

# High-sensitivity C-reactive Protein for Identification of Disease Phenotype, Active Disease, and Clinical Relapses in Crohn's Disease: A Marker for Patient Classification?

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**Background:** C-reactive protein (CRP) is a traditional nonspecific marker of inflammation, with Crohn's disease (CD) being associated with a strong CRP response. Thus far, no clear cutoff values have been determined. The authors' aim was to investigate whether high-sensitivity (hs)-CRP is useful for the identification disease phenotype, active disease, and relapse during follow-up, using a classification based on the hs-CRP value at diagnosis.

**Methods:** In all, 260 well-characterized, unrelated, consecutive CD patients (male/female: 120/140; duration:  $7.0 \pm 6.1$  years), with a complete clinical follow-up, were included. Hs-CRP, clinical activity according to the Harvey–Bradshaw Index, and clinical data (disease phenotype according to the Montreal Classification, extraintestinal manifestations, smoking habits, medical therapy, and surgical events) were prospectively collected between January 1, 2008 and June 1, 2010. Medical records prior to the prospective follow-up period were analyzed retrospectively.

**Results:** In all, 32.3% of CD patients had normal hs-CRP at diagnosis. Elevated hs-CRP at diagnosis was associated with disease location ( $P = 0.002$ ), noninflammatory disease behavior ( $P = 0.058$ ), and a subsequent need for later azathioprine/biological therapy ( $P < 0.001$  and  $P = 0.024$ ), respectively. The accuracy of hs-CRP for identifying patients with active disease during prospective follow-up was good (area under the curve [AUC]: 0.82, cutoff: 10.7 mg/L). AUC was better in patients with an elevated hs-CRP at diagnosis (AUC: 0.92, cutoff: 10.3 mg/L). In Kaplan–Meier and Cox-regression analyses, hs-CRP was an independent predictor of 3- ( $P = 0.007$ ) or 12-month ( $P = 0.001$ ) clinical relapses for patients in remission who had elevated hs-CRP at diagnosis. In addition, perianal involvement ( $P = 0.01$ ) was associated with the 12-month relapse frequency.

**Conclusions:** Our data suggest that hs-CRP positivity at diagnosis is associated with disease location and behavior, and in patients who are hs-CRP positive at diagnosis, is an accurate marker of disease activity and a predictor of short- and medium-term clinical flare-ups during follow-up.

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**Key Words:** CD, hs-CRP, clinical activity, flare-up, risk, prediction

Inflammatory bowel disease (IBD) follows a variable disease course; both Crohn's disease (CD) and ulcerative colitis (UC) are characterized by periods of remission and relapse.<sup>1</sup> However, disease flare-ups occur in a random way and the disease course is often unpredictable. Based on their half-life and changes over time, serum laboratory and fecal markers, alone or in combination, may be used

for the prediction of disease activity, as well as short- or medium-term relapses.

C-reactive protein (CRP) is a traditional, nonspecific marker of inflammation, and is one of the most important acute phase proteins in humans.<sup>2</sup> Under normal circumstances, CRP is produced by hepatocytes in low quantities. During inflammation, under the influence of interleukin (IL)-6, tumor necrosis factor (TNF)- $\alpha$ , and IL-1 $\beta$ , hepatocytes rapidly increase production of CRP.<sup>3</sup> It has a short half-life (19 hours) compared to other acute phase proteins.<sup>2</sup> The mechanisms leading to CRP elevation in CD are not fully understood. It has been proposed that in CD patients the accumulation of mesenteric fat—a major site of IL-6 and TNF- $\alpha$  synthesis—may contribute to CRP production. Another potential explanation is that, during disease flare-up, even with the lack of overt infection, significant bacterial migration occurs due to the transmural inflammation of the gut wall. Bacteremia is one of the strongest stimulators of CRP production.<sup>4</sup>

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CD is associated with a strong CRP response in both adult and pediatric populations. CRP is widely used in CD, since it shows acceptable correlation (coefficient: 0.32–0.36) with disease activity, as defined by clinical indices.<sup>5–8</sup> and also with endoscopic or histological activity.<sup>9</sup> Medical therapy does not seem to have a direct effect on hepatic CRP production; rather, changes in CRP levels during treatment appear to be a result of the effect of therapy on the underlying disease.

However, although CRP levels in patients with IBD have been measured for many years, several unanswered questions exist regarding the value of this protein as a clinical marker. Some years ago the GETAID group<sup>10</sup> proposed a simple biological score for predicting short-term relapse (6 weeks) in CD patients. Multivariate analysis selected two predictive markers for relapse: CRP >20 mg/L and erythrocyte sedimentation rate (ESR) >15 mm/h. The score's sensitivity and specificity were 89% and 43%, respectively, with a negative predictive value of 97%, suggesting that normal CRP and ESR rule out a relapse in the next 6 weeks with high certainty. Only one relatively small study investigated the role of high-sensitivity (hs)-CRP in IBD.<sup>7</sup> Ninety CD, 70 UC patients, and 160 controls were included. The correlation coefficient between hs-CRP and the disease activity score was weak but similar in both: UC (0.26) and CD (0.36).

