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Serum MMP-9: a novel biomarker for prediction of clinical relapse in patients with quiescent Crohn's disease, a *post hoc* analysis

Doron Yablecovitch, Uri Kopylov, Adi Lahat, Michal M. Amitai, Eyal Klang, Dana Ben-Ami Shor, Sandra Neuman, Nina Levhar, Ella Fudim, Benjamin Avidan, Ido Laish, Limor Selinger, Noam Zingboim-Orbach, Orit Picard, Miri Yavzori, Rami Eliakim and Shomron Ben-Horin

Abstract

Background: Matrix metalloproteinase-9 (MMP-9) is a novel marker of intestinal inflammation. The aim of this study was to assess if serum MMP-9 levels predict clinical flare in patients with quiescent Crohn's disease (CD).

Methods: This study was a *post hoc* analysis of a prospective observational study in which quiescent CD patients were included and followed until clinical relapse or the end of a 2-year follow-up period. Serial C-reactive protein (CRP) and fecal calprotectin (FC) levels were measured, and the patients underwent repeated capsule endoscopies (CEs) every 6 months. Small bowel inflammation was quantified by Lewis score (LS) for CE. A baseline magnetic resonance enterography was also performed, and MaRIA score was calculated. Serum MMP-9 levels in baseline blood samples were quantified by ELISA.

Results: Out of 58 eligible enrolled patients, 16 had a flare. Higher levels of baseline MMP-9 were found in patients who developed subsequent symptomatic flare compared with patients who did not [median 661 ng/ml, 25–75 interquartile range (IQR; 478.2–1441.3) *versus* 525.5 ng/ml (339–662.7), respectively, $p = 0.01$]. Patients with serum MMP-9 levels of 945 ng/ml or higher were at increased risk for relapse within 24 months [area under the curve (AUC) of 0.72 [95% confidence interval (CI): 0.56–0.88]; hazard ratio 8.1 (95% CI 3.0–21.9, $p < 0.001$)]. Serum MMP-9 concentrations showed weak and moderate correlation to baseline LS and FC, respectively ($r = 0.31$, $p = 0.02$; $r = 0.46$, $p < 0.001$). No correlation was found between serum MMP-9 to CRP and MaRIA score.

Conclusions: Serum MMP-9 may be a promising biomarker for prediction of clinical flare in CD patients with quiescent disease.

Keywords: Crohn's disease, biomarker, extracellular matrix, matrix metalloproteinase-9

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Correspondence to:
Doron Yablecovitch
Department of Gastroenterology, Sheba Medical Center, Tel Hashomer, Israel, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel
doronyab@gmail.com

Uri Kopylov
Adi Lahat
Dana Ben-Ami Shor
Sandra Neuman
Nina Levhar
Ella Fudim
Benjamin Avidan
Ido Laish
Limor Selinger
Noam Zingboim-Orbach
Orit Picard
Miri Yavzori
Rami Eliakim
Shomron Ben-Horin
Department of Gastroenterology, Sheba Medical Center and Sackler School of Medicine Tel-Aviv University, Tel-Aviv, Israel

Michal M. Amitai
Eyal Klang
Department of Diagnostic Imaging, Sheba Medical Center and Sackler School of Medicine Tel-Aviv University, Tel-Aviv, Israel

Introduction

Crohn's disease (CD) is a chronic and progressive inflammatory disease, frequently associated with accumulation of structural bowel damage.[1] Assessing intestinal inflammation remains a difficult challenge. The current reliable method is invasive, costly, and uncomfortable for the patient as it requires frequent endoscopies with biopsy sampling. Moreover, in CD patients, the site of the lesion is not always accessible to endoscopy.[2]

Clinical remission is poorly correlated with mucosal healing, and subclinical inflammation may persist with a major contribution to the risk of relapse.[3,4] The prediction of clinical deterioration in CD patients may lead to optimization of

therapeutic strategies and decrease subsequent complications. Therefore, a dire need exists for novel, accurate, and noninvasive markers for this purpose.[5,6]

Matrix metalloproteinases (MMPs) are a family of 24 zinc-dependent extracellular matrix-degrading endopeptidases. MMPs are key players in extracellular matrix (ECM) turnover by degrading collagens type I, II, III, or IV, in their native form. MMPs also degrade nonmatrix substrates, including cytokines, growth factors, chemokines, and junctional proteins.[7] MMPs are involved in multiple pathological processes such as tumor spread and metastasis, cardiovascular disease, rheumatoid arthritis, and initiation and maintenance of chronic inflammatory processes.[8–11]

