Chapter 1

Endoscopy and Histopathology

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Additional information is available at the end of the chapter

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1. Introduction

Endoscopy and histopathology are two morphological diagnostic procedures which allow direct examination of organs with optical methods. They can detect abnormalities of the normal anatomy and histology and provide a precise diagnosis. Based on the information derived from these investigations an adequate treatment, either medical or surgical can be proposed. The optical resolution of both methods is different. Classical endoscopy is using essentially the naked eye observation of the tissue which allows a diagnosis of an ulcer or a raised lesion for instance, while histopathology is reaching the cellular and sub-cellular level. The new endoscopic techniques however do increase the optical resolution. The major contributions of histopathology to endoscopy are situated in inflammatory and neoplastic diseases. Histopathology allows a more precise diagnosis of the type of inflammation and a better classification of tumours. This has again an impact upon treatment. For the diagnosis, histopathology can be an essential element, as illustrated by gluten sensitive enteropathy (although serology is also an essential element) or by identification of specific pathogens such as Giardia lamblia, Mycobacterium avium, cryptosporidia.... Histopathology can further be important for the confirmation of a diagnosis but very often it will provide a more precise diagnosis by determining the aetiology of inflammation as illustrated by autoimmune gastritis, or by typing a tumour (adenocarcinoma or lymphoma). In addition, histopathology can provide essential elements for further therapy strategy by demonstrating the presence or absence of risk factors for residual tumour in polypectomy or endoscopic mucosal resection. Indirectly, it offers the possibility of using additional techniques such as biomarkers for dysplasia and cancer or the demonstration of mutations such as KRAS in colorectal cancer or HER2 amplification in oesophageal and gastric cancer.[1, 2].These applications can have important therapeutic consequences. It has been shown for instance that activating mutations of the KRAS gene are associated with poor response to anti-EGFR therapies and that patients

with tumors that had high levels of HER2 protein expression derived the greatest benefit from treatment with trastuzumab..

2. What is the influence of endoscopy on the diagnostic yield of histopathology?

2.1. General requirements for the endoscopist and the pathologist

A close collaboration between the endoscopist and the pathologist is essential for an accurate diagnosis. This imposes on each of the partners some constraints.

Overall the endoscopist should provide the pathologists with a copy of the endoscopy report mentioning the sites of the biopsies, a macroscopic description of the lesions if present and the adjacent mucosa and essential clinical information such as the age of the patient, the immune status of the patient, duration of symptoms and treatment if any.

The pathologist should provide information of the quality of the biopsies (number and size and depth of the samples) in order to avoid false conclusions, a degree of probability of his initial diagnosis and if needed suggest particular conditions for further sampling or ancillary techniques such as immune histochemistry. Contentious cases should be selected for clinic pathological discussion.[3]

2.2. Sampling of biopsies

The diagnostic yield of histopathology depends upon the experience of the pathologist but also upon the quality of the biopsy samples and sampling error. The quality of the samples is influenced by a variety of elements such as the size and shape of the biopsy forceps, the nature and location of the disease, the experience of the endoscopist and the number of samples. During endoscopy samples can be obtained by way of different techniques. These include pinch biopsy, suction biopsy with a multipurpose tube (which provides larger samples), brush cytology, endoscopic fine needle aspiration (offering material from deep areas in the lesion) and snare excision or strip biopsy.

Pinch biopsy is the most common technique. Several types of biopsy forceps are available. A distinction can be made between those with elliptical and those with round cups. Generally the samples obtained with elliptical cups are larger. A forceps with round cups may be more appropriate for children in order to avoid complications. The size of the biopsy forceps determines partly the size (surface and depth) of the samples. The small forceps has a width of 1.8 mm when opened. The average forceps has a 2.4 mm diameter and allows to obtain samples containing the muscularis mucosae (and upper submucosa) in 60% of the cases. The larger Jumbo forceps has a 3.4 mm diameter. Samples obtained with this forceps are larger, but, they usually contain not more submucosa and the risk of complications (perforation and bleeding) may be more important, whereas it is minimal with the smaller forceps (if the patient has normal coagulation). A forceps can have a central spike so that it stays in position in the

mucosa, during the procedure. The spike can induce artefacts which should not be confused with erosions.

The anatomic location or certain types of lesions may be the reason why samples are of less good quality or superficial in nature. This is often so in areas immediately distal to a stricture, and at the papilla of Vater in the duodenum. The extrahepatic bile ducts and the pancreatic duct are other areas where biopsies are more difficult to obtain and hence usually smaller. If the biopsies of the papilla are taken following sphincterotomy, coagulation artefacts are likely to be present.

In order to obtain samples of appropriate depth air insufflation during the endoscopic examination should be limited. When over-insufflation occurs the mucosa is stretched and pushed towards the underlying submucosa and the samples are likely to be more superficial.