In a recent population-based study by the IBSEN group, CRP at diagnosis or during follow-up was predictive for long-term outcome, as measured by the need for colectomy or resective surgery. A significant association between CRP levels at diagnosis and risk of surgery was also found in patients with CD with terminal ileitis, where the risk increased when CRP levels were above 53 mg/L in this subgroup (odds ratio [OR]: 6.0, 95% confidence interval [CI]: 1.1–31.9).<sup>11</sup> In contrast, no association was found between disease phenotype and CRP levels at diagnosis. In addition, 25% of CD patients did not have elevated CRP levels at diagnosis as defined by the authors (CRP >10 mg/L). In earlier studies, approximately one-third of the patients with clinically active disease had normal CRP levels, while another one-third of the patients in clinical remission had elevated CRP (>5 mg/L).<sup>12</sup> However, a wide range of CRP values was observed and no clear cutoff values exist (8 mg/L, Sonic), 10 mg/L (Precise II, Charm), or 20 mg/L.<sup>13–15</sup>

The aims of this study were: first, to define the best cutoff value for distinguishing between active and inactive disease; second, to investigate whether hs-CRP is useful for the identification of patients with active disease and patients at risk for clinical relapse during follow-up; and third, whether prediction of disease phenotype is possible, using a classification based on the hs-CRP value at diagnosis, in a Caucasian referral CD cohort.

**TABLE 1. Clinical Characteristics of Patients with Crohn's Disease**

|                                  | CD (n = 260)          |
|----------------------------------|-----------------------|
| Male/female                      | 120/140               |
| Age at presentation (years)      | 26.9 ± 11.6           |
| Duration (years)                 | 7.0 ± 6.1             |
| Familial IBD <sup>a</sup>        | 21 (8.5%)             |
| Location (n) L1 (ileal)          | 37                    |
| L2 (colonic)                     | 60                    |
| L3 (ileocolonic)                 | 157                   |
| L4 (only upper GI)               | 6                     |
| Behavior (n) B1 (inflammatory)   | 143                   |
| at diagnosis B2 (stricturing)    | 53                    |
| B3 (penetrating)                 | 64                    |
| Perianal disease <sup>a</sup>    | 93 (35%)              |
| Frequent relapse <sup>a</sup>    | 83 (32.8%)            |
| Arthritis <sup>a</sup>           | 109 (42.1%)           |
| Liver <sup>a</sup>               | 7 (2.7%)              |
| Ocular <sup>a</sup>              | 29 (11.2%)            |
| Cutaneous <sup>a</sup>           | 21 (8.1%)             |
| Steroid use <sup>a</sup>         | 208 (80.6%)           |
| Azathioprine use <sup>a</sup>    | 192 (73.6%)           |
| Anti-TNF use <sup>a</sup>        | 75 (28.8%)            |
| Surgery/reoperation <sup>a</sup> | 88 (34.1%)/26 (29.5%) |
| Smoking habits (n) no            | 153                   |
| stopped before the diagnosis     | 5                     |
| yes                              | 86                    |

<sup>a</sup>n (%).

## PATIENTS AND METHODS

### Patients

In all, 260 well-characterized, unrelated, consecutive CD patients (male/female: 120/140, age at presentation: 26.9 ± 11.6 years, disease duration: 7.0 ± 6.1 years) with a complete clinical follow-up were included from two IBD referral centers (Budapest and Debrecen). The clinical characteristics are presented in Table 1.

### Methods

The diagnosis was based on the Lennard-Jones criteria.<sup>16</sup> The disease phenotype (age at onset, duration, location, and behavior) was determined according to the Montreal Classification.<sup>17</sup> Disease activity at the time of the diagnosis and during follow-up visits was calculated according to the Harvey-Bradshaw Index (HBI) index.<sup>18</sup> All patients had active disease at the time of the diagnosis. For the purposes of this study we followed the ECCO (European Crohn's and Colitis Organisation) guidelines<sup>19</sup> and defined HBI ≤4 as a state of remission, 5–7 as mild, and ≥8 as moderate to severe disease. The mean disease duration was 7 years. Detailed clinical

phenotypes were determined by thorough review of patients' medical records, which had been collected in a uniform format. Medical records that documented the presence of extraintestinal manifestations (EIM) (for example, arthritis: peripheral and axial; ocular manifestations: conjunctivitis, uveitis, iridocyclitis; skin lesions: erythema nodosum, pyoderma gangrenosum; and hepatic manifestations: primary sclerosing cholangitis [PSC]), frequency of flare-ups (frequent flare-up: >1 clinical relapse/year),<sup>20</sup> medication use (e.g., steroid and/or immunosuppressive use at any time), need for surgery (resections), the presence of familial IBD, smoking habits, and perianal involvement were retrospectively analyzed for the period prior to the prospective follow-up.

