

Early histological findings may predict the clinical phenotype in Crohn's colitis

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Abstract

Background and aims: Predicting the clinical course of Crohn's disease (CD) is relevant for treatment selection. Currently, such diagnostic tools are lacking. In a previous pilot study, morphometric tissue image analysis showed promise in predicting the clinical phenotype and need for surgery. In this study, we aimed to validate our previous results on a larger cohort.

Methods: Colonic biopsies from CD patients with colonic or ileocolonic disease and at least five years of post-biopsy clinical follow-up were analyzed. The results were used to predict post-biopsy clinical phenotypes and outcomes. Data analysis was performed using multivariate regression models, discriminant score (DS) computations and Neural Network (NNET).

Results: Multivariate analysis of morphometric variables differentiated between B1 and B2 phenotypes (sensitivity 81%, specificity 74%, accuracy on cross-validation 75%; area under the curve (AUC) of 0.74 (CI 0.6–0.84; NNET model sensitivity 87%, specificity 67% on the testing population)). Differentiation between B1 and B3 phenotypes was also possible (sensitivity 69%, specificity 76%, accuracy 70.5% on cross-validation; AUC 0.78 (CI 0.68–0.89); NNET model sensitivity 78%, specificity 77% on the testing population)). Differentiating between B2 and B3 phenotypes was not possible using morphometric variables. Multivariate analysis predicted surgery (sensitivity 67%, specificity 72.5%, accuracy 69%; AUC 0.72 (CI 0.61–0.82); NNET model sensitivity 80%, specificity 91% on the testing population)).

Conclusions: This study validates previous results and suggests that morphometric image analysis of early biopsies from Crohn's colitis patients may contribute to the prediction of future outcomes such as clinical phenotype and surgery. Prospective validation on larger cohorts is still needed.

Keywords

Inflammatory bowel disease, histology, morphometry, image analysis, phenotype prediction, Crohn's disease

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Introduction

Crohn's disease (CD) is characterized by chronic inflammation of the gastrointestinal tract. The inflammatory process is thought to result from an interaction between genetic background, environmental factors, the host immune system and the gut microflora.^{1,2} The clinical phenotype of CD can be classified into three main categories as previously described in detail.³ B1 phenotype implies an inflammatory type disease without stricturing or penetrating complications. B2 phenotype is dominated by luminal stricture formation and B3 phenotype by penetrating complication such as fistulas and abscesses. Disease course and phenotype may change over the years and up to 80% of patients may develop disease complications.^{4,5}

Histopathological findings are important tools in the diagnosis of CD. No findings are pathognomonic; however, some are considered highly suggestive when coupled with a likely clinical scenario, endoscopic findings and imaging results.⁶

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Current management of CD may include the use of early aggressive therapy with immunosuppressive, biological agents or a combination of both. This strategy may offer advantages by disease course modification,^{7–10} but entails significant short- and long-term complications such as increased risk of infections and malignancy.^{11–13} Balancing efficacy and risks is a major therapeutic challenge, which can be significantly assisted by the ability to predict disease course. Previously, multiple clinical, serological and genetic characteristics have been shown to correlate with disease phenotype and complications.^{14–22} However, despite these observations, to date, prediction of disease course and complications is suboptimal and presents a major medical need.

Morphometry is a quantitative field that investigates changes in shape, size, architectural complexity and orientation of objects. Several methods for extraction of morphological parameters from an object exist. These include length, angles, perimeter shape and distribution in the space. Architectural complexities are typically analyzed by fractal and lacunar geometry algorithms.²³ Morphometry has been used to predict lymph node metastasis, tumor progression and patient survival in squamous cell carcinoma of the vulva and renal cell carcinoma, and in the assessment of dysplasia and progression to adenocarcinoma in Barrett's esophagus.^{23–26}

In a previous pilot study, we were able to show that morphometric analysis may contribute to the prediction of the clinical phenotype and surgery in patients with Crohn's colitis.²⁷ In the present study, we sought to further evaluate and validate the histomorphometric features of early colonic biopsies from patients with Crohn's colitis and their relationship to evolving clinical phenotypes.

