YKL-40 as a Marker of Joint Involvement in Inflammatory Bowel Disease, Daniela Bernardi,1 Marta Podswiadek,2 Martina Zanninotto,1 Leonardo Punzi,1 and Mario Plebani1,2
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Rheumatic symptoms are the most common extra-intestinal manifestations of inflammatory bowel diseases (IBD). Both ulcerative colitis and Crohn disease (1,2) can be complicated by seronegative spondyloarthropathies, including two principal patterns of arthritis: spondylitis and peripheral arthritis. Spondyloarthropathies resembling idiopathic ankylosing spondylitis occur in 10% of patients with ulcerative colitis and, less frequently, in those with Crohn disease; peripheral, often asymmetric, arthropathies occur in 5–20% of IBD patients. Unlike arthropathy limited to five or fewer joints, polyarthropy and spondyloarthropathies do not reflect the activity of the underlying IBD.

The “gold standard” for assessing joint damage remains the plain radiograph, which images only the bone and allows reliable detection of changes within a time span of at least 12 months. Importantly, all such techniques image only damage that has already occurred. Even after repeated investigations, they are of limited use in informing the clinician of continuing or future damage.

Biochemical markers may provide a valuable tool for the frequent quantitative measurements required for the diagnosis and monitoring of joint disease. Highly sensitive C-reactive protein (CRP) assays provide information on the inflammatory process (3) but are poor markers for joint disease in IBD. Other markers for joint disease have been proposed (4) that are related to structures within joints.

The concentrations of YKL-40 in serum and synovial fluid are closely correlated in patients with joint disease (5), suggesting that most of the protein found in serum may be produced within the joint (6, 7). YKL-40 is produced not only by chondrocytes and synovial cells but also by macrophages, neutrophils, cancer cells, endothelial cells, smooth muscle cells in blood vessels, and by cells (probably hepatic stellate cells) in the fibrotic liver (8–13). The protein is a growth factor for fibroblasts, chondrocytes, synovial cells, and endothelial cells (12, 14, 15) and appears to be a nonspecific marker of inflammation, tissue remodeling, or ongoing fibrosis (16, 17). Its value in detecting joint damage is still under evaluation.

In the present study, we evaluated serum YKL-40 as a possible marker for both peripheral and axial arthropathies in patients with IBD, and we assessed its biological variation and compared it with that reported for CRP and serum amyloid A (SAA) (18, 19).

We studied 171 patients (median age, 47 years; range, 21–79 years), of whom 29 had psoriatic arthritis (PsA; cutaneous psoriasis and arthritis and tenosynovitis and/or enthesopathy), 66 had IBD (36 with Crohn disease and 30 with ulcerative colitis), and 76 had joint involvement in IBD (IBD; 44 with Crohn disease and 32 with ulcerative colitis) consecutively admitted to the Rheumatology Department of the University-Hospital of Padua between January 7 and December 20, 2002.

The diagnosis of IBD was based on clinical signs and symptoms, macro- and microscopic findings at endoscopy, and radiologic imaging. Patients were classified as active IBD (26%, of whom 4% had severe active disease) and nonactive IBD according to the international criteria for remission of IBD [for Crohn disease, a Crohn Disease Activity Index <150; for ulcerative colitis, a Powell-Tuck score <2 (20, 21)]. The arthropathies consisted of seronegative spondyloarthritis, with a clinical spectrum that included peripheral and axial involvement or isolated enthesopathy. Spondyloarthropathy, diagnosed on the...
basis of clinical and radiologic findings, was classified according to the European Spondyloarthropathy Study Group system. All patients were given mesalazin (1800 mg daily) or sulfasalazin (3–4 g daily); none were treated with corticosteroids. The control group consisted of 20 age-matched healthy individuals (median age, 50.5 years; range, 42–61 years).

