Handout

Minisymposium: Pathology of Gastrointestinal Tract

Cord Langner, MD

Diagnostic & Research Centre for Molecular BioMedicine

Institute of Pathology

Medical University of Graz

Neue Stiftingtalstraße 6

8010 Graz

Austria

Email: cord.langner@medunigraz.at

Gastritis

As the endoscopic diagnosis of gastritis is not reliable (some types of gastritis are barely visible upon endoscopy, if at all, and mucosal redness on gross inspection does not necessary reflect inflammation), diagnosis of gastritis is based upon the histological proof of gastric mucosa inflammation.

The following types of gastritis have been included in the classification proposed by the Kyoto Global Consensus Conference (2015):

- Autoimmune gastritis
- Infectious gastritis
  - *Helicobacter pylori* [HP]-induced gastritis
  - Bacterial gastritis other than HP
  - Gastritis phlegmone
  - Viral gastritis
  - Fungal gastritis
  - Parasitic gastritis
- Gastritis due to external causes (e.g. drug-induced gastritis, gastritis due to duodenal reflux)
- Gastritis due to specified causes (e.g. lymphocytic gastritis, eosinophilic gastritis)
- Gastritis due to other disease classified elsewhere (e.g. sarcoidosis, vasculitis, Crohn's disease)

The histological diagnosis of gastritis is not only descriptive. It should always include (at least suggest) the aetiology of disease. With the decline in HP prevalence in Western countries, gastric biopsies are more likely to have reactive gastropathy, HP-negative gastritis [includes post-HP or ex-HP gastritis], autoimmune gastritis, and less commonly active HP infection.

The following pattern-based algorithmic approach is recommended for the routine assessment of gastric biopsies. The initial examination should be done on low power (4ex), since in the majority of cases the aetiology of gastritis can easily be recognized without high magnification. However, when the most common types of gastritis have been ruled out (HP, post-/ex-HP, reactive gastropathy, autoimmune gastritis) thorough examination of the gastric biopsies at high magnification is recommended and may render the decisive aetiological clue.

It has to be emphasized that gastric biopsies are nowadays performed quite liberally (more or less during every endoscopic investigation of the upper gastrointestinal tract), so that the histological diagnosis of “normal stomach” may be one of the most common in daily routine.

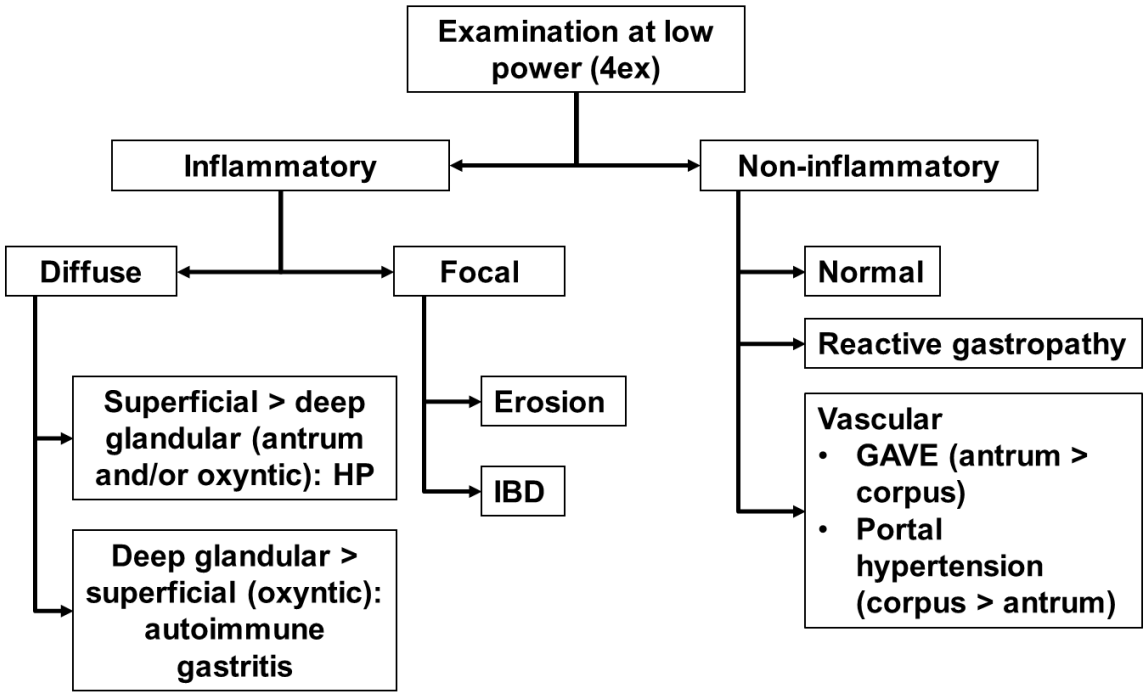

Figure 1 Algorithmic approach to the diagnosis of gastritis

Helicobacter gastritis

HP gastritis is usually acquired before age ten and for many remains antral predominant and asymptomatic for life with little or no progression. Nonetheless, with continued infection, gastric inflammation and damage advances proximally from the antrum into the corpus.

Early HP gastritis (as in duodenal ulcer patients) is antral predominant and often corpus sparing, with normal or increased acid secretion. Advanced gastritis (patients with gastric ulcer and the intestinal type gastric cancer) is typically an extensive pan-gastritis (with widespread intestinal metaplasia and hypo- or achlorhydria). A similar shift of HP density (and inflammation) to the corpus can be observed in patients under PPI therapy, underlining the need for a minimum of four gastric biopsies (2x antrum, 2x corpus).

In summary, gastritis patterns seen with HP infection include:

- Antral predominant gastritis (with or without atrophy and/or metaplasia)
- Corpus predominant gastritis (with or without atrophy and/or metaplasia)
- Pan-gastritis (with or without atrophy and/or metaplasia, includes multifocal-atrophic gastritis; MAG)
- Lymphocytic gastritis
- Russell body gastritis.

Upon histology, HP gastritis is usually chronic and active, involving the upper half of the gastric biopsy, accompanied by varying dgree of oedema (particularly in the corpus). The pathology report should be performed in accordance with the recommendations made in the Updated Sydney System, grading the extent of chronic (lymphocytes, plasma cells) and active (neutrophils) inflammation. Changes to the surface epithelium may occur and include acute changes (erosion, ulcer) as well as chronic changes (intestinal metaplasia).

HP negative gastritis is defined as inflamed gastric biopsies but the bacteria are not identified. False negative HP reporting due to sampling error or hostile microenvironment [extensive intestinal metaplasia and/or treatment with PPIs, which leads to only few bacteria deep within the oxyntic glands, often with “pseudo-intracellular” location] should be excluded.

Presence of HP can be assessed on H&E stained slides (provided there is enough haematoxylin), but special stains (e.g. modified Giemsa, silver staining) have been shown to increase the sensitivity of histological diagnosis. In many countries “upfront” staining with Giemsa or silver staining is part of the routine process. However, in countries with low HP prevalence, primary H&E-based diagnosis with subsequent HP immunostaining in selected cases [HP negative cases with active inflammation and/or at least moderate chronic inflammation] may be considered. This approach is in accordance with recent recommendations made by the Roger Haggit Society of gastrointestinal pathology.

Reactive gastropathy

Reactive gastropathy reflects mucosal injury caused by medication and/or bile reflux (“reflux gastritis”). The following histological changes can be observed:

- Foveolar hyperplasia (with mucin depletion and mild reactive nuclear changes, including increased nuclear/cytoplasmic ratio)
- Ascending smooth muscle fibres in the lamina propria
- Vasodilation and congestion of superficial mucosal capillaries
- Stromal oedema
- Paucity of both acute and chronic inflammatory cells

Important differentials in reactive gastropathy include: (i) distinguishing reactive changes from low grade dysplasia (can be facilitated by appreciating the apparently seamless transition from adjacent epithelium), (ii) degenerating gastric cells can acquire eccentric nuclei with vacuolar cytoplasm that can bear a close resemblance to signet-ring cells (in these cases, lack of a desmoplastic reaction can be helpful to avoid overdiagnosis).

Autoimmune gastritis

Classical autoimmune gastritis is a chronic disease leading to progressive destruction of the oxyntic mucosa with reduced acid production and reduced or absent intrinsic factor necessary for vitamin B12 absorption. In pernicious anaemia, approximately 90% of patients have antibodies to parietal cells and their components, including antibodies to the intrinsic factor, and proton pump H+, K+ -ATPase. Females are primarily affected.

Upon histology, classical autoimmune gastritis is characterized by a corpus predominant atrophic gastritis with relative sparing of the antrum. The infiltrate is dominated by lymphocytes (T cells), that lead to gland destruction (emperipolesis) with parietal cell apoptosis. Plasma cells and often also eosinophils take part in the inflammatory process that leads to progressive gland loss.

Stolte defined the morphological features of active pre-atrophic autoimmune gastritis as follows:

- Lymphocytic infiltration of the glands of the oxyntic mucosa
- Focal destruction in individual oxyntic glands
- Reactive hypertrophy of (residual) parietal cells

It is of note that atrophy may occur in an uneven distribution, leading to the formation of **oxyntic gland pseudopolyps**. Diagnosis of autoimmune gastritis may be missed when only these remnant islands of non-atrophic mucosa are biopsied.

Atrophy and/or intestinal metaplasia are the histological consequences of chronic gastritis. Their presence and extent need to be assessed and recorded in the pathology report as patients with chronic atrophic gastritis and/or intestinal metaplasia are at risk for development of gastric adenocarcinoma. According to recent data (including a systematic meta-analysis) incomplete intestinal metaplasia is of major importance and warrants special attention by the pathologist.

Introduced scoring systems include the “Operative Link on Gastritis Assessment” (**OLGA**), which evaluates the extent of gastric atrophy and the “Operative Link on Gastritis Assessment based upon Intestinal Metaplasia” (**OLGIM**), which evaluates the extent of intestinal metaplasia.

The OLGIM superior is superior to the OLGA system, because (i) assessment of intestinal metaplasia shows less interobserver variation and (ii) the predictive value for intestinal metaplasia is higher than for atrophy. According to a recent study, the likelihood for progression to gastric cancer of high versus low OLGIM stage is two times that of high versus low OLGA stages. As conseqeuence, the authors of the updated MAPS guidelines (MAPS II; Pimentel-Nunes et al. 2019) recommend OLGIM for staging of chronic gastritis.

On the clinical side, the updated MAPS II guidelines state that biopsies of at least two topographic sites (from both the antrum and the corpus, at the lesser and greater curvature of each) should be taken (and clearly labelled in two separate vials). If visible neoplastic suspicious lesions are identified additional biopsies should be taken, labelled and submitted in additional containers. Whether antrum and corpus biopsies really need to be submitted separately by our clinical partners is still under debate, as in the vast majority of cases antrum and corpus can be separated very easily under the microscope. Furthermore, immediate comparison of different areas is possible (antrum-dominant or corpus-dominant inflammation, atrophy and/or intestinal metaplasia) and identification of HP is usually easier and less time consuming. Here the MAPS II authors state: “Regarding the number of vials, even though separate vials may not be required among expert pathologists, as antral and corpus mucosa can be easily distinguished in the absence of severe atrophic changes, use of a single vial cannot be recommended in all cases. Future studies should evaluate specific scenarios when antrum, incisura, and corpus samples can be sent in the same vial.”

