Microinflammation in patients with Crohn's disease in clinical remission

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Abstract

Background and aims: It is not clear whether Crohn’s disease patients in clinical remission (Crohn’s disease activity index < 150) display normal concentrations of inflammation sensitive biomarkers. Our goal in this work was to explore the intensity of the microinflammatory response in a group of Crohn’s disease patients in clinical remission.

Methods: High sensitivity C-reactive protein, quantitative fibrinogen, erythrocyte sedimentation rate as well as platelet and leukocyte counts were examined in a group of 76 patients with Crohn’s disease in remission and in 228 matched controls.

Results: Crohn’s disease patients in clinical remission displayed a statistically significant (p < 0.001) elevated concentration of hs-CRP (4.83 ± 3.8 mg/l) compared to controls (1.05 ± 2.9 mg/l). All other bio-markers were also significantly higher in Crohn’s disease patients in remission compared to controls. Similar results were obtained in a subgroup of Crohn’s disease patients with very low disease activity — CDAI < 75.

Conclusions: Clinical remission is not equivalent to biochemical remission raising a question concerning the true definition of remission in Crohn’s disease.

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KEYWORDS
Crohn’s disease; Remission; Inflammatory markers

1. Introduction

Crohn’s disease (CD) is a chronic inflammatory intestinal disorder. Its treatment is based on administration of anti-inflammatory and immunomodulatory agents.1 Markers of acute phase response are used to detect and quantify the intensity of inflammatory processes 2 particularly, of the inflammatory bowel diseases.3 CD Activity Index (CDAI) is a clinical scoring system used for evaluating the activity of CD...
Inflammatory markers in Crohn's Disease in remission

in daily practice and in clinical research.\textsuperscript{4,5} An individual scoring $\leq 150$ is considered to be in clinical remission.\textsuperscript{2}

Undoubtedly, a major goal for care givers in the treatment of CD patients is to achieve remission. However, it is not entirely clear whether the definition of remission should be based on clinical scores, inflammation sensitive biomarkers or both.\textsuperscript{6,7} It is conceivable that the concentrations of inflammation sensitive biomarkers during clinical remission be close to normal or within normal limits. Yet, the definition of normal limits is complex since even minor inflammatory stimuli may elicit a rise in the concentration of the inflammation sensitive biomarkers.\textsuperscript{8}

We compared CD patients in clinical remission (CDAI $\leq 150$) to a large number of matched controls and found that despite their low clinical score, these patients have significant elevations in the concentration of inflammation sensitive biomarkers. This observation raises concern regarding the precision of the commonly used clinical scores, specifically, CDAI in identifying patients who are in true remission.

2. Materials and methods

2.1. Patients

Seventy-six consecutive male and female CD patients in remission were recruited and constitute our patient group. These patients were recruited at the Tel Aviv Sourasky Medical Center and Liver Diseases, Tel Aviv Sourasky Medical Center, Tel-Aviv, Israel during their routine follow up. Remission in patients was defined as CDAI of $< 150$.\textsuperscript{2} CDAI was determined by an inflammatory bowel diseases oriented gastroenterologist (ID).

2.2. Controls

For the control group apparently healthy men and women were recruited at the Tel Aviv Sourasky Medical Center Inflammation Survey (TAMCIS), which is a registered data bank of the Israeli Ministry of Justice.\textsuperscript{9-11} This is a cross sectional study to which we have invited apparently healthy employees of the Tel Aviv Medical Center and Tel Aviv Municipality. We further invited individuals with atherothrombotic risk factors who were in follow up in the outpatient clinics of the Medical Center to join our study. Excluded were any individuals with an underlying inflammatory disease (arthritis, IBD, etc.) or past history of malignancy, as well as, individuals with any infections or other inflammatory conditions, including infarction, surgery or angiography during the six months before their recruitment. Individuals treated with steroids or non-steroidal anti-inflammatory agents, except for aspirin (at doses lower than 325 mg/day), were likewise excluded.

All patients and controls signed a written informed consent according to the guidelines of the local ethics committee.

2.3. Cardiovascular risk factors

To control for low-grade inflammation information from the patient and control groups was collected on age, height, weight, smoking habits, family history of cardiovascular disease, and physical activity. Blood was drawn for lipid profile (total cholesterol level, low density lipoprotein, high density lipoprotein and triglycerides level) after an overnight fast. Hypertension was defined as systolic blood pressure $> 140$ mmHg or a diastolic blood pressure $> 90$ mmHg or the use of antihypertensive agents. Hypercholesterolemia was defined as total cholesterol level $> 200$ mg/dl or the use of lipid lowering agents. Positive family history of cardiovascular disease was defined as any report of a cardiovascular event in a first degree relative under 65 years old for women and under 55 years old for men. Current smoker was defined as anyone who reported habitual smoking during the past 3 months (at least). Regular physical activity was defined as performing more than 60 min a week of aerobic physical activity.

