphosphamide, and disulone.

Measurement of cartilage-related biomarkers

Three cartilage serum biomarkers were measured: COMP, C2C, and CS846. COMP was measured by sandwich enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies 17C10 and 16F12 as previously reported (11); C2C was measured with a commercially available competitive ELISA kit (Ibex, Montreal, Quebec, Canada); and CS846 was measured by a competitive radioimmunoassay described for serum by Poole *et al*. (7). The intra- and interassay coefficient of variation (CV) was 2.1% and 14.2% for COMP, and the manufacturer's reported intra- and inter-assay precision for C2C was <15% and <20%. The intra- and inter-assay CV for CS846 was 9.9±6.5% and 4.8±5.1 respectively.

Statistical analysis

Biomarker levels were compared between the active and inactive phases of RP using the Wilcoxon matched-pairs signed-rank test. They were also compared between patients who received steroids and those who did not by using the non parametric Mann-Whitney two-sample ranksum test. Continuous data are presented as median (interquartile range), categorical variables are presented as number (%). All tests were two-sided, p-values <0.05 were considered statistically significant. Data were analysed using the Stata Statistical software (StataCorp 2003. Release 8.0. College Station, Texas).

Table II. Serum biomarker levels in the active and inactive phases of relapsing polychondritis.

Biomarkers	Phase of Disease		p-value*
	Active phase (n=21)	Inactive phase (n=21)	Active phase *vs.* Inactive phase
COMP ng/ml	694.8 [446.4-754.6]	335.7 [241.8-479.3]	0.05
CS846 epitope µg/ml	0.227 [0.147-0.266]	0.189 [0.131-0.227]	0.51
C2C pmol/ml	82.5 [56.5-121.7]	95.6 [68.8-107.2]	0.49

Concentrations are expressed as median [interquartile range]:
*p-values of the Wilcoxon matched-pairs signed-rank test.

Results

Baseline characteristics of patients

Table I displays the main demographic, clinical and biological characteristics of patients. The patients ranged in age from 42 to 54 years, with a median age of 51.

Serum COMP is a marker of the active phase of RP

Serum COMP levels during the active phase were significantly higher than COMP levels during the inactive phase (p=0.05) (Table II). There was no significant difference between serum levels of C2C or CS846 between the active and the inactive phase.

Steroids did not alter the serum cartilage-related biomarkers

During the active phase of the disease, about half the patients were treated with steroids at the time of blood sampling for this study. We found no evidence of association between steroid intake and the variation in serum levels of COMP, or CS846, during the active and inactive phases of RP (Table III). However, during the active phase, C2C levels were significantly higher (p=0.011) in steroid treated patients compared with non-steroid treated patients.

Discussion

The principal outcome of this study was that serum COMP levels reflected disease activity in RP. We did not use a control population, since the objective was to compare the level of markers during active and inactive phases of the disease in the same patient. COMP is a 524 kd homopentameric non-collagenous glycoprotein predominantly found in the extracellular matrix of cartilage, ligaments and tendons (12). It can interact with collagens I, II, IX, and matrilins (13). Serum COMP levels have demonstrated prognostic value for disease progression in rheumatoid arthritis, psoriatic arthritis, osteoarthritis and joint trauma (14, 15). Its level also fluctuates with physical activity (14). The prognostic value of COMP in RP was first suggested in a case report of a 50 year-old man. With 3 years of follow-up, serum COMP levels increased while respiratory symptoms improved, suggesting that COMP degradation could be involved in the active disease process and COMP synthesis in the repair process (6). More interestingly, COMP levels and matrilin-1 varied inversely during monitoring of this patient (6). Our results suggest that increased serum COMP levels correlated with disease activity of RP representing increased COMP turnover that may or may not include an attempt at a repair process. Serum COMP may reflect turnover of elastic cartilage (nasal, auricular and tracheal cartilages) though it may also vary with the involvement of non-elastic hyaline articular cartilage. Previous work has shown high titers of antibodies against COMP during RP, although it was not possible to correlate their presence to tracheal or articular involvement (4).

C2C is a marker of collagenase activity in cartilage and the CS846 is present on intact proteoglycan aggrecan and is considered a reflection turnover. The

Table III. Associations between steroid intake and serum biomarker levels in the active and inactive phases of relapsing polychondritis.

	Active phase			Inactive phase		
	No steroids n=10	Steroids n=11	p^*	No steroids n=16	Steroids n=5	p^*
C2C	66.7 [44.6-81.4]	109 [92.6-135.9]	0.011	71.6 [53.1-107.2]	95.6 [68.8-100.6]	0.63
CS 846 epitope	0.25 [0.12-0.27]	0.17 [0.15-0.31]	0.72	0.18 [0.18-0.21]	0.19 [0.09-0.25]	0.83
COMP	726.9 [638.6-754.6]	558.2 [323.9-909.0]	0.67	539.1 [460.8-653.8]	475.6 [273.1-745.2]	0.90

*p-values of the Wilcoxon matched-pairs signed-rank test.

prognostic values of these markers has been suggested in OA and in RA (7, 8). In our series, changes in serum levels of these two markers (C2C and CS846) were not statistically different between active and inactive phases of disease.

Steroids were given during acute flares to reduce systemic and cartilage inflammation, as well as to prevent the frequency and severity of recurrence (1, 2). *In vitro* high doses of steroids may alter proteoglycan metabolism (16). However, in our study, steroids did not influence the levels of CS846 during either phase. In contrast, C2C levels were higher in patients with active disease treated with steroids compared with those not treated with steroids. The increased levels of C2C might therefore reflect disease severity during the active phase.

