Value of counting colonic mucosal Ig-containing cells in the differential diagnosis of chronic inflammatory bowel disease

C A Seldenrijk, S G M Meuwissen, N W Schipper, B C Morson, J Lindeman, C J L M Meijer

Abstract

Aims: To investigate whether counting cells containing immunoglobulin (Ig) subclass in colonic biopsy specimens of patients with chronic inflammatory bowel disease, in addition to conventional histological evaluation, can improve the differentiation of patients with Crohn's disease from those with ulcerative colitis.

Methods: The colonic and rectal biopsy specimens of 40 patients with chronic inflammatory bowel disease, comprising 20 patients with Crohn's disease and 20 with ulcerative colitis, were used and sections were stained specifically for IgA, IgM, and IgG heavy chains using an indirect immune peroxidase method. The immunoglobulin subclass containing cells were counted using an ocular grid counting method in a light microscope. A linear stepwise discriminant analysis was performed on Ig subclass containing cell counts in combination with 16 reproducible histological features. The results of this discriminant analysis were compared with the results of the discriminant analyses in which only the histological features were used.

Results: Applying stepwise discriminant analysis, two histological features (an excess of histiocytes in the lamina propria and the villous or irregular aspect of the mucosal surface) in combination with IgMmax were selected as the most discriminatory parameters that distinguish Crohn's disease from ulcerative colitis. IgMmax was defined as the maximum value of the mean percentage of IgM containing cells over all the biopsy locations. The use of this combination resulted in a better classification in 20% of the patients with Crohn's disease and in 9% of the patients with ulcerative colitis compared with the use of histological features alone.

Conclusions: Morphometric enumeration of Ig subclass containing cells in colonic mucosal biopsy specimens has diagnostic value as a means of differentiating individual patients with Crohn's disease from those with ulcerative colitis.

Differentiation of chronic inflammatory bowel disease (CIBD) in Crohn's disease and ulcerative colitis is important from the clinical and surgical point of view.^{1,2} Although the biopsy features of acute self limiting colitis and CIBD have been well described and criteria for distinguishing between them have been validated statistically,³ studies on the Crohn's disease features that distinguish from ulcerative colitis are scarce.⁴⁻⁷

In a previous study⁸ we determined which histological features in colonic biopsy specimens are reproducible. Moreover, using multivariate discriminant analysis, we selected the combination of excess histiocytes in the lamina propria, the aspect of the mucosal surface, and the presence of granulomas as the most useful features for making a reliable distinction between Crohn's disease and ulcerative colitis. With these features 70% of those with Crohn's disease and 75% of those patients with ulcerative colitis were correctly classified with a high a posteriori probability (>0.85). The false positive rate was 10% for Crohn's disease and 4% for ulcerative colitis.⁸

Several studies have shown differences between the number of immunoglobulin (Ig) containing cells in the lamina propria of colonic and rectal biopsy specimens of patients with Crohn's disease and those with ulcerative colitis.⁹⁻¹⁵ IgM containing cells tend to be increased in colonic biopsy specimens from patients with Crohn's disease compared with those with ulcerative colitis,⁹⁻¹¹ and this finding has subsequently been confirmed in surgical colonic resection specimens.^{15,16} These studies, however, are concerned only with differences in Ig subclass bearing cells in groups of patients with ulcerative colitis or Crohn's disease. The predictive value of counts of Ig containing cells in undiagnosed patients to distinguish Crohn's disease from ulcerative colitis has not been elucidated in these studies. To our knowledge, only Jenkins *et al*^{15,16} investigated whether Ig cell counting in addition to histological features was useful to differentiate between Crohn's disease and ulcerative colitis in individual patients, but their results were controversial. The aim of the present study was to determine whether counting Ig containing cells in colonic mucosal biopsy specimens of clinically well defined patients in addition to conventional

Department of Pathology, Free University Hospital of Amsterdam
C A Seldenrijk
N W Schipper
C J L M Meijer

Department of Gastroenterology, Free University Hospital of Amsterdam
S G M Meuwissen

Department of Pathology, St Mark's Hospital, London
B C Morson

Department of Pathology, The Slotervaart Hospital, Amsterdam
J Lindeman

Correspondence to:
C A Seldenrijk, Department of Pathology SSDZ, Reinier de Graafweg 7, 2625 AD Delft, The Netherlands

Accepted for publication 5 September 1991

histological evaluation⁸ could improve the differentiation of individual patients with Crohn's disease from those with ulcerative colitis.