The samples obtained with a forceps are usually limited to the mucosa. Normally they are not suitable for the assessment of submucosal or deeper lesions. This means that they are not good for instance for a diagnosis of “vasculitis”, except for small vessel disease. By the use of a "burrowing technique" whereby several biopsies are taken in the same area information of deeply situated lesions can eventually be obtained. An alternative are samples obtained with endoscopic ultrasound guided fine needle aspiration. They are usually smaller but they permit both morphologic and cytologic analysis of lesions within or adjacent to the gastrointestinal (GI) tract. They can be used for the assessment of neoplastic lesions, but because of the small size, they are not good for conditions such as vasculitis.[4]

2.3. Larger samples

Larger samples are obtained with endoscopic mucosal resection (EMR) or endoscopical submucosal resection (ESD) and snare polypectomy. These samples must be handled adequately by the endoscopist or/and in the pathology lab. The histopathological interpretation of these samples provides important information for subsequent management and assessment of the risk for residual cancer. A correct diagnostic process involves, tumour differentiation, precise determination of deep infiltration, lymphatic permeation and adequate determination of the section margin. Identification of this area is easy if the lesion is adequately oriented. In the case of polypectomy, the endoscopist could identify the section margin with India ink or with a pin if the lesion is removed in one piece. The specimen will be cut along the marker. (Fig. 1) In the case of EMR /ESD, ideally, the specimen should be oriented, pinned and stretched on card board in the endoscopy unit.(Fig 2) If the specimen is not removed in one piece, reconstruction of the specimens should be attempted. Painting of the base and margins is useful, as tumour extension to the deep margin implies surgery and remnants of the neoplastic epithelium at the lateral margins indicate re-excision or postoperative destruction.[5, 6] Good communication between the pathologist and the clinicians is important for the assessment of the efficacy of the treatment and for the design of the strategy of the additional treatment which is based upon the depth of invasion of the lesion. If the resection has been performed in piecemeal fashion and the specimen is received in two or more fragments, it may be impossible to determine the true margin of resection, if the endoscopist did not attempt to identify the true margin or placed the true margin in a separate container.[7]

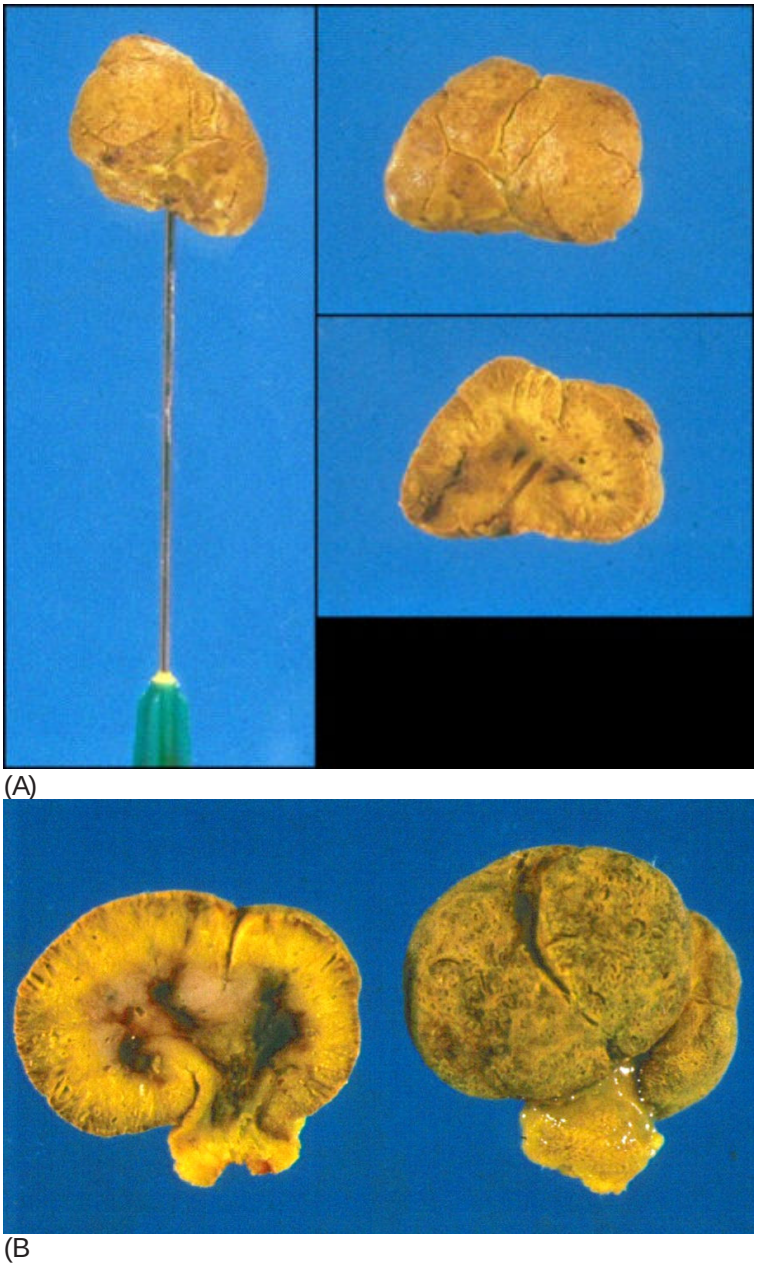

Figure 1. Polypectomy specimen correctly oriented using a needle showing the top (upper right) and the base (lower right) (A) and cut along the orientation (B)