The updated MAPS (MAPS II) recommendations with respect to patient staging and surveillance (that are of special importance for pathologists) are listed below:

- Patients with chronic atrophic gastritis or intestinal metaplasia are at risk for gastric adenocarcinoma (high quality evidence)
- Histologically confirmed intestinal metaplasia is the most reliable marker of atrophy in gastric mucosa (high quality evidence)
- Patients with advanced stages of gastritis, that is atrophy and/or intestinal metaplasia affecting both antral and corpus mucosa, should be identified as they are considered to be at higher risk for gastric adenocarcinoma (moderate quality evidence, strong recommendation)
- High grade dysplasia and invasive carcinoma should be regarded as the outcomes to be prevented when patients with chronic atrophic gastritis or intestinal metaplasia are managed (moderate quality evidence, strong recommendation)
- Patients with an endoscopically visible lesion harboring low or high grade dysplasia or carcinoma should undergo staging and treatment (high quality evidence, strong recommendation)
- In patients with dysplasia in the absence of an endoscopically defined lesion immediate high quality endoscopic reassessment with chromoendoscopy (virtual or dye-based) is recommended. If no lesion is detected with this high quality endoscopy, biopsies for staging of gastritis (if not previously done) and endoscopic surveillance within 6 months

(if high grade dysplasia) to 12 months (if low grade dysplasia) are recommended (low quality evidence, strong recommendation)

- For patients with mild to moderate atrophy restricted to the antrum there is no evidence to recommend surveillance (moderate quality evidence, strong recommendation)
- Patients with intestinal metaplasia at a single location have a higher risk of gastric cancer. However, this increased risk does not justify surveillance in most cases, particularly if a high quality endoscopy with biopsies has excluded advanced stages of atrophic gastritis (moderate quality evidence, strong recommendation)
- In patients with intestinal metaplasia at a single location but with a family history of gastric cancer, or with incomplete intestinal metaplasia, or with persistent HP gastritis, endoscopic surveillance with chromoendoscopy and guided biopsies in 3 years' time may be considered (low quality evidence, weak recommendation)
- Patients with advanced stages of atrophic gastritis (severe atrophic changes or intestinal metaplasia in both antrum and corpus, OLGA/OLGIM III/IV) should be followed up with a high quality endoscopy every 3 years (low quality evidence, strong recommendation). In a comment the authors of the MAPOS II recommendations state that "mild atrophy without intestinal metaplasia, even when affecting antrum and corpus, should not be considered to be an advanced stage of gastritis" (please note: this only holds true for atrophy, not for mild intestinal metaplasia present in the same locations)
- Patients with advanced stages of atrophic gastritis and with a family history of gastric cancer may benefit from a more intensive follow-up (e. g. every 1 – 2 years after diagnosis) (low quality evidence, weak recommendation)
- Patients with autoimmune gastritis may benefit from endoscopic follow-up every 3 – 5 years (low quality evidence, weak recommendation)

References

1. Anim JT, Al-Sobkie N, Prasad A, John B, Sharma PN, Al-Hamar I. Assessment of different methods for staining Helicobacter pylori in endoscopic gastric biopsies. Acta Histochem. 2000; 102: 129-137.
2. Batts KP, Ketover S, Kakar S, Krasinskas AM, Mitchell KA, Wilcox R, Westerhoff M, Rank J, Gibson J, Mattia AR, Cummings OW, Davison JM, Naini BV, Dry SM, Yantiss RK; Rodger C Haggitt Gastrointestinal Pathology Society. Appropriate use of special stains for identifying Helicobacter pylori: Recommendations from the Rodger C. Haggitt Gastrointestinal Pathology Society. Am J Surg Pathol. 2013; 37: e12-22.
3. Bettington M, Brown I. Autoimmune gastritis: novel clues to histological diagnosis. Pathology. 2013; 45: 145-149.
4. Capelle LG, de Vries AC, Haringsma J, Ter Borg F, de Vries RA, Bruno MJ, van Dekken H, Meijer J, van Grieken NC, Kuipers EJ. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. Gastrointest Endosc. 2010; 71: 1150-1158.
5. Correa P. A human model of gastric carcinogenesis. Cancer Res. 1988; 48: 3554-3560.
6. Dinis-Ribeiro M, Areia M, de Vries AC, Marcos-Pinto R, Monteiro-Soares M, O'Connor A, Pereira C, Pimentel-Nunes P, Correia R, Ensari A, Dumonceau JM, Machado JC, Macedo G, Malfertheiner P, Matysiak-Budnik T, Megraud F, Miki K, O'Morain C, Peek RM, Ponchon T, Ristimaki A, Rembacken B, Carneiro F, Kuipers EJ; MAPS Participants; European Society of Gastrointestinal Endoscopy; European Helicobacter Study Group; European Society of Pathology; Sociedade Portuguesa de Endoscopia Digestiva. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). Virchows Arch. 2012; 460: 19-46.
7. Dirschmid K, Platz-Baudin C, Stolte M. Why is the hyperplastic polyp a marker for the precancerous condition of the gastric mucosa? Virchows Arch. 2006; 448: 80-84.
8. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol. 1996; 20: 1161-1181.
9. Dixon MF, O'Connor HJ, Axon AT, King RF, Johnston D. Reflux gastritis: distinct histopathological entity? J Clin Pathol. 1986; 39: 524-530.
10. El-Zimaity H, Choi WT, Lauwers GY, Riddell R. The differential diagnosis of Helicobacter pylori negative gastritis. Virchows Arch. 2018; 473: 533-550.
11. Faller G, Kirchner T. Immunological and morphogenic basis of gastric mucosa atrophy and metaplasia. Virchows Arch. 2005; 446: 1-9.
12. González CA, Sanz-Anquela JM, Companioni O, Bonet C, Berdasco M, López C, Mendoza J, Martín-Arranz MD, Rey E, Poves E, Espinosa L, Barrio J, Torres MÁ,

Cuatrecasas M, Elizalde I, Bujanda L, Garmendia M, Ferrández Á, Muñoz G, Andreu V, Paules MJ, Lario S, Ramírez MJ; Study group, Gisbert JP. Incomplete type of intestinal metaplasia has the highest risk to progress to gastric cancer: results of the Spanish follow-up multicenter study. J Gastroenterol Hepatol. 2016; 31: 953-958.

13. González CA, Sanz-Anquela JM, Gisbert JP, Correa P. Utility of subtyping intestinal metaplasia as marker of gastric cancer risk. A review of the evidence. Int J Cancer. 2013; 133: 1023-1032.
14. Isajevs S, Liepniece-Karele I, Janciauskas D, Moisejevs G, Putnins V, Funka K, Kikuste I, Vanags A, Tolmanis I, Leja M. Gastritis staging: interobserver agreement by applying OLGA and OLGIM systems. Virchows Arch. 2014; 464: 403-407.
15. Jhala NC, Montemor M, Jhala D, Lu L, Talley L, Haber MM, Lechago J. Pancreatic acinar cell metaplasia in autoimmune gastritis. Arch Pathol Lab Med. 2003; 127: 854-857.
16. Kocsmár É, Szirtes I, Kramer Z, Szijártó A, Bene L, Buzás GM, Kenessey I, Bronsert P, Csanadi A, Lutz L, Werner M, Wellner UF, Kiss A, Schaff Z, Lotz G. Sensitivity of Helicobacter pylori detection by Giemsa staining is poor in comparison with immunohistochemistry and fluorescent in situ hybridization and strongly depends on inflammatory activity. Helicobacter. 2017; 22(4). doi: 10.1111/hel.12387. Epub 2017 Apr 12.
17. Lebwohl B, Green PH, Genta RM. The coeliac stomach: gastritis in patients with coeliac disease. Aliment Pharmacol Ther. 2015; 42: 180-187.
18. Li D, Bautista MC, Jiang SF, Daryani P, Brackett M, Armstrong MA, Hung YY, Postlethwaite D, Ladabaum U. Risks and Predictors of Gastric Adenocarcinoma in Patients with Gastric Intestinal Metaplasia and Dysplasia: A Population-Based Study. Am J Gastroenterol. 2016; 111: 1104-1113.
19. Lim JH, Kim N, Lee HS, Choe G, Jo SY, Chon I, Choi C, Yoon H, Shin CM, Park YS, Lee DH, Jung HC. Correlation between Endoscopic and Histological Diagnoses of Gastric Intestinal Metaplasia. Gut Liver. 2013; 7: 41-50.
20. Madisch A, Miehlke S, Neuber F, Morgner A, Kuhlisch E, Rappel S, Lehn N, Bayerdörffer E, Seitz G, Stolte M. Healing of lymphocytic gastritis after Helicobacter pylori eradication therapy--a randomized, double-blind, placebo-controlled multicentre trial. Aliment Pharmacol Ther. 2006; 23: 473-479.
21. Malfertheiner P. The intriguing relationship of Helicobacter pylori infection and acid secretion in peptic ulcer disease and gastric cancer. Dig Dis. 2011; 29: 459-464.
22. Matsunari O, Shiota S, Suzuki R, Watada M, Kinjo N, Murakami K, Fujioka T, Kinjo F, Yamaoka Y. Association between Helicobacter pylori virulence factors and gastroduodenal diseases in Okinawa, Japan. J Clin Microbiol. 2012; 50: 876-883.
23. Mera RM, Bravo LE, Camargo MC, Bravo JC, Delgado AG, Romero-Gallo J, Yepez MC, Realpe JL, Schneider BG, Morgan DR, Peek RM Jr, Correa P, Wilson KT, Piazuelo MB. Dynamics of Helicobacter pylori infection as a determinant of progression of gastric precancerous lesions: 16-year follow-up of an eradication trial. Gut. 2018; 67: 1239-1246.

24. Neumann WL, Coss E, Rugge M, Genta RM. Autoimmune atrophic gastritis--pathogenesis, pathology and management. Nat Rev Gastroenterol Hepatol. 2013; 10: 529-541.
25. Park JY, Cornish TC, Lam-Himlin D, Shi C, Montgomery E. Gastric lesions in patients with autoimmune metaplastic atrophic gastritis (AMAG) in a tertiary care setting. Am J Surg Pathol. 2010; 34: 1591-1598.
26. Pimentel-Nunes P, Libânio D, Marcos-Pinto R, Areia M, Leja M, Esposito G, Garrido M, Kikuste I, Megraud F, Matysiak-Budnik T, Annibale B, Dumonceau JM, Barros R, Fléjou JF, Carneiro F, van Hooft JE, Kuipers EJ, Dinis-Ribeiro M. Management of epithelial precancerous conditions and lesions in the stomach (MAPS II): European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter and Microbiota Study Group (EHMSG), European Society of Pathology (ESP), and Sociedade Portuguesa de Endoscopia Digestiva (SPED) guideline update 2019. Endoscopy. 2019; 51: 365-388.
27. Pittayanon R, Rerknimitr R, Klaikaew N, Sanpavat A, Chaithongrat S, Mahachai V, Kullavanijaya P, Barkun A. The risk of gastric cancer in patients with gastric intestinal metaplasia in 5-year follow-up. Aliment Pharmacol Ther. 2017; 46: 40-45.
28. Pittman ME, Kharar jian A, Wood LD, Montgomery EA, Voltaggio L. Prospective identification of Helicobacter pylori in routine gastric biopsies without reflex ancillary stains is cost-efficient for our health care system. Hum Pathol. 2016; 58: 90-96.
29. Rappel S, Müller H, Stolte M. Die aktive präatrophische Autoimmungastritis. Ein praxisorientiertes Konzept für Diagnostik und Therapie. Pathologe. 2001; 22: 19-24.
30. Rugge M, Correa P, Dixon MF, Fiocca R, Hattori T, Lechago J, Leandro G, Price AB, Sipponen P, Solcia E, Watanabe H, Genta RM. Gastric mucosal atrophy: interobserver consistency using new criteria for classification and grading. Aliment Pharmacol Ther. 2002; 16: 1249-1259.
31. Rugge M, Fassan M, Pizzi M, Zorzetto V, Maddalo G, Realdon S, De Bernard M, Betterle C, Cappellesso R, Pennelli G, de Boni M, Farinati F. Autoimmune gastritis: histology phenotype and OLGA staging. Aliment Pharmacol Ther. 2012;35: 1460-1466.
32. Rugge M, Genta RM. Staging and grading of chronic gastritis. Hum Pathol. 200; 36: 228-233.
33. Rugge M, Genta RM, Graham DY, Di Mario F, Vaz Coelho LG, Kim N, Malfertheiner P, Sugano K, Tsukanov V, Correa P. Chronicles of a cancer foretold: 35 years of gastric cancer risk assessment. Gut. 2016; 65: 721-725.
34. de Sablet T, Piazuelo MB, Shaffer CL, Schneider BG, Asim M, Chaturvedi R, Bravo LE, Sicinschi LA, Delgado AG, Mera RM, Israel DA, Romero-Gallo J, Peek RM Jr, Cover TL, Correa P, Wilson KT. Phylogeographic origin of Helicobacter pylori is a determinant of gastric cancer risk. Gut. 2011; 60: 1189-1195.
35. Shao L, Li P, Ye J, Chen J, Han Y, Cai J, Lu X. Risk of gastric cancer among patients with gastric intestinal metaplasia. Int J Cancer. 2018 Apr 29. doi: 10.1002/ijc.31571. [Epub ahead of print]

36. Song H, Ekheden IG, Zheng Z, Ericsson J, Nyrén O, Ye W. Incidence of gastric cancer among patients with gastric precancerous lesions: observational cohort study in a low risk Western population. BMJ. 2015; 351: h3867.
37. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, Haruma K, Asaka M, Uemura N, Malfertheiner P; faculty members of Kyoto Global Consensus Conference. Kyoto global consensus report on Helicobacter pylori gastritis. Gut. 2015; 64: 1353-1367.
38. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. N Engl J Med. 2001; 345: 784-789.
39. Veijola LI, Oksanen AM, Sipponen PI, Rautelin HI. Association of autoimmune type atrophic corpus gastritis with Helicobacter pylori infection. World J Gastroenterol. 2010; 16: 83-88.
40. de Vries AC, van Grieken NC, Looman CW, Casparie MK, de Vries E, Meijer GA, Kuipers EJ. Gastric cancer risk in patients with premalignant gastric lesions: a nationwide cohort study in the Netherlands. Gastroenterology. 2008; 134: 945-952.
41. Weck MN, Brenner H. Association of Helicobacter pylori infection with chronic atrophic gastritis: Meta-analyses according to type of disease definition. Int J Cancer. 2008; 123: 874-881.
42. Wolf EM, Plieschnegger W, Geppert M, Wigginghaus B, Höss GM, Eherer A, Schneider NI, Hauer A, Rehak P, Vieth M, Langner C. Changing prevalence patterns in endoscopic and histological diagnosis of gastritis? Data from a cross-sectional Central European multicentre study. Dig Liver Dis. 2014; 46: 412-418.
43. Wolf EM, Plieschnegger W, Schmack B, Bordel H, Höfler B, Eherer A, Schulz T, Vieth M, Langner C. Evolving patterns in the diagnosis of reactive gastropathy: data from a prospective Central European multicenter study with proposal of a new histologic scoring system. Pathol Res Pract. 2014; 210: 847-854.
44. Yue H, Shan L, Bin L. The significance of OLGA and OLGIM staging systems in the risk assessment of gastric cancer: a systematic review and meta-analysis. Gastric Cancer. 2018; 21: 579-587.

