Levels of C-reactive Protein Are Associated With Response to Infliximab Therapy in Patients With Crohn’s Disease

MATHIAS JÜRGENS,* JESTINAH M. MAHACHE JOHN,‡§ ISABELLE CLEYNEN,* FABIAN SCHNITZLER,* HERMA FIDDER,* WOUTER VAN MOERKERKE,* VERA BALLET,* MAJA NOMAN,* ILSE HOFFMAN,† GERT VAN ASSCHE,* PAUL J. RUTGEEHT,* KRISTEL VAN STEEN,‡§ and SEVERINE VERMEIRE*†

*Department of Gastroenterology, UZ Gasthuisberg, Leuven; ‡Systems and Modeling Unit, Montefiore Institute, University of Liege, Liège; §Bioinformatics and Modeling, GIGA-R, University of Liege, Liège; †Department of Pediatric Gastroenterology, UZ Gasthuisberg, Leuven, Belgium

BACKGROUND & AIMS: Infliximab is an antibody against tumor necrosis factor-alpha that is used to treat patients with moderate to severe Crohn’s disease (CD). C-reactive protein (CRP) is a marker used to identify and follow individuals with CD. We analyzed changes in levels of CRP in a large cohort of patients with CD undergoing treatment with infliximab.

METHODS: Serial levels of CRP were analyzed in 718 CD patients. Blood was collected before each infusion; a total of 8845 CRP levels were available for analysis. The correlations between CRP levels and need for dose adjustment, outcomes, and mucosal healing (based on endoscopic analysis of 253 patients) were evaluated. Therapy adjustment was considered successful if therapy continued without need for change. Subgroup analysis was performed by using data from 268 patients who received 8 weeks of maintenance therapy. RESULTS: More patients with high baseline levels of CRP responded to infliximab than patients with normal levels (90.8% vs 82.6%; P = .014). Early normalization of CRP levels correlated with sustained long-term response (P < .001). CRP levels remained significantly higher among patients who lost their response to infliximab, compared with those with a sustained response (P = .001). At time of loss of response, CRP levels were significantly increased (median, 11.2 mg/L) and did not return to baseline levels (median, 18.2 mg/L; P = .039). CRP correlated with mucosal healing (P = .033). CONCLUSIONS: CRP is a good marker of disease activity in patients treated with infliximab. Increased levels of CRP indicate mucosal inflammation and a likelihood of clinical relapse.

Keywords: Inflammatory Bowel Disease; Anti-TNF Therapy; Biomarkers; Diagnostics.

Crohn’s disease (CD) is an inflammatory bowel disease (IBD) “characterized by subacute or chronic necrotizing and cicatrizing inflammation,” as described by Burrill Crohn in 1932.1 Disabling symptoms of bloody diarrhea and abdominal cramps and relapsing flares of disease activity are marking features of this chronic disease, which might affect the entire gastrointestinal tract but also extraintestinal organs.2 The multifactorial pathogenesis of CD is composed of a genetic susceptibility, environmental factors, and an inappropriate immune response to the gut microbiota.3,4 Tumor necrosis factor alpha (TNF-α) has been shown to play a pivotal role in the onset and perpetuation of the inflammation.5,6

Infliximab (IFX; Merck Sharp & Dohme Corp, Whitehouse Station, NJ), a chimeric monoclonal antibody that binds with high specificity and affinity to TNF-α, was the first biological therapy that was approved for treatment of CD and ulcerative colitis (UC). Overall, 60%–70% of the patients report clinical benefit from IFX in short and long term.7–11 A detailed meta-analysis on efficacy and safety of TNF antagonists was presented by Peyrin-Biroulet et al.12 Supplementary to the clinical assessment, inflammatory proteins are used to evaluate severity and activity of inflammation and to follow up the response to therapy. The most commonly used acute phase protein is C-reactive protein (CRP). CRP is a pentameric molecule produced and released by hepatocytes on triggering by the cytokines interleukin (IL)-6, IL-1, and TNF-α. CRP plays an important role as noninvasive inflammatory marker in patients with IBD and especially in patients with CD. However, its up-regulation is heterogeneous; where a strong CRP response has been observed in CD, this response is only modest in UC.13–16 However, 20%–25% of CD patients will not mount a CRP response in the case of active inflammation, and the reasons for this are unknown.