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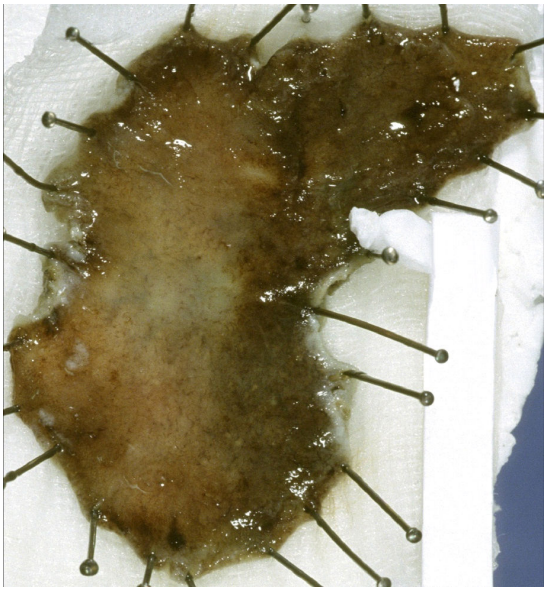

(A)

(B

Figure 2. Specimen from endoscopic mucosal resection pinned out and stretched (A) and cut at all levels (B)

2.4. Endoscopic ultrasound-guided fine-needle aspiration biopsy

Endoscopic ultrasound guided fine needle aspiration has become the most accurate modality for characterization of pancreatic cystic and solid lesions, differential diagnosis of indeterminate masses and locoregional staging of some digestive cancers (gastric, oesophagus, pancreas, biliary tract…). It should be performed in the primary mass but also in distant lymph nodes, or metastatic locations. EUS-FNA has a high sensitivity, specificity, positive predictive value and accuracy in the assessment of biliopancreatic tumours. The performance of this technique is dependent on the endoscopist and cytopathologist experience. It is well known that accuracy of FNA increases when the technique is performed by an experienced clinician and when the slides are reviewed by an experienced cytopathologist the collaboration between these two physicians is also very important.[8]

2.5. Number of samples

Several studies have shown that the diagnostic yield of histopathology is increased and sampling error is decreased by increasing the number of biopsies. This has been demonstrated for inflammatory diseases such as chronic idiopathic inflammatory bowel diseases (IBD) and for neoplastic diseases.[9] Therefore different guidelines for endoscopic sampling in various diseases have been developed.[10-12] ECCO guidelines propose to obtain "multiple" biopsies from five sites around the colon (including the rectum) and the ileum for a reliable diagnosis of Crohn's disease. Multiple biopsies imply a minimum of two samples from each site (Table) This is also true for a diagnosis of collagenous or lymphocytic colitis. Thickening of the subepithelial collagen table in collagenous colitis is indeed not homogeneous. Such guidelines are very important in clinical practice. They limit sampling error and compensate for the small size of the samples. However, the introduction of new technologies and modern endoscopes including zoom endoscopy, high magnification endoscopy and more sophisticated techniques such as laser-scanning confocal endoscopy and endo-cytoscopy (microscope incorporated in the endoscope) will change practice in the future by offering the possibility of targeted biopsies. In a recent study at our institute, chromo endoscopy (CE) and narrow band imaging (NBI) were used to detect dysplasia in ulcerative colitis. A total of 268 raised lesions were detected in 83 patients (156 lesions in 45 patients with CE and 112 lesions in 38 patients with NBI). On histology, 44 were shown to be neoplastic (26 lesions in 10 patients with CE and 18 lesions in 12 patients with NBI): 1 adenocarcinoma, 1 high grade dysplasia, 2 dysplasia associated lesion or mass, and 17 adenoma like mass. The new endoscopic techniques are also narrowing the gap between endoscopy and pathology. Laser scanning endoscopy provides a microscopy-level image without obtaining a biopsy specimen. Endo-cytoscopy is based on the technology of light contact microscopy. The tip of an endoscope is placed in direct contact with a dye-stained surface and then the surface is scanned with condensed normal white light, producing cellular-level imaging. Laser endoscopy increases the real time diagnostic yield and can be used to confirm dysplasia with high accuracy. Bio-endoscopy is another technique under consideration. It involves the use of monoclonal antibodies labelled with a fluorescent tag of reporter probes (molecules that enter cells) or fluorescent DNA probes for FISH in order to detect in situ molecular changes or chromosomal instability.[13-16]

Organ	Disease	Location	Area for biopsies	Evidence
Oesophagus	GERD/NERD	Distal esophagus	Z-line	?
			2 cm above Z-line	
			Cardia	
			Stomach (antrum) Hp	
	Eosinophilic oesophagitis	Entire oesophagus	Proximal, mid	+
			Distal esophagus	
	Barrett's oesophagus	Distal oesophagus	Four quadrant biopsy	++
			Every 1cm (short)	
			Every 2 cm (long)	
			(Seattle protocol)	
Stomach	Gastritis	Entire stomach	Corpus : 2	++
			Antrum 2	
			Angulus 1	
			(Sydney protocol)	
			+ duodenum	
Small intestine	Coeliac disease	Descending duodenum	Multiple (4)	++
Colon	Colitis	Ileum	Multiple	+
	Crohn's disease	Entire colon		
	Ulcerative colitis			
	Microscopic colitis			
Pouch	Pouchitis	5 cm from Ileoanal anastomosis Anterior and posterior wall		+

Table 1. Recommendations for biopsy strategies in inflammatory conditions of the gastrointestinal tract

While these new techniques can offer real time images and diagnosis, the interpretation of the images still depends on the morphological features of the lesions, as observed with microscopy and some lesions like sessile serrated adenomas are still beyond the reach of real time diagnosis. The endoscopist must therefore have a thorough knowledge of pathology.