Clinical data and blood samples were prospectively captured between January 1, 2008 and June 1, 2010 either during regular or extraordinary follow-up visits. Of note, due to Hungarian health authority regulations, a follow-up visit is obligatory for IBD patients at a specialized gastroenterology center every 6 months (the actual interval varies between 3–6 months). Otherwise, the conditions of the health insurance policy change and they forfeit their ongoing subsidized therapy. Consequently, the relationship between IBD patients and specialists is a close one. The follow-up period lasted 12 months in those patients who showed no relapse, or until the date of flare-up in patients with clinical relapse (HBI score >4,  $\Delta$ HBI score  $\geq 3$ , and change in medical therapy).<sup>18,21</sup> All relapses were of sufficient severity to warrant a change in treatment.

Hs-CRP levels were determined in all cases using the Integra 700 autoanalyzer system (Roche, Basel, Switzerland). The lower assay sensitivity limit was 0.1 mg/L and the reference range, according to the manufacturer, is 0–5 mg/L. The study was approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics.

The central coordination of sample and database management was completed at the 1st Department of Medicine, Semmelweis University (by L.S.K., P.L.L.).

## Statistical Methods

Variables were tested for normality using Shapiro Wilk's W test. *T*-test with separate variance estimates, analysis of variance (ANOVA) with post-hoc Scheffe test,  $\chi^2$ -test, and  $\chi^2$ -test with Yates correction and logistic regression analysis were used to evaluate differences within subgroups of CD patients. To test the accuracy and to identify cutoff values for hs-CRP to identify patients with active disease or clinical relapses, receiver operating characteristics (ROC) curves were generated by plotting sensitivity versus 1-specificity. Cutoff values were determined using individual data collected prospectively during the first visit after January 1, 2008. The same cutoff value was used to cluster patients into hs-CRP-positive and -negative subgroups based on the patients' hs-CRP value at diagnosis. Sensitivities, specificities, positive predictive values (PPV), and negative predictive values (NPV) were calculated to determine the predictive power of hs-CRP in distinguishing between active and inactive disease. Kaplan–

Meier survival curves were plotted for analysis of short- (3-month) and medium-term (12-month) clinical relapse, with LogRank and Breslow tests, to determine the predictive power of hs-CRP in identifying clinical relapse during the prospective follow-up period for patients who either were in clinical remission for at least 4 weeks at the first follow-up visit after January 1, 2008, or in whom a clinical remission was achieved for at least 4 weeks after January 1, 2008 and had at least 12 months follow-up by June 1, 2010 ( $n = 222$ ). Chronically active patients were excluded from this analysis. Additionally, forward stepwise Cox-regression analysis was used to assess the association between categorical clinical variables and probability of clinical relapse. Variables with  $P < 0.2$  were included in the multivariate testing.  $P < 0.05$  was considered significant. For the statistical analysis, SPSS v. 15.0 (Chicago, IL) was used.

## RESULTS

### Hs-CRP Positivity at Diagnosis and Accuracy of Hs-CRP for Identifying Active Disease During Prospective Follow-up

In all, 32.3% of the CD patients had normal hs-CRP at diagnosis. At the start of the prospective follow-up period (January 1, 2008), the accuracy of hs-CRP to identify patients with active disease was overall good (area under the curve [AUC]: 0.82, 95%; CI: 0.77–0.87; Fig. 1). The accuracy was better in patients who had elevated hs-CRP at diagnosis (AUC: 0.92, 95%; CI: 0.89–0.97) compared to patients with a normal value at diagnosis (AUC: 0.61, 95%; CI: 0.49–0.73; Fig. 1). The most accurate cutoff value for identifying active disease was 10.7 mg/L in the entire cohort and 10.3 mg/L in the subgroup of patients with elevated hs-CRP at diagnosis. For internal validation the ROC analysis was recalculated using the CRP and disease activity data obtained 6 months after the start of the follow-up period (AUC<sub>overall</sub>: 0.79, cutoff: 9.7 mg/L, AUC<sub>CRP positive at diagnosis</sub>: 0.87, cutoff: 10.1 mg/L). Therefore, sensitivity analysis was performed using the cutoff value of 10 mg/L (Table 2). If the cutoff identified by the ROC analysis (3.5 mg/L) was used for the CRP-negative subgroup, the accuracy of the marker to identify active disease later during the prospective follow-up period was better (sensitivity: 55%, specificity: 69%, PPV: 65%, NPV: 61%).