Methods

The colonic and rectal biopsy specimens of 40 patients with CIBD, comprising 20 with Crohn's disease (seven men, 13 women; mean age 31.7 years, range 21–62 years) and 20 with ulcerative colitis (13 men, seven women; mean age 37 years, range 15–70 years) were used in this immunohistochemical study. All these clinically defined patients had a colonoscopy or sigmoidoscopy and multiple biopsy specimens were taken, usually from the descending colon, sigmoid, and rectum, and if possible from transverse and ascending colon (table 1). For further clinical information the reader is referred to our previous study.⁸

At least three specimens were taken from each site; therefore, usually nine biopsy specimens per patient were studied, and from some, nine to 15 biopsy specimens. Biopsy specimens were taken from inflamed mucosa (when present), and not from ulcer margins or ulcer debris.

BIOPSY PROCESSING

As well as routinely (haematoxylin and eosin) stained sections, three consecutive sections were cut at 4 µm and stained specifically for IgA, IgM, and IgG heavy chains using an indirect immunoperoxidase method.¹⁷ In summary, immunoglobulin containing cells were shown using an indirect immunoperoxidase technique with rabbit antisera against human α (1 in 400), µ (1 in 400), and γ (1 in 400) heavy chains (Dakopatts) as first layer and peroxidase labelled mouse anti-rabbit immunoglobulin as second layer. Visualisation was done using 3.3 diaminobenzidine as substrate.

Appropriate controls were done according to the method of Sternberger.¹⁸ As a negative control, one section of each biopsy specimen was stained with a non-immune rabbit immunoglobulin fraction (Dakopatts) at the same dilution as the specific antiserum.

COUNTS OF Ig CONTAINING CELLS

This was done according to a new technique with high reproducibility that we recently developed.¹⁹ In brief Ig containing cells were counted with a light microscope equipped with an ocular counting grid containing 100 squares. The optical parameters were adjusted in such a way (magnification factor 200) that one square of the counting grid corresponded to 0.0025 mm² (the area of one square). The area of the lamina propria was calculated by multiplying the numbers of squares covering the lamina propria by 0.0025 mm². A mucosal field was selected in sections of each of the three biopsy specimens from each site with intact full thickness mucosa; this field was delineated by the muscularis mucosae, the surface epithelium, and the lateral boundaries of the ocular grid (fig 1). The selection of the mucosal field

was made on the subjective estimation that the field contained the largest number of Ig subclass cells in that biopsy specimen. The Ig subclass containing cells were counted in at least one field of each of the three biopsy specimens from each site. At each biopsy location the mean percentage of IgM, IgA, and IgG containing cells was calculated. The cell counts were expressed as percentages of the total number of Ig subclass containing cells per mm² of lamina propria. To stabilise the variation in the running mean of counts of Ig subclass containing cells per biopsy location at least 600 Ig containing cells had to be counted, as described in our previous study.¹⁹ Therefore, if after counting of the three mucosal fields the total number of Ig containing cell counts was less than 600, consecutive fields in each biopsy specimen were selected and Ig containing cells were counted until a total number of 600 Ig containing cells were counted per biopsy location. The reproducibility of this counting method is sufficient to detect differences in the mean counts of IgA and IgG containing cells of more than 10%, whereas the differences in the mean counts of IgM must exceed 5%.¹⁹

According to this protocol several parameters were recorded:

- 1 At each biopsy location, the mean percentage of IgM, IgA, and IgG containing cells in the three biopsy specimens (expressed as percentages of the total number of cells per mm² of lamina propria) was defined as:

$$\text{IgX}_i = \frac{\text{IgX}_{\text{biopsy1}} + \text{IgX}_{\text{biopsy2}} + \text{IgX}_{\text{biopsy3}}}{3} \quad (= \text{number of biopsy specimens})$$

X = counts of IgM, IgA, or IgG containing cells.

