Virchows Archiv A Pathol Anat (1993) 422:345–349

Virchows Archiv A
Pathological Anatomy and Histopathology

Increased PCNA/cyclin index correlates with severity of duodenitis defined by histological criteria

Wenancjusz Domagala[1, 3], **Krzysztof Marlicz**[2], **Dariusz Bielicki**[2], **Mary Osborn**[3]

[1] Department of Tumor Pathology, Medical Academy, Szczecin, Poland
[2] Gastroenterology Clinic, Medical Academy Szczecin, Poland
[3] Department of Biochemistry, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

Received December 9, 1992 / Accepted February 22, 1993

Abstract. The proliferative activity of crypt epithelial cells was studied in 64 duodenal biopsies using immunohistochemistry and proliferating cell nuclear antigen (PCNA)/cyclin monoclonal antibodies in alcohol-fixed paraffin-embedded sections. A positive correlation between duodenitis as defined by histological criteria and increased mean percentage of PCNA positive crypt cell nuclei (PCNA index) was found. The mean PCNA index in normal mucosa was $11.8 \pm 2.7\%$ (mean $\pm$ SD), in mild (grade 1) duodenitis $17.3 \pm 3.9\%$, in moderate (grade 2) $30.6 \pm 6.9\%$, and in severe (grade 3) duodenitis $41.1 \pm 8.5\%$. The inclusion of PCNA index, which is easily measured in paraffin-embedded sections, in the existing histopathological grading systems of duodenitis may improve their clinical relevance.

Key words: Proliferating cell nuclear antigen – Cyclin – Duodenitis

Introduction

One reason for the poor correlation between the existing histopathological grading systems for duodenitis and the clinical features associated with this disease, may be that the degree of proliferative activity of mucosal epithelial cells is not taken into account. Whitehead et al. (1975) noted that epithelial changes in duodenitis reflected a change in cell turnover in the mucosa. This can be inferred from the increased mitotic activity seen in the crypts, the tendency of immature cells to move closer to the surface and a decrease in the goblet cell population. However, objective and reproducible assessment of these variables is difficult in routine sections. Proliferative activity of cells can be assessed by counting mitoses, by autoradiography with incorporation of tritiated thymidine (Lipkin 1987) by flow cytometry or by immunocytochemistry using cell cycle specific monoclonal antibodies that preferentially label proliferating cells (Gerdes 1990). For most such antibodies frozen sections are required, so they are less useful in the routine pathology laboratory. Antibodies to proliferating cell nuclear antigen (PCNA) have the advantage that they can be applied to paraffin sections, provided the tissue has been appropriately fixed (Hall et al. 1990). PCNA (Miyachi et al. 1978) identical to "cyclin" (Bravo et al. 1982) has been identified as the DNA polymerase delta accessory protein. Expression of PCNA/cyclin is associated with cell proliferation (Hall et al. 1990) and two types of nuclear PCNA/cyclin staining are seen: a granular pattern probably originating from S-phase PCNA, and a more diffuse nuclear staining mainly restricted to non-S-phase cells (Van Dierendonck et al. 1991).

Proliferative activity of cells in duodenal biopsies has not yet been studied with the use of immunohistochemistry. In the present study, we evaluated immunocytochemical staining of PCNA/cyclin in duodenal mucosal biopsies. We compared staining results in normal duodenal mucosa and in duodenitis.