The general aim of this study was to investigate whether CRP is helpful in optimizing therapy with IFX in the individual patient with moderate to severe CD. We particularly investigated patients on IFX maintenance therapy whether CRP is an accurate marker to predict reactivation of disease activity, which could allow timely therapy adjustment. Therefore, we analyzed CRP changes over time with respect to long-term outcome. We studied whether clinical relapses are preceded by CRP increases, whether success of treatment adjustment can be reliably assessed by using CRP, and whether CRP could predict success of therapy adjustments. The relation of endoscopic mucosal improvement under IFX therapy and CRP was further assessed.

Methods

Patients and Treatment

Between November 1994 and July 2009, 718 patients with moderate to severe CD receiving therapy with IFX and having given consent to the VLECC registry (Biobank contain-
ing serum, DNA, and clinical characteristics of IBD patients followed at the University Hospital Gasthuisberg, Leuven, Belgium) were eligible for study (Table 1). Within this registry, serial serum samples were obtained and stored at –20°C. Diagnosis of CD was confirmed by endoscopic, radiologic, and histologic criteria, and indication for IFX treatment was assessed by experienced clinicians on the basis of a synopsis of clinical, biological, and endoscopic findings. Ten percent of patients (n = 75) were not continued on maintenance IFX because of primary nonresponse. Of the 643 initial responders, therapy was given episodically in 363 patients (administration

of infusions in case of worsened disease activity only). Of those, 195 were continued episodically, and 168 patients were later switched to 8-weekly maintenance (5 mg/kg). Maintenance therapy from the start was administered in 280 patients. Twelve of the 280 patients interrupted therapy for more than 3 months for various reasons (pregnancy, moving, surgery) and restarted afterward. Because introducing intervals of more than 12 weeks influences CRP over time, these 12 patients were not taken into account for analysis (Figure 1).

C-reactive Protein Measurement

Serial blood samples were taken before each IFX infusion, and CRP was measured as part of standard follow-up by using an enzyme-linked immunosorbent assay (Roche, Basel, Switzerland). A CRP level below 3 mg/L is the cutoff for this assay CRP and was therefore considered normal. A median of 7 (mean, 11.8) CRP values were available per patient.

All clinical data and CRP values at date of each infusion (taken before the infusions) were retrospectively collected from the patients’ records and were correlated to clinical outcome including relapse of disease activity, the need for therapy adjustment, and endoscopic findings.

In total, serial CRP values from 8845 infusions were available for analysis.

Definitions

Short-term response (primary clinical response) was previously defined as initial clinical improvement of symptoms after start of IFX therapy and was assessed by experienced clinicians at week 4 or 10 (the latter in case of 0-2-6-weeks induction).17 Treatment was discontinued in patients with lack of response to the therapy. Early normalization was defined as normalization of CRP after start of therapy, either at week 4 (for patients receiving no 0-2-6 induction scheme) or at week 10