Methods

Patients

CD patients with colonic involvement were selected from our database according to clinical phenotypes as outlined in the Montreal classification.³ Patients had either isolated colonic disease or a combination of small-bowel and colonic disease. We analyzed biopsies obtained at the first documented colonoscopy for each patient, which was around the time of diagnosis in the majority of cases. All patients had at least five years of post-biopsy follow-up. Clinical data were extracted from patient files and included the clinical phenotype, medical and surgical history. The clinical data were coded and subsequently analyzed and correlated with the histological findings. The observer who performed the morphometric analysis was completely blinded to the clinical variables and patient identity. The study was approved by the institutional review board.

Morphometric analysis

New slides were prepared from the original paraffin blocks. The histological stains included: hematoxylin and eosin (H&E) for diagnosis, confirmation and evaluation of the inflammatory cells type and numbers; pancytkeratin (CK) to highlight and quantify the mucosal crypts; and Mason-Tri-chrome (TC) and reticulin (RET) for the presence of mature and young collagen, respectively. Staining was performed according to standard procedures. Slides were scanned using the 2.1 Dotslide virtual microscopy system (Olympus, Germany and Japan). From each slide, three to four representative images were collected. The number of scanned fields and the magnification used were identical for all slides with each histological stain. The ImagePro plus 7.0 program (Media Cybernetics, USA) was used to analyze and quantify the collagen fibers, the inflammatory cells and the crypt architecture. The MATLAB program (MathWorks, USA) was used to analyze the fractal and lacunar dimensions and the orientation of the crypts in order to assess architectural complexity and distortions of the mucosal crypts. Figure 1 shows the morphometric analysis of different quantitative features in the colonic mucosa.

Statistical analysis

Univariate analysis was performed and morphometric variables associated with specific clinical characteristics and evolving clinical phenotype were identified. Variables identified as significant on univariate analysis were then used in a multivariate model (discriminant analysis) to adjust for confounding variables and to single out independent and significant predictors of disease phenotype. Validation of the results was obtained by using the "leave one out method." Using the independent variables and their coefficients, discriminant scores (DS) were computed and receiver operating characteristic (ROC) analysis of the DS was calculated to obtain an area under the curve (AUC). In order to better handle the complexity and the large number of variables, a neural network (NNET) model with a feed forward architecture was trained using the morphometric variables data in order to develop a mathematical classifier that best predicts the clinical phenotypes. Our model used for function fitting a two-layer feed forward network with a sigmoid transfer function both in the hidden and the output layers. The output data consisted of the patients' clinical phenotype. We used 50%–70% of the cases as the training population for our model, and 30%–50% of the cases as the testing population. Statistical analysis was performed with SPSS version 23 (SPSS Inc, Chicago, IL, USA).