The association between the median hs-CRP and disease activity groups was also tested. The median hs-CRP was 2.87 mg/L (IQR: 1.17–7.15 mg/L) in patients in remission, while in patients with active disease it was 22.0 mg/L (IQR: 8.05–56.0 mg/L;  $P < 0.001$ ). The median hs-CRP gradually increased according to disease severity (mild: 20.7 mg/L, IQR: 11.5–41 mg/L, moderate-to-severe: 25.1 mg/L; IQR: 6.3–67.8 mg/L;  $P < 0.001$  between mild, moderate-to-severe vs. remission) in the entire cohort. The

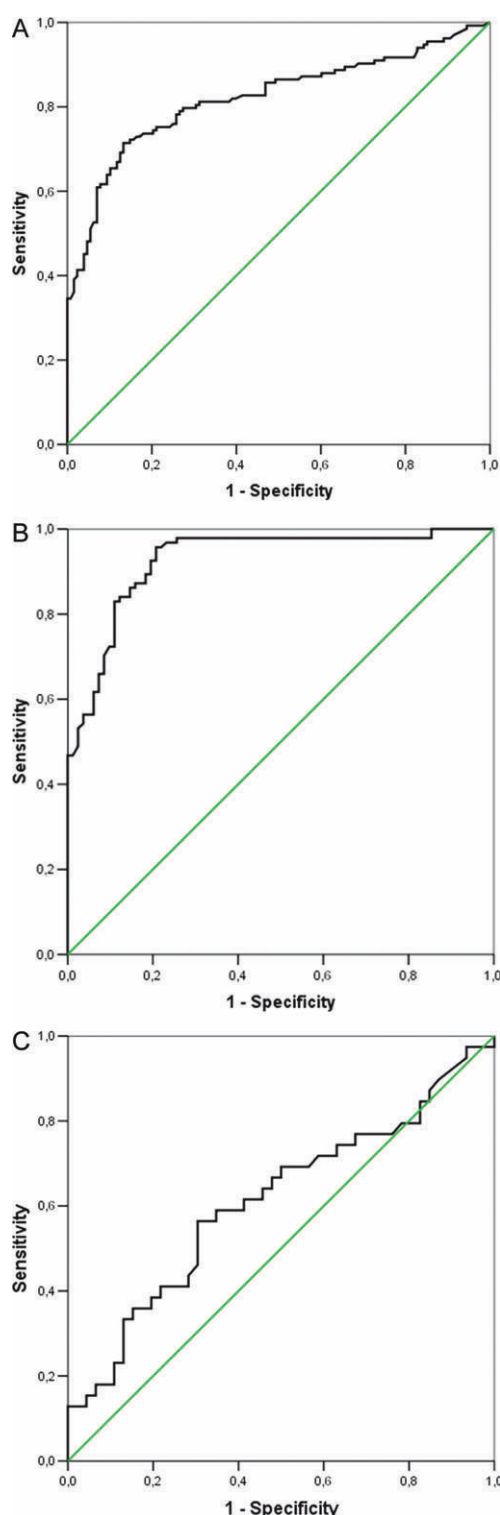


FIGURE 1. Predictive power of hs-CRP for identifying active disease in patients with Crohn's disease; all patients (A), patients with elevated hs-CRP at diagnosis (B), and patient without an elevated hs-CRP at diagnosis (C). (A) AUC = 0.82, cutoff: 10.7 mg/L. (B) AUC: 0.92, cutoff: 10.3 mg/L. (C) AUC: 0.61, cutoff: 3.5 mg/L. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

difference in patients with elevated hs-CRP at time of diagnosis was significant among all disease severity states (remission: 4.76 mg/L, IQR: 1.5–9.4 mg/L; mild: 27.7 mg/L, IQR: 16.5–51.3 mg/L; moderate-to-severe: 41.6 mg/L, IQR: 23.9–88 mg/L;  $P < 0.001$  between mild, moderate-to-severe vs. remission;  $P = 0.034$  between mild vs. moderate-to-severe, by ANOVA and post-hoc Scheffe). In contrast, a significant difference was not observed in patients with normal hs-CRP at the time of diagnosis (data not shown).

### Association of Hs-CRP and Clinical Relapse During Prospective Follow-up

Actual hs-CRP values in patients in clinical remission predicted relapse with reasonable accuracy, both at 3 months (AUC: 0.67, 95% CI: 0.54–0.80; sensitivity: 65%; specificity: 71%; PPV: 70%; NPV: 67%; cutoff: 10.1 mg/L) and 12 months (AUC: 0.63, 95% CI: 0.54–0.73; sensitivity: 52%; specificity: 75%; PPV: 68%; NPV: 62%; cutoff: 8.8 mg/L) in the subgroup of patients with an elevated hs-CRP at time of diagnosis. In contrast, the predictive power of hs-CRP to predict clinical relapse during the follow-up period in patients in whom the hs-CRP was not elevated at diagnosis was limited (at 3 months: AUC: 0.53; sensitivity: 0%; specificity: 89%; and at 12 months: AUC: 0.53; sensitivity: 5%; specificity: 87%; PPV: 26%).