Within the MMPs family, MMP-9 (also known as gelatinase B) has been suggested as a possible marker of intestinal inflammation.[10,12] MMP-9 is an inducible protease that was shown to be upregulated in several inflammatory conditions. It has been found to be the most abundant MMP in inflamed intestinal tissue of patients with inflammatory bowel disease (IBD). MMP-9 is secreted mainly by neutrophils and other cell types such as mesenchymal cells, fibroblasts, and several inflammatory cells like monocytes or lymphocytes.[13–15]

The increase in MMP-9 proteolytic activity correlates with histological and endoscopic scores, and with the extent of tissue damage.[16–20] Moreover, MMP-9 was suggested as potential marker of mucosal inflammation in several studies.[12,21–28] However, in all these studies, MMP-9 was evaluated cross-sectionally with concurrent inflammatory activity, but, to our knowledge, there are hitherto no reports of its diagnostic utility to predict the future course of IBD.

Therefore, present study evaluated serum MMP-9 as a predictor of future exacerbations in CD patients with quiescent disease.

Methods

Patient population

The study was a *post hoc* analysis of a prospective observational study aimed at identifying predictors of clinical relapse in CD patients with quiescent disease. The patients were followed until clinical flare or the end of the 2-year study.[29] The study population included adult CD patients (>18 years) with known small bowel (SB) disease in remission, or mild disease symptoms, as evaluated by a CD activity index (CDAI) of <220. All patients were in corticosteroid-free remission for 3–24 months, and were treated with a stable medication dose [30 days for adalimumab and 5-aminosalicylic acid (5-ASA) agents, 60 days for methotrexate, thiopurines, and infliximab]. CD treatment was unchanged during follow up. The patients were followed prospectively by clinical evaluation and biomarker [C-reactive protein (CRP)/fecal calprotectin (FC) levels] once every 3 months, video capsule endoscopy (VCE) at baseline and every 6 months thereafter, and by magnetic resonance enterography (MRE) examinations at baseline and upon study conclusion. Clinical relapse was defined as an increase of >70 points on CDAI from baseline, and a CDAI > 150, or the need for rescue medication for CD necessitated by disease worsening as determined by physician global assessment (PGA). Patients were excluded from the study if they were unable to provide informed consent; suffered from severe unstable comorbidities such as kidney, liver, metabolic, neurologic, or cardiorespiratory disorders at enrollment; current or history of aspirations or dysphagia; implanted metal objects or cardiac pacemaker, claustrophobia, preventing performance of magnetic resonance imaging; or known or suspected severe stricture or intestinal obstruction. All patients signed an informed consent, and the study was approved by the institutional ethics review board (SMC 13-0218).

Inflammatory biomarkers and disease activity measures

Serum MMP-9 concentrations at baseline were determined using human MMP-9 enzyme-linked immunosorbent assay kit (ELISA; R&D systems, Minneapolis, MN, USA) in accordance with manufacturer's instructions. The test procedure was standardized using standards provided with the kit. All standards and samples were analyzed in duplicate. The results were measured in units of ng/ml.

Complete blood count (CBC), CRP, and FC were measured every 3 months. FC levels were evaluated using the Quantum Blue calprotectin kit (Bühlmann Laboratories AG, Basel, Switzerland). The reported value range is 30 (detection level) to

300 μg/g (no further quantification was possible above 300 μg/g). Levels >100 μg/g were considered positive. CRP levels were regarded as elevated if >5 mg/l. Patients underwent physician's assessment and CDAI estimation for disease activity every 3 months.