Gastric Cancer

Gastric cancer develops predominantly in patients with chronic-active gastritis, following an atrophy/intestinal metaplasia – dysplasia – invasive adenocarcinoma sequence (Correa's cascade). More rarely, sporadic gastric cancer may develop from **adenomatous precursor lesions** which include three distinct subtypes:

- Intestinal type (tubular or tubulovillous) adenoma (most common): positive for MUC2 and CDX-2
- Foveolar type (tubular or tubulovillous) adenoma (exceedingly rare): positive for MUC5AC
- Pyloric gland adenoma: positive for MUC6 (MUC5AC within the surface epithelium) and pepsinogen 1

The latter is underrecognized but may show transition to well differentiated adenocarcinoma in 30-50% of cases. It is found usually in stomachs with chronic gastritis, preferably autoimmune gastritis, and is also quite common in Lynch syndrome and may also be found in patients with familial adenomatous polyposis.

Though gastric cancer can now be classified using molecular data (compare below), many clinical trials still use **Lauren's classification** system which was introduced already in 1965 and represents the first attempt to classify gastric cancer.

Specifically, Lauren's classification system divides gastric cancer chiefly into two types, that is intestinal and diffuse, according to the presence or absence of glandular formation, also taking account the macroscopic appearance of the tumour (well circumscribed for the intestinal type versus poorly circumscribed for the diffuse tyoe). Tumours with approximately equal quantities of intestinal and diffuse components are called mixed type carcinomas.

The specific recognition of the tumour margin (well delineated versus poorly delineated) makes Lauren's classification particularly important for the surgical treatment of gastric cancer patients, Specifically, patients with intestinal type cancers, in particular when located within the antrum, do no longer need complete gastrectomy, which has major impact on the quality of life of affected individuals.

Category	Intestinal type gastric cancer	Diffuse type gastric cancer
Macroscopy	Well circumscribed Antrum > corpus	Poorly circumscribed Corpus > antrum
Histology	Glandular (or solid, depending on the grade of differentiation) Preserved cell adhesion (depending on the grade of differentiation) Often in association with intestinal metaplasia	Non-cohesive tumour cell growth (non-glandular) Lack of cell adhesion Includes cancers with signet-ring cell morphology
Clinical characteristics	Elderly patients Males > females More prevalent in high-risk areas	Younger patients Females > males More prevalent in low risk areas
Aetiology	Helicobacter pylori (Correa's cascade)	Chronic inflammation Genetic background (hereditary diffuse gastric cancer, HDGC)
Metastasis	Liver > peritoneum	Peritoneum > liver
Biomarkers	CDX-2 and MUC2 HER2 MSI	*CDH1* mutation (loss of E-cadherin expression) MUC5AC MSS

Table 1 Lauren's classification system of gastric cancer

The 3rd edition of the WHO classification (2000) included adenocarcinoma (intestinal versus diffuse type, according to the Lauren's classification) and recognized additional subtypes,

such as papillary, tubular, mucinous, signet-ring cell, adenosquamous, squamous cell, small cell, and undifferentiated carcinoma.

The major change of the **4th edition of the WHO classification (2010)** was to leave the concept of the Lauren's classification and to define the following categories:

- Adenocarcinoma
  - Papillary adenocarcinoma
  - Tubular adenocarcinoma
  - Mucinous adenocarcinoma
  - Poorly cohesive carcinoma (including signet-ring cell carcinoma and other variants)
  - Mixed adenocarcinoma
- Adenosquamous carcinoma
- Carcinoma with lymphoid stroma (medullary carcinoma)
- Hepatoid adenocarcinoma
- Squamous cell carcinoma
- Undifferentiated carcinoma
- Neuroendocrine neoplasms
  - Neuroendocrine tumour (NET)
    - NET G1
    - NET G2
  - Neuroendocrine carcinoma
    - Small cell NEC
    - Large cell NEC
  - Mixed adenoneuroendocrine carcinoma (MANEC)

Although most gastric cancers are sporadic, familial aggregation is known to occur in up to one third of patients.

Hereditary cancer syndromes, in which gastric cancer may be found but in which the stomach is not the predominantly affected organ, include the following:

- Lynch syndrome (HNPCC) with mismatch repair deficiency and microsatellite instability (germline mutation in mismatch repair genes, *MLH1*, *MSH2*, *MSH6*, *PMS2*); predominantly cancers of the colon and female genital organs (endometrium)
- Li-Fraumeni syndrome (germline mutation in *TP53*)
- Peutz-Jeghers syndrome (germline mutation in *STK11*)
- Juvenile polyposis (germline mutations in *SMAD4*, *BMPR1A*)
- Familial adenomatous polyposis (FAP, mutation in *APC*)

Three **distinct hereditary/familial gastric cancer syndromes** have been identified:

- Hereditary diffuse gastric cancer (HDGC, CDH1 germline mutation mainly, but not exclusively)
- Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS, APC promoter germline mutation)
- Familial intestinal gastric cancer (FIGC, no cause identified to date)

Patients with HDGC are young (usually < 40 years). Affected females are at increased for lobular breast cancer.

Pathologists need to be ware of the peculiar gastric precursor lesions (pagetoid spread, in situ signet-ring cell carcinoma), which may the only lesions present in a prophylactic gastrectomy specimen of a mutation carrier.

The morphology of neoplastic lesions in GAPPS are of gastric phenotype. Precursor lesions show different morphology: most of them are equivalent to fundic gland polyps (with or without dysplasia), others look like hyperplastic polyps, and also serrated features may be observed.

Several **molecular classification systems of gastric cancer** have been introduced which are promising for developing personalized therapy. Using molecular analysis, in situ hybridization techniques (and also immunohistochemistry) gastric cancer can be classified broadly into four groups based upon Epstein–Barr virus (EBV) positivity, microsatellite instability, chromosomal instability and genomic stability (Comprehensive molecular characterization of gastric adenocarcinoma by the Cancer Genome Atlas Research Network. Nature 2014).

EBV-positive gastric cancer is defined by the presence of virus in tumor cells and/or in dysplastic epithelial cells. EBV-associated tumors have a higher prevalence of DNA hypermethylation compared to other tumors. EBV-positive tumors display CpG methylation, *CDKN2A* promoter hypermethylation, *PIK3CA* mutations (80%), *JAK2* amplification and increased PD-L1/2 expression. Genetic alterations of P53 are rare. EBV-positive gastric cancer is more prevalent in younger patients, predominantly male, and tend to be diffuse-type in proximal portions of the stomach with better overall survival. EBV infection is strongly associated with lymphoepithelioma-like gastric carcinoma and carcinoma in gastric remnant.

Microsatellite instable (MSI)-tumors are strongly correlated with *MLH1* hypermethylation. MSI gastric cancer lack *BRAF* mutations commonly seen in MSI sporadic MSI colorectal cancer. These tumors can be identified using antibodies against MLH1, PMS2, MSH2, and MSH6. MSI-gastric cancers often express the immune checkpoint molecules PD-L1 and PD-1. MSI gastric cancer is more prevalent in older patients in the distal stomach with a lower number of lymph node metastasis, and better overall survival. Accurate classification of MSI

gastric cancers is clinically important as they may not require standard (neo-)adjuvant radio-chemotherapy, while PD-L1 and PD-1 inhibitors may be considered suitable for treatment.

Chromosomal instable (CIN) gastric cancer subtype is the largest group (almost 50% of all gastric cancer), and often shows an intestinal phenotype. These tumors frequently harbour *TP53* mutations, *ERBB2* (*HER2*) amplification, and *K-RAS* mutation, while *PIK3CA* mutation is lowest in this subtype.

Genomically stable (GS) carcinomas are associated with diffuse-type histology and tend to be diagnosed at an earlier age. These tumors frequently have *CDH1* mutations, *RHOA* gene mutations and recurrent interchromosomal translocation, with CLDN18 and ARHGAP fusion).

EBV-positive	Microsatellite instable (MSI)	Chromosomal instable	Genomically stable
Lymphoepithelioma-like gastric carcinoma and carcinoma in gastric remnant *PIK3CA* mutation PD-L1/2 overexpression EBV-CIMP *CDKN2A* silencing Immune cell signalling	Intestinal type according to Lauren, susually well to moderately differentaied (G1/G2 > G3) Hypermutation Gatric-CIMP *MLH1* silencing Mitotic pathways May show PD-L1/2 overexpression	Intestinal type according to Lauren, marked aneuploidy, rarely well differentiated (G2/G3 > G1) TP53 mutations ERBB2 (HER2) amplification RTK-RAS activiation	Diffuse-type according to Lauren *CDH1* mutation (reduced cell adhesion) *RHOA* mutation *CLDN18-ARHGAP* fusion

Table 2 Molecular classification of gastric cancer (modified after Cancer Genome Atlas Research Network. Nature 2014)

The ToGA trial (Phase III trastuzumab Gastric cancer study) demonstrated a survival benefit with trastuzumab plus chemotherapy (capecitabine or 5-fluorouracil and cisplatin) in patients with HER2-positive gastric cancer whether locally advanced, recurrent and/or metastatic gastric or gastro-oesophageal junction cancer. The benefit was confined to those

with IHC 2+/3+ positivity. Fluorescence in situ hybridization should be performed in equivocal (2+) cases. Unfortunately, HER2 is overexpressed in only 20% of intestinal type gastric cancers and 7% of diffuse-type carcinomas.

Score	Seen at objective	Resections	Biopsies	HER2 status
0	NA	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity / no membranous reactivity in any tumour cell	Negative
1 +	40x	Faint / barely perceptible membranous reactivity in ≥ 10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with faint/barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2 +	10-20x	Weak-moderate complete basolateral / lateral membranous reactivity in ≥ 10% of tumour cells	Tumour cell cluster with weak to moderate complete basolateral / lateral membranous reactivity irrespective of percentage of tumor cells stained	Equivocal
3 +	2.5-5x	Strong complete basolateral / lateral membranous reactivity in ≥ 10% of tumour cells	Tumour cell cluster with strong complete basolateral / lateral membranous reactivity irrespective of % of tumour cells stained	Positive

Table 3 Criteria for HER2 evaluation in gastric cancer.

Regression grading after neoadjuvant treatment

Neoadjuvant (radio-)chemotherapy has successfully been included in multimodal strategies for the treatment of locally advanced gastrointestinal malignancies, that is oesophageal, gastric and rectal cancers. The benefit for the patients can be summarized as follows:

- Tumour downsizing / downcategorizing / downstaging
- Higher distance to resection margin
- Higher rate of complete resection (R0)
- Lower rate of systemic tumour cell dissemination (micrometastasis, ITC)
- Lower rate of intraoperative tumour cell dissemination
- Better outcome (local recurrence, survival)

Successful neoadjuvant treatment induces tumour shrinkage, which can be observed on macroscopic and microscopic level.

Changes on the macroscopic level are highly variable and range from “no change” to complete cancer regression. The latter may cause major problems for the pathologist, as it may be particularly challenging to identify the previous tumour area, that is, the so-called tumour bed on gross inspection. Usually, the wall of the organ is thickened and the consistency increased. The overlying mucosa may be entirely normal.

Depressed ulcers are common, which may give the impression of complete regression, but this does not need to be the case and the extent of remnant cancer is usually underestimated. This is due to the fact that cancer regression is usually more pronounced in the tumour centre (including the luminal parts of the lesion), while cancer cells often better sustain treatment in the periphery (including the invasion front). To avoid unpleasant situations, pathologists need to ink the entire circumferential margin (CRM) before slicing. Whenever possible, the tumour bed should be embedded totally and – ideally – be investigated in connection with the CRM.