Materials and methods

Duodenal biopsies from 64 unselected patients were studied. In each patient, gastroduodenoscopy was performed in the morning, after 12 h fasting, using a fibreoptic gastroscope (Olympus XQ20). One or two biopsies were taken from the duodenal bulb. The biopsies were fixed immediately in 75% ethanol for 2 to 6 h and embedded in paraffin. The degree of duodenitis was diagnosed histologically according to the criteria of Whitehead et al. (1975) on haematoxylin and eosin stained paraffin sections. In short, it is based on an analysis of morphological changes in villi, superficial epithelium, crypts and lamina propria. The increased cellularity of the lamina propria is the main feature of mild (grade 1) duodenitis. In moderate (grade 2) duodenitis, there are in addition abnormalities of the superficial epithelium including metaplastic epithelium of gastric surface mucin-secreting type. The severe (grade 3) duodenitis is characterized by the critical feature of erosion of the abnormal epithelium associated with effacement and loss of villi

Correspondence to: M. Osborn, Department of Biochemistry, Max Planck Institute for Biophysical Chemistry, Postfach 2841, W-3400 Göttingen, Germany

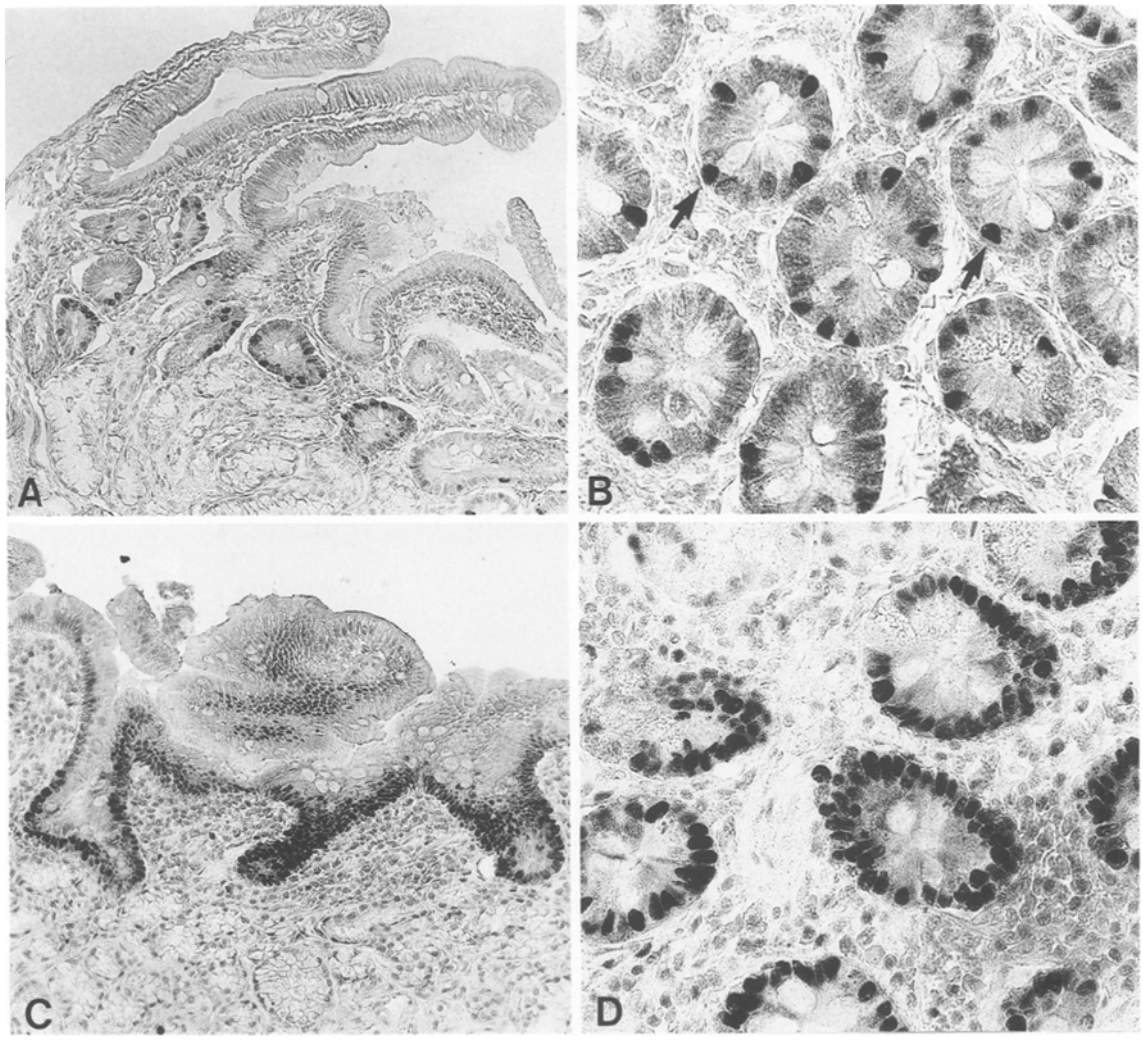

Fig. 1 A, B. Biopsy of normal duodenal mucosa. A few PCNA/cyclin positive nuclei are seen in cross-sections through the crypts (*arrows*) (**A**, **B**). Note that epithelial cells on tip and sides of villi are PCNA/cyclin negative (**A**). **C–D**. Severe duodenitis. Numerous PCNA/cyclin positive nuclei throughout the entire length of the crypts (**C**) and in cross-sections through the crypts (**D**). Streptavidin-biotin-complex. **A** and **C**, ×150; **B** and **D**, ×400

and heavy inflammatory infiltrate (Whitehead et al. 1975). Endoscopically and histologically, 16 patients had a normal duodenum and the remaining 48 had duodenitis. Of these, 11 were ulcer related. In 7 of 11 ulcer related cases *Helicobacter pylori* was found.