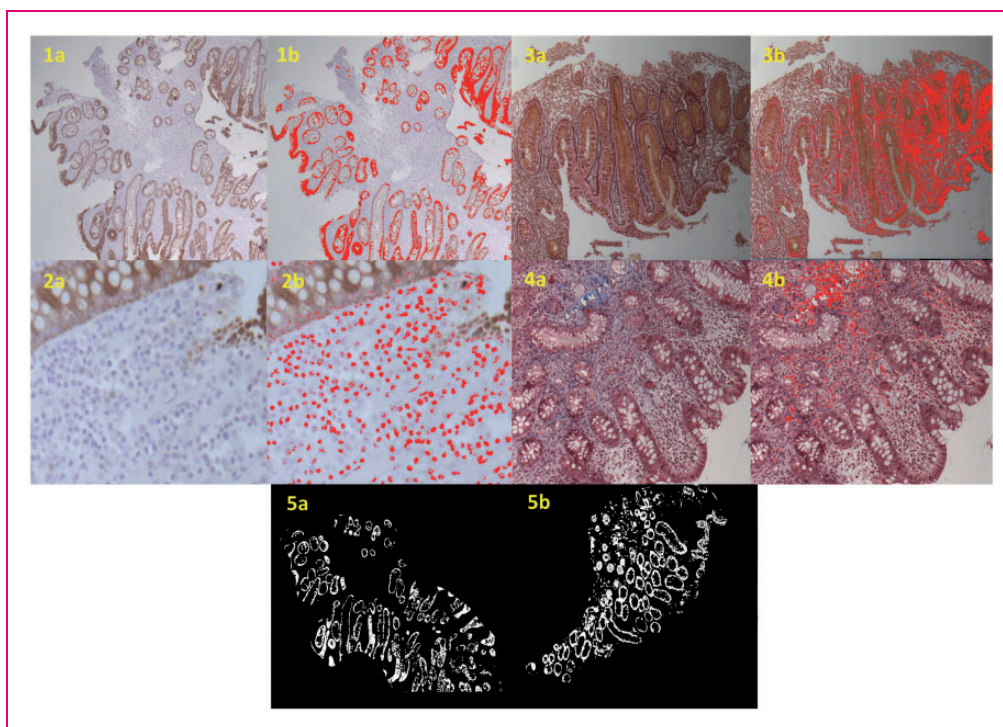


Figure 1. Examples of morphometric measurements. The left image in each pair represents the clean stained histological specimen whereas the right image displays the quantified variable during image analysis (1a + 1b: CK stain highlighting the crypts; 2a + 2b: CK stain negative highlights the inflammatory cells; 3a + 3b: reticulin stain highlighting young collagen; 4a + 4b: Mason TC stain highlighting mature collagen; 5a + 5b: image masking highlights the mucosal architecture). CK: pancytokeratin; TC: Tri-chrome.

Results

Patients

Patient characteristics are summarized in Table 1. The patient groups did not differ in average age at diagnosis, the average post-biopsy clinical follow-up or the gender. Differences were seen between groups in clinical data such as surgery, smoking and extraintestinal manifestations. Four patients in our cohort received 5 aminosalicylate (SASA) or steroid treatment prior to biopsy (within one to two years). In the rest of the cases, biopsy was performed before any CD medications were started. The time lag between the diagnosis and the first documented biopsy was available only as an ordinal (not interval) variable measured in years (not months). As a result, the calculated median disease duration at time of biopsy was 0 years (less than one year, interquartile range (IQR) 0–3).

Morphometry and its correlation to clinical variables and disease phenotypes

Univariate analysis showed multiple morphometric variables associated with specific clinical characteristics (Table 2): Smoking was associated with smaller, more

Table 1. Patient characteristics.

	Phenotype ^a			p value
	B1	B2	B3	
Age at diagnosis ^b	26.7 ± 13.9	27 ± 12.5	25.4 ± 12.6	NS
Age group ^a				
<17	12 (25%)	4 (21%)	9 (23%)	NS
17-40	24 (51%)	11 (58%)	18 (46%)	NS
>40	11 (24%)	4 (21%)	12 (31%)	NS
Mean post-biopsy follow-up (years)	11	14	13	NS
Smoking	25 (52%)	4 (22%)	12 (39%)	NS
CD extent ^a				
Colon	18 (37%)	6 (32%)	10 (33%)	NS
Ileo-colon	30 (62%)	13 (68%)	20 (66%)	NS
Peri-anal disease	8 (17%)	6 (21%)	15 (52%)	0.007
Surgery	10 (21%)	10 (29%)	19 (68%)	<0.001
Extra-intestinal manifestations	23 (48%)	3 (19%)	9 (32%)	NS

^aAccording to Montreal classification.

^bMean ± standard deviation.

CD: Crohn's disease.

complex-shaped crypts. Smoking was also associated with a lower degree of mature collagen optical density; peri-anal disease was associated with more inflammatory cells and lower degree of mature collagen optical density; the older age group (17–40) according to the Montreal classification had a smaller crypt area. Univariate analysis demonstrated an association between the number of inflammatory cells and the optical density of mature collagen and disease phenotypes (Table 3).

