Journal of Crohn's and Colitis (2014) 8, 702–707

Available online at www.sciencedirect.com

ScienceDirect

SHORT REPORT

Pre-clinical Crohn's disease: Diagnosis, treatment and six year follow-up

Dario Sorrentino [a,c,*], Claudio Avellini [b], Marco Geraci [d], Salvatore Vadalà [a]

[a] Chair of Gastroenterology, Department of Clinical and Experimental Medical Sciences, University of Udine School of Medicine, Udine, Italy
[b] Chair of Pathology, University of Udine School of Medicine, Udine, Italy
[c] IBD Center, Division of Gastroenterology, Virginia Tech—Carilion School of Medicine, Roanoke, VA, USA
[d] MRC Centre of Epidemiology for Child Health, UCL Institute of Child Health, London, UK

Received 9 October 2013; received in revised form 27 November 2013; accepted 13 December 2013

KEYWORDS
Pre-clinical Crohn's disease;
Early diagnosis;
Natural history;
Infliximab;
T-regs

Abstract

Diagnosis of Crohn's disease is usually made at a symptomatic stage. However diagnosis at a pre-clinical stage might provide valuable information on etiology/pathogenesis and allow early intervention to alter its natural history. We describe here the case of a 27 year old woman who was diagnosed with Crohn's disease at a completely asymptomatic stage and followed up for more than six years. She was part of an ongoing screening study in first degree relatives of Crohn's disease patients. At diagnosis, colonoscopy showed modest inflammation and few superficial ulcerations and erosions in the ileo-cecal valve and the terminal ileum. Fecal calprotectin was only modestly elevated. Intestinal permeability was also increased. During follow-up and while still asymptomatic the patient was sequentially treated with therapeutic doses of 5-ASA, budesonide, azathioprine and infliximab in an attempt to stop disease progression. Only infliximab appeared capable of inducing profound mucosal healing—however the disease recurred several months after the medication was ceased. Over time, quantification by immunohistochemistry of a number of cell types and cytokines revealed a positive correlation between CD4-CD25-FOXP3 (Treg) cell number and inflammation, a finding potentially consistent with tissue resistance to Tregs' activity.

1. Introduction

Diagnosis of Crohn's disease (CD) is invariably made at an advanced, symptomatic stage.[1] Yet diagnosis of preclinical CD may shed light on the early pathogenetic features of CD. In addition, disease course prediction may impact on treatment/disease prevention.[2] Screening programs for CD in the general population are essentially considered unfeasible.[3] However, first degree relatives (FDRs) of CD patients could be tested for the purpose with a relatively high yield.[4] Since *symptomatic* CD is localized in the colon, ileocolon or terminal ileum in ≥90% of patients[5] by inference the disease must originate in sites which can be reached by the

* Corresponding author at: IBD Center—Division of Gastroenterology, Virginia-Tech Carilion School of Medicine, 3 Riverside Circle, Roanoke, VA 24016, USA. Tel.: +1 540 526 1053; fax: +1 540 983 8242.
E-mail address: drsorrentino@carilionclinic.org (D. Sorrentino).

1873-9946/$ - see front matter © 2013 European Crohn's and Colitis Organisation. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.crohns.2013.12.008

ileocolonoscope in the vast majority of cases. Based on these premises we have initiated such screening study several years ago. Among the screened individuals we identified and diagnosed with early CD a 27 year old woman who was then carefully followed for several years with endoscopy, blood and fecal tests. In addition, intestinal tissue samples were analyzed over time for a number of cell types and cytokines believed to play a role in CD pathogenesis.

2. Material and methods

2.1. Endoscopy

Endoscopy and biopsies were performed under light sedation with standard Olympus (Segrate—Italy) or Pentax (Milano—Italy) equipment. During each colonoscopy the ileum was intubated and explored for at least 25 cm.

Endoscopic damage was scored using a simplified Crohn's Disease Endoscopic Index of Severity (CDEIS)[6]: a score of 1 was given for each ulceration; for ulcers larger than 2 mm such scores were multiplied by 2; for deep (as opposed to superficial) ulcers the score was further multiplied by 2; for any number of erosions or in the presence of edema or hyperemia 0.5 points were added to the total score. Endoscopic lesions were scored in the ileocecal valve and terminal ileum by review of the film of the colonoscopy the day after the examination by an independent investigator who was unaware of the patient treatment. Each time, pictures were taken at the same anatomical location.