On the histological level, tumour necrosis, fibrosis of the tumour bed and acellular mucin lakes (in adenocarcinomas) are typical morphological features of tumour regression. Cancer cells often show condensed, enlarged, sometimes bizarre nuclei that may contain nuclear inclusions. The cytoplasm is hypereosinophilic or vacuolated. Multinucleated and cancer giant cells may occur. Neoadjuvant treatment affects also non-neoplastic epithelium: ulceration, reactive / regenerative and also metaplastic changes are well recognized features. On the stroma level the following changes may be observed: Resorption, mixed inflammatory infiltrate, granulation tissue, haemorrhage, foamy histiocytes, cholesterol clefts, calcification, foreign body reaction / granuloma formation as well as vascular changes (obliterative vasculopathy).

Please note, neoadjuvant treatment induces architectural changes that preclude tumour grading. That is, information on tumour grade should be obtained from pre-treatment

biopsies. In addition, a pre-treatment diagnosis of adenocarcinoma (NOS) should, not be changed into a diagnosis of mucinous adenocarcinoma on the basis of mucin lakes present after neoadjuvant treatment. This can only be done, when MRI data suggest that biopsies may not have been representative for the entire lesion.

There are principally two possibilities to assess the extent of tumour regression in a post-treatment cancer specimen:

- Comparing the proportion of residual tumour to the extent of therapy-induced fibrosis or scarring (consistent with the tumour bed)
- Estimating the percentage of residual tumour in relation to the former tumour site (consistent with the tumour bed)

Both approaches have been applied in a long list of schemes, published by different authorities (from different countries) for different organs. In the following, the presumably most important will briefly be presented and discussed. It is obvious that regional, that is, national requirements influence the choice of a specific grading scheme (the name of which should be provided in the pathology report).

Regression grading in gastric cancer is widely performed following the proposal made by Becker et al. (2003, 2011). This scheme (Table 4) is based upon an estimation of the percentage of vital tumour tissue in relation to the macroscopically identifiable tumour bed. Three major grades are used, with subdivision of the first category. The scheme can likewise be used for adenocarcinomas of the gastroesophageal junction and distal oesophagus (Barrett's adenocarcinoma).

Response of the primary tumour does not guarantee recurrence-free long-term survival, but histopathological complete responders have better prognosis compared to patients with partial or subtotal tumour regression. In one study, 20% of patients with histological complete response recurred during follow-up.

Category	Description
TRG 1	Complete or subtotal regression TRG 1a: 0% residual tumour TRG 1b: <10% residual tumour
TRG 2	Partial tumour regression (10-50% residual tumour)
TRG 3	Minimal or no tumour regression (>50% residual tumour)

Table 4 The Becker scheme is widely used for assessing the extent of regression in gastric carcinoma after neoadjuvant treatment (TRG = tumour regression grade).

In 2012, Becker et al. proposed a multifactorial prognostic score that accurately classifies three groups of gastric carcinoma patients with different outcomes after neoadjuvant chemotherapy and surgery. This score includes the tumour regression grade, but also the ypT- and ypN-categories. Prognostication seems to be better than using the regression grade alone.

Recommendations for a standardized work-up of cancer specimens after neoadjuvant treatment are currently lacking. Recently, Langer and Becker (Virchows Arch 2018) published detaled recommendations for the routine work-up of cancer specimens and reporting of tumour regression grade (TRG). These are summarized in Table 5.

Work-up of Cancer Specimens after Neoadjuvant Treatment and Reporting of Tumour Regression Grade (TRG)	
Phase 1 (documentation and description)	Photographic documentation (for orientation and documentation of blocks and of histologically proven residual tumour) Macroscopic description according to standard macroscopy: tumour site (three-dimensional), distance to margins
Phase 2 (macroscopic work-up, grossing)	Inking of relevant circumferential margins (most important for oesophagus and rectum, but also for proximal stomach cancer) Complete embedding of macroscopically identifiable tumour bed (e.g. oriented from proximal to distal, for tumour beds ≤ 5 cm) If tumour bed > 5 cm take blocks following the longitudinal and vertical largest dimension at first step (as significant regression is unlikely); if not or less tumour residual tumour by histology, embed remaining tumour bed in a second step
Phase 3 (histological work-up)	All slides stained by haematoxylin and eosin (H&E), selected blocks by periodic acid-Schiff (PAS) or Elastica van Gieson (EvG), if appropriate Immunohistochemistry may be helpful for discrimination between histiocytes and altered tumour cells, but conventional light microscopy is usually sufficient for the identification of residual tumour If no residual tumour in first section, prepare three step sections to confirm complete response (lymph nodes: step sections if signs of regression without residual tumour in first section) Establish tumour regression (TRG) grade according to the appropriate TRG scheme (for the respective cancer)

Table 5 Proposal for standardized work-up and reporting of cancer specimens after neoadjuvant treatment (modified after Langer & Becker, Virchows Archiv 2018)

References

1. Ahn S, Lee SJ, Kim Y, Kim A, Shin N, Choi KU, Lee CH, Huh GY, Kim KM, Setia N, Lauwers GY, Park DY. High-throughput Protein and mRNA Expression-based Classification of Gastric Cancers Can Identify Clinically Distinct Subtypes, Concordant with Recent Molecular Classifications. Am J Surg Pathol. 2017; 41: 106-115.
2. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010; 376: 687-697.
3. Becker K, Mueller JD, Schulmacher C, et al. Histomorphology and grading of regression in gastric carcinoma treated with neoadjuvant chemotherapy. Cancer. 2003; 98: 1521-1530.
4. Becker K, Langer R, Reim D, et al. Significance of histopathological tumor regression after neoadjuvant chemotherapy in gastric adenocarcinomas: a summary of 480 cases. Ann Surg. 2011; 253: 934-939.
5. Becker K, Reim D, Novotny A, et al. Proposal for a multifactorial prognostic score that accurately classifies 3 groups of gastric carcinoma patients with different outcomes after neoadjuvant chemotherapy and surgery. Ann Surg. 2012; 256: 1002-1007.
6. Camargo MC, Kim WH, Chiaravalli AM, Kim KM, et al. Improved survival of gastric cancer with tumour Epstein-Barr virus positivity: an international pooled analysis. Gut. 2014; 63: 236-243.
7. Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature. 2014; 513:2 02-9.
8. Carneiro F. Hereditary gastric cancer. Pathologe. 2012;33 Suppl 2: 231-234.
9. Correa P. A human model of gastric carcinogenesis. Cancer Res. 1988; 48: 3554-3560.
10. de Boer WB, Ee H, Kumarasinghe MP. Neoplastic Lesions of Gastric Adenocarcinoma and Proximal Polyposis Syndrome (GAPPS) Are Gastric Phenotype. Am J Surg Pathol. 2018; 42: 1-8.
11. Gullo I, Devezas V, Baptista M, Garrido L, Castedo S, Morais R, Wen X, Rios E, Pinheiro J, Pinto-Ribeiro I, Ferreira RM, Preto J, Santos-Antunes J, Marques M, Campos M, Almeida F, Espinheira MDC, Amil Dias J, Figueiredo C, Oliveira C, Trindade E, Carneiro F. Phenotypic heterogeneity of hereditary diffuse gastric cancer: report of a family with early-onset disease. Gastrointest Endosc. 2018; 87: 1566-1575.
12. Hackeng WM, Montgomery EA, Giardiello FM, Singhi AD, Debeljak M, Eshleman JR, Vieth M, Offerhaus GJ, Wood LD, Brosens LA. Morphology and genetics of pyloric gland adenomas in familial adenomatous polyposis. Histopathology. 2017; 70: 549-557.

13. Hissong E, Ramrattan G, Zhang P, Zhou XK, Young G, Klimstra DS, Shia J, Fernandes H, Yantiss RK. Gastric Carcinomas with Lymphoid Stroma: An Evaluation of the Histopathologic and Molecular Features. Am J Surg Pathol. 2018; 42: 453-462.
14. Hofmann M, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, Ochiai A, Rüschoff J, Henkel T. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology. 2008; 52: 797-805.
15. Huang KH, Wang RF, Yang MH, Wu CW, Fang WL, Li AF, Chi CW, Kao HL. Advanced gastric cancer patients with lymphoid stroma have better survival than those without. J Surg Oncol. 2013; 107: 523-528.
16. Kawazoe A, Kuwata T, Kuboki Y, Shitara K, Nagatsuma AK, Aizawa M, Yoshino T, Doi T, Ohtsu A, Ochiai A. Clinicopathological features of programmed death ligand 1 expression with tumor-infiltrating lymphocyte, mismatch repair, and Epstein-Barr virus status in a large cohort of gastric cancer patients. Gastric Cancer. 2017; 20: 407-415.
17. Langer R, Becker K. Tumor regression grading of gastrointestinal cancers after neoadjuvant therapy. Virchows Arch. 2018; 472: 175-186.
18. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand. 1965; 64: 31-49.
19. Li J, Woods SL, Healey S, Beesley J, et al. Point Mutations in Exon 1B of APC Reveal Gastric Adenocarcinoma and Proximal Polyposis of the Stomach as a Familial Adenomatous Polyposis Variant. Am J Hum Genet. 2016; 98: 830-842.
20. Ma C, Patel K, Singhi AD, Ren B, Zhu B, Shaikh F, Sun W. Programmed Death-Ligand 1 Expression Is Common in Gastric Cancer Associated with Epstein-Barr Virus or Microsatellite Instability. Am J Surg Pathol. 2016; 40: 1496-1506.
21. Ma J, Shen H, Kapesa L, Zeng S. Lauren classification and individualized chemotherapy in gastric cancer. Oncol Lett. 2016; 11: 2959-2964.
22. McDuffie LA, Sabesan A, Allgäeuer M, Xin L, Koh C, Heller T, Davis JL, Raffeld M, Miettienen M, Quezado M, Rudloff U. β-Catenin activation in fundic gland polyps, gastric cancer and colonic polyps in families afflicted by 'gastric adenocarcinoma and proximal polyposis of the stomach' (GAPPS). J Clin Pathol. 2016; 69: 826-33.
23. Oliveira C, Pinheiro H, Figueiredo J, Seruca R, Carneiro F. Familial gastric cancer: genetic susceptibility, pathology, and implications for management. Lancet Oncol. 2015; 16: e60-70.
24. Riquelme I, Saavedra K, Espinoza JA, Weber H, García P, Nervi B, Garrido M, Corvalán AH, Roa JC, Bizama C. Molecular classification of gastric cancer: Towards a pathway-driven targeted therapy. Oncotarget. 2015; 6: 24750-24779.
25. Rüschoff J, Dietel M, Baretton G, Arbogast S, Walch A, Monges G, Chenard MP, Penault-Llorca F, Nagelmeier I, Schlake W, Höfler H, Kreipe HH. HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. Virchows Arch. 2010; 457: 299-307.

26. Rüschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, van de Vijver M, Viale G. HER2 testing in gastric cancer: a practical approach. Mod Pathol. 2012; 25: 637-650.
27. Spoto CPE, Gullo I, Carneiro F, Montgomery EA, Brosens LAA. Hereditary gastrointestinal carcinomas and their precursors: An algorithm for genetic testing. Semin Diagn Pathol. 2018;35: 170-183.
28. Tamura T, Ohira M, Tanaka H, Muguruma K, Toyokawa T, Kubo N, Sakurai K, Amano R, Kimura K, Shibutani M, Maeda K, Hirakawa K. Programmed Death-1 Ligand-1 (PDL1) Expression Is Associated with the Prognosis of Patients with Stage II/III Gastric Cancer. Anticancer Res. 2015; 35: 5369-5376.
29. Tan P, Yeoh KG. Genetics and Molecular Pathogenesis of Gastric Adenocarcinoma. Gastroenterology. 2015; 149: 1153-1162.
30. Van Cutsem E, Bang YJ, Feng-Yi F, Xu JM, Lee KW, Jiao SC, Chong JL, López-Sanchez RI, Price T, Gladkov O, Stoss O, Hill J, Ng V, Lehle M, Thomas M, Kiermaier A, Rüschoff J. HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. Gastric Cancer. 2015; 18: 476-484.
31. Van der Post RS, Carneiro F. Emerging Concepts in Gastric Neoplasia: Heritable Gastric Cancers and Polyposis Disorders. Surg Pathol Clin. 2017; 10: 931-945.
32. Van der Post RS, Vogelaar IP, Carneiro F, et al. Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet. 2015; 52: 361-374.
33. Vieth M, Kushima R, Borchard F, Stolte M. Pyloric gland adenoma: a clinico-pathological analysis of 90 cases. Virchows Arch. 2003; 442: 317-321.
34. Vieth M, Kushima R, Mukaisho K, Sakai R, Kasami T, Hattori T. Immunohistochemical analysis of pyloric gland adenomas using a series of Mucin 2, Mucin 5AC, Mucin 6, CD10, Ki67 and p53. Virchows Arch. 2010; 457: 529-536
35. Vieth M, Montgomery EA. Some observations on pyloric gland adenoma: an uncommon and long ignored entity! J Clin Pathol. 2014; 67: 883-890.
36. Vieth M, Vogel C, Kushima R, Borchard F, Stolte M. Pyloric gland adenoma-- how to diagnose? Cesk Patol. 2006; 42: 4-7.
37. Worthley DL, Phillips KD, Wayte N, Schrader KA, Healey S, Kaurah P, Shulkes A, Grimpen F, Clouston A, Moore D, Cullen D, Ormonde D, Mounkley D, Wen X, Lindor N, Carneiro F, Huntsman DG, Chenevix-Trench G, Suthers GK. Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS): a new autosomal dominant syndrome. Gut. 2012; 61: 774-779.