Imaging and capsule endoscopy studies

Upon enrollment, all patients underwent an MRE. MR image acquisition was performed using a protocol as previously described.[4] All patients with active SB disease detected on MRE went through a patency capsule (PC) test. If the PC was not expelled from the SB within 30 h, the patient was withdrawn from the study. In patients with isolated SBCD, a PillCam SB3 capsule (Given Imaging, Yoqneam, Israel) was used. In patients with known ileo-colonic CD, a colonic capsule procedure (PillCam colon2 capsule, Given Imaging, Yoqneam, Israel) was conducted. The SB data retrieved from a colonic capsule was reviewed and analyzed in a process similar to that for the SB capsule. All images were examined using the RAPID 8 software (Given Imaging, Yoqneam, Israel). To ensure visualization of the entire SB, the adaptive frame rate mode was activated. Mucosal inflammation was quantified using the Lewis score (LS). The definition of mucosal healing was LS < 135, mild-to-moderate inflammation as LS of 135–790, and moderate-to-severe inflammation as LS > 790.[30] LS was calculated manually when using colonic capsule. The capsule endoscopy videos were read by a board-certified gastroenterologist with over 10 years of experience in the procedure.

Statistics

Categorical variables were described as frequency and percentage. Continuous variables were described as median and interquartile range (IQR). Associations between MMP-9 levels and continuous variables were assessed using Spearman's correlation coefficient. Associations between MMP-9 levels and categorical variables were assessed using Mann–Whitney *U* test. In additional analysis, FC was categorized by cut-off level of 250 μg/g, as *p* proposed in previous studies.[31,32] Receiver operating characteristic (ROC) analysis and the Youden index were used to find an optimal cut-off value. Categorical variables were compared between those above the cutoff value and those below using Chi-square test or Fisher's exact test, and continuous variables were compared using Mann–Whitney *U* test. Survival without disease relapse was analyzed by Kaplan–Meier curve and log rank test. Logistic regression was used to calculate the propensity score. Age, gender, CRP, FC, medication, and smoking status at presentation were included in the propensity score. Univariate Cox regressions were used to evaluate the association between MMP-9, patient characteristics, medication, and inflammatory markers at presentation with disease relapse. Multivariate Cox regression was used to describe the association between MMP-9 and disease relapse using propensity score. All statistical tests were two sided. $p < 0.05$ was considered as statistically significant. All statistical analysis was performed using SPSS (IBM SPSS Statistics for Windows, version 25, IBM Corp, Armonk, NY, USA). Area under the curve (AUC) was evaluated using survival-ROC package version 1.0.3 in R: a language and environment for statistical computing (The R foundation for statistical computing version 3.3.3, 2017).

Results

A total of 90 patients were screened for eligibility and underwent PC study to verify small bowel patency, of whom 29 failed screening (due mostly to retained PC, $n = 17$); 61 patients were thus enrolled and underwent capsule endoscopy. After exclusion of 3 patients due to technical causes, there were 58 patients with analyzable samples. The clinical and demographic characteristics of the patients included in the study are described in Table 1.

Associations between baseline characteristics, treatment at presentation, and serum MMP-9 are presented in Table 2. Serum MMP-9 concentrations displayed moderate ($r = 0.46$, $p < 0.001$) and weak ($r = 0.31$, $p = 0.02$) correlation with baseline FC and with capsule endoscopy LS, respectively. Serum MMP-9 was not significantly correlated with age, CRP, MaRIA score of MRE, and CDAI. Smoking status, gender, oral treatment with 5-ASA, and treatment with anti-TNF agents were not associated with MMP-9 levels. Patients receiving immunomodulators medication had significantly higher serum MMP-9 levels at presentation. In categorical analyses, patients who presented with FC ⩾250 μg/g had significantly higher serum MMP-9 levels compared with patients with lower FC ($p = 0.012$,

Table 1. Patient demographics and baseline characteristics.

		n **= 58**
Median age at enrollment, years (IQR)		29.5 (24–37.5)
Gender	Female	25 (43%)
	Male	33 (57%)
Smoking		11(19%)
Median disease duration, years (IQR)		4 (2–9.5)
Median CDAI (IQR)		42 (24–110)
Disease location	L1	36 (62%)
	L2	1 (2%)
	L3	21 (36%)
Disease behavior	B1	39 (67%)
	B2	11 (19%)
	B3	8 (14%)
Perianal disease		10 (17%)
Mild symptoms at enrollment (CDAI 150–220)		6 (10%)
Previous intestinal resection		10 (17%)
Medication at enrollment	None	9 (16%)
	Oral 5-ASA	9 (16%)
	Immunomodulators	27 (46%)
	Anti-TNF	22 (38%)
Median CRP (mg/l), (IQR)		2.2 (0.9–5.9)
Median FC (μg/g), (IQR)		93 (35–204)

CDAI, Crohn's disease activity index; CRP, C-reactive protein; FC, Fecal calprotectin; IQR, Interquartile range 25–75; TNF, tumor necrosis factor.