In a Kaplan–Meier analysis, hs-CRP was shown to be associated with the probability of 3- and 12-month clinical relapse (at 3 months:  $P_{\text{LogRank}} = 0.001$ ,  $P_{\text{Breslow}} = 0.001$ ; and at 12 months:  $P_{\text{LogRank}} = 0.002$ ,  $P_{\text{Breslow}} = 0.001$ ; cutoff: 10 mg/L; Fig. 2) for patients in clinical remission. The probability of a relapse at 3 and 12 months in the entire cohort was 6.7% and 22% for CRP-negative and 28.5% and 48.4% for CRP-positive patients (absolute  $\Delta = 15.3\%$  and 19.5%). Accuracy of hs-CRP was better for patients with elevated hs-CRP at diagnosis. The probability of a

TABLE 2. Predictive Power of Hs-CRP\* to Distinguish Between Active (HBI > 4) and Inactive Crohn's Disease (CD)

|                                              | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|----------------------------------------------|-----------------|-----------------|---------|---------|
| All CD patients                              | 71              | 85              | 83      | 75      |
| CD patients with a positive CRP at diagnosis | 95              | 79              | 83      | 95      |
| CD patients with a negative CRP at diagnosis | 13              | 96              | 74      | 52      |

PPV, positive predictive value; NPV, negative predictive value.

\*Cutoff for hs-CRP 10mg/L.

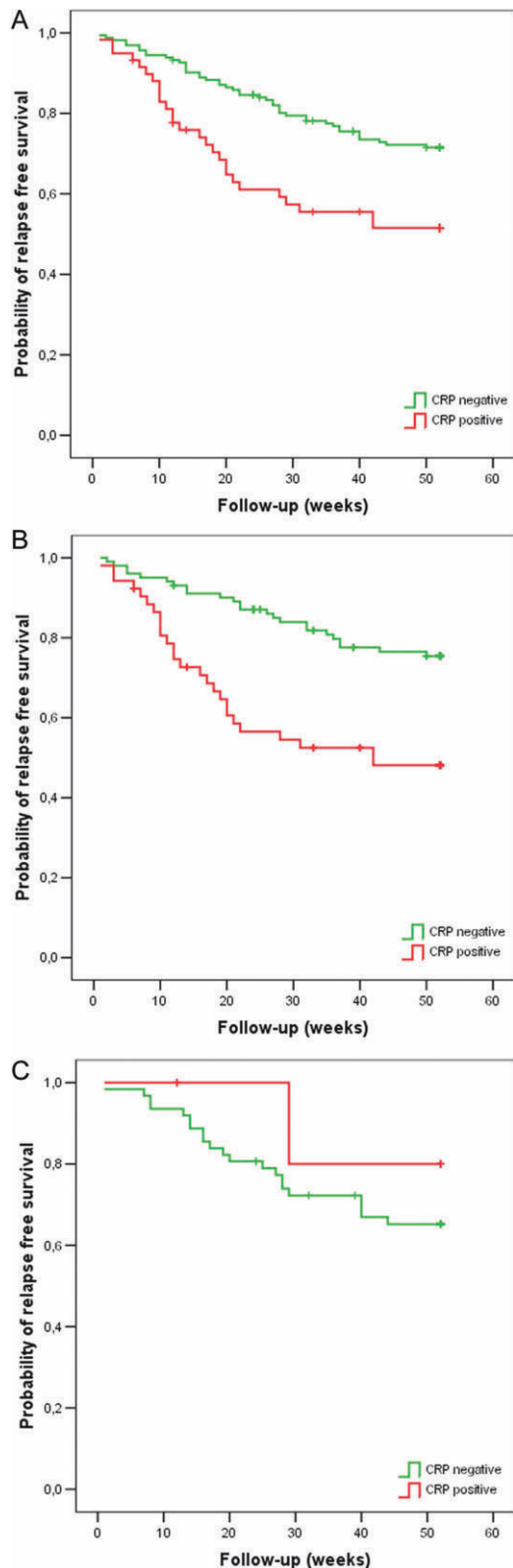


TABLE 3. Summary of Cox Model: Factors Affecting Time to 3-month Clinical Relapse

|                                  | <i>P</i> | Hazard Ratio | 95% CI    |
|----------------------------------|----------|--------------|-----------|
| hs-CRP (>10mg/L)                 | 0.007    | 3.11         | 1.37-7.08 |
| Location                         | 0.33     | 1.39         | 0.71-2.70 |
| Noninflammatory disease behavior | 0.49     | 0.74         | 0.31-1.75 |
| Perianal involvement             | 0.41     | 1.44         | 0.61-3.39 |
| Frequency of relapses (>1/year)  | 0.51     | 1.34         | 0.57-3.15 |

relapse at 3 and 12 months in this subgroup was 6.9% and 25% for CRP-negative and 24.6 and 51.9% for CRP-positive patients (absolute  $\Delta = 18.1\%$  and  $27.3\%$ ). In addition, frequency of relapses ( $P_{\text{LogRank}} = 0.049$  and  $P_{\text{Breslow}} = 0.038$ ) and perianal involvement ( $P_{\text{LogRank}} = 0.001$  and  $P_{\text{Breslow}} = 0.001$ ) was associated with the probability of a clinical relapse within 12 months.