Classical pathology of inflammatory bowel disease and dysplasia

Chronic inflammatory bowel disease (IBD) includes, strictly spoken, two diseases, that is, ulcerative colitis and Crohn's disease. Both represent a steadily growing burden for health care systems. The pathologist's role in the management of the disease includes assistance in initial diagnosis, assessment of activity, differential diagnosis and diagnosis of dysplasia and/or cancer.

Analysis of multiple biopsies allows a correct diagnosis of IBD in 66-75% of newly diagnosed patients. Providing additional endoscopic and clinical data to the pathologist increases the diagnostic accuracy, allowing a final diagnosis in more than 90% of cases, respectively.

The histological features useful for a diagnosis of IBD may be grouped into four categories:

- Mucosal (crypt) architecture
- Lamina propria cellularity
- Infiltration by neutrophils
- Epithelial changes

Abnormalities in mucosal (crypt) architecture include crypt distortion, branching, reduced crypt length (shortening) and reduced crypt density (both can be referred to as crypt atrophy) and surface epithelium irregularities (pseudovillous change). These changes are particularly pronounced in ulcerative colitis (57-100% of cases), but may also occur in Crohn's disease (27-71% of cases).

Within the stroma, there is a transmucosal increase of inflammatory cells with basal plasmacytosis. Neutrophils (cryptitis / crypt abscess formation) are markers of disease activity. Epithelial changes include epithelial damage (mucosal breaks, erosions and ulcers) and mucin depletion (at active sites) as well as metaplastic changes (as markers of chronicity).

The key histological features of ulcerative colitis are the following:

- Diffuse (continuous) mucosal disease that begins in the rectum and spreads variably to the proximal colon (it is usually worse distally)
- Severe diffuse mucosal architectural abnormalities (crypt shortening and distortion, decreased crypt density)
- Severe diffuse transmucosal increase of (predominantly mononuclear) inflammatory cells with basal plasmacytosis
- Epithelial abnormalities, such as surface epithelial damage and mucin depletion as well as Paneth cell metaplasia (in biopsies obtained distal to the hepatic flexure)
- Tissue fragments both within the same biopsy and within separately submitted specimens tend to show the same degree of inflammation

The key histological features of Crohn's disease are the following:

- Segmental (discontinuous) transmural disease ("skip lesions" with fissures, fistulae) with variable rectal involvement and variable disease severity (usually worse proximally)
- Focal (discontinuous) crypt architectural abnormalities (focal crypt atrophy and distortion)
- Focal (discontinuous) inflammation (focal mononuclear expansion of the lamina propria, focal cryptitis). Focal or patchy inflammation may be observed in biopsies submitted from different parts of the bowel or may be present within tissue fragments of the same biopsy, not rarely within a single biopsy specimen
- Aphthous erosions/ulcers and deep fissures, any location
- Epithelioid cell granulomas (not crypt related) in approximately 20% of mucosal biopsies (up to 50% in resections) – they need to be differentiated from so-called cryptolytic granulomas (unspecific foreign body reaction to ruptured crypts, may occur in several types of colitis)

While ulcerative colitis is usually restricted to the large bowel (apart from so-called backwash ileitis and rare upper tract involvement, mainly in children and adolescents, but also in adults), Crohn's disease may affect the whole gastrointestinal tract: Crohn's disease affecting both small and large bowel is seen in about 40-50% of cases, isolated small bowel or isolated large bowel disease in 30-35% and 15-25% of cases respectively. Upper tract involvement is common in Crohn's disease and may be detected in 50-75% of cases, usually in the form of focally enhanced gastritis (with and without granulomatous reaction).

The **differential diagnosis between ulcerative colitis and Crohn's disease** may be challenging, since overlapping morphological features are seen in 10-15% of cases. In unclear

cases diagnosis of indeterminate colitis (for resection specimens) or IBD unclassified, IBDU (for biopsies) should be made. It has to be acknowledged that there is no single pathognomonic histological feature, and the diagnosis typically rests on a combination of clinical, laboratory, endoscopic, and histological observations, with ulcerative colitis showing more severe architectural and inflammatory abnormalities than Crohn's disease.

Please note: Differential diagnosis may be particularly challenging under therapy, since mucosal healing in ulcerative colitis may cause discontinuous inflammation and rectal sparing.

	Infectious colitis	UC active phase	UC in remission	Crohn's disease
Crypt architectural abnormalities / basal plasmacytosis	- / (+)	+++	++/+	+/(+)
Metaplastic Paneth cells / mucin depletion	-	++	++ / (+)	(+)
Mononuclear cells ↑	(+)	+++	-	(+)
Neutrophils	+++	+++	-	++
Granulomas / giant cells	(+)	(+)	-	++
Continous morphologic changes	(+)	+++	++ / (+)	-
Discontinous morphologic changes	+	-	- / (+)	++

Table 1 Histological features of inflammatory bowel disease and differential diagnosis

IBD occurring in patients with primary sclerosing cholangitis (PSC) has a peculiar phenotype. In the large bowel, the disease prevails in the proximal colon (in particular when PSC was diagnosed before IBD), often showing continuous inflammation. The rectum is usually not involved, which is in contrast to the terminal ileum which usually shows marked inflammatory changes. It is of of note, that cancer risk is invreased compared with “classical” IBD, dysplastic lesions likewise occurring mainly on the right side.

The pathologist’s report should include information on the **grade of disease activity**. This mainly holds true for ulcerative colitis (and is less important for Crohn’s disease due to its discontinuous nature). Different scoring systems have been developed, of which the “Nancy Index” is appealing as it can easily be applied:

- **Grade 0:** no significant histological disease (no or mild increase of chronic inflammatory infiltrate)
- **Grade 1:** moderate or marked increase ofchronic inflammatory infiltrate with no acute inflammatory infiltrate
- **Grade 2:** Mildly active disease characterized by an acute inflammatory cell infiltrate with few or rare neutrophils in lamina propria or in the epithelium that are difficult to see
- **Grade 3**: Moderatively active disease characterized by moderate to severe acute inflammatory cell infiltrate with presence of multiple clusters of neutrophils in lamina propria and/or in epithelium that are easily apparent (without ulceration)
- **Grade 4:** Severely active disease characterized by presence of ulceration (the presence of only epithelial stripping should not be considered as ulceration)

Please note that endoscopic mucosal healing does not automatically imply histological healing. The latter, however, is important for prediction of disease course, that is, patient management.

Ulcerative colitis and Crohn’s disease need to be **differentiated from other types of colitis**, such as infectious colitis (mainly active inflammation which may be discontinuous, no basal plasmacytosis, preserved crypt architecture), segmental colitis associated with diverticulosis (SCAD, syn. diverticular colitis which may show IBD-like changes typically within the sigmoid colon in elderly individuals), and also different types of drug-induced colitis.

Of particular interest in the latter group are immunomodulators, such as mycophenolate mofetil (MMF) and the new kis on the block: Immune checkpoint modulators, such als ipilmumab, nivolumab and pembolizumab, which are used to boost the patient’s own anti-tumour immune activity (so-called immunotherapy), resulting in a plethora of immune-mediated side effects. The gastrointestinal tract is commonly (and usually severely) involved, with two distinct patterns: lymphocytic colitis-like and active colitis-like, the latter possibly

showing mild architectural disturbances. A diagnostic clue is looking for apoptotic bodes which are commonly increased and easy to detect in drug-mediated disease.

Finally, patients with IBD, particularly those with ulcerative colitis (but also Crohn's disease patients with large bowel involvement), are at increased risk for colon cancer. Long disease duration, extensive bowel involvement, young age at onset and severity of microscopic inflammation have been identified as main risk factors.

On the histological level, **dysplasia (intraepithelial neoplasia)** represents the best and most reliable marker of malignancy risk. It develops only in areas with chronic inflammation and can be divided into four diagnostic categories: negative (regenerating epithelium), indefinite and positive for low-grade and high-grade dysplasia.

Grossly, dysplastic lesions may be polypoid, non-polypoid or endoscopically not visible (3rd ECCO Cosensus on Ulcerative Colitis, 2017). The former terms "DALM" (dysplasia associated lesion or mass) and "flat dysplasia" have been abandoned by the respective parties and should no longer be used. The following features may be used to differentiate IBD-associated dysplasia from regenerating epithelium.

	Colitis-associated dysplasia	Regenerating epithelium
Crypt architecture	Altered (budding, branching, cribriforming, crowding or back-to-back growth)	Preserved
Cytologic atypia	Moderate (to marked)	Mild (to moderate)
N/C ratio	Increased	Normal
Nuclei	Hyperchromatic, stratification	No stratification
Nucleoli	Prominent, enlarged (or multiple)	May be prominent, but usually not enlarged
Mitoses	Frequent, pathological mitoses	Frequent, normal looking
Surface maturation	No	Yes
Increased lamina propria inflammation	Variable	Usually present (neutrophils!)

Table 2 Histological features to distinguish colitis-associated dysplasia from regenerating epithelium

Differential diagnosis should not be based upon a single morphological feature, that is, different variables should be interpreted in conjunction, taking into account also the macroscopic aspect of the lesion. Polypoid colitis-associated dysplasia is often poorly delineated, extending into the adjacent non-polypoid mucosa. Lesions are often asymmetrical, showing an irregular outline or surface. Of note, this is in contrast to the gross appearance of sporadic adenomas which encounter as more regular and often symmetrical polypoid lesions. If diagnosis of dysplasia cannot be made with certainty the term “indefinite for dysplasia” may be used.

Low-grade dysplastic lesions show palisading and/or stratification of elongated nuclei with retained polarity. The nuclear/cytoplasmic (N/C) ratio is mildly increased. High-grade dysplastic lesions are characterized by marked pleomorphism (hyperchromatic rounded nuclei arramged in varying rows, with macro-nucleoli and increased mitosis) and more promunced architectural disturbance (with gland crowding and back-to-back pattern).

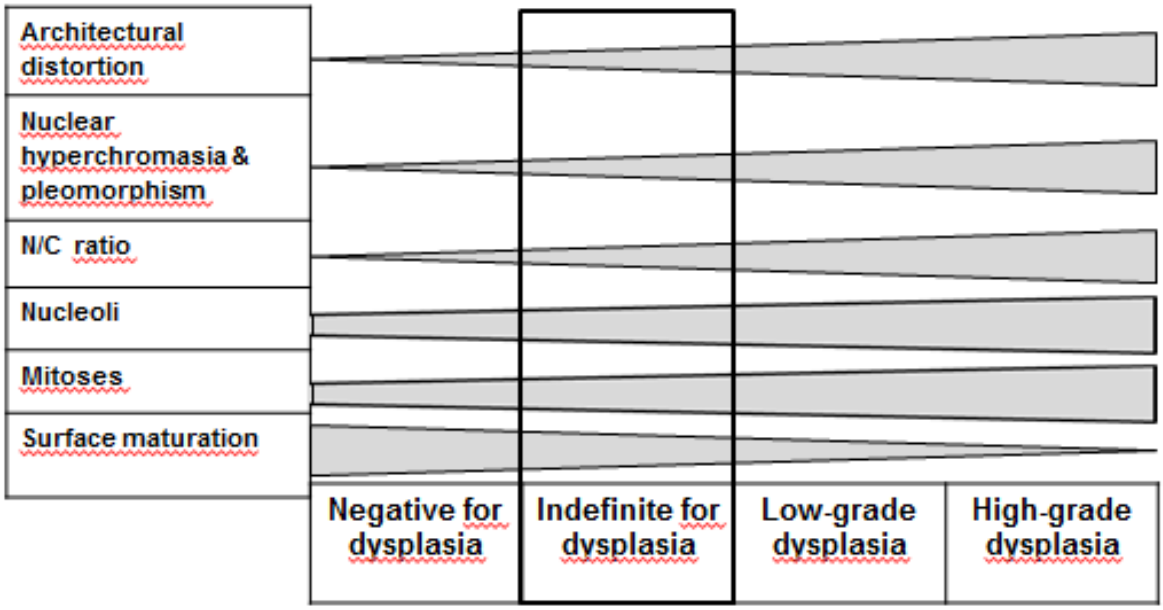

Figure 1 The features that can be used for differential diagnosis and distinction of low-grade from high-grade dysplasia show a morphological continuum.