On multivariate analysis (Table 4) crypts and collagen optical densities were independent predictors for differentiating between B1 and B2 phenotypes (sensitivity 81%, specificity 74%, accuracy on cross-validation

75%). A ROC analysis of the discriminant score (DS) yielded an AUC of 0.74 (95% confidence interval (CI) 0.6–0.84) for this comparison. A NNET model was also able to differentiate between B1 and B2 phenotypes (sensitivity 87%, specificity 67% on the testing population). Morphometric measurements of crypts and collagen area and optical densities were found to be independent predictors for differentiating between B1 and B3 phenotypes (sensitivity 69%, specificity 76%, accuracy 70.5% on cross-validation). ROC analysis of the DS revealed an AUC of 0.78 (95% CI 0.68–0.89). A NNET model applied to this comparison yielded a sensitivity of 78%, and a specificity of 77% on the testing population. Differentiating between B2 and B3 phenotypes was not possible using morphometric variables. Multivariate analysis also predicted surgery (sensitivity 67%, specificity 72.5%, accuracy on cross-validation 69%). Morphometric measurements of collagen area were independent predictors in this analysis. ROC analysis of the DS for surgery revealed an AUC of 0.72 (95% CI 0.61–0.82). A NNET model predicted surgery with a sensitivity of 80% and a specificity of 91% on the testing population. Figures 2–4 show a

Table 2. Association between morphometric variables and specific clinical characteristics of the cohort.

Morphometric variable		Mean	STD	p value
Smoking status				
Relative area occupied by crypts	No	0.178	0.08	0.023
	Yes	0.140	0.07	
Mean complexity of crypts	No	0.386	0.07	0.049
	Yes	0.36	0.05	
Minimal optical density (Tri-chrome)	No	104.2	23.5	0.038
	Yes	94.8	19.3	
Peri-anal disease				
Inflammatory cells per mm ²	No	4994	2947	0.046
	Yes	6542	4323	
Optical density (Tri-chrome)	No	12.4	5.7	0.041
	Yes	9.8	5.2	
Age group				
Overall crypt area	<17 years	964455	1181175	0.04
	17–40 years	481326	467595	

STD: standard deviation.

Table 4. Independent predictors found in multivariate analysis for distinguishing between disease phenotypes and the need for surgery.

Table 4	Variable	p value
B1 versus B2	Mean percentage crypt area	0.009
	Optical density reticulín	0.002
	Mason TC optical density	<0.001
B1 versus B3	Mean percentage crypt area	0.002
	Mason TC optical density	0.001
Surgery	Crypt area	0.003
	Maximal area of TC	0.021

TC: Mason Tri-chrome.

Table 3. Univariate analysis of the correlation between morphometric variables and the evolving clinical phenotypes.

Morphometric variable	B1 phenotype	B2 phenotype	B3 phenotype	p value (ANOVA)	p value (Bonferroni)
Minimum number of inflammatory cell per mm ²	3342 ± 1782 95% CI (2824–3859)	3709 ± 2330 95% CI (2586–4832)	4653 ± 2960 95% CI (3567–5739)	0.005	0.047 (B1 VS B3)
Mean overall optical density, Tri-chrome	100 ± 25 95% CI (92–107)	108 ± 24 95% CI (96–120)	115 ± 24 95% CI (106–124)	0.02	0.02 (B1 VS B3)
Mean optical density (Red), Tri-chrome	129 ± 26 95% CI (121–136)	132 ± 29 95% CI (118–145)	144 ± 27 95% CI (135–153)	0.04	0.04 (B1 VS B3)
Minimal overall optical density, Tri-chrome	85 ± 23 95% CI (78–88)	92 ± 22 95% CI (81–102)	99 ± 22 95% CI (91–107)	0.03	0.03 (B1 VS B3)

Values are mean ± standard deviation.

ANOVA: analysis of variance; CI: confidence interval.