2.2. Histology and immunohistochemistry

Histological scoring was based on methods previously described[7,8] which include epithelial damage, architectural changes, infiltration of mononuclear and polymorphonuclear cells in lamina propria, polymorphonuclear cells in surface epithelium, presence of ulcer and granuloma and proportion of abnormal biopsies.

Tissue was taken in the immediate vicinity of lesions (erosions and ulcerations) in diseased areas and preserved in formalin for H&E staining and immunohistochemistry. An antibody panel was used to measure the following cytokines, cell surface markers and chemokine receptors: IL1B, IL4, IL6, IL10, IL12, IL17, IL22, IL23N, IL23p19, TNF-α, TGF-β, IFN-γ, CD4, FOXP3, CCR4, CCR6 (Abcam, Prodotti Gianni, Milan-Italy), IL21 (Acris Antibodies, Space Imports Milan-Italy) and CD25 (Imgenex, San Diego, CA, USA), at time 0, 18, 30, 38, 50, 57, and 73 months. CD4, CD25 and FOXp3 are markers of Treg cells[9] and CD4-CCR4-CCR6 are expressed in Th17 cells.[10]

From formalin fixed and paraffin embedded biopsies, appropriate 4 μm thick sections were immunostained using antigen retrieval.[11] Immunostaining was accomplished in triplicate on a Dako Autostainer Link 48, using Envision + Detection (Dako, Carpinteria, CA, USA). The slides were examined by a single pathologist (C.A.). After low power scanning to identify the distribution of the inflammatory cells, the whole specimen in each slide was examined at high power view (40×), on a minimum of 8 fields, evaluating the immunochemical reactivity as absolute number of reactive cells per mm^2. Two different biopsy specimens (ileal and colonic mucosa) from patients with normal ileocolonoscopy were used as negative controls for each patient's specimen. The slides were treated as described above.

2.3. Fecal calprotectin

Fecal calprotectin (FC) was measured by a commercially available ELISA test (Calprest, Eurospital, Trieste—Italy) after protein extraction on a weighted stool sample a few days before bowel preparation for colonoscopy.

2.4. Permeability test

Lactulose and mannitol (10 and 5 g respectively) were dissolved in 50 mL of water and administered after an overnight fast. Over the following 6 h urines were collected in plastic containers with chlorhexidine (1 mL of a 2% solution) added as preservative. The urine volume was measured and an aliquot stored at −20 °C until analysis. Sugars were measured in the urine after sample preparation using standard sugar solutions by HPLC as described by Marsilio et al.[12] The results are given as the percentage of orally administered quantity and expressed as lactulose/mannitol (L/M) ratio. The normal threshold was set at 0.025, mean value of the control population + 2 SD.

2.5. Statistical analysis

Two correlation analyses were performed using the endoscopic score, the histologic score, FC and the above cell types and cytokines. Firstly, a functional principal component analysis (fPCA)[13] was performed in order to reduce the dimensionality of the data to a smaller number of factors (principal components) while including the temporal dimension. Hence, both cross-correlation and temporal autocorrelation are accounted for by fPCA. A smoothing spline (B-spline of order 4) was used for modeling the nonparametric relationship $y = f(t)$, where y is the variable of interest, t denotes time (t = 0, 18, 30, 38, 50, 57, 73 months) and f is a nonparametric function. For comparison purposes, data were standardized on a scale from 0 (min) to 1 (max).

Secondly, the pairwise Pearson's correlation coefficient and its p-value were calculated.

3. Case report

D.M. is the 27 year old sister of a CD patient. As a part of a larger study – approved by the University of Udine Ethical Committee – we proposed to screen her for CD with ileocolonoscopy and other tests as needed. She was asymptomatic and denied alcohol, NSAID's or other medication use, recent traveling or infections, pregnancy and exposure to tuberculosis. Neoplasia, celiac, rheumatologic, endocrine, cardiovascular, lung, liver, renal, ocular, skin and genital diseases and food allergies/intolerance were also excluded.

Ileocecal valve and terminal ileum images (taken at the same location) as well as valve histology are shown in Fig. 1. The initial exam (Fig. 1A) showed a hyperemic valve with 2 small superficial ulcerations. The terminal ileum also showed hyperemia and few erosions. Histology showed a focal