This calculation may be the result of IgM_{rectum}, IgA_{rectum}, IgG_{rectum}, IgM_{sigmoid}, etc.

- 2 In each patient the mean percentage IgX (X = IgM, IgA, or IgG) containing cells from all biopsy locations was defined as:

the sum of

$$\Sigma \text{IgX}_r + \text{IgX}_s + \text{IgX}_{dc} + \text{IgX}_{tc} + \text{IgX}_{ac}$$

$$\text{IgX} = \frac{\text{sum of IgX values}}{\text{number of biopsy locations}}$$

r = rectum; s = sigmoid; dc, tc, and ac = descending, transverse and ascending colon, respectively.

Figure 1 Full thickness mucosal field of a colonic biopsy specimen with an ocular grid of 100 counting points.

Table 1 Univariate analysis of mean percentage of Ig containing cells per biopsy location

Location	Ig type	Crohn's disease		Ulcerative colitis		p Values for Crohn's disease/ Ulcerative colitis
		n = **	Mean % Ig*	n = **	Mean % Ig	
Rectum	IgM	16	13	16	9	0.1697
	IgG		22		25	0.3381
	IgA		65		66	1.0000
Sigmoid colon	IgM	15	14	17	7	0.0058
	IgG		22		26	0.2717
	IgA		64		66	0.8110
Descending colon	IgM	14	17	14	7	0.0001
	IgG		26		21	0.1108
	IgA		57		72	0.0113
Transverse colon	IgM	10	15	4	10	0.1178
	IgG		22		25	0.2278
	IgA		63		65	0.7228
Ascending colon	IgM	5	13	3	19	0.2410
	IgG		26		26	0.5577
	IgA		31		55	0.4344

3 In each patient IgMmax was determined, defined as the maximum value of the mean percentage of IgM containing cells from all biopsy locations with the corresponding IgG (c-IgG max) and IgA (c-IgA max) of that biopsy location. For example, if the mean IgM_{rectum} (as defined under parameter 1) is 10% and the mean IgM_{sigmoid} is 20%, the IgMmax of that patient would be 20%. The c-IgG max and c-IgA max are mean percentage IgG_{sigmoid} and mean percentage IgA_{sigmoid}.

Twelve per cent of the biopsy specimens (45/387) could not be counted for several reasons, such as inadequate orientation, ulcer material only, or too much background staining. But there were sufficient biopsy specimens to include each patient in the study except for one patient with ulcerative colitis in whom the biopsy specimens of all three locations showed too much background staining; therefore a total of 19 patients with ulcerative colitis were analysed.

STATISTICAL ANALYSIS

Univariate analysis

The discriminative power of the mean IgX, mean IgX in a patient, and the IgMmax, with corresponding IgG and IgA to distinguish patients with Crohn's disease from those with ulcerative colitis was investigated through univariate analysis using the non-parametric Mann-Whitney test (Programs P3S of the BMDP statistical software package).²⁰ Results with a p value of <0.05 were regarded as significant.

Multivariate analysis

To determine whether counting of Ig containing cells in addition to histological evaluation is

useful to differentiate Crohn's disease from ulcerative colitis in patients classified as having chronic inflammatory bowel disease (n = 40) two (linear) stepwise discriminant analyses (Program P7M of the BMDP statistical software package) were carried out.²⁰ First, a stepwise discriminant analysis was performed on the mean IgX_i, mean IgX per patient, and IgMmax per patient with the corresponding IgG and IgA to differentiate chronic inflammatory bowel disease in Crohn's disease and ulcerative colitis. Second, a stepwise discriminant analysis was done on these Ig parameters in combination with 16 reproducible histological features described in a previous paper.⁸ The classification results of these two discriminant analyses were compared with the results of the discriminant analysis in which only histological features were used.⁸