Immunohistochemistry using antibody preparations directed against p53 may be of help in selected cases. Two distinct pathological staining patterns have been described

- p53 overexpression (due to impaired protein degradation)
- total lack of p53 staining (due to protein truncation; internal positive control of active non-mutated p53 necessary).

According to international guidelines, confirmation of dysplasia by an independent expert gastrointestinal pathologist is recommended.

It may be added that cancer in patients with IBD may development also “spontaneously”, that is, indepdent from the chronic inflammatory disease. Previously, the distinction between colitis-associated (colitis-dependent) and colitis-independent dysplastic (neoplastic) lesions was of eminent importance as treatment differed significantly: Patients with colitis-associated dysplasia almost invariably underwent colectomy, while colitis-independent dysplasia (sporadic adenomas) was treated by polypectomy.

Colitis-associated and colitis-independent dysplastic (neoplastic) lesions do not only differ on the morphological (macroscopical and histological) level but also on the molecular level. As shown above, the early mutation of the *TP53* gene in the colitis-associated pathway may be used diagnostically.

Still, the distinction is nowadays only an “academic” one, since both types of dysplasia are treated more or less the same way, provided colits-associated dysplastic lesions are visible and can be removed totally by endoscopy.

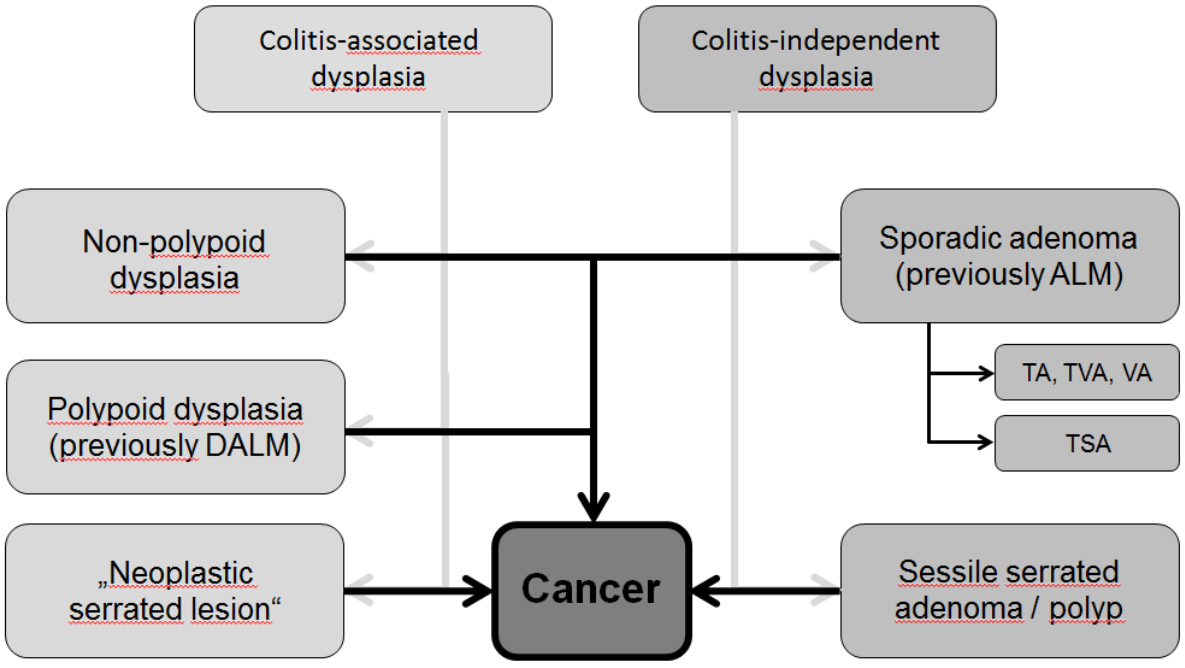

Figure 2 Colitis-associated (colitis-dependent) and colitis-indepdent dysplasia may both lead to colorectal cancer. The distinction is today however more or less only academic. The role of colitis-associated “neoplastic serrated lesions” still needs to be defined.

References

1. Adler BL, Pezhouh MK, Kim A, Luan L, Zhu Q, Gani F, Yarchoan M, Chen J, Voltaggio L, Parian A, Lazarev M, Lauwers GY, Pawlik TM, Montgomery EA, Jaffee E, Le DT, Taube JM, Anders RA. Histopathological and immunophenotypic features of ipilimumab-associated colitis compared to ulcerative colitis. J Intern Med. 2018; 283: 568-577.
2. Annese V, Daperno M, Rutter MD, Amiot A, Bossuyt P, East J, Ferrante M, Götz M, Katsanos KH, Kießlich R, Ordás I, Repici A, Rosa B, Sebastian S, Kucharzik T, Eliakim R; European Crohn's and Colitis Organisation. European evidence based consensus for endoscopy in inflammatory bowel disease. J Crohns Colitis. 2013; 7: 982-1018.
3. Assarzadegan N, Montgomery E, Anders RA. Immune checkpoint inhibitor colitis: the flip side of the wonder drugs. Virchows Arch. 2018; 472: 125-133.
4. Beaugerie L, Itzkowitz SH. Cancers complicating inflammatory bowel disease. N Engl J Med. 2015; 372: 1441-1452.
5. Beswick L, Ye B, van Langenberg DR. Toward an Algorithm for the Diagnosis and Management of CMV in Patients with Colitis. Inflamm Bowel Dis. 2016; 22: 2966-2976.
6. Bryant RV, Winer S, Travis SP, Riddell RH. Systematic review: histological remission in inflammatory bowel disease. Is 'complete' remission the new treatment paradigm? An IOIBD initiative. J Crohns Colitis. 2014; 8: 1582-1597.
7. Chen JH, Pezhouh MK, Lauwers GY, Masia R. Histopathologic Features of Colitis Due to Immunotherapy With Anti-PD-1 Antibodies. Am J Surg Pathol. 2017;4: 643-654.
8. Galandiuk S, Rodriguez-Justo M, Jeffery R, Nicholson AM, Cheng Y, Oukrif D, Elia G, Leedham SJ, McDonald SA, Wright NA, Graham TA. Field cancerization in the intestinal epithelium of patients with Crohn's ileocolitis. Gastroenterology. 2012; 142: 855-864.
9. Joo M, Odze RD. Rectal sparing and skip lesions in ulcerative colitis: a comparative study of endoscopic and histologic findings in patients who underwent proctocolectomy. Am J Surg Pathol. 2010; 34: 689-696.
10. Jouret-Mourin A, Faa G, Geboes K (eds). Colitis – A Practical Approach to Colon and Ileum Biopsy Interpretation (2nd edition). Springer 2018
11. Kaye PV, Haider SA, James PD, Soomro I, Catton J, Parsons SL, Ragunath K, Ilyas M. Novel staining pattern of p53 in Barrett's dysplasia--the absent pattern. Histopathology. 2010; 57: 933-935.
12. Ko HM, Harpaz N, McBride RB, Cui M, Ye F, Zhang D, Ullman TA, Polydorides AD. Serrated colorectal polyps in inflammatory bowel disease. Mod Pathol. 2015; 28: 1584-1593.
13. Laine L, Kaltenbach T, Barkun A, McQuaid KR, Subramanian V, Soetikno R; SCENIC Guideline Development Panel. SCENIC international consensus statement on surveillance and management of dysplasia in inflammatory bowel disease. Gastrointest Endosc. 2015; 81: 489-501.
14. Langner C. Colorectal normal histology and histopathologic findings in patients with chronic diarrhea. Gastroenterol Clin North Am. 2012; 41: 561-580

15. Langner C, Magro F, Driessen A, Ensari A, Mantzaris GJ, Villanacci V, Becheanu G, Borralho Nunes P, Cathomas G, Fries W, Jouret-Mourin A, Mescoli C, de Petris G, Rubio CA, Shepherd NA, Vieth M, Eliakim R, Geboes K; European Society of Pathology; European Crohn's and Colitis Foundation. The histopathological approach to inflammatory bowel disease: a practice guide. Virchows Arch. 2014; 464: 511-527.
16. Leedham SJ, Graham TA, Oukrif D, McDonald SA, Rodriguez-Justo M, Harrison RF, Shepherd NA, Novelli MR, Jankowski JA, Wright NA. Clonality, founder mutations, and field cancerization in human ulcerative colitis-associated neoplasia. Gastroenterology. 2009; 136: 542-550.
17. Loftus EV Jr, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. Gut. 2005; 54: 91-96.
18. Magro F, Langner C, Driessen A, Ensari A, Geboes K, Mantzaris GJ, Villanacci V, Becheanu G, Borralho Nunes P, Cathomas G, Fries W, Jouret-Mourin A, Mescoli C, de Petris G, Rubio CA, Shepherd NA, Vieth M, Eliakim R; European Society of Pathology (ESP); European Crohn's and Colitis Organisation (ECCO). European consensus on the histopathology of inflammatory bowel disease. J Crohns Colitis. 2013; 7: 827-851.
19. Magro F, Gionchetti P, Eliakim R, Ardizzone S, Armuzzi A, Barreiro-de Acosta M, Burisch J, Gecse KB, Hart AL, Hindryckx P, Langner C, Limdi JK, Pellino G, Zagórowicz E, Raine T, Harbord M, Rieder F; European Crohn’s and Colitis Organisation [ECCO]. Third European Evidence-based Consensus on Diagnosis and Management of Ulcerative Colitis. Part 1: Definitions, Diagnosis, Extra-intestinal Manifestations, Pregnancy, Cancer Surveillance, Surgery, and Ileo-anal Pouch Disorders. J Crohns Colitis. 2017; 11: 649-670.
20. Makapugay LM, Dean PJ. Diverticular disease-associated chronic colitis. Am J Surg Pathol. 1996; 20: 94-102.
21. Marchal Bressenot A, Riddell RH, Boulagnon-Rombi C, Reinisch W, Danese S, Schreiber S, Peyrin-Biroulet L. Review article: the histological assessment of disease activity in ulcerative colitis. Aliment Pharmacol Ther. 2015; 42: 957-967.
22. Marchal-Bressenot A, Salleron J, Boulagnon-Rombi C, Bastien C, Cahn V, Cadiot G, Diebold MD, Danese S, Reinisch W, Schreiber S, Travis S, Peyrin-Biroulet L. Development and validation of the Nancy histological index for UC. Gut. 2017; 66: 43-49.
23. Marchal-Bressenot A, Scherl A, Salleron J, Peyrin-Biroulet L. A practical guide to assess the Nancy histological index for UC. Gut. 2016; 65: 1919-1920.
24. Matkowskyj KA, Chen ZE, Rao MS, Yang GY. Dysplastic lesions in inflammatory bowel disease: molecular pathogenesis to morphology. Arch Pathol Lab Med. 2013; 137: 338-350.
25. McCarthy AJ, Lauwers GY, Sheahan K. Iatrogenic pathology of the intestines. Histopathology. 2015; 66: 15-28.

26. McCurdy JD, Enders FT, Jones A, Killian JM, Loftus EV Jr, Bruining DH, Smyrk TC. Detection of Cytomegalovirus in Patients with Inflammatory Bowel Disease: Where to Biopsy and How Many Biopsies? Inflamm Bowel Dis. 2015; 21: 2833-2838.
27. Odze RD. Diagnostic problems and advances in inflammatory bowel disease. Mod Pathol. 2003; 16: 347-358.
28. Odze RD. A contemporary and critical appraisal of 'indeterminate colitis'. Mod Pathol. 2015 ;28 Suppl 1:S30-46.
29. Odze RD, Goldblum J, Noffsinger A, Alsaigh N, Rybicki LA, Fogt F. Interobserver variability in the diagnosis of ulcerative colitis-associated dysplasia by telepathology. Mod Pathol. 2002; 15: 379-386.
30. Patil DT, Odze RD. Biopsy diagnosis of colitis: an algorithmic approach. Virchows Arch. 2018; 472: 67-80.
31. Sager K, Alam S, Bond A, Chinnappan L, Probert CS. Review article: cytomegalovirus and inflammatory bowel disease. Aliment Pharmacol Ther. 2015; 41: 725-733.
32. Schaeffer DF, Win LL, Hafezi-Bakhtiari S, Cino M, Hirschfield GM, El-Zimaity H. The phenotypic expression of inflammatory bowel disease in patients with primary sclerosing cholangitis differs in the distribution of colitis. Dig Dis Sci. 2013; 58: 2608-2614.
33. Shen J, Gibson JA, Schulte S, Khurana H, Farraye FA, Levine J, Burakoff R, Cerda S, Qazi T, Hamilton M, Srivastava A, Odze RD. Clinical, pathologic, and outcome study of hyperplastic and sessile serrated polyps in inflammatory bowel disease. Hum Pathol. 2015; 46: 1548-1556.
34. Soucy G, Wang HH, Farraye FA, Schmidt JF, Farris AB, Lauwers GY, Cerda SR, Dendrinos KG, Odze RD. Clinical and pathological analysis of colonic Crohn's disease, including a subgroup with ulcerative colitis-like features. Mod Pathol. 2012; 25: 295-307.
35. de Vries AB, Janse M, Blokzijl H, Weersma RK. Distinctive inflammatory bowel disease phenotype in primary sclerosing cholangitis. World J Gastroenterol. 2015; 21: 1956-1971.
36. Zagórowicz E, Bugajski M, Wieszczy P, Pietrzak A, Magdziak A, Mróz A. Cytomegalovirus Infection in Ulcerative Colitis is Related to Severe Inflammation and a High Count of Cytomegalovirus-positive Cells in Biopsy Is a Risk Factor for Colectomy. J Crohns Colitis. 2016; 10: 1205-1211.
37. Zenlea T, Yee EU, Rosenberg L, Boyle M, Nanda KS, Wolf JL, Falchuk KR, Cheifetz AS, Goldsmith JD, Moss AC. Histology Grade Is Independently Associated With Relapse Risk in Patients With Ulcerative Colitis in Clinical Remission: A Prospective Study. Am J Gastroenterol. 2016; 111: 685-690.
38. Zidar N, Ferkolj I, Tepeš K, Štabuc B, Kojc N, Uršič T, Petrovec M. Diagnosing cytomegalovirus in patients with inflammatory bowel disease—by immunohistochemistry or polymerase chain reaction? Virchows Arch. 2015; 466: 533-539.