Figure 1a). Patients with LS ⩾ 790 (i.e. moderate-to-severe mucosal inflammation by conventional LS) had higher median level of serum MMP-9 compared with patients with LS of 135–789, who, in turn, had higher median levels compared with patients with LS < 135 (i.e. no mucosal inflammation). However, this trend did not reach statistical significance ($p = 0.09$, Figure 1b).

Baseline serum MMP-9 levels for prediction of 2-year risk of flare

Overall, 16/58 (28%) of patients relapsed over the 24-month study period, with a median time to relapse of 4.5 months (IQR 3–18). No difference was found between baseline clinical and demographic variables between relapsers and nonrelapsers.

Median baseline levels of serum MMP-9 in the relapse group were significantly higher than MMP-9 levels in the nonrelapse group (661 ng/ml, IQR 478.2–1441.3 *versus* 525.5 ng/ml, IQR 339–662.7, $p < 0.001$, Figure 2). Serum MMP-9 had a fair ability to discriminate between patients who subsequently experienced a relapse and those who did not [AUC 0.72, 95% confidence interval (CI) 0.56–0.88, $p = 0.012$, Figure 3]. A cutoff level of 945 ng/ml of serum MMP-9 showed sensitivity of 44% and specificity of 100% for detecting relapse within 24 months. The positive predictive value (PPV) and negative

predictive value (NPV) were 100% and 78%, respectively.

Kaplan–Meyer analysis of survival without relapse during the follow-up period showed that patients with baseline serum MMP-9 > 945 ng/ml had much shorter duration to relapse compared with patients with baseline MMP-9 ⩽ 945 ($p < 0.001$, Figure 4). The risk of relapse was significantly increased in patients with MMP-9 > 945 ng/ml compared with those below this level (hazard ratio 8.1, 95% CI 3.0–21.9). A formal multivariate analysis was statistically precluded by the relatively limited number of patients who flared ($n = 16$). Therefore, controlling for possible confounders (age, gender, CRP, FC, medications, and smoking status at presentation) was employed using propensity score. This analysis still yielded significant HR of 6.22 for flare in patients with MMP-9 > 945 ng/ml (95% CI 2.1–17.9, $p = 0.001$). We did not include the MaRIA MRE score in this propensity scoring as it was not correlated with MMP-9 levels or with future risk of flare, and also because it is not readily available for clinicians in routine practice. Further sensitivity-analysis of the 13/16 relapsed patients defined exclusively by an increase in CDAI after exclusion of patients relapsing by PGA criteria, demonstrated a higher discrimination ability with AUC of 0.77 (95% CI 0.58–0.93, $p = 0.006$). The same threshold value (945 ng/ml) resulted in 24 months sensitivity of 54% and specificity of 100% for detecting relapse, and PPV and NPV of 100% and 84.2%, respectively.

Discussion

This *post hoc* analysis of a prospective study demonstrates, for the first time, that serum MMP-9 can identify adult patients with quiescent CD who are at risk of future disease exacerbation. Baseline MMP-9 levels were significantly elevated in patients who relapsed within 24 months compared with nonrelapsers, and a cut-off value of MMP-9 above 945 ng/ml identified patients with high risk of flare during a follow-up period of 24 months. Furthermore, an association was found between serum MMP-9, FC, and LS, which are well validated and widely used tools for assessing bowel inflammation in patients with CD.

Table 2. Association between serum MMP-9 levels, patients characteristics and inflammatory indices.

	MMP-9 (ng/ml)	*p*
Age	–0.1	0.453
CRP	0.198	0.136
FC (μg/g)		0.012*
<250	524 (352–679)	
⩾250	660 (569–1245)	
LS		0.09
<135	323 (211–878)	
135–789	528 (373–725)	
⩾790	623 (565–818)	
MaRIA score	0.05	0.722
CDAI	0.135	0.314
Smoker		0.519
No	565 (344–736)	
Yes	563 (408–878)	
Sex		0.556
Male	563 (416–732)	
Female	615 (278–756)	
Oral 5-ASA		0.147
No	575 (394–761)	
Yes	429 (238–699)	
Immunomodulators		0.035*
No	524 (344–636)	
Yes	645 (381–876)	
Anti-TNF		0.95
No	549 (379–779)	
Yes	563 (339–705)	

Data are presented as Spearman's rank correlation coefficient (r_s) or median and IQR.
*Statistically significant *p*-values.
5-ASA, 5-aminosalicylic acid; CDAI, Crohn's disease activity index; CRP, C-reactive protein; FC, fecal calprotectin; IQR, interquartile range 25–75; LS, Lewis score; MMP-9, matrix metalloproteinase-9; TNF, tumor necrosis factor.