Table 1. Patients’ Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/male</td>
<td>434/284</td>
</tr>
<tr>
<td>Median age (y) (IQR)</td>
<td>40 (32–50)</td>
</tr>
<tr>
<td>Median age at diagnosis (y) (IQR)</td>
<td>23 (18–30)</td>
</tr>
<tr>
<td>Median disease duration until first IFX (mo) (IQR)</td>
<td>91 (26–189)</td>
</tr>
<tr>
<td>Median follow-up (mo) (IQR)</td>
<td>47 (22–80)</td>
</tr>
<tr>
<td>Indication for IFX</td>
<td></td>
</tr>
<tr>
<td>Luminal disease (%)</td>
<td>471 (65.6)</td>
</tr>
<tr>
<td>Fistulizing disease (%)</td>
<td>208 (29.0)</td>
</tr>
<tr>
<td>Mainly extraintestinal symptoms (%)</td>
<td>39 (5.4)</td>
</tr>
<tr>
<td>Therapy scheme</td>
<td></td>
</tr>
<tr>
<td>Primary nonresponders (%) not continued in maintenance</td>
<td>75 (10.4)</td>
</tr>
<tr>
<td>Episodic (%)</td>
<td>195 (27.2)</td>
</tr>
<tr>
<td>Started episodically and switched to maintenance</td>
<td>168 (23.4)</td>
</tr>
<tr>
<td>Maintenance only (%)</td>
<td>280 (39.0)</td>
</tr>
<tr>
<td>Maintenance interrupted (drug holiday &gt;12 wk) (%)</td>
<td>12 (1.7)</td>
</tr>
<tr>
<td>Concomitant medication</td>
<td></td>
</tr>
<tr>
<td>Azathioprine/6-mercaptopurine/methotrexate (%)</td>
<td>462 (64.3)</td>
</tr>
<tr>
<td>Corticosteroids (%)</td>
<td>447 (62.3)</td>
</tr>
</tbody>
</table>

Figure 1. Flow chart of study cohort.
Sustained clinical benefit was defined as the period of clinical benefit from the therapy without the need for any dose or interval adjustment or any other changes in therapy. A minimum period of 14 weeks was taken to allow for patients to complete their induction treatment and to assess the further evolution. In case of loss of response, optimization of anti-TNF treatment was always considered as primary intention. The choice of the therapy adjustment, dose escalation to 10 mg/kg, shortening the infusion interval, or repeating the induction scheme with 3 infusions at weeks 0, 2, and 6 was at the discretion of the treating physician. Therapy adjustments were clinically successful if symptoms disappeared and therapy could be continued thereafter again in a regular fashion. In case of corticosteroids, immunomodulators, or other medications that needed to be initiated, the patient was considered to have failed therapy. Endoscopic mucosal status was assessed on the basis of the presence of ulcers and erosions. Complete mucosal healing was defined as the absence of any mucosal lesions or signs of active inflammation. A marked improvement of mucosal conditions but still no complete healing was defined as partial healing.

**Statistical Analysis**

All data were arranged and processed in tables by using Microsoft Office Excel (Microsoft Corp, Redmond, WA) and analyzed with SPSS 15.0 (SPSS Inc, Chicago, IL). Comparative models as chi-squared test, Fisher exact test, t test, or Mann–Whitney test, respectively, were used for cross-sectional analysis of categorical data. Receiver operating characteristic analysis was used to calculate sensitivity and specificity of the positive predictive value (PPV) (SPSS 15.0).

Time to normalization after start of IFX was calculated by using Kaplan–Meier analysis. Longitudinal analysis of the CRP evolution over time was implemented by using the SAS 9.1.3 (SAS Inc, Cary, NC) procedure mixed. For this analysis, only the 280 patients with initial response to IFX who continued on maintenance therapy were considered, leaving a total of 3736 CRP values. These mixed effects analyses were adjusted for several covariates, including gender, disease location, previous surgery, concomitant therapy, age at diagnosis, disease duration until first IFX, and weight at first IFX infusion. The necessity to include random effects in the models was checked by using a mixture of chi-squared distribution. All hypotheses were tested at 5% level of significance.

**Results**

**C-reactive Protein and Short-Term Outcome**

For short-term analysis, 654 baseline CRP values from patients who received IFX from the beginning at our center were available. In 35 patients only baseline CRP (4.9%) and in 64 patients (8.9%) only CRP values during follow-up therapy were available because of participation of patients in clinical trials for which no CRP values were provided (n = 22), or because IFX was started outside our hospital (n = 42) and only the follow-up took place in our center. The majority of patients (533/654, 81.5%) showed elevated CRP level (>3 mg/L) at baseline, and 121 patients (18.5%) had normal CRP level before start of IFX. Of the 533 patients with increased baseline CRP, 484 (90.8%) clinically responded to the IFX induction scheme, and 49 (9.2%) did not show response. Among the 121 patients with normal CRP level, 100 (82.6%) showed response, and 21 nonresponders (17.4%) were identified (P = .014 for comparison between patients with increased CRP as compared with those with normal CRP). However, median baseline CRP in the responders (CRP, 8.8 mg/L; interquartile range [IQR], 1.5–35.3) did not differ from the nonresponders (CRP, 12.3 mg/L; IQR, 4.9–32.2; P = .188; Supplementary Figure 1). Concomitant immunomodulators (azathioprine, methotrexate) were used by 29.2% of the responders and 31.1% of nonresponders (P = .788) at first IFX, which thus did not influence primary response.