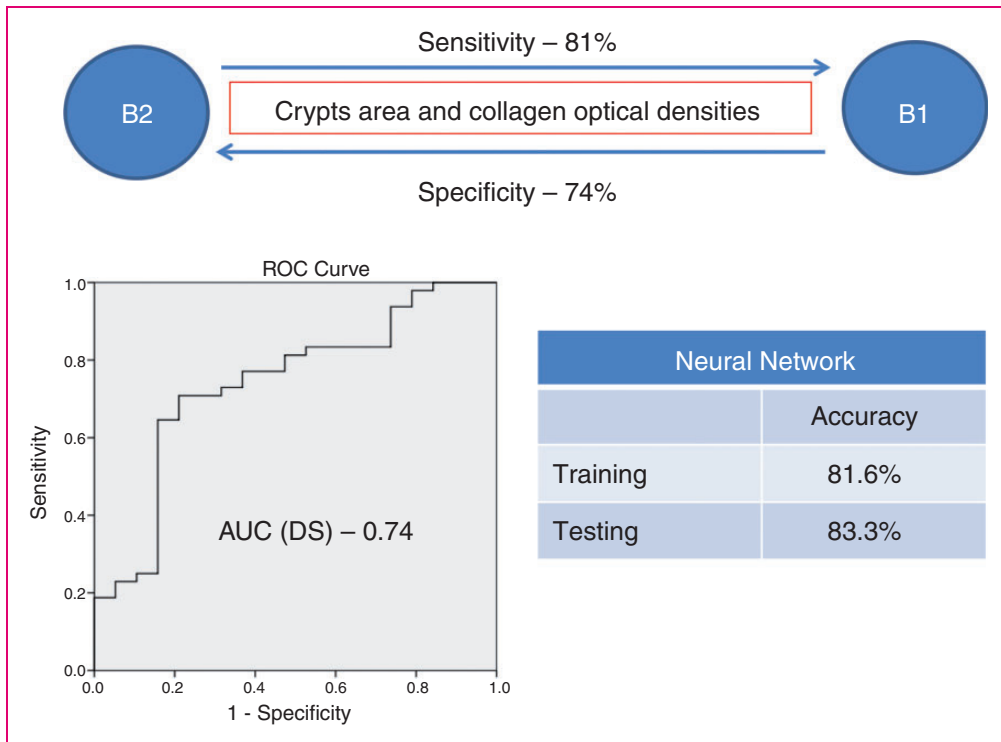


Figure 2. A schematic presentation of multivariate analysis and NNET model for predicting between B1 and B2 phenotypes. NNET: neural network; AUC: area under the curve; DS: discriminant score.