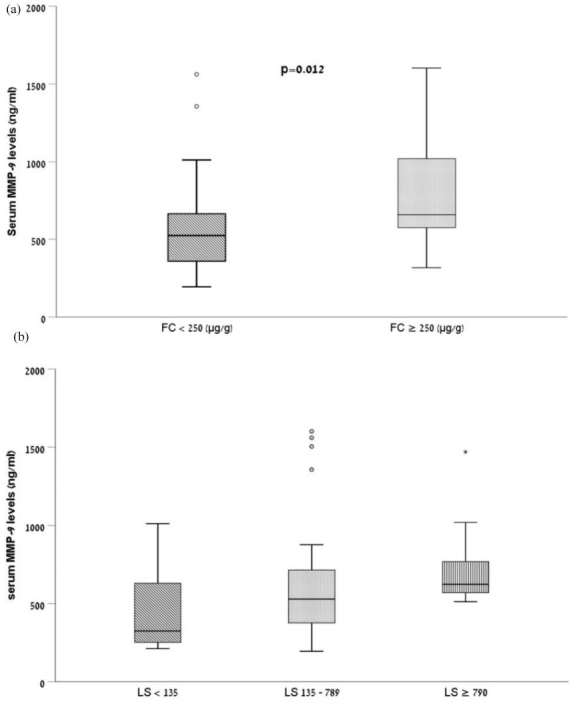

Figure 1. Box-plot representation of serum MMP-9 concentrations in patients with quiescent CD with respect to (a) FC levels and (b) LS. Baseline serum MMP-9 concentration in patients with FC $\geqslant$250 µg/g differed significantly from patients with FC $<$ 250 µg/g ($p = 0.012$). Baseline serum MMP-9 concentration in patients with LS $>$ 790 tend to be higher than patients with LS 135–789 and patients with LS $<$ 135 but did not reach statistical significance ($p = 0.09$). The limits of the box represent the first and third quartiles; the black crossbar line represents the median.
CD, Crohn's disease; CFC, fecal calprotectin; LS, Lewis score; MMP-9, matrix metalloproteinase-9.

Discovery of novel biomarkers that also have a defined pathophysiological role in the disease would improve clinical evaluation and therapeutic strategy. Aberrant tissue remodeling with excessive degradation or accumulation of ECM components is a key event in IBD.[10] ECM remodeling processes are executed by MMPs, which are considered predominant proteases involved in IBD.[11,33] MMPs possess a conserved catalytic domain with a Zn^{2+} at the active site and a prodomain that confers latency and requires cleavage during secretion to obtain activity.[34–36] Although MMP-9 is not a canonical inflammatory molecule, accumulating evidence suggests that MMP-9 is not only an outcome of inflammatory pathways, but also a propagator of inflammation.[10,14] Serum MMP-9 may be derived from increased expression and release from the inflamed intestinal tissues, or from activated neutrophils as they migrate from blood vessels into inflamed tissues.[37] The neutrophil is the most important source of MMP-9 during the active inflammatory phase. Upon cleavage by MMP-9, the potent neutrophil activating chemokine IL-8 increases its potency tenfold. This promotes further neutrophil infiltration and activation, leading to enhanced MMP-9 secretion, thus creating a vicious cycle.[38] MMP-9 is also involved in generating the collagen-derived fragment proline-glycine-proline (PGP) in the gut, which is a chemo-attractant for neutrophils. PGP, as well as MMP-9 levels are elevated in the intestine of CD patients, and neutrophils from CD patients are capable of producing higher amounts of PGP *via* MMP-9 secretion than healthy controls.[16,17] Thus, our observation of significant correlation between serum MMP-9 and FC is not surprising considering that MMP-9 is normally stored, poised for rapid release, in neutrophil granules, which are also the predominant source of calprotectin.[37]