Results

UNIVARIATE ANALYSIS

The results of the Ig subclass-containing cell counts are shown in table 1. In patients with Crohn's disease there was a relative increase in the mean percentage of IgM containing cells in all locations compared with patients with ulcerative colitis, except in the ascending colon. In Crohn's disease the mean percentages of IgM containing cells in the sigmoid and descending colon (14% and 17%, respectively) were significantly different (p = 0.0058 and p = 0.0001, respectively) compared with ulcerative colitis (7% and 10%, respectively).

In Crohn's disease the range of the mean percentage of IgG and IgA containing cells was 22–26% and 57–65%; in ulcerative colitis these values varied from 21–26% and 66–72%, res-

Table 2 Univariate analysis of mean Ig subclass containing cells per patient and IgMmax per patient

	n** =	Crohn's disease	n** =	Ulcerative colitis	p Values for Crohn's disease/ Ulcerative colitis
Mean IgM ⁺	20	14	19	8	0.005
Mean IgG		24		25	0.8141
Mean IgA		62		67	0.2083
IgMmax ⁺⁺	20	18	19	9	0.0000
clgG max		25		26	0.1916
clgA max		58		65	0.4804

*Mean percentage Ig containing cells per biopsy location; **number of patients in which the Ig-containing cells were counted; ⁺mean percentage of Ig subclass containing cell per patient; ⁺⁺IgMmax is the maximum value of the mean percentage of IgM containing cells found over all biopsy locations in patient; clgG max and clgA max are the corresponding values of the mean percentage of IgG and IgA containing cells in that biopsy location.

pectively. These differences were not significant (table 1).

Both the percentage of IgMmax ($p < 0.0001$) and mean IgM ($p = 0.0005$) contributed significantly to distinguishing Crohn's disease from ulcerative colitis (table 2).

MULTIVARIATE ANALYSIS

Ig counting alone

If only Ig subclass containing cell counting was used, IgMmax was selected as the strongest feature to discriminate between Crohn's disease and ulcerative colitis. The addition of other Ig subclass containing cell counts did not result in a better classification. Jackknifed classification of the analysis was performed to evaluate the discriminant function. As in our previous study⁸ values of 0.15 and 0.85 were used as cutoff points for the a posteriori probabilities. A patient was predicted to have ulcerative colitis when the ulcerative colitis a posteriori probability was larger than 0.85, and was diagnosed as having Crohn's disease when the a posteriori probability is smaller than 0.15. Indeterminate CIBD was classified when the a posteriori probability lay between or was equal to 0.15 and 0.85.

Using IgMmax as the discriminant feature eight of 20 (35%) patients with Crohn's disease and seven of 19 (37%) of those with ulcerative colitis were correctly classified (fig 2) and (table 3). Twelve out of 20 (60%) patients with Crohn's disease and 12 of 19 (63%) of those with ulcerative colitis were classified as CIBD indeterminate. No false positive diagnoses were made.

Histological features in combination with Ig subclass cell counting

When discriminant analysis was applied to Ig subclass containing cell counting together with conventional reproducible histological features, three discriminants were selected. The presence of an excess of histiocytes emerged as the most discriminant feature, followed by the aspect of the mucosal surface (normal/irregular/villous). Instead of granu-

lomas (selected as the third feature when only histopathological features were included⁹), IgMmax was selected as the third most discriminatory feature. Additional histological features or Ig subclass containing cell counts did not offer a higher diagnostic accuracy for the distinction between Crohn's disease and ulcerative colitis.

According to the jackknifed classification, the combination of these three criteria permitted an accurate diagnosis with a high a posteriori probability (> 0.85) in 18 of 20 (90%) patients with Crohn's disease and 16 of 19 (84%) with ulcerative colitis (fig 3 and table 3). Two out of 19 patients with ulcerative colitis were wrongly diagnosed as having Crohn's disease; no false positive cases of ulcerative colitis were recorded. Two out of 20 (10%) patients with Crohn's disease ($p = 0.526$ and $p = 0.423$) and one out of 19 (5%) patients with ulcerative colitis ($p = 0.283$) were classified as CIBD indeterminate.