Colorectal Polyps

Although often viewed as a single disease, colorectal cancer more accurately represents a family of diseases with different precursor lesions.

Conventional (tubular, tubulovillous and villous) adenomas are the most common neoplastic lesions occurring in the large intestine. They are circumscribed benign lesions defined by the presence of dysplastic epithelium. Dysplasia (syn. intraepithelial neoplasia) can be low or high grade, depending on the degree of architectural complexity, extent of nuclear stratification, and severity of abnormal nuclear morphology.

Most adenomas are smaller than 1 cm and have tubular architecture. Villous architecture is defined as leaf- or finger-like projections of epithelium overlying a small amount of lamina propria. Tubulovillous adenomas are defined by a mixture of tubular and villous structures with arbitrary percentages in different studies, typically between 25% and 75% villous component

- Tubular adenoma: <25 villous component (>75% tubular component)
- Tubulovillous adenoma: 25-75% villous component
- Villous adenoma >75% villous component (<25% tubular component)

Conventional adenomas share APC mutations (prompting constitutive activation of the Wnt/β-catenin signalling pathway) and arise from dysplastic aberrant crypt foci.

In sporadic tumours, neoplastic progression typically follows the traditional chromosomal instability (CIN) pathway. The first step is an inactivation of the Wnt signalling pathway, which is usually accomplished by a somatic mutation in the *Adenomatous Polyposis Coli* (*APC*) gene. Of note, germline in *APC* lead to familial adenomatous polyposis (FAP). Conventional adenomas are also the precursors of Lynch syndrome-associated microsatellite-instable (MSI-H) cancers.

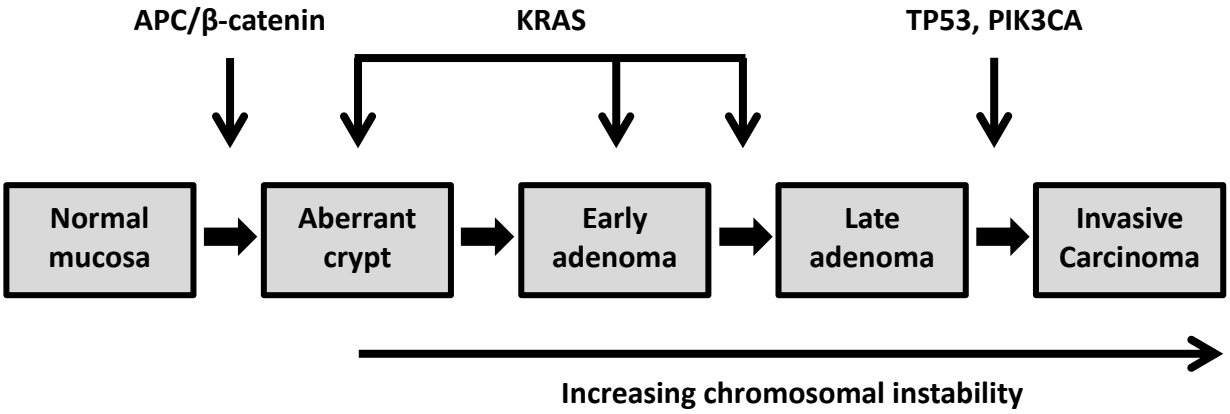

Figure 1 Multistep genetic model of colorectal carcinogenesis (adenoma carcinoma sequence). The initial step is the formation of a dysplastic aberrant crypt focus (ACF). Progression to larger adenomas and early carcinomas requires activating mutations of the proto-oncogene KRAS, mutations in TP53, and/or loss of heterozygosity at chromosome 18q. Mutational activation of PIK3CA occurs late in the adenoma carcinoma sequence in a small proportion of colorectal cancers. Chromosomal instability (CIN) is observed in benign adenomas and increases in conjunction with tumour progression.

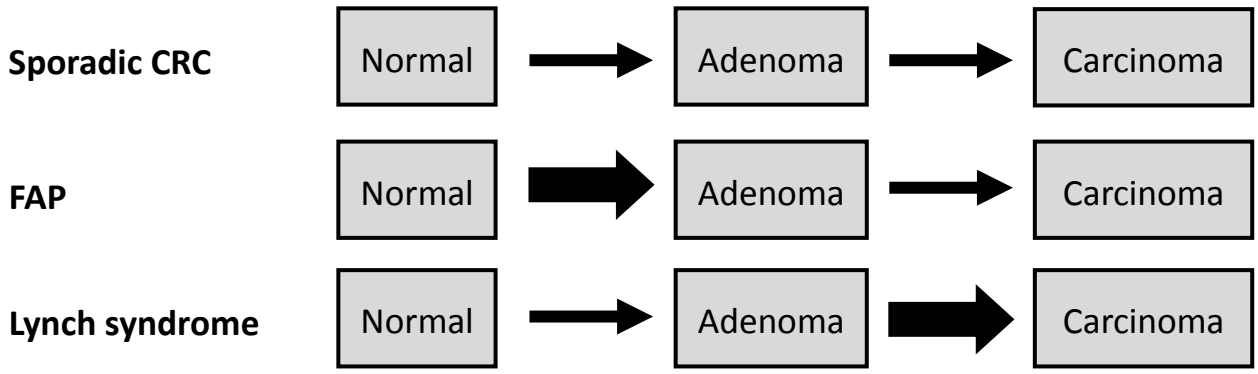

Figure 2 Relative effects of germline mutations on tumour initiation and progression: In sporadic colorectal cancers, both adenoma formation and cancer development are rate-limiting steps. In familial adenomatous polyposis (FAP), germline inactivation of one APC allele markedly accelerates adenoma formation, while adenomas most probably progress to cancer at a rate similar to that of sporadic adenomas. This is in contrast to Lynch syndrome. Here, the germline inactivation of one of the mismatch repair genes, coupled with somatic inactivation of the remaining allele in an initiated lesion greatly increases the mutation rate and, subsequently, the rate of progression from adenoma to cancer.

Approximately one third of colorectal cancers develop from serrated precursor lesions. These are a heterogeneous group of lesions characterized morphologically by a serrated (saw-toothed or stellate) architecture of the epithelial compartment. Lesions that are included in this group are the hyperplastic polyp, sessile serrated adenoma/polyp, and traditional serrated adenoma (Table 1).

	Hyperplastic polyp	Sessile serrated lesion	Traditional serrated adenoma
Prevalence	Very common	Common	Rare
Location	Left colon > right colon	Right colon > left colon	Left colon > right colon
Macroscopy	Flat, sessile	Flat, sessile	Sessile, pedunculated

Table 1 Clinicopathological characteristics of colorectal serrated lesions.

Hyperplastic polyps are common, accounting for 70-95% of all serrated lesions or 25-30% of resected large intestinal polyps. They occur most often in the left colon, particularly in the sigmoid colon and rectum, as diminutive, pale, sessile lesions. It is not uncommon for distal hyperplastic polyps to be multiple. BRAF or KRAS is frequently mutated, and these mutations are most probably the initiating event in the majority of lesions.

Histologically, hyperplastic polyps are characterized by straight crypts, with “serration” typically restricted to the upper half (unaltered basal proliferative compartment). Distinct subtypes are recognized that differ in terms of morphology (mucin content), distribution, and molecular characteristics, i.e. microvesicular and goblet cell-rich hyperplastic polyps.

The term “**sessile serrated adenoma**” was coined by Torlakovic and Snover in 1996. There were, and still are, pathologists who prefer the name “sessile serrated polyp” rather than sessile serrated adenoma, since these lesions lack typical adenoma-like dysplasia. Both terms are currently considered synonyms and both are acceptable diagnostic terms. The compromise in the 2010 WHO Classification of Tumours of the Digestive System was to name these polyps sessile serrated adenomas/polyps (SSA/P). Still, the discussion never ended and ultimately “**sessile serrated lesion**” (SSL) emerged as new player and represents the preferred term in the upcoming WHO classification.

Regardless of the name, the important thing is for pathologists and clinicians to recognize that these are carcinoma-associated serrated polyps, that is, precursor lesions of colorectal cancer. In contrast to hyperplastic polyps SSLs are more likely to be located in the right colon (75%), accounting for approximately 5-25% of all serrated lesions. The average size of SSLs is larger than that of hyperplastic polyps. More than half of the lesions measure >5 mm and 15–20% of the lesions are >10 mm.

Upon histology, SSLs are characterized mechanistically by movement of the proliferative zone away from its usual location in the base of the crypts, resulting in maturation, which may develop toward the base of the crypts leading to distorted crypt architecture, that is, greater architectural complexity (in comparison with hyperplastic polyps).

Morphological features include crypt dilation with mucus retention (responsible for the “mucus cap” that is characteristically seen upon endoscopy), lateral growth at the base of crypts (leading to L-shaped [boot-shaped] and inverted T-shaped [anchor-like] crypts), and exaggerated serration along the middle and lower parts of the crypts. Peculiarly, some of these big serrated polyps overlie large amounts of submucosal adipose tissue resembling a lipoma. Others may show gland misplacement (herniation) into the submucosa, which should not be mistaken for invasion.

Strict adherence to the presented macroscopical (i.e. lesion size and location) and histological features prevents misclassification. In particular large right-sided hyperplastic polyps >5mm have a high chance of reclassification to SSA after expert review.

Minimum criteria for diagnosis: In accordance with current WHO guidelines, a lesion should be diagnosed as SSL if more than two or three contiguous crypts demonstrate features of SSL. However, recently an international consensus panel recommended that the presence of at least one unequivocal architecturally distorted, dilated and/or horizontally branched crypt, particularly if it is associated with inverted maturation, is sufficient for a diagnosis of SSL. This recommendation was confirmed by subsequent systematic morphological analysis demonstrating that serrated polyps (hyperplastic polyps or SSLs) with any SSL-like crypts had clinical features more in common with fully developed / classical SSL than hyperplastic polyps and that this diagnostic cut-off showed good reproducibility between pathologists.

Cytological dysplasia is not present in uncomplicated SSL, but develops with progression toward carcinoma. In addition to conventional adenoma-like dysplasia (compare above) more cuboidal cells with eosinophilic cytoplasm and vesicular nucleoli with prominent nucleoli may occur, referred to as “serrated-type dysplasia”.

In the past, these lesions have been referred to as “mixed polyp” (e.g. mixed hyperplastic polyp / tubular adenoma), a term that is nowadays discouraged. In accordance with WHO guidelines these lesions should now be termed “SSL with cytological dysplasia”. The significance of the grade of dysplasia (low or high grade) has not been fully evaluated.

On the molecular level, cytological dysplasia develops as a consequence of epigenetic silencing of the MLH1 gene due to promoter methylation ("serrated route to colorectal cancer"). Gene silencing can be visualized by MLH-1 immunohistochemistry: dysplastic glands often lack nuclear expression of the protein (in conjunction with loss of PMS2). As immediate consequence of MLH1 gene inactivation high level microsatellite instability (MSI-H) is observed upon molecular analysis. There is usually markedly increased proliferative activity (Ki67, MIB-1) in dysplastic SSLs.

