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Serum calprotectin as a biomarker for Crohn's disease

M.-A. Meuwis[a], G. Vernier-Massouille[b], J.C. Grimaud[c], Y. Bouhnik[d], D. Laharie[e], E. Piver[f], L. Seidel[g], J.F. Colombel[h], E. Louis[a,*], the GETAID (Groupe d'Étude Thérapeutique Des Affections Inflammatoires Digestives)

[a] *Hepato-Gastroenterology and Digestive Oncology Department, Liège University Hospital, CHU and GIGA-R, University of Liège, Liège, Belgium*
[b] *Department of Gastroenterology, Université Lille Nord de France and CHRU Lille, 59000 Lille, France*
[c] *Service de Gastro-Entérologie Hôpital Nord et Faculté de Médecine de Marseille, 13915 Cedex 20, Marseille, France*
[d] *Gastroenterology and IBD Department, Hôpital Beaujon-APHP-Paris-Diderot University, 92110 Clichy, France*
[e] *CHU de Bordeaux, Hôpital Haut-Lévêque, Service d'Hépato-Gastroentérologie, Université de Bordeaux, Laboratoire de Bactériologie, 33000 Bordeaux, France*
[f] *CHRU Tours Biochimie et Biologie Moleculaire, INSERM U966, Université François Rabelais de Tours, Faculté de Médecine, 10 Bd. Tonnellé, 37000 Tours, France*
[g] *Medical Informatics and Biostatistics, University of Liège, Belgium*
[h] *Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1069 New York, NY 10029, USA*

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Abstract

Background and aims: In Crohn's disease, correlation between clinical assessment and disease activity at tissue level is weak. Our aim was to evaluate the value of serum calprotectin as a biomarker for Crohn's disease.
Methods: The STORI trial patients (n = 115) were studied at baseline, in clinical remission before infliximab withdrawal, or at the time of relapse after infliximab withdrawal. Forty healthy controls were also studied. Serum calprotectin level was measured by ELISA. Data were analyzed through correlation analyses, Kaplan Meier curves and Cox model, using available Crohn's Disease Activity Index (CDAI), Crohn's Disease Endoscopic Index of Severity (CDEIS), fecal calprotectin and C-reactive protein levels (hsCRP).
Results: Median serum calprotectin was 8892 ng/mL (range: 410–125,000 ng/mL) in Crohn disease patients as compared with 1318 ng/mL (range: 215.8–3770 ng/mL) in controls ($P < 0.0001$). Serum calprotectin was significantly higher for active disease (median = 19,584 ng/mL) than for inactive

Abbreviations: IBD, Inflammatory Bowel Disease; CD, Crohn's disease; hsCRP, high sensitivity CRP; CDAI, Crohn's Disease Activity Index; CDEIS, Crohn's Disease Endoscopic Index of Severity.

* Corresponding author.
E-mail address: edouard.louis@ulg.ac.be (E. Louis).

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disease (median = 8353 ng/mL) ($P < 0.0001$). Serum calprotectin correlated with hsCRP (r = 0.4092, $P < 0.0001$) and CDAI (r = 0.4442, $P < 0.0001$), but not with CDEIS, on the contrary to fecal calprotectin (r = 0.6458, 0.5515, 0.2577 with $P < 0.0001$, $P < 0.0001$, $P = 0.019$ respectively). In multivariate analysis, serum calprotectin used as a discrete variable (threshold: 5675 ng/ml), appeared complementary to hsCRP (>5 mg/l) and fecal calprotectin (>250 μg/g) to predict relapse after infliximab withdrawal ($P = 0.0173$, 0.0024 and 0.0002; HR: 3.191, 3.561 and 4.120).
Conclusions: As a CD biomarker, serum calprotectin has a similar profile as hsCRP. It is also complementary to fecal calprotectin and hsCRP for prediction of relapse after infliximab withdrawal.