2.4. Laboratory methods

Blood count was performed using the Beckman STKS Coulter (Beckman Coulter, Nyon, Swiss, Beckman Coulter, Miami, FL,

<table>
<thead>
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<th>Variables</th>
<th>CD patients</th>
<th>Controls</th>
<th>p value</th>
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<tr>
<td>Age (years)</td>
<td>34.6±13.8</td>
<td>35.6±12.5</td>
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<td>Female (%)</td>
<td>47.3</td>
<td>49.1</td>
<td>NS</td>
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<tr>
<td>BMI (kg/m$^2$)</td>
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<td>23±3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>28.9</td>
<td>29.4</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>1.3</td>
<td>3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of IHD (%)</td>
<td>5.3</td>
<td>6.1</td>
<td>NS</td>
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<tr>
<td>Regular physical activity (%)</td>
<td>31.6</td>
<td>44.7</td>
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BMI — body mass index; CD — Crohn’s disease; IHD — ischemic heart disease; NS — non significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CD patients</th>
<th>Controls</th>
<th>p value</th>
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</thead>
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<tr>
<td>Cholesterol (mg/dl)</td>
<td>174±36</td>
<td>183±32</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>116±65</td>
<td>95±63</td>
<td>0.02</td>
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<tr>
<td>HDL (mg/dl)</td>
<td>56±16</td>
<td>58±16</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>95±26</td>
<td>106±26</td>
<td>0.002</td>
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</table>

CD — Crohn’s disease; HDL — high density lipoprotein; LDL — low density lipoprotein; NS — non significant.
USA); erythrocyte sedimentation rate was performed by the method of Westergren, quantitatively fibrinogen by the method of Clauss, and Sysmex 6000 autoanalyzer (Sysmex Corporation, Hyogo, Japan). The quantitative concentration of high sensitivity C-reactive protein (hs-CRP) was determined by the Boering BN II Nephelometer (DADE Boering, Marburg, Germany) by a method according to Rifai whereupon the lowest hs-CRP concentration obtained by this method is 0.17 mg/L. Lipid profile was performed using the routine biochemical assays of the medical center.

2.5. Statistical analyses

All data were summarized and displayed as the mean ± standard deviation (SD) for the continuous variables (age, body mass index, inflammation markers, etc.), and as the percentage in each group for categorical variables (smoking and other cardiovascular risk factors, etc.). The cross-tabs and descriptive procedures were used to produce frequencies of categorical variables and the mean ± SD of continuous variables. The one-sample Kolmogorov–Smirnov test was used to verify that the logarithmic transformation is normally distributed. For all continuous variables the independent sample t-test was used to evaluate the difference between the groups, while for all categorical variables the chi-square test was used to assess the difference in significance between the groups. The level of significance used for all above analyses was two-tailed (p < 0.05). SPSS statistical package was used to perform all statistical evaluation (SSPS Inc., Chicago, Illinois, USA).

3. Results

Seventy six CD patients in clinical remission (CDAI < 150) were recruited and compared with 228 carefully matched controls (3 controls for each patient were evaluated). The clinical details of patients and controls such as age, BMI and hypertension are presented in Table 1. Patients and controls displayed similar age, gender, body mass index (BMI) and percent of atherothrombotic risk factors; and differed in that more subjects in the patients group followed a sedentary life style compared to control subjects. In addition, patients had lower cholesterol concentrations and mildly elevated triglyceride levels as opposed to controls (Table 2). Values of inflammatory variables are presented in Table 3.

It is clearly evident that CD patients in remission displayed enhanced inflammatory activity as opposed to controls and this was not due to the low grade inflammation associated with atherothrombotic risk (Table 1, 2). In fact, the concentration of hs-CRP in patients was 4.6 times higher than that observed in the controls (p < 0.001). The distribution of hs-CRP in patients and controls is presented in Fig. 1.