In a proportional Cox-regression analysis, only hs-CRP ( $P = 0.007$ ) was independently associated with the probability of clinical relapse at 3 months (Table 3). In contrast, hs-CRP ( $P = 0.001$ ) and perianal involvement ( $P = 0.01$ ) were identified as independent predictors for clinical relapse at 12 months (Table 4). Similar to the results stated above, hs-CRP was not predictive of clinical relapse at 3 and 12 months in patients without an elevated hs-CRP at time of diagnosis.

Association Between Hs-CRP at Diagnosis and Disease Behavior at Diagnosis and Medical Therapy During Follow-up

Elevated hs-CRP at time of diagnosis was associated with disease location (ileal disease: 43.2%, colonic disease 70%, ileocolonic disease: 72.6%,  $P = 0.002$ ), noninflammatory disease behavior (inflammatory: 36.5%, stenosing/penetrating: 48.9%,  $P = 0.058$ ), and need for azathioprine (AZA) / biological therapy during the later course of the disease but not with the need for surgery/reoperation. AZA use in patients with elevated hs-CRP at diagnosis was 81.1% (vs. without 56.5%,  $P < 0.001$ ). Biological therapy in patients with elevated hs-CRP at diagnosis was 33.9% (vs. without 20.0%,  $P = 0.024$ ). The mean hs-CRP was also significantly higher in patients with colonic/ileocolonic

FIGURE 2. Association between hs-CRP and clinical relapse in CD during follow-up; all patients (A), patients with elevated hs-CRP at diagnosis (B), and patient without an elevated hs-CRP at diagnosis (C). (A)  $P_{\text{LogRank}} = 0.002$  and  $P_{\text{Breslow}} = 0.001$ . Patients at risk. CRP-positive ( $n = 163$ ). CRP-negative ( $n = 59$ ). (B)  $P_{\text{LogRank}} < 0.001$  and  $P_{\text{Breslow}} < 0.001$ . Patients at risk. CRP-positive ( $n = 101$ ). CRP-negative ( $n = 52$ ). (C)  $P_{\text{LogRank}} = \text{NS}$  and  $P_{\text{Breslow}} = \text{NS}$ . Patients at risk. CRP-positive ( $n = 62$ ). CRP-negative ( $n = 7$ ). [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

**TABLE 4.** Summary of Cox Model: Factors Affecting Time to 12-month Clinical Relapse; All Patients (A), Patients with Elevated Hs-CRP at Diagnosis (B), and Patients Without an Elevated Hs-CRP at Diagnosis (C)

|                                  | <i>P</i> | Hazard Ratio | 95% CI    |
|----------------------------------|----------|--------------|-----------|
| <b>A</b>                         |          |              |           |
| hs-CRP (>10mg/L)                 | <0.001   | 2.26         | 1.38-3.72 |
| Location                         | 0.45     | 1.15         | 0.80-1.67 |
| Noninflammatory disease behavior | 0.77     | 0.93         | 0.56-1.54 |
| Perianal involvement             | 0.01     | 1.90         | 1.14-3.17 |
| Frequency of relapses (>1/year)  | 0.27     | 1.33         | 0.80-2.21 |
| <b>B</b>                         |          |              |           |
| hs-CRP (>10mg/L)                 | <0.001   | 2.88         | 1.64-5.08 |
| Location                         | 0.42     | 1.20         | 0.76-1.90 |
| Noninflammatory disease behavior | 0.30     | 0.73         | 0.40-1.32 |
| Perianal involvement             | 0.06     | 1.77         | 0.97-3.21 |
| Frequency of relapses (>1/year)  | 0.40     | 1.30         | 0.71-2.36 |
| <b>C</b>                         |          |              |           |
| hs-CRP (>10mg/L)                 | 0.49     | 0.49         | 0.06-3.92 |
| Location                         | 0.94     | 0.98         | 0.52-1.83 |
| Noninflammatory disease behavior | 0.11     | 2.30         | 0.81-6.52 |
| Perianal involvement             | 0.21     | 1.85         | 0.71-4.80 |
| Frequency of relapses (>1/year)  | 0.37     | 1.55         | 0.59-4.09 |

disease (L2: 35.5, 95% CI: 23.1–48.1 mg/L and L3: 35.8, 95% CI: 29.2–42.3 mg/L) compared to patients with ileal disease (L1: 17.8, 95% CI: 6.9–28.6 mg/L,  $P = 0.05$  by ANOVA and  $P = 0.06$  between L1 and L3 with post-hoc Scheffe test) as well as in patients with complicated disease phenotype (B2: 39.3, 95% CI: 23.9–54.6 mg/L and B3: 42.7, 95% CI: 29.6–55.8 mg/L) compared to patients with inflammatory disease (B1: 27.1, 95% CI: 21.9–32.1 mg/L, ANOVA  $P = 0.026$  and  $P = 0.05$  between B1 and B3 with post-hoc Scheffe test). Of note, overall follow-up was shorter in the subgroup of patients who were hs-CRP-positive at diagnosis ( $6.3 \pm 5.0$  years vs. hs-CRP-negative:  $8.6 \pm 7.7$  years,  $P = 0.014$ ).