Almost half (45.8%) of patients with primary clinical response showed early CRP normalization (224/484), compared with only 13 of the 49 nonresponders (26.0%; P = .007).

**C-reactive Protein and Long-Term Outcome**

For long-term analysis, only patients on maintenance IFX therapy (n = 268) were included. Of those, 197 (73.5%) had increased CRP level at baseline, and 92 (46.7%) normalized after induction at week 4 of 10. In another 29 patients (14.7%), CRP level normalized after 10 weeks. The median time to normalization of CRP after start of IFX was 8.7 weeks (IQR, 5.0–45.6). Survival analysis demonstrated that patients who normalized CRP early after 4 of 10 weeks had better long-term benefit (P < .001; Figure 2). In fact, early CRP normalization had a PPV of 63.0% for sustained clinical benefit.

Clinical relapse despite maintenance infusions occurred in 97 patients (36.2%), leading to the need for dose adjustment. Patients who clinically relapsed and needed therapy adjustment had higher baseline CRP (median, 22.7; IQR, 10.1–42.1) compared with patients who did not relapse (median, 12.0; IQR, 6.8–30.0; P = .012; Supplementary Figure 2). Even adjusted for gender, disease location, previous surgery, concomitant therapy, age at diagnosis, disease duration until first IFX, and weight at first IFX infusion, loss of response and the subsequent need for dose adjustment were associated with higher CRP levels compared with patients who did not lose response (estimated, 5.13; standard error, 2.13; P = .016; Figure 3A). Concomitant medication as azathioprine and methotrexate (estimated, 2.04; stan-
dard error, 2.58; \( P = 0.429 \) as well as disease location (estimated, 1.644; standard error, 5.605; \( P = 0.769 \)) did not affect the CRP evolution over time.

At the time of clinical relapse, CRP was increased (median, 11.2 mg/L) compared with CRP levels after first infusion at week 4 of 10 (median, 3.2 mg/L; \( P < .001 \)), although the values did not reach the levels of baseline (median, 18.2 mg/L; \( P = 0.039 \); Supplementary Figure 3). The CRP increase preceded clinical relapse in 69.1% (67/97) of the patients, with a median of 8.4 weeks (IQR, 0 –14.7). At time of relapse, increased CRP level (which was not known at moment of clinical investigation) was noticed in 72.0%.

In 79 patients (81.4%) dose adjustment was successful, whereas 18 patients (18.6%) had to discontinue IFX after failure of intervention. CRP levels at the time of loss of response and before therapy adjustment were not associated with the success of the intervention (\( P = .485 \)). However, 33.0% (32 of 97 patients) with successful interventions normalized their CRP level in contrast to 21.1% (4 of 19) of patients in whom interventions failed (\( P = .082 \)). We did not observe differences in success for the various types of therapy adjustments.

Loss of response leading to discontinuation of IFX therapy strongly affected CRP kinetics. Patients who lost response and discontinued IFX had significantly higher CRP levels over time than patients who were continued on IFX (estimated, 14.2897; standard error, 3.07; \( P < .001 \); Figure 3B).

**C-reactive Protein and Mucosal Healing**

In 253 patients of the study cohort, endoscopic data before and after IFX were available, and 99 of these patients were treated in an 8-weekly maintenance regimen from the start. Of the latter, 46 of 99 (46.5%) achieved complete mucosal healing, and 27.3% (27/99) and 26.2% (26/99) had partial healing and no healing, respectively.