Our study has several limitations. It is a retrospective study. Thus, it is difficult to assess whether the constitutive and baseline levels of cartilage biomarkers reflected ongoing and/or past destruction of the cartilage. Secondly, it was not possible to weight the influence of other treatments such as disulone or cyclophosphamide administrated during the active phase of the disease. Thirdly, we studied only a limited number of cartilage biomarkers. Studying combinations of markers of degradation and of synthesis of the same molecule has been shown to be more useful in terms of prognosis than the use of a single molecule in patients with knee osteoarthritis (17). Therefore, in the future, it would be interesting to measure the values of both CS846 together with aggregan keratan sulfate serum level (as a marker of aggrecan degradation), as well as C2C or CTX–II (degradation epitopes) and CPII (marker of biosynthesis). Finally, the number of patients is rather limited, but RP is a rare condition (2). The clinical presentation of RP is heterogeneous and a correlation between biomarkers and organ involvement is therefore difficult. Despite these limitations, elevation of serum COMP level during the active phase of RP is a noteworthy observation.

In conclusion, elevated concentrations of serum COMP suggest a high level of cartilage turnover during active phases of RP. Serum COMP may be useful for monitoring cartilage damage in RP, and serum C2C may be of use in examining response to treatment with steroids in RP. Further prospective studies are required to confirm this result.

References

1. GERGELY P JR, POÓR G: Relapsing polychondritis. *Best Pract Res Clin Rheumatol* 2004; 18: 723-38.
2. LETKO E, ZAFIRAKIS P, BALTATZIS S, VOUDOURI A, LIVIR-RALLATOS C, FOSTER CS: Relapsing polychonditis: a clinical review. *Semin Arthritis Rheum* 2002; 31: 384-95.
3. KRAUS VB, STABLER T, LE ET, SALTARELLI M, ALLEN NB: Urinary type II collagen neoepitope as an outcome measure for relapsing polychondritis. *Arthritis Rheum* 2003; 48: 2942-8.
4. HANSSON AS, HEINEGÅRD D, PIETTE JC, BURKHARDT H, HOLMDAHL R: The occurrence of autoantibodies to matrilin 1 reflects a tissue-specific response to cartilage of the respiratory tract in patients with relapsing polychondritis. *Arthritis Rheum* 2001; 44: 2402-12.
5. HANSSON AS, HOLMDAHL R: Cartilage-specific autoimmunity in animal models and clinical aspects in patients – focus on relapsing polychondritis. *Arthritis Res* 2002; 4: 296-301.
6. SAXNE T, HEINEGÅRD D: Serum concentration of two cartilage matrix proteins reflecting different aspect of cartilage turnover in relapsing polychondritis. *Arthritis Rheum* 1995; 38: 294-6.
7. POOLE AR, IONESCU M, SWAN A, DIEPPE PA: Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of the cartilage proteoglycan aggrecan. Implications for pathogenesis. *J Clin Invest* 1994; 94: 25-33.
8. POOLE AR, IONESCU M, FITZCHARLES MA, BILLINGHURST RC: The assessment of cartilage degradation *in vivo*: development of an immunoassay for the measurement in body fluids of type II collagen cleaved by collagenases. *J Immunol Methods* 2004; 294: 145-53.
9. MICHET CJ JR, MCKENNA CH, LUTHRA HS, O'FALLON WM: Relapsing polychondritis. Survival and predictive role of early disease manifestations. *Ann Intern Med* 1986; 104: 74-8.
10. STABLER T, PIETTE JC, CHEVALIER X, MARINI-PORTUGAL A, KRAUS VB: Serum cytokine profiles in relapsing polychondritis suggest monocyte/macrophage activation. *Arthritis Rheum* 2004; 50: 3663-7.
11. VILÍM V, OLEJÁROVÁ M, MACHÁCEK S, GATTEROVÁ J, KRAUS VB, PAVELKA K: Serum levels of cartilage oligomeric matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. *Osteoarthritis Cartilage* 2002; 10: 707-13.
12. MÜLLER G, MICHEL A, ALTENBURG E: COMP (cartilage oligomeric matrix protein) is synthesized in ligament, tendon, meniscus, and articular cartilage. *Connect Tissue Res* 1998; 39: 233-44.
13. MANN HH, OZBEK S, ENGEL J, PAULSSON M, WAGENER R: Interactions between the cartilage oligomeric matrix protein and matrilins. Implications for matrix assembly and the pathogenesis of chondrodysplasias. *J Biol Chem* 2004; 279: 25294-8.
14. POSEY KL, HECHT JT: The role of cartilage oligomeric matrix protein (COMP) in skeletal disease. *Curr Drug Targets* 2008; 9: 869-77.
15. SKOUMAL M, HABERHAUER G, FINK A *et al*.: Increased serum levels of cartilage oligomeric matrix protein in patients with psoriasis vulgaris: a marker for unknown peripheral joint involvement? *Clin Exp Rheumatol* 2008; 26: 1087-90.
16. THONAR EJ, SCHNITZER TJ, KUETTNER KE: Quantification of keratan sulfate in blood as a marker of cartilage metabolism. *J Rheumatol* 1987; 14: 23-4.
17. GARNERO P, PIPERNO M, GINEYTS E, CHRISTGAU S, DELMAS PD, VIGNON E: Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. *Ann Rheum Dis* 2001; 60: 619-26.