The association between hs-CRP positivity ( $P = 0.01$ , OR: 1.1, 95% CI: 1.02–1.23) at diagnosis and subsequent need for AZA therapy remained significant in a logistic regression model adjusted for disease duration ( $P_{\text{per year}} = 0.18$ ), location ( $P = 0.247$ ), behavior ( $P = 0.041$ , B1: reference; B2:  $P = 0.73$ ; B3:  $P = 0.01$ ; OR: 3.17, 95% CI: 1.29–7.79).

## DISCUSSION

In the present study we have shown during prospective follow-up that hs-CRP measurement alone proved to

be a highly accurate marker of disease activity, with an ideal cutoff value of 10 mg/L, in patients with elevated hs-CRP at diagnosis. In addition, hs-CRP was an independent predictor for short- and medium-term clinical flare-ups during prospective follow-up. Classification based on hs-CRP value at diagnosis was useful for the identification of a subgroup of patients with a more complicated disease phenotype and need for earlier aggressive therapy (immunosuppressant/biological) during follow-up.

In a recent population-based, prospective study by the IBSEN group,  $\approx 25\%$  of CD patients did not have elevated CRP at diagnosis as defined by the authors (CRP >10 mg/L).<sup>11</sup> In the current study, performed at a referral CD population, the percentage of patients with negative hs-CRP at diagnosis was similar, 32.3%. In earlier studies, approximately one-third of patients with clinically active disease had normal CRP levels, while another third of patients in clinical remission had elevated CRP (>5 mg/L).<sup>12</sup> Of note, in recent double-blind clinical trials with biologicals the reported CRP-negativity rate at inclusion may be even higher, ranging from 52.3%–68% despite clinically active disease<sup>13–15</sup>; however, the best cutoff value still has not been defined.

In this study an ROC analysis was performed to determine the cutoff value of hs-CRP to identify active disease. The accuracy of hs-CRP to identify patients with active disease during the course of the disease was overall good (AUC: 0.82) in the entire cohort with a cutoff value of 10.7 mg/L. Therefore, a cutoff value of 10 mg/L was chosen for this study to classify patients as CRP-negative or -positive at time of diagnosis. The ROC analysis was performed separately for the two latter groups, and the accuracy of detecting active disease was much better in patients with elevated hs-CRP at diagnosis (AUC: 0.92; sensitivity: 95%; specificity: 79%; PPV: 83%), with a similar cutoff value for this subgroup (10.3 mg/L). In contrast, for patients with normal hs-CRP value at diagnosis accuracy was poor (AUC: 0.61; sensitivity: 13%; specificity: 96%; PPV: 74%) at the 10 mg/L cutoff, and a clinically useful lower cutoff was not available for this subgroup. Therefore, if confirmed, the use of hs-CRP is probably unnecessary in CD.

The association between the magnitude of CRP response and disease severity was also studied. In a French study,<sup>22</sup> the authors assessed the value of CRP in identifying moderate to severely active CD patients by using the van Hees index. Good accuracy (AUC: 0.85) was reported between the van Hees index  $\geq 150$  and CRP values (cutoff: 21.6 mg/L). Similarly, in a relatively small Tunisian study,<sup>23</sup> the authors reported a cutoff of 19 mg/L for the identification of patients with a Crohn's Disease Activity Index (CDAI) >220. The sensitivity was 76.4%, while the specificity was only 56.2%. Accordingly, in the present study, the

median hs-CRP was gradually elevated according to the disease severity. This difference was more striking in the subgroup of patients with elevated hs-CRP at diagnosis.

In addition, elevated hs-CRP at diagnosis was associated with colonic or ileocolonic disease location ( $P = 0.002$ ), noninflammatory disease behavior ( $P = 0.058$ ), and the need for AZA or biological therapy during the course of the disease in patients included in the study. Similarly, in a recent study from Brisbane the proportion of L1 location was higher in the subgroup of patients with a low CRP ( $<10$  mg/L) during the course of the disease.<sup>24</sup> Somewhat dissimilarly, no significant association between CRP and clinical phenotype at diagnosis was reported in a population-based study by the IBSen group. Nevertheless, a CRP value above 10 mg/L was numerically less prevalent in patients with L1 (70%) disease compared to L2 (74%) or L3 (80%). The median and mean CRP values followed the same tendency. In addition, a significant positive association between CRP levels at diagnosis and risk of surgery was reported in patients with L1 disease when the CRP levels were above 53 mg/L in this subgroup (OR 6.0, 95% CI: 1.1–31.9).