1. Introduction

In Crohn's disease (CD), the correlation between simple clinical assessment and disease activity at the tissue level is weak. Hence the prediction of tissue healing, disease relapse and disease progression remains a challenge. Despite active research in the field, few biomarkers have proved to be useful for the assessment and monitoring of CD. Fecal calprotectin is one of the most informative. Calprotectin is an heterodimer made of Myeloid Related Protein 8 and 14 (MRP8 and MRP14) and represents about 40% to 60% of the proteins in the neutrophil cytosol.[1,2] Fecal concentration of calprotectin is increased in the presence of intestinal inflammation and its measurement has been used to differentiate between irritable bowel syndrome and inflammatory bowel disease (IBD).[3] Fecal calprotectin also correlates with the endoscopic activity of CD as measured by the Crohn's Disease Endoscopic Index of Severity (CDEIS), with small bowel inflammation at MRI assessed by a semi-quantitative scale, and with response to therapy.[4,5] Fecal calprotectin can also predict clinical relapse of CD with a sensitivity and specificity ranging between 60 and 100%.[5,6] Nevertheless, collecting stools is a hurdle for the patient and a blood marker may be more convenient for routine practice. In rheumatoid arthritis, serum calprotectin is well correlated to disease activity,[6,7] but very few data are available in IBD.[8,9] The STORI trial prospectively assessed the time to relapse and the predictive factors of relapse after infliximab withdrawal, in a cohort of patients treated for more than one year by combined therapy with an anti-metabolite and in stable remission without steroids for more than six months.[7] In univariate analysis, fecal calprotectin was the strongest predictor of the time-to-relapse.

The aim of our work was to assess the performances of serum calprotectin in the STORI cohort. In particular we looked at its correlation with clinical disease activity (Crohn's disease activity index (CDAI)), biologic activity (C-reactive protein (CRP) and fecal calprotectin), endoscopic activity (CDEIS) as well as at its ability to predict CD relapse and response to treatment.

2. Material and methods

2.1. Study design and patients clinical information

The design of the STORI study has been described in details elsewhere.[7] Briefly, the patients were prospectively recruited in several hospitals in France and Belgium, between 2006 and 2009. The study protocol was accepted by the ethics committee of the St Louis Hospital, Paris and all other participating centers in 2005. All the patients provided their informed consent before screening both for the core study and ancillary studies. Patients who were included in the study were in clinical remission with a CDAI <150 under a combined treatment with infliximab (IFX) and anti-metabolites (azathioprine (>2 mg/kg), 6-mercaptopurine (>1.5 mg/kg) or methotrexate (>15 mg weekly)) for at least one year and had been corticosteroid-free over the last 6 months. At baseline, CDEIS was evaluated by ileocolonoscopy and serum and stool samples were collected on the same day (the stool sample was collected the day of blood sampling but was not necessarily the first stool of the day). Patients were followed up with a CDAI calculation on a two monthly basis. Relapsing patients presented a CDAI above 250 points or between 150 and 250 points with a 70 points increase from baseline over two consecutive weeks. In case of relapse, the patients were retreated with IFX, resuming scheduled maintenance infusions. Stool and blood samples were collected at the time of relapse and one month after first IFX retreatment, on the same day. Median follow-up time ± SE was 28 ± 2 months. Out of the 115 patients studied, 52 were relapsers. Table 1 summarizes clinical data of the included patients. Moreover, some healthy controls (n = 40) were also tested for serum calprotectin to determine value in a normal population (see Table 1). These healthy subjects were prospectively recruited, after giving their informed consent, in the setting of a broader study approved by the ethics committee at Liège University Hospital in 2005. Those were patients undergoing colonoscopy for colorectal cancer screening and confirmed as healthy by specialized gastroenterologists.

2.2. Sample parameters measurements

Sera were stored at −80 °C and analyzed using the turbidimetric technique for high sensitivity CRP (hsCRP) quantitation, ranging from 0.06 to 160 mg/L and serum calprotectin quantitation using the CALPROLAB™ ELISA ALP (Lysaker, Norway) according to manufacturer's recommendations. Limit of quantification (LOQ) for this kit is 5 ng/mL and max coefficient of variation (CV) for inter-assay is 10% for EDTA plasma samples. The same kit lot number was used for all the samples tested. Overall 103 serum samples were available for these analyses at baseline, 31 at relapse, and 24 after infliximab retreatment.

Stools samples were stored at −80 °C and fecal calprotectin was measured using PhiCal® ELISA (Lysaker, Norway) test after