Mouse monoclonal PCNA antibody (clone PC10, Medac, Hamburg, Germany), originally isolated by Waseem and Lane (1990), was used as the primary antibody for immunocytochemistry at a dilution of 1:60. Sections were deparaffinized in xylene, transferred to 100% and 95% ethanol for 3 min each and air-dried. Sections were incubated for 1 h at room temperature with the primary antibody and incubated with biotinylated rabbit anti-mouse second antibody and streptavidin-peroxidase, followed by a substrate-chromogen mixture with aminoethylcarbazole as a chromogen (Histostain-SP Kit, Zymed Laboratories Inc., San Francisco, Calif., USA). Sections were slightly counterstained with haematoxylin.

Results

Well-orientated longitudinal sections through the entire crypt from the bottom to the tips of neighboring villi are rare in biopsy specimens; in most cases, cross-sections of crypts are seen. Moreover, the point at which the villus ends and a crypt begins cannot be standardized (Whitehead et al. 1975). Therefore we decided to count the number of PCNA labelled cells in all cross-sections of crypts containing at least one positive cell, using the method of Hansen et al. (1977). All epithelial cell nuclei with intensely red, granular staining patterns corresponding to S-phase PCNA (Van Dierendonck et al. 1991) were counted as PCNA/cyclin positive. Nuclei with weak, diffuse staining were not counted as positive. The number of cells with labelled nuclei, and the total number of cells in all cross-sections of crypts containing one or more PCNA/cyclin positive cells were counted and the mean PCNA index (the percentage of labelled cells) was calculated. In all biopsies several sections, not closer than one in four consecutive sections, were analysed. Between 500 and 1000 cells were usually counted per biopsy. Approximately a quarter of the biopsies yielded an insufficient number of cross-sections through