3. Specimen handling

Specimen handling should be done carefully in order to allow optimal diagnostic work up. It implies proper identification of the patient, including the age, specification of the site of origin, fixation and in some instances, orientation. Adequate fixation by an appropriate fixative is

central to any histological preparation. Tissue that is inadequately fixed will lead to difficulties for cutting, staining and performing ancillary tests. These problems are not correctable in a later stage. Unfortunately there is no "all purpose" fixative. The choice of the appropriate fixative is based on the type of tissue being fixed and on projected needs for ancillary tests, such as special stains, immune histochemistry, in situ hybridization, and electron microscopy. Routine Haematoxylin and eosin staining of multiple sections is adequate in most cases but insufficient in particular situations such as a diagnosis of Hirschsprung's disease or metabolic storage disorders.(Fig 3) For such indications freshly frozen tissue for enzyme histochemistry for the demonstration of acetylcholinesterase activity in nerves, or the identification of fat are needed or tissue fixed in glutaraldehyde for transmission electron microscopy. If possible, the endoscopist should be aware of the clinical indication for the biopsy, and, if necessary contact the pathology laboratory in order to know whether a special fixation is needed. In general formalin (10% neutral buffered formalin, i.e. a 10% v/v solution of 40% formaldehyde gas in water) allows good fixation and application of immune histochemistry as well as molecular analyses. Bouin fixation should therefore be “proscribed”. Furthermore it is important to control the duration of fixation. Samples need to be immersed in the fixative immediately and the duration of fixation can have an impact on the quality of the results of ancillary techniques such as immune histochemistry. A minimum of 6 hours and no longer than 48 hours is recommended for adequate molecular biology procedures such as for HER2 immune histochemistry in gastric cancer.[17] Frozen sections will allow application of most ancillary techniques. Freezing must be done properly (by immersion in liquid nitrogen for instance) and quickly in order to avoid the formation of ice cristals. Rapid adequate freezing and prevention of tissue degeneration is equally essential when molecular techniques based on DNA analysis are considered.

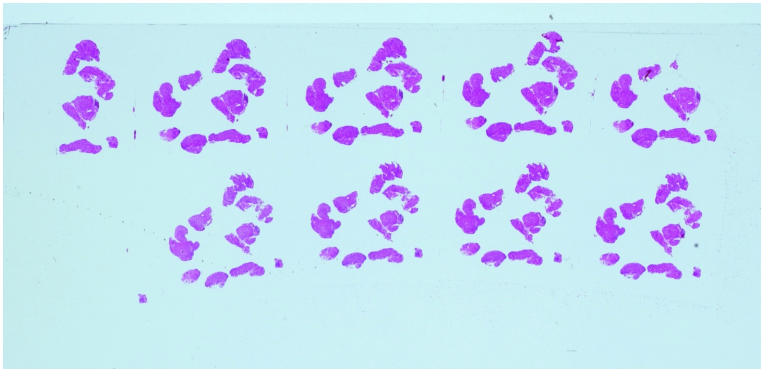

Figure 3. Multiple sections from multiple endoscopic biopsies allow a more complete microscopic analysis

Proper orientation of the tissue samples is important for a correct diagnosis of malabsorptive states such as celiac disease, where the ratio villous height – crypt depth must be assessed and for specimens from endoscopic resections of polyps or early neoplastic lesions.

4. Immune histochemistry and other ancillary techniques

In most instances histopathology identifies the nature of the lesion or tumour. Neoplastic – malignant - tumours are most frequently epithelial. A smaller number is neoplastic but non-epithelial, and includes lymphoproliferative disorders and soft tissue tumors. Histopathology is an adequate tool for solving differential diagnostic problems and typing of tumours. The differential diagnosis between anaplastic carcinomas, large-cell lymphoma, epithelioid stromal tumours and neuroendocrine tumours can be difficult but immunohistochemical stainings with antibodies against cytokeratins (CK), a marker for epithelial cells, CD117 a marker for gastrointestinal stromal tumors, chromogranin, a marker for endocrine cells and a common leucocyte marker can solve the problem. Antibodies to intermediate filaments such as the CKs can be potentially useful in other situations. CKs comprise a subfamily of more than 20 members. The relatively limited distribution of some CKs such as CK7 and CK20 and examination of coordinate expression of these two CKs can help in the differential diagnosis of carcinomas of unknown primary site.(Fig. 4)

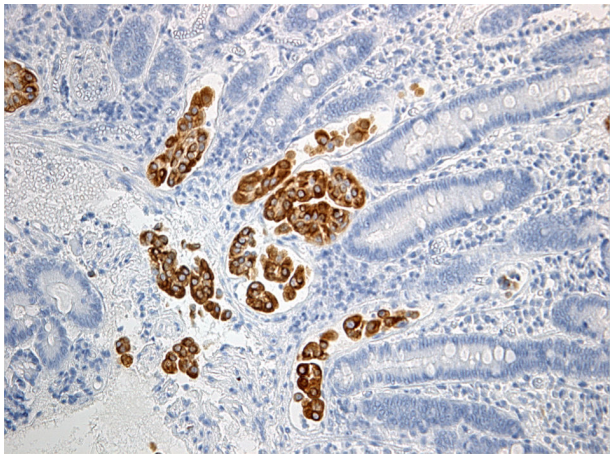

Figure 4. Microphotograph showing a rectal biopsy with Cytokeratin 7 positive immunohistochemistry demonstrating the presence of a breast cancer metastasis.