To assess biological variability, we recruited 10 apparently healthy members of our laboratory staff (median age, 36 years; range, 29–44 years) after applying the following inclusion criteria: within 20% of ideal body weight; not on medication or consuming significant quantities of alcohol; and for women, a regular menstrual cycle and no use of oral hormonal contraceptives. During the study period, all participants were asked to continue with their dietary habits and usual activities and to keep their weight stable to within ±1.0 kg. Following the guidelines in the Helsinki Declaration II, we thoroughly explained the design and execution of the experiment to the participants and obtained informed consent in all cases. Blood samples were obtained once a week for 4 weeks from all participants.

YKL-40, SAA, and CRP were determined in all serum samples in a masked fashion. Serum YKL-40 was measured by a quantitative immunoassay (7) according to the manufacturer’s instructions (Quidel Corporation, formerly Metra Biosystems). All YKL-40 assays were performed in duplicate. Serum CRP was determined by a high-sensitivity assay (22) (N High Sensitivity CRP; Dade Behring Diagnostic), using a particle-enhanced immunonephelometric technique performed on a Dade Behring Nephelometer II. The method is standardized against the IFCC/Community Bureau of Reference of the Commission of the European Communities (BRC)/College of American Pathologists reference preparation (23). For the quantitative determination of SAA, we used a nephelometric assay (BN II; Dade Behring Diagnostic) (22).

All samples, drawn by the same phlebotomist, were allowed to clot and then centrifuged at 3000g for 15 min at room temperature within 1 h of collection. Sera were separated within 3 h after collection to avoid a time-dependent increase in YKL-40 because degranulation of neutrophils occurs during prolonged storage (24). Samples were stored at −20°C until testing at the end of the collection period.

Because the distributions of YKL-40, CRP, and SAA data were not gaussian (Kolmogorov–Smirnov test), we performed a log transformation before statistical analysis. Differences in biological marker concentrations between groups were assessed by ANOVA, and statistical significance was set at \( P < 0.05 \). ROC analysis (25) was performed with DDU Astute Software (26). Nested ANOVA was applied, and the analytical (CV\(_{\text{A}}\)), within-subject (CV\(_{\text{w}}\)), and between-subject (CV\(_{\text{b}}\)) components of biological variability were calculated (27). We calculated the index of individuality (CV\(_{\text{I}}\)/CV\(_{\text{c}}\)) (28) and the critical difference \([2.77 \times CV_{\text{c}}^2 + CV_{\text{w}}^2]^{1/2}\), i.e., the minimum difference between consecutive measurements in the same patient that is significant at \( P = 0.05 \).

The median values and 2.5 and 97.5 percentiles of the evaluated biochemical markers in the PsA group, IBD patients with (JIBD) and without (IBD) joint involvement, and in controls are summarized in Table 1.

YKL-40 values in JIBD patients were significantly higher \((P = 0.000003)\) than in IBD patients without joint involvement, whereas, as expected, we observed no differences between the same groups for CRP and SAA. We observed no significant differences between the JIBD and PsA groups for YKL-40, CRP, or SAA. Median concentrations of all markers were significantly higher \((P < 0.05)\) in the JIBD group than in healthy adults (Table 1).

In PsA patients YKL-40 and CRP, but not SAA, concentrations were significantly higher than in IBD patients. In IBD patients and controls, YKL-40 and SAA concentrations overlapped. As expected, all marker concentrations were significantly higher in PsA patients than in controls. Patients with inactive bowel disease, the biochemical markers showed performances comparable to those of the total group of the patients. In particular, YKL-40 showed significant differences \((P = 0.05)\) between JIBD (median, 67 mg/L; range, 37–350 mg/L) and IBD values (median, 48 mg/L; range, 25–300 mg/L) as well as control values (median, 55 mg/L; range, 25–90 mg/L).

The areas (SE) under ROC curves (Fig. 1) were 0.82 (0.06) for YKL-40, 0.75 (0.08) for CRP, and 0.69 (0.07) for SAA, with \( z \)-scores of 5.5, 3.3, and 2.6, respectively. The area under ROC curve for YKL-40 (0.82) was significantly higher than the area for SAA (0.69), with a \( z \)-score of 2.