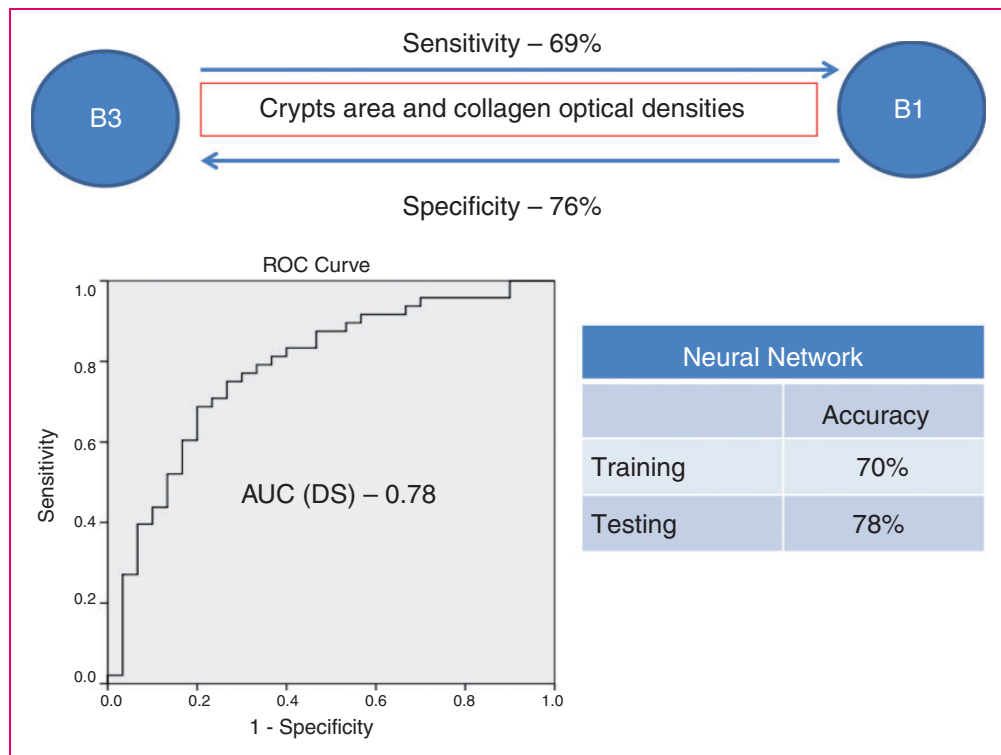


Figure 3. A schematic presentation of multivariate analysis and NNET model for predicting between B1 and B3 phenotypes. NNET: neural network; AUC: area under the curve; DS: discriminant score.

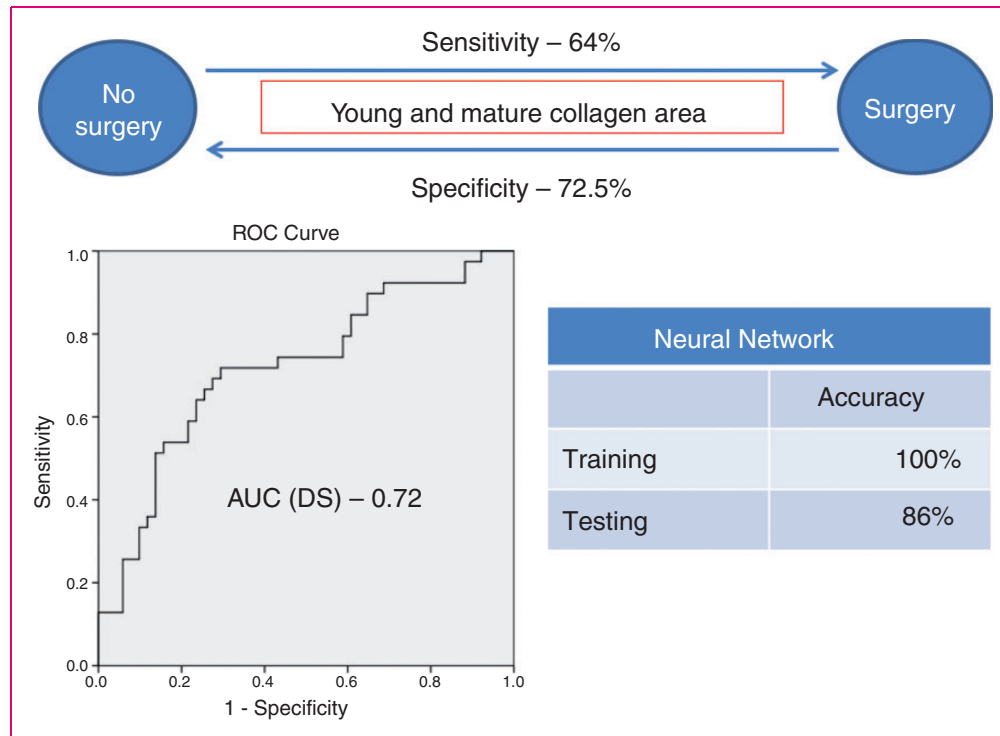


Figure 4. A schematic presentation of multivariate analysis and NNET model for predicting the need for surgery. NNET: neural network; AUC: area under the curve; DS: discriminant score.

schematic representation of the multivariate analysis between the clinical phenotypes.