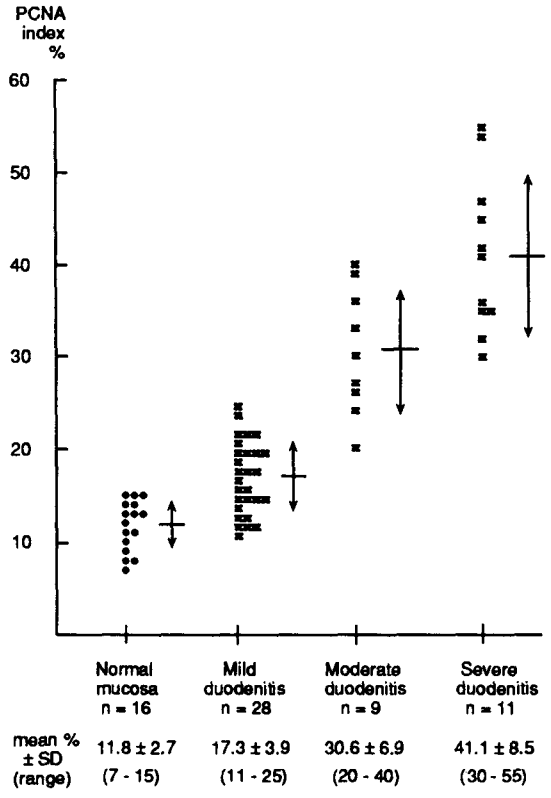

Fig. 2. Pathological diagnosis and percentage of PCNA/cyclin positive cells (PCNA index) in duodenal crypt epithelium; normal mucosa (●); duodenitis (×)

the crypts and were not included in the study. The Wilcoxon rank sum test was used for statistical comparisons. In normal duodenal mucosa, labelled cells were present only in the lower and middle crypt and few PCNA positive crypt cell nuclei were seen in cross-sections (Fig. 1 B). In contrast, in mild and especially in severe duodenitis the PCNA positive cells were found throughout the entire length of the crypt (Fig. 1 C) and numerous PCNA positive nuclei were seen in cross-sections (Fig. 1 D). In a few instances of severe duodenitis, scattered PCNA positive cells were seen at the sides of villi.

The results are summarized in Fig. 2. There were significant increases in the mean PCNA index in non-specific and ulcer related moderate and severe duodenitis as compared with normal mucosa. The PCNA index in histologically normal duodenal epithelium ranged from 7 to 15% (mean $11.8 \pm 2.7\%$) (mean $\pm$ SD), in mild duodenitis from 11 to 25% (mean $17.3 \pm 3.9\%$), in moderate duodenitis from 20 to 40% (mean $30.6 \pm 6.9\%$) and in severe duodenitis from 30 to 55% (mean $41.1 \pm 8.5\%$). All differences were significant. Between normal epithelium and mild, moderate and severe duodenitis ($p < 0.005$), between mild versus moderate versus severe duodenitis ($p < 0.005$) and between moderate versus severe duodenitis ($p < 0.01$). Thus there was a clear trend towards an increased percentage of labelled nuclei with increasing severity of duodenitis. There were significant differences of mean PCNA index between normal

Table 1. PCNA index versus histologically defined degree of duodenitis

PCNA defined degree of duodenitis	*n*	Histological diagnosis			
		Normal mucosa	Mild	Duodenitis Moderate	Severe
Normal ≤15% PCNA positive nuclei	27	16	11	0	0
Moderate 16–29% PCNA positive nuclei	21	0	17	4	0
Severe ≥30% PCNA positive nuclei	16	0	0	5	11

mucosa and moderate and severe duodenitis. However, between normal mucosa and mild duodenitis or moderate and severe duodenitis considerable overlap was noted. In almost 50% of cases diagnosed histologically as mild duodenitis the PCNA index was within the range of normal mucosa. Also in roughly half of the biopsies with moderate duodenitis, the PCNA index was within the lower value of the range of severe duodenitis. Taking 15% of PCNA positive cells as the highest cut-off level for normal duodenal mucosa and 30% as the lowest level for severe duodenitis, we divided all cases into three categories defined by their PCNA index: normal mucosa (≤15% PCNA positive cells), moderate duodenitis (16–29%) and severe duodenitis (≥30% PCNA positive cells) (Table 1).

There were no differences in the mean PCNA index in ulcer related duodenitis when compared with non-specific duodenitis; however the numbers are too small to permit statistical analysis. There were 3 cases of severe, 1 of moderate and 7 of mild ulcer related duodenitis. The mean PCNA index was 39.3 and 17.1 in severe and mild ulcer related duodenitis when compared with 41.8 and 17.3 in non-specific duodenitis. The 3 ulcer related cases of severe duodenitis were also positive for *H. pylori*. Among ulcer related cases with mild duodenitis three were positive for *H. pylori* (mean PCNA index 18.6 versus 17.2 for *H. pylori* negative cases).