For all evaluated markers, most of the variability observed depended on the biological factors (CV\(_{\text{A}}\) and CV\(_{\text{c}}\)). Within-run analytical variation was 0.03 for YKL-40, 0.05 for CRP, and 0.04 for SAA, whereas within-subject variation was lower for YKL-40 (0.23) than for CRP (0.42) and quite similar to the value for SAA (0.25). Interindividual CVs were higher for CRP (0.92) and SAA (0.61) than for YKL-40 (0.17). The index of individuality was low (<0.6) for CRP and SAA and high (1.36) for YKL-40, suggesting the utility of reference values for YKL-40. The reference change values for YKL-40 (65%) and SAA (69%) showed the need for relatively large differences between sequential results to indicate significant change, but both were lower than the critical difference for CRP (118%).

The increased serum concentrations of YKL-40 in JIBD compared with IBD patients and the absence of influence by bowel inflammation suggest that YKL-40 may be a marker of articular damage in patients with IBD.

<table>
<thead>
<tr>
<th>Group</th>
<th>YKL-40, mg/L</th>
<th>CRP, mg/L</th>
<th>SAA, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>PsA</td>
<td>86 (26–300)</td>
<td>11.1 (1–199)</td>
<td>19.4 (1–662)</td>
</tr>
<tr>
<td>JIBD</td>
<td>68 (30–350)</td>
<td>6.0 (0.5–86)</td>
<td>11.8 (1–537)</td>
</tr>
<tr>
<td>IBD</td>
<td>41 (20–187)</td>
<td>1.0 (0.1–22)</td>
<td>4.6 (1–94)</td>
</tr>
<tr>
<td>Controls</td>
<td>55 (25–90)</td>
<td>0.6 (0.1–3)</td>
<td>3.9 (1–12)</td>
</tr>
</tbody>
</table>

\*\*\* Significantly different vs controls: * \( P < 0.05 \); ** \( P = 0.00002 \).
\*\*\* Significantly different vs CRP: * \( P < 0.05 \); ** \( P = 0.00001 \).
\*\*\* Significantly different vs PsA: * \( P = 0.000003 \); ** \( P < 0.05 \).
though other reports have suggested an important role of ongoing fibrogenesis in the bowel, disease type (17), and generic autoimmune response (16) in increasing serum concentrations of YKL-40, our data underline the practical usefulness of the marker for demonstrating an articular involvement in IBD. Our findings show that YKL-40 may be proposed as a marker of joint damage in IBD patients because it has improved performance compared with CRP and SAA in the detection of joint involvement. We found that CRP is a sensitive marker of inflammatory processes, but it lacks specificity for joint diseases, as does SAA.

Finally, data on biological variation indicate that the critical difference for YKL-40 is lower than that for CRP; thus, YKL-40 may be better than CRP for evaluating the significance of changes in serial results. Furthermore, biological data indicate the potential utility of YKL-40 reference values in assessing joint turnover in patients.

L. Punzi and M. Podswiadek were responsible for patient care and clinical data collection. D. Bernardi, M. Zaniotto, and M. Plebani were responsible for biochemical marker measurements, statistical treatment of data, and revision of the manuscript.

References

Circadian Rhythm of Salivary Cortisol, 17α-Hydroxyprogesterone, and Progesterone in Healthy Children, Michael Gröschl,* Manfred Rauh, and Helmuth-Günther Dörr (Klinik mit Poliklinik für Kinder und Jugendliche, Friedrich-Alexander-University Erlangen-Nürnberg, Loschgestrasse 15, 91054 Erlangen; Germany; * author for correspondence: fax 49-9131-8533714, e-mail michael.groeschl@kinder.imed.uni-erlangen.de)

Very few reference intervals for salivary steroids in children have been established to date (1). Even the manufacturers of salivary steroid assays do not provide sufficient reference data for their products. This lack of information is surprising because the measurement of salivary steroids has been accepted as being noninvasive and stress-free (2, 3). In particular, psychiatric and neuroendocrinologic experiments are frequently designed with saliva as the medium of choice for steroid analysis (4–6).