In order to strengthen our results we then focused on a group of 43 CD patients in remission who displayed with especially low disease activity score (CDAI ≤ 75). To ensure validity of results this group of CD patients was compared to 258 healthy controls (whereupon each patient was matched to 6 controls). Demographic variables and atherothrombotic risk factors were similar in both groups (Table 4). Lipid

![Figure 1](image1.png)

**Table 4** Demography and vascular risk factors in 43 Crohn's disease patients in remission (CDAI score ≤ 75) and 258 matched controls

<table>
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<td>33.8±13.7</td>
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</tr>
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<td>Female (%)</td>
<td>46.5</td>
<td>47.6</td>
<td>NS</td>
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<tr>
<td>Smokers (%)</td>
<td>32.6</td>
<td>32</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>2.3</td>
<td>3.5</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of IHD (%)</td>
<td>9.3</td>
<td>6.2</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>0</td>
<td>8.1</td>
<td>NS</td>
</tr>
<tr>
<td>Regular physical activity (%)</td>
<td>34.9</td>
<td>46.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI – body mass index; CD – Crohn's disease; IHD – ischemic heart disease; NS – non significant.
profiles were similar as well (data not shown). Interestingly, despite complete clinical remission (CDAI < 75), results show that the levels of inflammation sensitive biomarkers were still, significantly elevated compared to those in matched controls (Table 5).

While it is conceivable that various anti-inflammatory medications may have influenced the results of the inflammatory biomarkers, we have ruled out this possibility in our study: from our group of CD patients we compared the results obtained from patients treated with anti inflammatory medications to those untreated with medications. Of our whole study group of 76 patients, 43 were on various non-steroidal anti-inflammatory drugs, 21 were taking cytotoxic medications, while 8 were on systemic steroids and some patients received combinations of the above-mentioned medications. The mean ± SD CDAI scores of the treated and untreated patients were 75 ± 42 and 64 ± 35 respectively. In Table 6 we present the data of the 56 treated and 20 untreated patients who differed only in the concentration of platelets in the treated patients group.

In Table 7, we compare the inflammatory sensitive biomarkers of 56 treated patients with anti-inflammatory drugs and 20 untreated patients who differed only in the concentration of platelets in the treated patients group. The inflammatory sensitive markers were presented in Table 7. No difference in the concentration of the various inflammation sensitive biomarkers was noted between the groups except for a higher concentration of platelets in the treated patients group. Moreover, no significant differences were noted between the two groups regarding their lipid profiles (data not shown).

4. Discussion

At present the notion is that CD patients in remission present with a low grade of inflammatory activity and yet, patients in clinical remission may demonstrate high inflammatory activity on blood tests. This contradiction challenges the current goal of the clinician and suggests that it should be refocused not only toward clinical remission but perhaps also towards biochemical remission. It is not yet clear whether patients in clinical remission who also express high levels of inflammatory markers are at a high risk for exacerbations or other complications of CD. However, we do know that chronic inflammation on its own is associated with morbidity. Thus, even subclinical inflammatory activity should be controlled. In fact, recent studies suggest that low grade inflammatory activity can be detected in the general population. This microinflammation is related to multiple etiologies including obesity, smoking, hyperlipidemia, hypertension, diabetes, physical and mental stress, minor trauma, hormonal changes, low grade infections, as well as diet composition (for review see Kushner et al.). Therefore, it has become difficult to define what the “true normal” values for a given individual should be. This has special relevance for the definition of biochemical, as opposed to clinical remission in patients with a CDAI score of < 150.

In order to overcome these obstacles, mentioned above, we used a large number of controls for each patient with CD in remission. We believe this to be the appropriate approach towards ascertaining the expected “background” levels of inflammation for a given individual. Controls were matched for as many as possible variables including age, gender, BMI, smoking status and atherothrombotic risk factors. Importantly, our results showed that CD patients in clinical remission displayed with clearly elevated concentrates of inflammation sensitive biomarkers. Moreover, similar results were also attained in a subgroup of CD patients with a CDAI of only < 75. Contrary perhaps to initial perception this raises a concern regarding correlation, if any, between clinical scores and the level of inflammation sensitive biomarkers in CD patients.
Another relevant finding in this regard is that inflammation sensitive biomarkers were not significantly altered among CD patients not treated with anti-inflammatory drugs compared to those treated. It can therefore be argued that these untreated patients presenting with a CDAI of < 150 are probably in a prolonged state of clinical as well as biochemical remission.

We conclude that patients with CD in clinical remission present clearly elevated concentrations of inflammation sensitive biomarkers including hs-CRP, quantitative fibrinogen, erythrocyte sedimentation rate as well as leukocyte and platelet counts. These findings raise questions related to the tools we employ in daily practice for the definition of remission of Crohn's disease, and may well have clinical implications regarding therapeutic interventions in this particular group of patients.

Acknowledgements

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NM carried out the study, collected data, and performed data analyses. LZ collected data. YA collected data. IS participated in study design and coordination. SB conceived the study, participated in study design and data collection. NA participated in data analyses. LZ collected data. YA collected data. IS participated in study design and data collection. SB conceived the study, interpreted data and wrote the manuscript. ID aided in patients’ recruitment and interpretation of data. All authors read and approved the final manuscript.

References