Discussion

In this study immunohistochemistry with antibodies to PCNA has been used to reveal proliferative activity of crypt epithelial cells in biopsies of normal human duodenal mucosa and duodenitis. We found a clear link between the grade of duodenitis and the percentage of PCNA positive cells in the crypts in alcohol-fixed, paraffin-embedded duodenal biopsies. Therefore we propose that the PCNA index (the percentage of PCNA positive cells in the crypts) be used as an additional criterion to assess the grade of duodenitis. We do not advocate PCNA index as the sole criterion for histopathological

diagnosis of duodenitis, although Table 1 does show that one can stratify duodenitis fairly well by the PCNA index. Future studies are needed to determine whether this variable improves the clinical relevance of existing histological grading systems of duodenitis.

Studies of cell proliferation in human duodenal mucosa have been difficult to perform because of a need for radioautography after in vitro labelling with tritiated thymidine (Lipkin 1987; MacDonald et al. 1964). One could not expect such methods to be of use for routine diagnosis in a surgical pathology laboratory. Nevertheless, in three reports published to date on epithelial cell proliferation in human duodenal biopsies using tritiated thymidine and autoradiography (Bransom et al. 1981; Gorelick et al. 1983, Bransom et al. 1984) an increased proliferation index in crypt epithelial cells in duodenitis when compared with normal mucosa, has been noted. However, there is disagreement whether this occurs both in ulcer and non-ulcer related duodenitis (Bransom et al. 1981) or only in the latter (Gorelick et al. 1983). We found an increased PCNA index in both. In one report lower levels of tritiated thymidine incorporation were found in ulcer related duodenal mucosa (Zagorulko and Puzyrev 1974). Similarly, in experimental animal models, decreased proliferation in duodenal ulcers (Jarvis et al. 1973) and a correlation between the severity of duodenitis, no matter whether ulcer related or non-specific, and the increased number of thymidine labelled nuclei was found (Smedley and Wastell 1988; Smedley et al. 1990). This parallels the findings in gastritis, where severity of chronic gastritis was also correlated with an increased proliferation index as measured by tritiated thymidine incorporation (Hansen et al. 1977) or by immunohistochemistry with Ki67 antibody on frozen gastric biopsies (Niedobitek et al. 1988). Thus our results and the evidence in the literature, indicate that increased proliferation rate of epithelial cells is one way in which duodenal or gastric mucosa respond to injury. Although our values for the labelling index are higher than those in autoradiography studies in human biopsies, the trend for increased rate of proliferation of crypt epithelial cells with increased grade of duodenitis remains the same. Similarly, higher values for labelling indices of gastric mucosa were obtained by immunohistochemistry with Ki67 antibody on frozen gastric biopsies (Niedobitek et al. 1988) than by autoradiography (Hansen et al. 1975). It has also been shown that the fraction of PCNA positive cells revealed by immunohistochemistry is higher than the fraction of thymidine or bromodeoxyuridine labelled cells (Galand and Degraef 1989; Garcia et al. 1989).