When this combination of conventional histological features was applied with additional IgMmax counts (table 3), a higher diagnostic accuracy rate was achieved compared with the use of histological features alone (table 3). The overall classification improved by 20% (four of 20) for Crohn's disease and 12.5% (one of 19) for patients with ulcerative colitis. Consequently the number of patients diagnosed as having CIBD indeterminate decreased from 20% (8/40) to 7.5% (7/39). Even a small reduction in the number of false positive diagnoses occurred; 5% (two cases were wrongly classified) instead of 7.5% (three patients were falsely classified, two with Crohn's disease and one with ulcerative colitis) when conventional histological features with additional IgMmax counts were applied.

Discussion

In this study we have extended our previous histopathological work⁸ with an immunohistochemical evaluation of colonic mucosal biopsy specimens to investigate whether containing Ig cell counts improved the diagnostic accuracy rate of Crohn's disease and ulcerative colitis compared with the use of histopathological features alone. It is important to emphasise that before the discriminative power of these parameters to differentiate Crohn's disease from ulcerative colitis were evaluated, the reproducibility of the counting method Ig subclass containing cells¹⁹ as well as the reproducibility of the histopathological features⁸ were extensively tested. As far as we know this is the first study in which reproducible histological features in combination with Ig subclass containing cell counts were used in a discriminant analysis to determine their diagnostic value to distinguish an individual patient with Crohn's disease from a patient with ulcerative colitis.

Applying multivariate discriminant analysis to Ig counts and the reproducible histopathological features (as described in our previous paper⁸), two histological features (an excess of histiocytes and irregular or villous mucosal

Figure 2 Jackknifed classification results using IgMmax as the discriminant. The CIBD a posteriori probabilities 0.15 and 0.85 were chosen as cutoff points:

● = Crohn's disease; ○ = ulcerative colitis.

Table 3 Comparison of results of stepwise discriminant analysis with IgMmax counts alone (A), histological features + IgMmax cell counting (B), and histological features alone (C)

A Variable entered: IgMmax	Correct diagnosis	Incorrect diagnosis	CIBD indeterminate	Total
Crohn's disease	8 (35%)	0 (0%)	12 (60%)	20
Ulcerative colitis	7 (19%)	0 (0%)	12 (63%)	19
Total	15 (38%)	0 (0%)	24 (62%)	39
B Variables entered: excess of histiocytes in lamina propria/irregular or villous mucosal surface/ IgMmax				
Crohn's disease	18 (90%)	0 (0%)	2 (10%)	20
Ulcerative colitis	16 (84%)	2 (11%)	1 (5%)	19
Total	34 (87%)	2 (5%)	3 (8%)	39
C Variables entered: excess of histiocytes/irregular or villous mucosal surface/granulomas				
Crohn's disease	14 (70%)	2 (10%)	4 (19%)	20
Ulcerative colitis	15 (75%)	1 (4%)	4 (19%)	20
Total	29 (72.5%)	3 (7.5%)	8 (20%)	40

surface), in combination with IgMmax (defined as the maximum value of the mean percentage of IgM containing cells over all biopsy locations) were selected as criteria with the highest predictive value to differentiate Crohn's disease from ulcerative colitis. The excess of histiocytes and the villous or irregular aspect of the mucosal surface was not necessarily made on the same biopsy specimen which gave the IgMmax. Using this combination, an improvement in the diagnostic accuracy was achieved, in particular for Crohn's disease (20%), but also, although to a lesser extent, for ulcerative colitis (9%) compared with the use of histological features alone. Moreover, the number of incorrect diagnoses and the number of patients classified as CIBD indeterminate decreased to 2.5% and 12%, respectively. The classifications were made with high a posteriori probabilities (> 0.85).