Discussion

The present study describes the use of morphometric analysis for quantitative measurements of various elements in endoscopic colonic biopsies in order to evaluate their associations and potential role in predicting disease course and complications.

We found an association between smoking and crypt size and complexity and level of collagen optical density. This observation implies that smoking may have an effect on tissue inflammation and fibrosis early in the disease course. An association was also seen between the presence of peri-anal disease and the amount of inflammatory cells and levels of collagen optical density. This is very interesting as both smoking and peri-anal disease have been shown to be associated with more severe disease and inflammation.^{21,22} Further research into this area and the potential molecular mechanisms behind these phenomena is needed.

Currently, prediction of disease phenotype is suboptimal. Genetic factors such as *NOD2* mutations are associated with a fibro-stenosing phenotype and an increased need for surgery.¹⁴ However, genetic variables, naturally available upon diagnosis, are useful in

only approximately one-third of patients, and are even less relevant in certain geographical areas. A prominent example of this limitation is the low prevalence of *NOD2/CARD15* mutations in the eastern Asian CD patient population as compared to Western populations.²⁸ Serology may be an additional useful tool. Antibodies to anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-CBIRL (anti-flagelin) are associated with small-bowel and penetrating disease, respectively.^{15–17} Antibodies to perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) correlate with an ulcerative colitis (UC)-like disease.¹⁸ Furthermore, multiple positive serologies are associated with propensity for disease complications.^{19,20} However, although serological findings may be present at least to some extent at diagnosis, only limited data are available regarding the association of serology at early disease stages and late outcomes.^{29,30} Clinical characteristics such as cigarette smoking, early use of steroid and the presence of peri-anal disease have also been shown to be suggestive of a complicated disease course.^{21,22} However, despite these observations, to date, prediction of disease course and complications is suboptimal and presents a major medical need. In this study, several morphometric variables emerged as significant for prediction of disease phenotype and the need for surgery in the multivariate analysis and NNET model

(crypts and collagen optical densities for comparison between B1 and B2 phenotypes; crypts and collagen area and optical densities for comparison between B1 and B3 phenotypes; collagen area for prediction of surgery). Using two different mathematical models provided an adequate validation of the potential predictive ability of morphometry in this cohort. These observations, in combination with known predictors, may enhance our ability to predict disease course and complications.

We recognize this study's limitations: Firstly, this was a retrospective study of a small group of patients. A small number of patients in our cohort (four) received 5ASA or steroid therapy prior to biopsy, which theoretically could have influenced the histological findings. However, since this is a minority, we believe it did not significantly influence the average result of the different variable for each phenotype group. Validation of our results using additional, larger blinded cohorts and eventually in a bona fide prospective manner is needed. Secondly, we examined biopsies from patient with colonic CD involvement and did not include small-bowel biopsies. In addition, some patients had isolated colonic disease whereas others within our cohort had a combination of colonic and small-bowel disease, which may already confer a different clinical phenotype. We did not have detailed information regarding patients' past complications (i.e. stricture location and extent, or nature of penetrating complications). However, since complicated disease can occur both in the small bowel and the colon, we felt these patients could be grouped into one cohort. Patients with isolated colonic disease represent only 25%–50% of CD patients, and further studies are needed in order to characterize and quantify the histological findings in other gastrointestinal segments in patients with combined small-bowel and colonic disease and in patients with isolated small-bowel disease.

In conclusion, this study validates our preliminary results that quantitative morphometric data may enable differentiation and prediction of clinical phenotypes and outcomes such as surgery. Because histological sampling is almost universally used in the workup of CD patients, this novel approach may be combined with other biologic variables and clinical predictors to significantly increase the ability to classify and predict the clinical course of CD colitis patients, thus improving their management. Prospective validation on larger cohorts is still needed.

Declaration of conflicting interests

Yehuda Chowers is a member of the Rambam Committee of Interns and Residents (CIR) and has served as a consultant and received lecture fees from Abbott, Schering Plough, Janssen and Takeda.

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