Several histological grading systems for duodenitis have been proposed (Beck et al. 1965; Whitehead et al. 1975; Lance et al. 1978; Greenlaw et al. 1980; Jenkins et al. 1985) but none takes into account the proliferation index of the epithelial cells in crypts. They are based on analysis of such histological criteria as the extent of mononuclear cell infilrate in the lamina propria, and changes involving villous processes, mucous secretion, superficial epithelium and crypts. Our findings provide an objective means by which to analyse cell proliferation in routine duodenal biopsies. We found that as the histological grade of duodenitis increased so did the percentage of PCNA positive proliferating cells in duodenal crypts. There is some overlap between the percentages of proliferating cells seen in normal mucosa and in mild duodenitis as well as in moderate and severe duodenitis. It may be that within the mild and moderate categories of duodenitis there is a genuine scatter of PCNA index. However, it may also be that Whitehead's mild and moderate categories need to be redefined, perhaps with the help of the PCNA index. It is not clear whether all cases of mild duodenitis as defined by Whitehead's histological criteria really belong to this group since the correlation with clinical symptoms is poor and there is no other objective point of reference. It has already been suggested that some cases which would be classified as mild duodenitis according to the Whitehead classification may in fact represent the extreme within the range of appearances of normal mucosa (Roca et al. 1975; Jenkins et al. 1985). However, some biopsies with high PCNA index may have severe duodenitis although no erosion is seen (this is required for the diagnosis of severe duodenitis in Whitehead's classification, Whitehead et al. 1975). Such a view is supported by reports on animal models of duodenitis and on human duodenal biopsies which showed that ulcers formed in mucosa with the fastest rates of mucosal cell renewal (Smedley et al. 1990), and that erosions and ulcers arose when the crypts passed into "high output failure" and were unable to compensate for further epithelial cell loss (Bransom et al. 1981). In any case, since ulcers formed in duodenal mucosa with the fastest rates of proliferation (Smedley et al. 1990). PCNA index may serve to define a group of patients with duodenitis with a high risk of developing ulcers or erosion.

Millions of cells are lost daily in the normal intestinal mucosa, therefore any change in the normal proliferate events may affect intestinal function profoundly. The rates of cell proliferation may be influenced by various factors present in the micro-environment of the cell population (Bullough 1962). For instance, inflammatory changes may induce increased functional and physical cell destruction which in turn may stimulate cells in the upper region of the crypt, where usually cells undergo maturation and differentiation, to undergo further proliferation. It is apparent from our results that even in mild and moderate duodenitis there is response of epithelium of the crypts in the form of an increased proliferation rate. In searching for a histological grading of duodenitis which correlates with clinical symptoms, information on the proliferation of epithelial cells in crypts and villi should be included. With monoclonal antibodies to PCNA/cyclin which work in paraffin sections one can measure proliferative activity of epithelial cells in duodenal biopsies routinely and use the PCNA index as a diagnostic criterion.

Acknowledgements. We thank Dr. George Striker for help with the statistical analysis and Susanne Isenberg for expert technical assistance.

References

Beck IT, Kahn DS, Lacerte M, Solymar J, Callegarini U, Geokas MC (1965) Chronic duodenitis: a clinical pathological entity? Gut 6:376–383

Bransom CJ, Boxer ME, Palmer KR, Clark JC, Underwood JCE, Duthie HL (1981) Mucosal cell proliferation in duodenal ulcer and duodenitis. Gut 22:277–282

Bransom CJ, Boxer ME, Clark JC, Smith JAR, Johnson AG (1984) Epithelial cell proliferation in duodenal ulcer. Scand J Gastroenterol 19:515–520

Bravo R, Fey SJ, Bellatin J, Larsen PM, Arevalo J, Celis JE (1982) Identification of a nuclear and of a cytoplasmic polypeptide whose relative proportions are sensitive to changes in the rate of cell proliferation. Exp Cell Res 136:311–319

Bullough WS (1962) The control of mitotic activity in adult mammalian tissues. Biol Rev 37:307–342

Galand P, Degraef C (1989) Cyclin/PCNA immunostaining as an alternative to tritiated thymidine pulse labelling for marking S phase cells in paraffin sections from animal and human tissues. Cell Tissue Kinet 22:383–392

Garcia RL, Coltrera MD, Gown AM (1989) Analysis of proliferative grade using anti-PCNA/cyclin monoclonal antibodies in fixed, embedded tissues. Am J Pathol 134:733–739