Published data on the enumeration of Ig containing cells in colonic mucosa of patients with chronic inflammatory bowel disease show striking discrepancies (table 4). Factors that can account for these discrepancies in Ig containing cell counts include the fixation method,¹⁸ the staining technique (immunoperoxidase^{11 13 22} compared with immunofluorescence^{9 14 18 23}) and method of quantification. Notwithstanding conflicting results of

some studies,^{5 9 14 22} several other studies reported a tendency towards an increase in mean numbers of IgM containing cells in Crohn's disease^{6 10 11 13 15 22 24 25}. This increase in the mean numbers of IgM containing cells has not only been shown in active disease,^{15 22} but also to a lesser extent in inactive Crohn's disease¹¹ and in histologically normal gastric and duodenum biopsy specimens of patients with Crohn's disease.²⁴ In most of these studies the observed ranges of Ig containing cells overlapped considerably and this limited their use as a sole discriminatory feature to differentiate Crohn's disease from ulcerative colitis. This agrees with our results as shown in figs 2 and 3. Using only the maximum value of the mean percentage of IgM containing cells over all the biopsy locations (IgMmax), we did not achieve an improvement of the classification compared with the use of histological features alone (table 3).

Whether additional Ig subclass counting is useful as well as conventional histological features in the differential diagnosis of Crohn's disease and ulcerative colitis has never been elucidated in those studies. Only Jenkins has performed such a morphometric study on rectal biopsy specimens.⁵ He found that 73% of the cases could be accurately classified on jackknifed testing by histological measurements alone (standard deviation of intercrypt distance, of crypt lengths, of vertical cell densities and the number of lamina propria cells per unit area). Including counts of IgM containing cells (expressed per mm mucosal length) improved the classification of ulcerative

Figure 3 Jackknifed classification results using two histological features (an excess of histiocytes in the lamina propria and the aspect (irregular/villous) of the mucosal surface) in combination with IgMmax. The ulcerative colitis a posteriori probabilities 0.15 and 0.85 were chosen as cutoff points: ● = Crohn's disease; ○ = ulcerative colitis.

Table 4 Percentage of mean Ig containing cells over all biopsy locations in large bowel reported by different authors*

	IgA	IgM	IgG
Crohn's disease:			
Skinner <i>et al</i> ²⁶	87.0	4.5	8.5
Baklien <i>et al</i> ²¹	62.3	10.5	27.2
Rosekrans <i>et al</i> ¹⁶	54	33	13
Present study	62	14	24
Ulcerative colitis:			
Skinner <i>et al</i> ²⁶	79.2	7.5	13.3
Söltoft <i>et al</i> ²⁸⁺⁺	52.8	11.8	35.2
Baklien <i>et al</i> ²¹	52.1	4.4	43.3
Rosekrans <i>et al</i> ¹⁶	57	5	38
Present study	67	8	25

*The data from other studies were recalculated to make comparison possible.
++Rectal biopsy specimens only.

colitis by almost 15%; the number of false positive Crohn's disease diagnoses decreased by 15%.⁵ No change in the number of correct diagnoses of Crohn's disease was recorded. In a recent study, however, Jenkins *et al* were not able to confirm these results⁶; in their opinion there is a wide range of counts in chronic inflammatory bowel disease and this limits the use of Ig subclass counting in the differentiation between Crohn's disease and ulcerative colitis.

In contrast to these data, in our study IgMmax was selected as the most discriminatory parameter in addition to two histological features. This can be explained by the fact that in our study multiple biopsy specimens from different locations were investigated. It indicates that there are focal differences in the increase in the number of IgM containing cells. This has already been shown by Keren *et al*,²³ who showed in sites of active inflammation in CIBD that there was a much bigger increase in the mean numbers of IgM containing cells compared with inactive CIBD. Therefore, when using IgMmax, locations with the most active inflammation are probably selected.