Immune histochemistry and cytogenetic analysis is essential for the management of lymphomas. Primary intestinal lymphomas should be sub-typed in B cell and T cell malignancies and classified according internationally validated classifications such as the recently published WHO.

Evaluating the proliferation fraction of the tumour cells using a marker such as Ki67 or MIB1 may provide some additional information on the biological behaviour of lymphomas. This is also true for endocrine tumours and gastrointestinal stromal tumours. Further ancillary techniques may include staining with antibodies against p53 for Barret's oesophagus or colitis-

associated dysplasia. Currently a number of markers are under investigation for a more accurate identification of early neoplasia.

Histochemistry (histological special stains) searching for mucins or other substances, and occasionally electron microscopy and genetic markers can also be applied on biopsy samples. Many stainings can be performed on routinely formalin fixed material. Increasingly there is some overlap, between immune histochemistry and molecular techniques since genetic markers can be demonstrated also by immune histochemistry. This is for instance true for large-bowel cancers with microsatellite instability (MS), where the products of the DNA repair genes hMLH1, hMSH2 and MSH6, or the lack of them, can be demonstrated immune histochemically. These products do not however cover the whole range of MS. DNA or RNA extraction and genetic analysis remains important and there may even be a growing need.

5. The oesophagus

5.1. Inflammatory conditions

At present, there is no ideal scenario for a biopsy series for the diagnosis of gastro-oesophageal reflux disease (GORD). In general, it is accepted that changes in the squamous mucosa are usually found in the distal oesophagus close to the squamo-columnar junction. Biopsies from the squamous mucosa should be completed with biopsies from the cardia. Histological changes indicative of gastro-oesophageal reflux are indeed found at both sides of the squamo-columnar junction.[18-21] The diagnosis of this condition, called carditis, which occurs in the absence of signs of gastritis in the antrum and corpus due to Helicobacter pylori or other causes of gastritis implies also biopsies of antrum and corpus in order to exclude the presence of these causes. A biopsy run for GORD should therefore ideally include samples from the distal oesophagus, particularly from the Z-line and at 2 cm above, from the cardia distal to the Z-line and from the stomach.[22, 23] However, in most cases, peptic oesophagitis due to GORD - the most common inflammatory condition of the oesophagus - does not require biopsy diagnosis for those patients presenting with typical symptoms and macroscopic endoscopic alterations.[24]

Biopsies are mainly useful in patients presenting with normal endoscopy and abnormal acid exposure (non-erosive reflux disease – NERD), in patients with typical symptoms and normal endoscopy and pH-metry or in patients with atypical symptoms. The presence of “dilated intercellular spaces (DIS)” or of a combination of DIS with other microscopic features such as basal zone hyperplasia observed in GORD may confirm the suspected diagnosis of reflux.[21] There are however several other types of oesophagitis. The presence of an intense eosinophil infiltration must orient towards a diagnosis of eosinophilic oesophagitis. Eosinophilic oesophagitis can present a typical endoscopic pattern known as “ringed oesophagus” but the oesophagus can appear normal in up to 20% of the patients. It is important to recognise that the eosinophilic infiltration may have a heterogeneous distribution within the oesophagus. Therefore, when considering eosinophilic oesophagitis, it is critical to have biopsies from multiple areas, including the distal, mid, and proximal oesophagus.[25] Biopsies of the

oesophagus are further indicated in the presence of oesophageal ulcers, erosions or an atypical aspect or topography and whenever an infectious aetiology is suspected. They can help to identify infections such as moniliasis, herpes and cytomegalovirus disease. (Fig. 5)