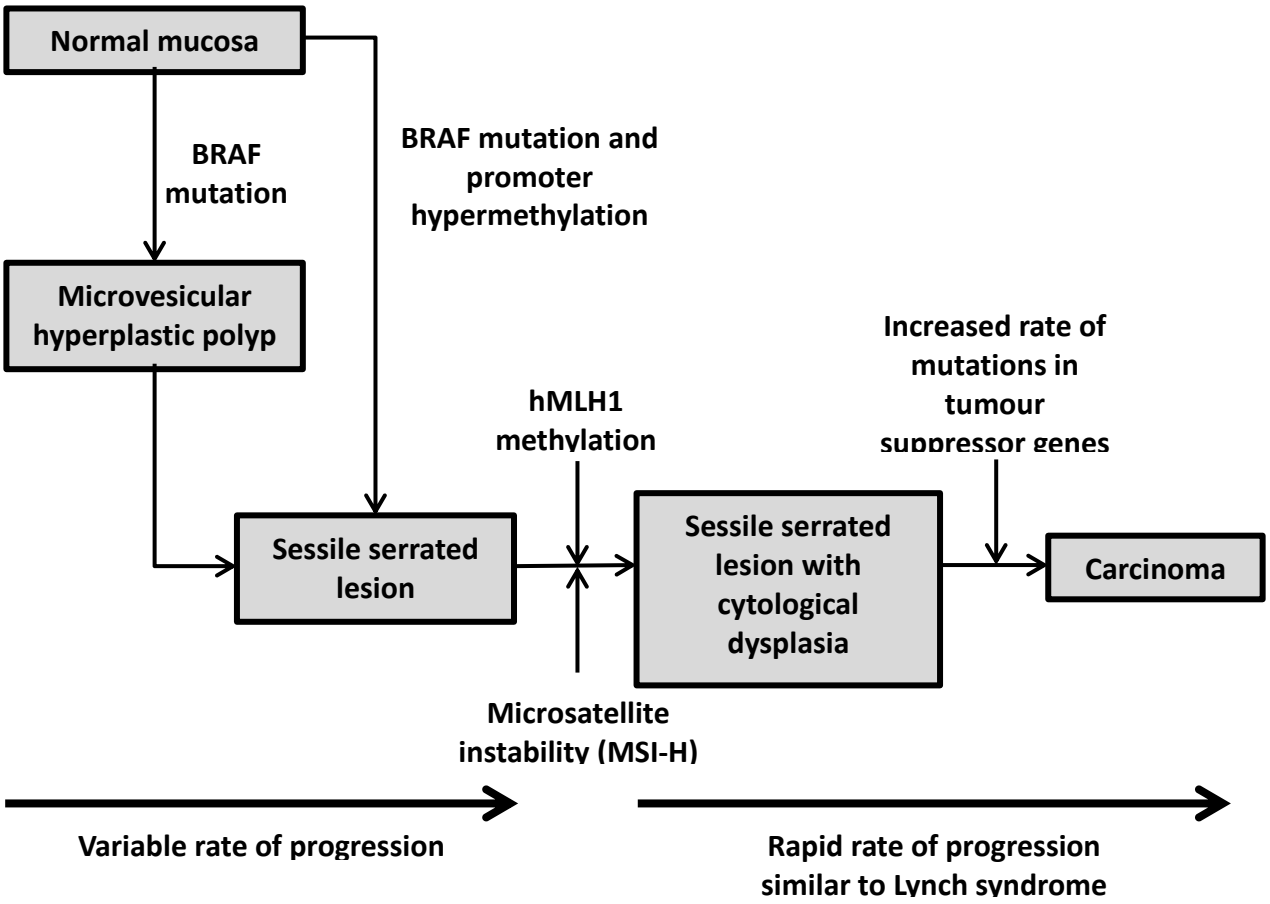

Figure 3 This scheme illustrates the development of sporadic colorectal adenocarcinoma with MSI-H phenotype from sessile serrated adenoma/polyp (SSAL) due to epigenetic silencing (promoter methylation) of the hMLH1 gene, referred to as "serrated route to cancer".

Traditional serrated adenomas (TSAs) are much less common than the other serrated lesions, accounting for approximately 1% of colorectal polyps. The majority of lesions are detected in the distal colon. Macroscopically, TSAs are typically exophytic, tubulovillous or

“pinecone-like” polypoid lesions and this is the key low-power architectural impression.. Mean size at diagnosis ranges from 9 to 14 mm.

Upon histology, there is a constellation of characteristic features, such as striking granular eosinophilic cytoplasm, slit-like luminal serration, presence of ectopic crypt foci (ECF) and elongated, pencillate nuclei with evenly dispersed coarse chromatin and small inconspicuous nucleoli. ECFs were previously thought to be a prerequisite for diagnosis. However, they may not always be seen in TSAs, particularly in smaller lesions (<10 mm) and have also been documented in conventional adenomas showing villous differentiation, albeit to a lesser frequency than observed in TSAs.

Bettington et al. suggest the following three criteria for diagnosis of TSA of which at least two should be present, with at least one feature evident in >50% of the polyps:

- Typical cytology (abundant brightly eosinophilic cytoplasm with centrally placed, pencillate nuclei)
- Slit-like epithelial serration (referred to narrow slits in the epithelium similar to normal small intestinal mucosa)
- Ectopic crypt formation referred to epithelial buds with their bases not seated adjacent to the muscularis mucosae

Several morphological variants have been described, including flat TSA, filiform TSA and mucin-rich / goblet cell-rich TSA. Morphological overlap with conventional (tubulovillous and villous) adenomas has been described and the diagnostic term “serrated tubulovillous adenoma” proposed for these lesions, respectively.

TSAs are unquestionably neoplastic, but the subject of dysplasia in TSA is still matter of debate: Most authors regard TSAs as “dysplastic”, although they lack the classical features of adenomatous dysplasia known from tubular, tubulovillous and villous adenomas. In fact, the presence of this type of dysplasia in an otherwise typical TSA has been associated with tumour progression. Others, however, refer to the comparably bland cytology in conjunction with a low Ki67 labelling index and regard TSAs as “not inherently (intrinsically) dysplastic”.

It is of note that TSAs often occur in conjunction with hyperplastic polyps and, more commonly SSLs, thereby suggesting that at least some TSAs originate from precursor lesions, i.e. pre-existing hyperplastic polyps and/or SSLs. On the molecular level, these lesions seem to be strikingly different, indicating two molecular phenotypes of TSA: one associated with BRAF mutations and the other with KRAS mutations. The former is associated with hyperplastic polyps and SSLs, while the latter is not; but this lesion may more often develop conventional adenomatous dysplasia, indication timely differences in neoplastic progression.

References

1. Bettington ML, Chetty R. Traditional serrated adenoma: an update. Hum Pathol. 2015; 46: 933-938.
2. Bettington M, Liu C, Gill A, Walker N, Leggett B, Whitehall V, Rosty C. BRAF V600E immunohistochemistry demonstrates that some sessile serrated lesions with adenomatous dysplasia may represent collision lesions. Histopathology. 2019 Mar 2. doi: 10.1111/his.13851. [Epub ahead of print]
3. Bettington M, Rosty C, Whitehall V, Leggett B, McKeone D, Pearson SA, Walker N. A morphological and molecular study of proposed early forms of traditional serrated adenoma. Histopathology. 2018; 73: 1023-1029.
4. Bettington M, Walker N, Clouston A, Brown I, Leggett B, Whitehall V. The serrated pathway to colorectal carcinoma: current concepts and challenges. Histopathology. 2013; 62: 367-386.
5. Bettington M, Walker N, Rahman T, Vandeleur A, Whitehall V, Leggett B, Croese J. High prevalence of sessile serrated adenomas in contemporary outpatient colonoscopy practice. Intern Med J. 2017; 47: 318-323.
6. Bettington M, Walker N, Rosty C, Brown I, Clouston A, Wockner L, Whitehall V, Leggett B. Critical appraisal of the diagnosis of the sessile serrated adenoma. Am J Surg Pathol. 2014; 38: 158-166.
7. Bettington ML, Walker NI, Rosty C, Brown IS, Clouston AD, McKeone DM, Pearson SA, Klein K, Leggett BA, Whitehall VL. A clinicopathological and molecular analysis of 200 traditional serrated adenomas. Mod Pathol. 2015; 28: 414-427.
8. Bettington M, Walker N, Rosty C, Brown I, Clouston A, McKeone D, Pearson SA, Klein K, Leggett B, Whitehall V. Serrated tubulovillous adenoma of the large intestine. Histopathology. 2016; 68: 578-587.
9. Bettington M, Walker N, Rosty C, Brown I, Clouston A, McKeone D, Pearson SA, Leggett B, Whitehall V. Clinicopathological and molecular features of sessile serrated adenomas with dysplasia or carcinoma. Gut. 2017; 66: 97-106.
10. Chetty R. Traditional serrated adenoma (TSA): morphological questions, queries and quandaries. J Clin Pathol. 2016; 69: 6-11.
11. Chetty R, Hafezi-Bakhtiari S, Serra S, Colling R, Wang LM. Traditional serrated adenomas (TSAs) admixed with other serrated (so-called precursor) polyps and conventional adenomas: a frequent occurrence. J Clin Pathol. 2015; 68: 270-273.
12. Choi EY, Appelman HD. A Historical Perspective and Exposé on Serrated Polyps of the Colorectum. Arch Pathol Lab Med. 2016; 140: 1079-1084.
13. Gibson JA, Odze RD. Pathology of premalignant colorectal neoplasia. Dig Endosc. 2016; 28: 312-323.

14. Hafezi-Bakhtiari S, Wang LM, Colling R, Serra S, Chetty R. Histological overlap between colorectal villous/tubulovillous and traditional serrated adenomas. Histopathology. 2015; 66: 308-313.
15. Kalimuthu SN, Chelliah A, Chetty R. From traditional serrated adenoma to tubulovillous adenoma and beyond. World J Gastrointest Oncol. 2016; 8: 805-809.
16. Kalimuthu SN, Serra S, Hafezi-Bakhtiari S, Colling R, Wang LM, Chetty R. Mucin-rich variant of traditional serrated adenoma (MrTSA): A distinct morphological variant. Histopathology. 2017; 71: 208-216.
17. Langner C. Serrated and non-serrated precursor lesions of colorectal cancer. Dig Dis. 2015; 33: 28-37.
18. Leedham SJ, Chetty R. Wnt disruption in colorectal polyps - the traditional serrated adenoma enters the fray. J Pathol. 2016; 239: 387-390.
19. Liu C, Bettington ML, Walker NI, Dwine J, Hartel GF, Leggett BA, Whitehall VLJ. CpG Island Methylation in Sessile Serrated Adenomas Increases With Age, Indicating Lower Risk of Malignancy in Young Patients. Gastroenterology. 2018; 155: 1362-1365.
20. Liu C, Walker NI, Leggett BA, Whitehall VL, Bettington ML, Rosty C. Sessile serrated adenomas with dysplasia: morphological patterns and correlations with MLH1 immunohistochemistry. Mod Pathol. 2017; 30: 1728-1738.
21. Pai RK, Bettington M, Srivastava A, Rosty C. An update on the morphology and molecular pathology of serrated colorectal polyps and associated carcinomas. Mod Pathol. 2019 Apr 25. doi: 10.1038/s41379-019-0280-2. [Epub ahead of print]
22. Rex DK, Ahnen DJ, Baron JA, Batts KP, Burke CA, Burt RW, Goldblum JR, Guillem JG, Kahi CJ, Kalady MF, O'Brien MJ, Odze RD, Ogino S, Parry S, Snover DC, Torlakovic EE, Wise PE, Young J, Church J. Serrated lesions of the colorectum: review and recommendations from an expert panel. Am J Gastroenterol. 2012; 107: 1315-1329.
23. Rau TT, Agaimy A, Gehoff A, Geppert C, Jung K, Knobloch K, Langner C, Lugli A, Groenbus-Lurkin I, Nagtegaal ID, Rüschoff J, Saegert X, Sarbia M, Schneider-Stock R, Vieth M, Zwarthoff EC, Hartmann A. Defined morphological criteria allow reliable diagnosis of colorectal serrated polyps and predict polyp genetics. Virchows Arch. 2014; 464: 663-672.
24. Schachschal G, Sehner S, Choschzick M, Aust D, Brandl L, Vieth M, Wegscheider K, Baretton GB, Kirchner T, Sauter G, Rösch T.. Impact of reassessment of colonic hyperplastic polyps by expert GI pathologists. Int J Colorectal Dis. 2016; 31: 675-683.
25. Snover DC. Update on the serrated pathway to colorectal carcinoma. Hum Pathol. 2011; 42: 1-10.
26. Torlakovic E, Gomez JD, Driman DK, Parfitt JR, Wang C, Benerjee T, Snover DC. Sessile serrated adenoma (SSA) vs. traditional serrated adenoma (TSA). Am J Surg Pathol. 2008; 32: 21-29.
27. Torlakovic E, Snover DC. Serrated adenomatous polyposis in humans. Gastroenterology 1996; 110: 748-755.
28. Väyrynen SA, Väyrynen JP, Klintrup K, Mäkelä J, Tuomisto A, Mäkinen MJ. Ectopic crypt foci in conventional and serrated colorectal polyps. J Clin Pathol. 2016; 69: 1063-1069.