Our results are somewhat in line with recent results demonstrating a correlation between fecal MMP-9 and FC, and higher precision for fecal MMP-9 compared with FC in detecting endoscopic ulcerations in CD patients.[24] Moreover, fecal MMP-9 was a better biomarker than FC in detecting endoscopic activity in a cohort of patients with ulcerative colitis (UC).[23] These findings were in accordance with data from Annahazi and colleagues, who found fecal MMP-9 as an accurate predictor for mucosal healing.[22] To our knowledge, except for the EMBARK study, which demonstrated the combination of FC, serum MMP-9, and serum interleukin-22 in CD patients to strongly correlate with endoscopy and imaging-defined inflammation by CTE,[26] this is the first report of significant association between serum MMP-9 and FC.

An important finding in our research is the association of baseline serum MMP-9 values with mucosal inflammation also when evaluated by VCE. To date, this study is the first to demonstrate a significant, albeit weak, correlation between serum MMP-9 and LS, which reflects the inflammatory burden in CD patients with SB involvement. This is of particular significance considering that VCE provides accurate assessment of SB inflammation, and that the persistence

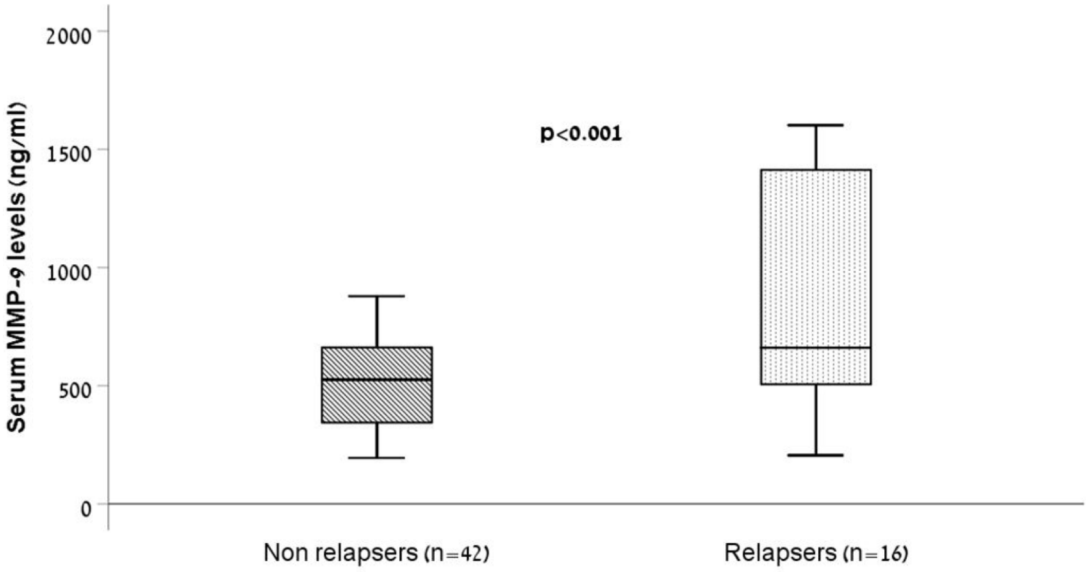

Figure 2. Box-plot representation of baseline serum MMP-9 measurements in patients with quiescent CD who subsequently flared during follow-up period of 24 months *versus* those who did not. Baseline serum MMP-9 levels in relapsers differed significantly from nonrelapsers ($p < 0.001$). The limits of the box represent the first and third quartiles; the black crossbar line represents the median.
CD, Crohn's disease; MMP-9, matrix metalloproteinase-9.