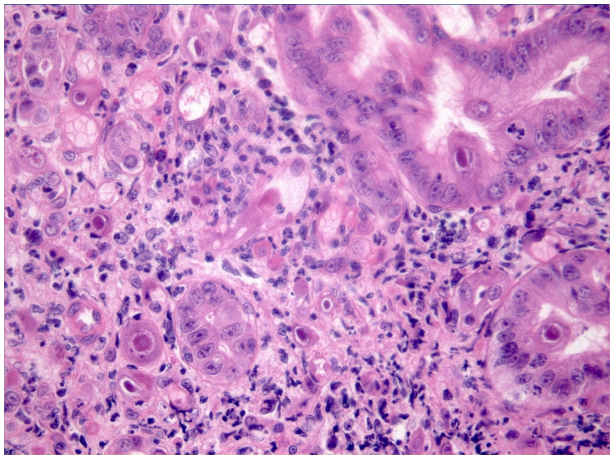

Figure 5. Gastric biopsy showing several Cytomegalovirus nuclear inclusions

Barrett's oesophagus presents a special problem. One definition of Barrett's oesophagus implies "endoscopic abnormalities suggestive of Barrett's oesophagus (endoscopically suspected oesophageal metaplasia) and the presence of columnar epithelium in biopsies. Barrett's oesophagus is a preneoplastic condition.[26] Effective management of the risk for oesophageal adenocarcinoma in Barrett's oesophagus requires precise detection of intestinal-type metaplasia and dysplasia. The detection of intestinal metaplasia is subject to significant sampling error.[27] (Fig. 6) Intestinal metaplasia increases with segment length of the Barrett's mucosa and detection improves with the number of biopsies taken.[28] Intestinal metaplasia can be missed easily when only one or two biopsies are obtained. Therefore it has been proposed to take multiple, closely spaced biopsies. One protocol proposes four-quadrant biopsies every 1 cm for circumferential metaplastic segments (in short segment Barrett's oesophagus) or 2 cm (in long segment Barrett's oesophagus).[3] In another study it was proposed that 8 random biopsies should be obtained. With 1 – 4 biopsies the yield of intestinal metaplasia was 35%.[6, 28] At the histological level, the detection of intestinal metaplasia can be increased by using mucin stains. or immunohistochemistry. It has been suggested that the presence of acidic mucins (blue on alcian blue stain) is a characteristic feature even in the absence of goblet cells. However, this theory has not been confirmed. Comparable disputed results have been obtained with immunohistochemical stains for CKs and MUC antigens.

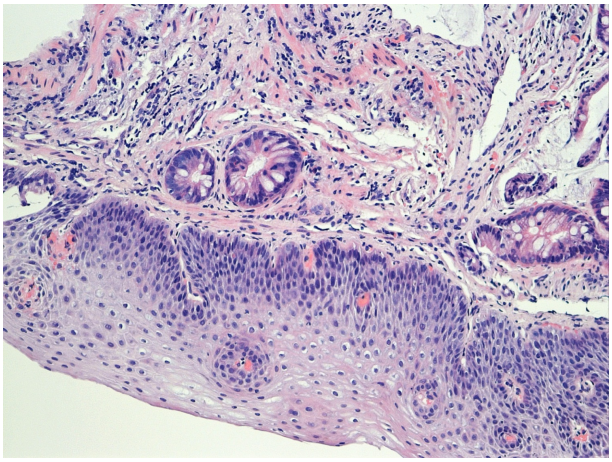

Figure 6. Oesophageal biopsy showing intestinal metaplasia in glands buried underneath the squamous epithelium

5.2. Neoplastic conditions

As the major risk of patients with Barrett's oesophagus is to develop an adenocarcinoma, there has been considerable interest in defining a subgroup of patients at risk. At the present time, the identification of dysplasia in endoscopic mucosal biopsies is the standard method to detect these patients. Systematic four-quadrant biopsy is considerably more effective for the detection of dysplasia in Barrett than non-systematic biopsy sampling.[29] Non-adherence to a protocol during surveillance leads to under-diagnosis or missed diagnosis because of sampling error.[30] However, problems with inter-observer agreement, particularly for low–grade dysplasia, on biopsy specimens have raised concern about the ability of pathologists to provide a consistent and accurate diagnosis upon which management decisions can be based.[31, 32] In order to reduce sampling errors, guidelines for the surveillance have been established by national and international societies. In the future, the diagnostic yield for dysplasia will however essentially be improved and sampling errors will be reduced by targeted biopsies. These can be obtained with the help of endoscopic procedures such as chromo-endoscopy and light- or laser-induced fluoroscopy.[33, 34]

High-grade dysplasia and early cancer can be treated by mucosal destructive or ablative techniques. Some techniques such as photodynamic therapy and laser therapy do not allow any histological study as their goal is complete destruction of the neoplastic tissue. Follow up biopsies can however show remnants of metaplastic and even neoplastic tissue buried underneath squamous epithelium. The frequency of buried metaplastic glands may be as high as 51% of cases. These glands may be difficult to identify on small endoscopic biopsies.

Endoscopic mucosal resection (EMR) is an ablative technique originally developed as a diagnostic procedure (strip-off biopsy) in the early 1980s but has now gained considerable attention as a potential curative form of therapy for patients with high grade dysplasia and

superficial cancers. It is also a good tool for histological staging because the procedure allows to remove intact mucosa and submucosa enabling complete evaluation of mucosal and submucosal invasion. EMR as a diagnostic tool has been shown to be superior to mucosal biopsy and inter-observer agreement of Barrett's oesophagus related dysplasia is significantly better compared with biopsy specimens.[35] The presence of a double layer of muscularis mucosae, which is a hallmark of Barrett's oesophagus, is an important landmark. Only when invasion extends beyond the deeper layer (the genuine muscularis mucosae), a diagnosis of submucosal invasion is justified.[36]

Endoscopic biopsies are also commonly used for the diagnosis of cancer of the oesophagus and the distinction between squamous cell carcinoma and adenocarcinoma. Two samples can provide a positive diagnosis in 95.8% of cases. The addition of four samples increases the positive yield to 100%. There is no statistically significant difference in the yield according to the site and type of growth.[37] However, in strictures the diagnosis can be difficult. In this situation, the additional use of brush cytology may increase the diagnostic yield. Soft tissue tumours and lymphomas are less common in the oesophagus. The so-called Abrikosoff tumour or granular cell tumour, a relatively rare lesion, may present a problem as the overlying squamous epithelium can show hyperplasia which might be confused with neoplastic changes. The tumour itself is composed of aggregates of round cells with a characteristic granular cytoplasm showing S100 positivity with immunohistochemical stains. If the biopsy samples are too superficial, the diagnosis can however be difficult. Fine needle aspiration biopsy could be used for the former, although most soft tissue tumours of the oesophagus are not malignant. Brush cytology can be helpful for the diagnosis of infections.