In this study neither patients with acute infectious colitis nor patients with acute infectious colitis superimposed on CIBD were included. However, some investigators²⁷ have claimed that counting Ig subclass containing cells in colonic biopsy specimens provides a useful additional criterion in the differential diagnosis of acute infectious colitis and CIBD.²⁶ They underline that caution is necessary in patients with acute infectious colitis that runs a protracted course, because the number of IgG containing cells in colonic biopsy specimens from these specimens may be increased as in patients with active CIBD. Therefore it remains to be elucidated whether enumeration of Ig subclass containing cells in colonic biopsy specimens really does have additional value for the individual patient in the differentiation of acute infectious colitis with a protracted course and an infectious colitis superimposed on CIBD.

A relative increase in the number of IgM containing cells through the whole gastrointestinal tract in patients with Crohn's disease suggests a defect in the switch of IgM to IgA containing plasma cells. Because IgA containing cells are present in the lamina propria and the switch of IgM to IgA is under the control of several types of suppressor cells, it might be that in Crohn's disease a certain subpopulation of suppressor cells is absent or that they have a functional defect. Moreover, until now no satisfactory explanation for the increase of IgM containing cells in the gastrointestinal tract of patients with Crohn's disease has been offered.

In conclusion our findings support previous suggestions^{6 10 11-13} that counting Ig subclass containing cells has diagnostic value, differentiating patients with Crohn's disease from those with ulcerative colitis. Using the combination of an excess of histiocytes, the pattern (irregular/villous) of the mucosal surface and IgMmax (defined as the maximum value of the mean percentage of IgM containing cells over all biopsy specimens in a patient) we were able

to improve the diagnostic accuracy of Crohn's disease and ulcerative colitis compared with the use of histological features alone. We favour a quantitative approach to determine the percentage of Ig subclass containing cells in the colonic mucosal biopsy specimens of those patients with CIBD in whom, on histological criteria alone, a differentiation between Crohn's disease and ulcerative colitis is difficult.

We thank Mr E Boots, Mr E Noteboom, Miss T Tadema and Mr W Vos for their excellent technical assistance.