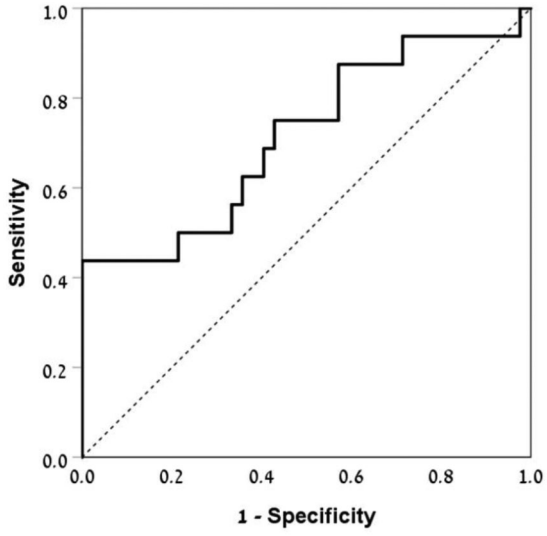

Figure 3. ROC curve illustrating the performance of baseline serum MMP-9 levels for the prediction of relapse in patients with quiescent CD over 24 months follow up. The AUC was 0.72 (95% CI 0.56–0.88, $p = 0.012$).
AUC, area under the curve; CD, Crohn's disease; CI, confidence interval; MMP-9, matrix metalloproteinase-9; ROC, receiver operating characteristic.

of subclinical mucosal inflammation may be associated with adverse outcomes in CD. As demonstrated earlier, such residual inflammation may occur in the absence of clinical activity.[4] These preliminary results are in accordance with a study showing that a reduction in the cellular expression of MMP-9 was associated with endoscopic and histologic mucosal healing.[39]

In this study, serum MMP-9 levels at presentation were significantly higher in patients receiving immunomodulators compared with those who did not. Previous report demonstrated that immunosuppressive drugs downregulate MMP-9 expression.[39] However, we do not have sera of these patients before starting immunomodulators treatment, and we cannot estimate the effect of the treatment on serum MMP-9 levels in these patients. One may postulate that this finding could be secondary to immunomodulator-treated patients having a more severe underlying disease, reflected in a trend for higher MMP-9 levels, even when symptomatically in remission. However, direct comparative studies are needed to corroborate or refute this association.

MMP-9 has been broadly explored in relation to IBD tissue damage, and, as mentioned above, was suggested as a factor in number of pathological pathways. However, although previous studies claimed that inhibition or genetic deletion of MMP-9 improved experimental colitis, the

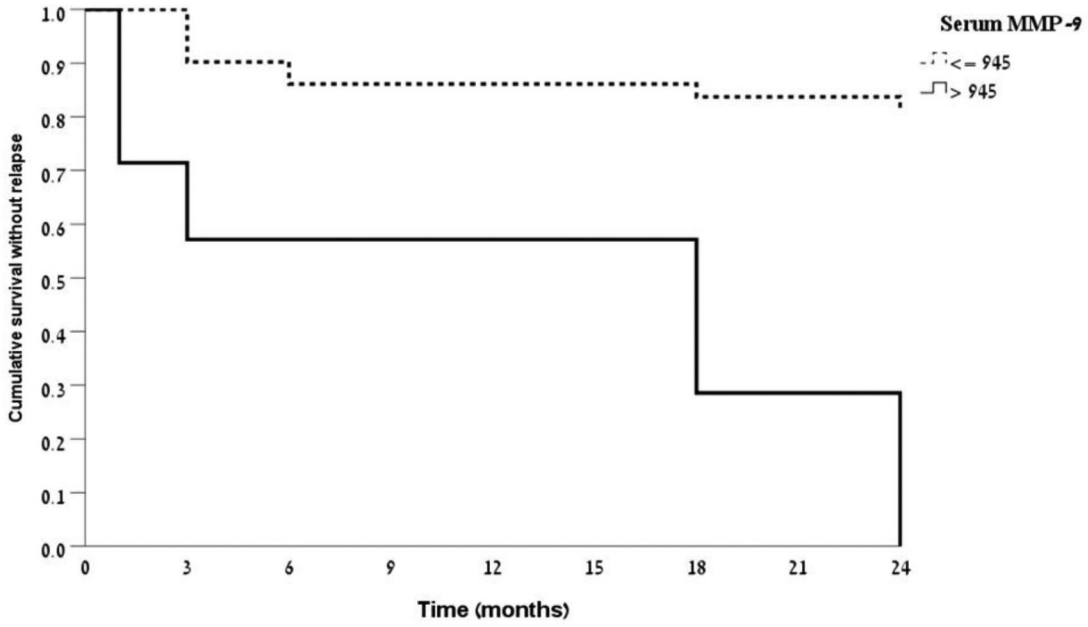

Figure 4. Kaplan–Meier analysis of survival without a flare for patients with quiescent CD and baseline serum MMP-9 levels below or above 945 ng/ml. Log rank test for equality of survivor functions, p value <0.001.
CD, Crohn's disease; MMP-9, matrix metalloproteinase-9.