The present work has demonstrated that serum hs-CRP in patients with elevated hs-CRP at diagnosis was useful in predicting clinically relevant relapse during prospective follow-up in patients in clinical remission. In addition, hs-CRP ( $P = 0.007$ ) was identified as the only independent predictor of clinical relapse at 3 months. Not surprisingly, the role of clinical factors was more important in predicting a medium-term relapse (12-month) rate. Besides hs-CRP ( $P = 0.001$ ), perianal involvement ( $P = 0.01$ ) was identified as independent predictor for 12-month clinical relapse in a Cox-regression analysis. In a recent study, the GETAID group prospectively followed 71 CD patients in medically induced remission. Biological markers were measured every 6 weeks. Two biological markers were predictive for relapse: CRP ( $>20$  mg/L) and ESR ( $>15$  mm/h). Based on these findings, a binary biologic predictive score (BPS) was derived. A positive score (at least one of the two markers positive) was associated with an 8-fold increased risk for relapse compared to patients negative for both markers. The sensitivity and specificity of a positive score were 89% and 43%, respectively.<sup>10</sup> In addition, very recently the Leuven group has reported an association between baseline CRP level, changes in serum CRP (cutoff: 3 mg/L), and response to infliximab therapy, sustained response, and mucosal healing.<sup>25</sup> Similarly, our group has recently reported that besides early clinical response, CRP normalization (cutoff: 10 mg/L) was the best predictor for medium-term clinical efficacy and mucosal healing during adalimumab therapy.<sup>26</sup>

Nevertheless, in the present study we show that hs-CRP remains a valid marker of disease activity and relapse during the course of the disease, years after diagnosis, in patients originally hs-CRP-positive. In contrast, in patients originally

hs-CRP-negative the use of CRP as a marker of disease activity during follow-up is limited, confirming that hs-CRP positivity may be an indicator of the underlying inflammatory process, which seems to be stable in each patient. The findings of our study confirm that hs-CRP positivity may be an important marker of classification in CD patients.

The authors are aware of the possible limitations of this study. First, endoscopic/histological activity was not assessed in the present study. Instead, a more vigorous, composite endpoint was utilized, as suggested by the recent position paper by the International Organization for Inflammatory Bowel Diseases<sup>27</sup> to identify clinically relevant relapses (HBI  $>4$ ,  $\Delta$ HBI  $>3$  and change in medical therapy) beyond the use of HBI or CDAI only, which is known to correlate poorly with the result of endoscopic or histological activity.<sup>28</sup> By doing so, the everyday clinical decision-making algorithm, where clinicians have to rely, in most cases, on clinical symptoms and laboratory tests, was approximated. Of note, CRP shows acceptable correlation with endoscopic or histological activity (Jones et al, Schoepfer et al<sup>28,29</sup>) following calprotectin, CRP, or hs-CRP ( $>5$  mg/L) was the second best marker for the detection of endoscopically active disease. Second, a number of studies have investigated the accuracy of additional serum and fecal markers or a combination of markers, including procalcitonin, lipopolysaccharide-binding protein (LBP), soluble (s)CD14<sup>8</sup>, and fecal calprotectin.<sup>30</sup> One could argue that the combination of these markers could be more accurate; however, the accuracy of using these markers in combination was not studied.<sup>28</sup> In addition, fecal calprotectin was shown to predict relapse in CD.<sup>30,31</sup> Finally, these authors<sup>8</sup> recently reported that serum LBP and sCD14 are markers of disease activity in CD with a similar accuracy as hs-CRP. Accuracy was increased for the combination of these markers. In addition, LBP, sCD14, and a high frequency of previous relapses were independent predictors for clinical flare-up within 1 year. Still, it is difficult to define the best cutoff values for the above markers based on these studies; for CRP or hs-CRP a wide range of values was used and no clear cutoff exists (5, 8, 10, or 20 mg/L).<sup>10–15</sup>

However, one of the strengths of the present study is that it included a cohort of well-characterized CD patients with complete clinical follow-up. Additionally, clinical data as well as the predictive power of hs-CRP values were analyzed during a prospective follow-up period. Furthermore, we did not accept using the “normal” hs-CRP value provided by the manufacturer to detect clinically meaningful elevated hs-CRP but we vigorously calculated cutoff values, to maximize accuracy in identifying active disease and predicting short-term relapses during the prospective follow-up period. In addition, this study demonstrated that classification based on the hs-CRP value at diagnosis highly affects the predictive potential of the hs-CRP during the follow-up; therefore, the marker should only be used in

patients hs-CRP-positive at diagnosis. If confirmed, hs-CRP alone may be sufficient in this patient subgroup to aid clinicians in more objectively identifying active disease and predict clinically relevant relapses during follow-up.

In conclusion, hs-CRP, in patients who were hs-CRP-positive at diagnosis, is an accurate marker of disease activity and predictor of short- and medium-term clinical flare-ups during follow-up. In these patients, routine measurement of hs-CRP alone may be sufficient to aid clinicians to more objectively measure disease activity and predict clinically relevant short-term relapse. In addition, hs-CRP level at time of diagnosis was associated with complicated disease phenotype and the need for a more aggressive medical therapy during follow-up.

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