6. The stomach

6.1. Inflammatory conditions

Throughout the GI tract, mucosal features such as redness, oedema, swelling, bleeding, erosions and ulcers can be observed. They reflect inflammation and tissue damage but may also be due to mucosal atrophy and epithelial metaplasia. Metaplasia is most readily detected endoscopically in the distal oesophagus but it is also common in the stomach. In the latter it may appear as small red depressions simulating erosions or aphthoid ulcers, as an irregular nodular area or as larger geographic red areas. The red colour and a depressed or nodular appearance can be explained by thinning of the mucosa due to atrophy and increased visibility of the vessels. Pathology is useful to confirm the endoscopic abnormality and probable diagnosis, or to exclude such abnormalities or give another explanation. A depressed red spot can indeed be a genuine erosion but it may also represent a vascular ectasia or a small area of mucosal atrophy. Inflammatory conditions in the stomach include gastritis and reactive gastropathy (chemical gastropathy, bile reflux). The latter is characterized by epithelial damage and a minimal inflammatory cell reaction. Several types of gastritis can be distinguished and histopathology plays a major role in this distinction. An aetiology-based classification was proposed in the Sydney system at the World Congress of Gastroenterology

in 1990 and updated in 1994.[38, 39] The Sydney system also established the need for taking different biopsies of the gastric mucosa.[38] The guidelines include a) two biopsies of the corpus and two of the antrum for an overall assessment of the distribution of the gastritis and the distinction between antral gastritis, corpus gastritis and pan gastritis; b) one biopsy of the angulus because atrophic gastritis and intestinal metaplasia are related with the development of gastric cancer and occur most commonly at the angulus; c) the same area is the most appropriate area to look for the presence of dysplasia. In small children, this approach may however not be appropriate. Two samples from the stomach may be sufficient. Biopsy diagnosis should include the morphological site or sites, the morphological lesions present, and any potential cause. The sensitivity and specificity for the diagnosis of Helicobacter pylori gastritis are high, varying between 88 and 99% for the former and 90 and 100% for the specificity. The negative predictive value is near 100% for antral biopsies. Active gastritis, or gastritis with neutrophils is often Helicobacter pylori positive and will imply treatment, whether activity is mild, moderate or severe. Grading atrophy and intestinal metaplasia is less reproducible.[40] Staging of gastritis has been proposed among others by the so-called OLGA system but may be difficult to apply in routine practice.[41] Grading and staging could however be useful for the identification of patients at risk for cancer. In addition to the gastric biopsies ii seems reasonable to obtain, during the first diagnostic examination, also duodenal biopsies to look for the presence of mucous surface (gastric) metaplasia, a requirement for Helicobacter pylori colonisation of the duodenum which can induce duodenal ulcers, or for epithelial lymphocytosis. If the stomach biopsies are normal and duodenitis is found on histopathology, a Helicobacter pylori-induced duodenitis is highly unlikely. If the patient has lymphocytic gastritis of the antrum and epithelial lymphocytosis in the duodenum, a diagnosis of celiac disease should be suspected. Follow-up biopsies for gastritis can be considered when a treatment for HP has been given in order to assess eradication or when intestinal metaplasia and atrophy are very extensive.

Whenever special forms of gastritis are suspected multiple biopsies are needed. Histopathology can identify a variety of pathogens in infectious gastritis. Many of the special types lack endoscopic abnormalities. Lymphocytic gastritis can present as a hypertrophic variant with erosions and thickening of the gastric wall suggestive of Menetrier's disease. It can be diffuse or corporeal and correspond in these forms to varioliform gastritis. It can also be limited to the antrum and in this case includes various conditions (reflux gastritis, HP gastritis or coeliac disease) must be considered. [42] The histopathology of gastroduodenal Crohn's disease includes a wide spectrum of changes, including the presence of granulomas as well as focally enhanced (active) gastritis.[43] A correct diagnosis of Crohn's disease of the stomach can be reached more accurately when multiple samples of the suspected sites (n=5) and of normal sites are available. Granulomas can be detected in biopsies from macroscopically abnormal mucosa as well as in biopsies from normal mucosa. The frequency of detecting granulomas varies between 4.6% and 26% depending upon the presence of endoscopic lesions, the number of biopsies and the number of sections examined. Multiple biopsies will increase the diagnostic yield. Focally enhanced or focally active gastritis is typified by small collections of lymphocytes and histiocytes surrounding a small group of foveolae or gastric glands, often with infiltrates of neutrophils. Several studies have found that focally enhanced gastritis is common in adult

Crohn's disease patients. However, studies that used control groups have reported a prevalence of focally enhanced gastritis in non-IBD patients in up to 19.4%. Therefore, this type of gastritis may not be a good marker for the diagnosis of IBD or IBD-related gastritis in adults. [44, 45] It may still be a good marker in children although it may not reliably distinguish between Crohn's disease and ulcerative colitis. Some studies have found that focally enhanced gastritis is present in up to 20% of paediatric ulcerative colitis patients, suggesting that this type of gastritis is a marker of IBD in general in children.