Gerdes J (1990) Ki-67 and other proliferation markers useful for immunohistological diagnostic and prognostic evaluations in human malignancies. Semin Cancer Biol 1:199–206

Gorelick FS, Deluca VA, Sheahan DG, Marignani P, Goldblatt RS, Winnan J, Livstone EM (1983) Duodenal epithelial thymidine uptake in patients with duodenal ulcer or endoscopic duodenitis. Dig Dis Sci 28:392–396

Greenlaw R, Sheahan DG, De Luca V, Miller D, Myerson D, Myerson P (1980) Gastroduodenitis: a broader concept of peptic ulcer disease. Dig Dis Sci 25:660–671

Hall PA, Levison DA, Woods AL, Yu CCW, Kellock DB, Watkins JA, Barnes DM, Gillett CE, Camplejohn R, Dover R, Waseem NH, Lane DP (1990) Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. J Pathol 162:285–294

Hansen OH, Pedersen T, Larsen JK (1975) A method to study cell proliferation kinetics in human gastric mucosa. Gut 16:23–27

Hansen OH, Johansen A, Larsen JK, Pedersen T, Svendsen LB (1977) Relationship between gastric acid secretion, histopathology, and cell proliferation kinetics in human gastric mucosa. Gastroenterology 73:453–456

Jarvis HR, Sheahan DG, Sprinz H (1973) Acute duodenal ulcer in the guinea-pig due to fasting. Lab Invest 28:501–513

Jenkins D, Goodall A, Gillet FR, Scott BB (1985) Defining duodenitis: quantitative histological study of mucosal responses and their correlations. J Clin Pathol 38:1119–1126

Lance P, Filipe MI, Wastell C (1978) Non-ulcer dyspepsia, duodenitits and cimetidine. In: Wastell C, Lance P (eds) Cimetidine. The Westminster Hospital Symposium, Churchill Livingstone, London, pp 126–129

Lipkin M (1987) Proliferation and differentiation of normal and diseased gastrointestinal cells. In: Johnson LR (ed) Physiology of the gastrointestinal tract, 2nd edn, Raven Press, New York, pp 255–284

MacDonald WC, Trier JS, Everett NB (1964) Cell proliferation and migration in the stomach, duodenum, and rectum of man: radioautographic studies. Gastroenterology 46:405–417

Miyachi K, Fritzler MJ, Tan EM (1978) Autoantibody to a nuclear antigen in proliferating cells. J Immunol 121:2228–2234

Niedobitek F, Habedank S, Volkheimer G, Mostertz P, Pietrkiewicz C (1988) Zur immunhistochemischen Darstellung der Proliferationszone der Magenschleimhaut. Z Gastroenterologie 26:303–309

Roca M, Truelove SC, Whitehead R (1975) Histological state of gastric and duodenal mucosa in healthy volunteers. Gut 16:404

Smedley FH, Wastell C (1988) Cimetidine and mucosal cell renewal in the duodenum. Eur Surg Res 20:320–324

Smedley FH, Rimmer J, Samanidis, Wastell C (1990) Cell renewal in non-specific duodenitis, ulcer related duodenitis and duodenal ulcer. Experiments in Mastomyms (Praomys natalensis). Digestion 45:72–79

Van Dierendonck JH, Wijsman JH, Keijzer R, Van de Velde CJH, Cornelisse CJ (1991) Cell cycle-related staining patterns of antiproliferating cell nuclear antigen monoclonal antibodies. Am J Pathol 138:1165–1172

Waseem NH, Lane DP (1990) Monoclonal analysis of the proliferating cell nuclear antigen (PCNA). J Cell Sci 96:121–129

Whitehead R, Roca M, Meikle DD, Skinner J, Truelove SC (1975) The histological classification of duodenitis in fibreoptic biopsy specimens. Digestion 13:129–136

Zagorulko MP, Puzyrev AA (1974) On proliferation of the epithelium of the duodenal mucosa in ulcerative disease. Arkh Patol 36:31–35