- 1 Pathology of the colon, small intestine, and anus. In: Norris HT, ed. *Contemporary issues in surgical pathology*. Vol 2. New York: Churchill Livingstone, 1983:1-21.
- 2 Morson BC, Dawson IMP. *Gastrointestinal pathology*. Oxford: Blackwell Scientific Publication, 1979:523-61.
- 3 Surawicz CM, Belic L. Rectal biopsy helps to distinguish acute self-limited colitis from idiopathic inflammatory bowel disease. *Gastroenterology* 1984;86:104-13.
- 4 Goldman H. Acute versus chronic colitis: how and when to distinguish by biopsy. *Gastroenterology* 1984;86:199-201.
- 5 Jenkins D, Goodall A, Drew K, Scott BB. What is colitis? Statistical approach to distinguish clinically important change in rectal biopsy specimens. *J Clin Pathol* 1988; 41:72-9.
- 6 Jenkins D. Computing and histopathology of intestinal inflammation. In: FR Vicery, ed. *Computers in gastroenterology*. London: Springer Verlag, 1988:193-204.
- 7 Thompson EM, Price AB, Altman DG, Sowter C, Slavin G. Quantitation in inflammatory bowel disease using computerized interactive image analysis. *J Clin Pathol* 1985; 38:631-8.
- 8 Seldenrijk CA, Morson BC, Meuwissen SGM, Schipper NW, Lindeman J, Meijer CJLM. Histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease. *Gut* 1991;32:1514-20.
- 9 Scott BB, Goodall A, Stephenson P, Jenkins D. Rectal mucosal plasmacells in inflammatory bowel disease. *Gut* 1983;24:519-24.
- 10 Green FHY, Fox H. The distribution of mucosal antibodies in the bowel of patients with Crohn's disease. *Gut* 1975;16:125-31.
- 11 Rosekrans PCM, Meijer CJLM, van der Wal AM, Cornelissen CJ, Lindeman J. Immunoglobulin containing cells in inflammatory bowel disease of the colon: a morphometric and immunohistochemical study. *Gut* 1980;21:941-7.
- 12 Meuwissen SGM, Feltkamp-Vroom TM, Brutel de la Rivière, von den Borne AEGK, Tytgat GN. Analysis of the lymphoplasmacytic infiltrates in Crohn's disease with special reference to identification of lymphocytic subpopulations. *Gut* 1976;17:770-80.
- 13 van Spreuwel JP, Meijer CJLM, Rosekrans PCM, Lindeman J. Immunoglobulin-containing cells in the gastrointestinal pathology: diagnostic implications. In: Sommers SC, Rosen PR, Fechner RE, eds. *Pathology annual*. Norwalk, Connecticut: Appleton-Century-Crofts, 1986:295-311.
- 14 Söltoft J. Immunoglobulin-containing cells in normal jejunal mucosa and in ulcerative colitis and regional enteritis. *Scand J Gastroenterol* 1969;38:353-60.
- 15 Meijer CJLM, Bosman FT, Lindeman J. Evidence for predominant involvement of the B-cell system in the inflammatory process in Crohn's disease. *Scand J Gastroenterol* 1979;14:21-32.
- 16 Rosekrans PCM, Meijer CJLM, Lindeman J. Immunoglobulin containing cells in colonic and rectal mucosa in Crohn's disease. In: Pena AS, Weterman IT, Booth CC, Strober W, eds. *Recent advances in Crohn's disease. Development in gastroenterology*. Vol 1; 1981:94-9.
- 17 Seldenrijk CA, Drexhage HA, Meuwissen SGM, Pals ST, Meijer CJLM. Dendritic cells and scavenger macrophages in chronic inflammatory bowel disease. *Gut* 1989;30:484-91.
- 18 Bosman FT, Lindeman J, Kuipers G, van der Wal AM, Kreuning J. The influence of fixation on immunoreactivity of plasma cells in routinely processed intestinal biopsy specimens. In: Van Noorden S, Polak JM, eds. *Immunocytochemistry: practical applications in pathology and biology*. Bristol: Wright PSG, 1983:11-42.
- 19 Seldenrijk CA, Hendriks G, Smeulders AWM, *et al*. Reproducibility of counting immunoglobulin containing cells in colonic mucosal biopsies. *Anal Quant Cytol Histol* 1988;2:94-100.
- 20 Dixon WJ, ed. *BMDP Biomedical Computer Programs*. Berkeley: University of California Press, 1983.
- 21 Baklien K, Brandtzaeg P. Comparative mapping of the distribution of immunoglobulin-containing cells in ulcerative colitis and Crohn's disease of the colon. *Clin Exp Immunol* 1975;22:197-209.
- 22 Jewell DP, Patel C. Immunology of inflammatory bowel disease. *Scand J Gastroenterol* 1985;20:119-26.

- 23 Keren DF, Appelman HD, Dobbins WO, *et al.* Correlation of histopathological evidence of disease activity with the presence of immunoglobulin-containing cells in the colons of patients with inflammatory bowel disease. *Hum Pathol* 1984;15:757–63.
- 24 van Spreeuwel JP, van der Wal AM, Weterman J, Kreuning J, Meyer CJLM. Morphological and immunohistochemical findings in upper gastrointestinal biopsies of patients with Crohn's disease of the ileum and colon. *J Clin Pathol* 1982;35:934–40.
- 25 Baklien K, Brandtzaeg P. Immunohistochemical characterisation of local immunoglobulin formation in Crohn's disease of the ileum. *Scand J Gastroenterol* 1976;11:447–57.
- 26 Skinner JM, Whitehead R. The plasmacells in inflammatory disease of the colon: a quantitative study. *J Clin Pathol* 1974;27:643–6.
- 27 Van Spreeuwel JP, Lindeman J, Meijer CJLM. A Quantitative study of immunoglobulin containing cells in the differential diagnosis of acute colitis. *J Clin Pathol* 1985;38:774–7.
- 28 Söltoft J, Binder V, Gudmand-Høyer E. Intestinal immunoglobulins in ulcerative colitis. *Scand J Gastroenterol* 1973;8:293–9.