Baseline CRP values were not different between patients who obtained complete healing (median CRP, 10.0 mg/L; IQR, 3.2–22.3), partial healing (median CRP, 13.9 mg/L; IQR, 7.0–31.7), or no healing (median CRP, 9.1 mg/L; IQR, 2.8–42.5).

CRP values within 2 weeks before endoscopy after IFX initiation were available in 75 patients. Twenty-one of those did not show any improvement compared with their baseline endoscopy (28.0%), 19 (25.3%) had partial healing, and 35 (46.7%) presented with complete healing of the mucosa.

The CRP level at time of endoscopy was significantly correlated with the degree of healing; the median CRP was 11.5 mg/L (IQR, 6.5–40.8) in patients without healing, 3.1 mg/L (IQR, 1.5–9.5) in patients with partial healing (\( P = .026 \)), and 3.2 mg/L (IQR, 1.1–5.6) in patients with complete healing (\( P = .021 \)) compared with no healing (Figure 4).

Likewise, the change in CRP level between baseline and time of endoscopy correlated significantly with the status of healing (median \( \Delta \)CRP, 4.6 mg/L [IQR, 1.7–31.1] in no healing; 15.8 mg/L [IQR, 6.2–25.2] in partial healing; and 8.9 mg/L [IQR, 3.1–19.4] in complete healing) (\( P = .017 \) and \( P = .001 \), respectively).

Of 21 patients with CRP level lower than 3 mg/L at time of endoscopy, 20 had at least mucosal improvement (\( n = 9 \)) or complete healing (\( n = 11 \)). Receiver operator characteristic analysis showed a PPV for detecting healing in case of CRP <3 mg/L of 95.2% (Supplementary Figure 4).

With a cutoff of CRP <5 mg/L and <10 mg/L, the PPVs were 93.9% (31/33 patients) and 83.7% (36/43), respectively.

**Discussion**

CRP is one of the most important acute phase proteins and is produced by hepatocytes on stimulation by viral or
bacterial stimuli. The cascade of signal transduction leading to CRP release from hepatocytes is particularly triggered by the cytokines TNF, IL-1β, and IL-6. Although CRP response is heterogeneous in IBD, with only a modest correlation with disease activity in UC, CRP has a much stronger correlation with disease activity in CD patients. In this study, the value of CRP as a noninvasive inflammatory marker in the long-term follow-up of IFX-treated CD patients was investigated. Our results show that CRP is a clinically useful surrogate marker, and evolution of CRP might help in predicting outcome and optimizing therapy. Although not specifically studied, these results most likely can be extrapolated to other anti-inflammatory induction agents used in CD-like corticosteroids and adalimumab.

There are a number of important observations we made in this study and which we believe have implications for clinical practice. First, the CRP level at baseline already gave first information on the likelihood of primary response to IFX. Increased CRP level before start of therapy was associated with higher response rates in contrast to normal CRP levels. Therefore, in patients who are candidates for anti-TNF but who have a normal CRP level, further imaging is required before starting treatment to rule out other reasons for the symptoms (strictures, bacterial overgrowth, irritable bowel syndrome) (proposed flow chart in Figure 5). This was recently also emphasized by the results of the SONIC study in which patients with normal CRP level and especially with absence of endoscopic lesions had lower response and remission rates, which were no longer different between the treatment arms.19

In view of the long-term follow-up, we confirmed that early normalization of CRP levels predicts sustained response with PPV of 63%. These findings confirm our previous results in a smaller cohort of CD patients and were also observed in patients with UC treated with IFX.17,20,21 Therefore, early determination of CRP level after induction therapy might give a good prognostic estimate, and when CRP level normalizes, a favor-
able long-term outcome can be assumed. If, on the other hand, after induction therapy CRP level does not improve or decreases less than 25% from baseline, a new assessment of the patient should be done, and therapy should be stopped in case of no mucosal improvement. When the lesions have improved but not healed, anti-TNF serum levels could help to see whether therapy needs to be optimized (Figure 5). The longitudinal analysis of CRP level over time further showed higher CRP levels in patients who lost response compared with those with sustained response. The highest CRP levels over time were found in patients who relapsed under therapy and finally needed to stop therapy completely. However, we could not identify an absolute CRP value above which therapy needed to be discontinued in all patients. This needs to be assessed on an individual level and interpreted in view of previous CRP values for that patient.