causality of MMP-9 could not be confirmed in a more recent study in murine models.[40] Moreover, the therapeutic potential of anti-MMP-9 antibodies failed to show efficacy in UC and CD.[41,42]

The close correlation between MMP-9 with endoscopic and histological scores hints at the potential to predict clinical deterioration.[19,20,27,28] Several studies associated MMP-9 activity with the disruption of epithelial barrier integrity observed in patients with CD.[43,44] Impaired intestinal barrier function has been proposed to precede the onset of inflammation and exacerbation in patients with CD.[45] As such, it could be assumed that the increased baseline serum MMP-9 levels in patients with a future flare within the follow-up period may reflect molecular events within the ECM that precede tissue damage associated with CD and lead to clinical exacerbation. However, the molecular events were not explored in the present study, and will need to be further defined by future studies. The predictive ability of serum MMP-9 was comparable to the results of FC to predict relapse in several previously published prospective studies, although some of these studies showed conflicting results and most of them assessed only short-term relapse.[46,47] This points to the need for further efforts in the search for noninvasive markers for disease activity. In this context, the advantages of a blood marker over a fecal marker, with respect to patient and caregiver convenience, have to be acknowledged. Collecting stools may be an obstacle for the patient, and a blood sample may be preferable for routine practice.[48]

Limitations of this study include the relatively modest cohort size, which is nonetheless among the largest CD cohorts ever to undergo comprehensive prospective follow up including serial MREs and VCEs. Only patients who had stable disease for 3–24 months prior to inclusion entered the study. Thus, whether the results can be extended to CD patients with longer remission period remains to be proven. Another limitation of the current *post hoc* analysis is the lack of zymography analysis of the serum samples. Zymography may reveal, individually, the activated forms and degradation products of MMP-9, whereas with ELISA, these molecules are measured in totality.

Finally, serum MMP-9 was measured only at baseline and not at different time points, and analysis of its possible correlation with intestinal fibrosis, was not assessed, given that intestinal strictures were exclusion criteria for this cohort. Thus, further studies are needed to assess if the

change in MMP-9 levels over time could better predict future relapse, and to investigate the correlation of MMP-9 with intestinal wall fibrosis, given its role in ECM remodeling.

In conclusion, elevation of serum MMP-9 precedes a clinical relapse, and may be a useful marker for predicting flare in patients with quiescent CD. These current findings extend previous observations and highlight that serum MMP-9 constitutes an important inflammatory marker for CD. Further studies are warranted to confirm these findings and to determine the role of serum MMP-9 as a prognostic and surrogate biomarker in CD.

Author contributions

Guarantor of the article: Shomron Ben Horin

DY, study design, data collection and analysis, manuscript drafting; UK, AL, DBS, SN, NL, EF, BA, IL, LS, NZO, OP, MY, - data collection and analysis, reviewed the manuscript for important scientific content; EY, MMA, reading of imaging data, reviewed the manuscript for important scientific content; SBH, study conception, initiation and design, reviewed the manuscript for important scientific content; RE, study conception, initiation and design, reading of the capsule studies, reviewed the manuscript for important scientific content. All authors reviewed and approved the final version of the manuscript

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Conflict of interest statement

Doron Yablecovitch, none to declare; Uri Kopylov, Speaker fees/advisory Janssen Medtronic Abvvie Takeda MSD; Research support, Janssen Takeda Medtronic; Adi Lahat, none to declare; Michal M. Amitai, none to declare; Eyal Klang, none to declare; Dana Ben-Ami Shor, none to declare; Sandra Neuman, none to declare; Nina Levhar, none to declare; Ella Fudim, none to declare; Benjamin Avidan, none to declare; Limor Selinger, none to declare; Noam Zingboim-Orbach, none to declare; Orit Picard, none to declare; Miri Yavzori, none to declare; Rami Eliakim, Consultation/lecture fee Medtronic/Given Imaging, consultant fee from Abbvie and Takeda; Shomron Ben Horin, Research support or consultancy/Advisory board fees from MSD, AbbVie, Janssen, CellTrion, Pfizer, GSK, and Takeda

ORCID iDs

Doron Yablecovitch https://orcid.org/0000-0001-6191-9270

Uri Kopylov https://orcid.org/0000-0002-7156-0588

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