A large variety of stressors can rapidly affect the adrenal cortex, causing increased adrenal steroid concentrations. For example, hypoglycemia (7) or physical exercise (8) are potent physiologic stressors, whereas fear (9), feelings of inferiority (10, 11), or experiences at school (12, 13) can affect the adrenal cortex activity as psychologic stressors. The taking of blood can also influence adrenal steroid concentrations in children; saliva collection, however, is almost stress-free (14). The use of saliva for steroid analysis in children is therefore an excellent alternative to blood.

The aim of our study was to establish age-dependent reference values for salivary cortisol, 17α-hydroxyprogesterone (17OHP), and progesterone in a large cohort of healthy children. The availability of such reference intervals will improve the applicability of saliva analysis as a diagnostic tool in pediatric endocrinology.

We collected 252 saliva profiles from healthy children and adolescents (125 boys; age range, 4 days to 15 years; 127 girls; age range, 6 days to 13 years) with normal body length/height and weight. None of the girls had developed a regular menstrual cycle. The parents of the children gave informed consent.

Saliva was collected either with the Salivette®, using polyester swabs (Sarstedt), from children >1 year of age or with modified medical pacifiers (Büttnner-Frank; see Fig. 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem. org/content/vol49/issue10/) from infants <1 year. The teats of the pacifiers were perforated, and strips of filter paper, as used for blood-spot collection, were inserted into the teats. The absorbent material had no effect on the steroid analysis in the RIAs, in contrast to the cotton Salivette, particularly for 17OHP (15) and progesterone (16).

Samples were taken three times a day (0700, 1300, and 1900) at home. After sample collection, the saliva was immediately frozen and sent to the laboratory in a cooler. We tried to exclude any potential stressful events; thus the samples were taken only on so-called “quiet” days at home, e.g., days with no school-related stress or sports, or on weekends. All of the children participating in the study lived in an intact familial environment.

We used adapted commercial RIAs (DSL), as described in detail elsewhere (17, 18), that require 50 μL of saliva per tube. The sensitivities and intra- and interassay CVs were 0.2 nmol/L (6% and 9.6%) for cortisol, 9 pmol/L (6% and 8.6%) for 17OHP, and 9 pmol/L (5.1% and 7.7%) for progesterone. Mean recoveries of analytes that had been added to saliva samples were 89–94% for the three steroids.

The samples were measured in duplicate without extraction. The conditions during preanalysis guaranteed maximum stability of the samples (19).

Variations in salivary steroid values in relation to age, gender, and time of day were tested using ANOVA (Bonferroni-adjusted post hoc test). For descriptive and statistical analysis, the children were divided into age groups of <4 weeks, 1–12 months, and 1–2, 2–4, 5–7, 8–10, 11–13, and 14–15 years.

For graphical presentation, percentiles were calculated by transformation of the age-dependent data according to the recommendations of the IFCC (20). An appropriate (square-root or ln) transformation was used to adjust data to a gaussian distribution. The appropriate transformation was identified by an iterative process for each data set. Percentiles (3rd and 97th) were determined from the mean and the SD of the transformed data and then transformed back to the original scale. Polynomial regression of the percentiles on age was then performed using PRISM software (GraphPad Inc.).

Several figures and a detailed table are presented in the online Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem. org/content/vol49/issue10/.

We observed no gender-related differences in the salivary steroid concentrations in the different age groups included in this study (P = 0.45). The data for boys and girls were therefore combined. There were no significant differences between the age groups >1 year of age (P >0.05) for any of the three steroids. Percentiles for these data sets were calculated without further division into age groups according to: Percentile = SD × 1.96 ± Mean (20).

A detailed summary of the steroid concentrations is presented in Table 1.