Interestingly, the increase of CRP level as sign of disease reactivation was observed already a median of 8 weeks before the loss of response became clinically apparent. Given that CRP measurement was regularly performed in 8-weekly intervals, this indeed might be a drawback of this study; however, these patients did not experience symptoms yet because no therapy adjustment was made, but after the CRP increase, therapy adjustment became necessary. It is unknown at the moment whether increase of biological markers in the absence of symptoms justifies therapy adjustment and might prevent loss of response. Measuring serum levels in case of CRP increase might give additional information and help to assess and decide further therapeutic actions. Future prospective studies randomizing patients in clinical remission but with increased inflammatory markers to early dose escalation or not will learn whether this approach could prevent clinical relapse. In the meantime, if a patient on maintenance anti-TNF shows a CRP increase and this is accompanied with symptoms, therapy should be optimized. On the other hand, symptoms under maintenance without CRP increase or CRP increase without symptoms should be further evaluated (Figure 5) before therapy is changed.

As more data became available showing the impact of mucosal healing on the long-term outcome of CD,18,22,23 we also focused on the relation of CRP to mucosal improvement after initiation of IFX therapy. CRP showed good association with endoscopic findings of complete or absence of healing. In the clinical situation of patients in whom remission is induced with IFX, a normal CRP level during follow-up had an excellent PPV of 95% toward endoscopic healing. Therefore, in a patient who has proved to mount CRP responses in case of endoscopic activity, normalization of CRP level after starting anti-TNF parallels healing of the mucosa with a high certainty. Nevertheless, we believe endoscopy remains necessary and indispensable before starting new therapies and during long-term maintenance in cases of surveillance.

In conclusion, our study showed that CRP is a useful marker in the management and follow-up of CD under anti-TNF therapy. Individual CRP profiles of CD patients are important indicators for long-term outcome of therapy including the prediction of loss of response. CRP was highly predictive for mucosal improvement after start of IFX and therefore represents a good surrogate marker for the status of the mucosal inflammation.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of Clinical Gastroenterology and Hepatology at www.cghjournal.org, and at doi:10.1016/j.cgh.2011.02.008.

References


Reprint requests
Address requests for reprints to: Séverine Vermeire, MD, PhD, University Hospital Gasthuisberg, Leuven, Herestraat 49, B-3000 Leuven, Belgium. e-mail: Severine.Vermeire@uz.kuleuven.be; fax: +32-16-34-43-99.

Conflicts of interest
The authors disclose the following: Matthias Jürgens and Fabian Schnitzler received grants from Centocor, Inc. Paul Rutgeerts, Geert Van Assche, Severine Vermeire, Fabian Schnitzler, and Matthias Jürgens have received lecture fees from Schering-Plough Pharma. The remaining authors disclose no conflicts.

Funding
Jestinah M. Mahachie John and Kristel Van Steen acknowledge research opportunities offered by the Belgian Network BioMAGNet (Bioinformatics and Modelling from Genomes to Networks), funded by the Interuniversity Attraction Poles Programme (Phase VI/4), initiated by the Belgian State, Science Policy Office. Wouter Van Moerkercke was supported by the FWO (Belgium). Matthias Jürgens was supported by a grant from Centocor Inc.
Supplementary Figure 1. Baseline CRP levels and primary clinical response.

Supplementary Figure 2. Baseline CRP and clinical relapse and subsequent need for interventions.

Supplementary Figure 3. CRP level decreases significantly after start of treatment and increases at time of relapse, however, without reaching baseline levels again.

Supplementary Figure 4. Receiver operating characteristic curve of the PPV of normal CRP (<3 mg/L) for mucosal healing.