Biopsies are less indicated for the diagnosis of vascular abnormalities. They can however be useful for the diagnosis of "gastric antral vascular ectasia" (GAVE). GAVE is a rare condition (prevalence approximately 3/10000 upper endoscopies), characterised by red spots in linear array in the antrum of the stomach. Based on the striped features from the antrum at endoscopy, the disorder has been called the "watermelon" stomach. The histological lesion consists of numerous dilated vessels in the mucosa, often with microthrombi, with fibromuscular hyperplasia and fibrohyalinosis of the perivascular lamina propria. The mucosa shows no or mild chronic inflammation or atrophy with intestinal metaplasia.[46] GAVE must be distinguished from "portal hypertensive gastropathy" and from "gastric vascular ectasia".[47]

6.2. Neoplastic conditions

In patients with marked atrophic gastritis or pernicious anaemia, the possibility of endocrine cell hyperplasia and dysplasia needs to be considered, and immunostains can readily answer this question. In patients with endocrine tumours (carcinoïds), the issue is whether these are sporadic, associated with atrophic gastritis, or even multiple endocrine neoplasia (MEN) and Zollinger-Ellison syndrome. Biopsies of adjacent gastric body mucosa will show whether there is hyperplasia of parietal cells without atrophy as in Zollinger Ellison and MEN, atrophy as seen in pernicious anemia, or normal mucosa as seen in sporadic endocrine tumours.

The macroscopic differential diagnosis between benign and malignant ulcers of the stomach is correct, on average, in only 75% of cases (52% to 94% of cases depending on the series reported in the literature).[3] Hence, the differential diagnosis can depend upon histology. Chromo-endoscopy with targeted biopsies will change the guidelines in the future.

In the series reported in the literature, the proportion of cancer-positive biopsies varies between 49% and 56% and about 25% of the biopsies are considered inadequate. A method of biopsy by quadrants with a technique that avoids the lesion to be covered by the bleeding from earlier biopsies reduces the number of unusable biopsies to 5.7% and increases the proportion of cancer-positive biopsies to 67%. An average of 7 - 10 biopsies is required to reach enough sensitivity and in order to avoid false negative results.[48, 49] When gastric lymphoma is suspected multiple biopsies are also required. If the lesion presents as an ulcer, biopsies from the edge (as for carcinoma) and the ulcer base should be obtained. Proper fixation (in order to allow additional tests such as immuno-histochemistry and Polymerase Chain Reaction) is absolutely indicated.

Histopathology is also very useful for the identification of metastases or secondary malignant involvement of the GI tract a problem which is becoming more common. Breast and melanoma are the most frequently found. Approximately 1 metastasis is observed per 3847 upper GI endoscopies and 1 lower metastasis per 1871 colonoscopies. The stomach and duodenum are the most common locations. Immune histochemistry for cytokeratin patterns and other markers can help to identify the primary origin if needed.

Overall a microscopic diagnosis of polyps (elevated lesions) depends on the type of the lesion and the size and number of biopsies. Polyps of epithelial origin can be diagnosed with classical pinch biopsies. They include benign lesions such as fundic gland polyps and neoplastic lesions such as adenomas or neuro-endocrine dysplasia. A complete evaluation may need larger snare biopsies and implies orientation. This is also needed for EMR specimens from early – superficial gastric cancer and adenomas. As in Barrett's oesophagus, a good orientation is essential for the assessment of the risk factors for residual tumour and the need for additional surgery. In contrast with the oesophagus, soft tissue tumours are more common in the stomach. These are usually gastrointestinal stromal tumours (GIST). These tumours show a positive staining with antibodies directed against CD117, DOG1 and often also for CD34 (87% positive cases in the stomach). They produce polypoid lesions with a smooth or ulcerated surface as a result of a submucosal process. Such a process can be inflammatory or tumoral and will often not be diagnosed adequately when the surface is intact and only mucosal biopsies are available (because of the superficial nature of these biopsies).

7. The duodenum

7.1. Inflammatory conditions

In the duodenum, inflammatory lesions include Helicobacter-associated disease, and other infections, malabsorption, drug-associated disease and the pathology of the papilla of Vater. Many GI diseases or systemic diseases (Helicobacter pylori, Crohn's disease, vasculitis, eosinophilic infiltrates) affect both the stomach and duodenum. Therefore, if duodenal biopsies are taken for any reason it is good to include biopsies of the antrum, in addition. Any duodenitis, inevitably raises the question of whether the condition may be associated with Helicobacter or drugs and biopsies of the antrum can solve this issue readily. Histopathology of the duodenum alone is indeed less useful for the diagnosis of Helicobacter pylori. Cytology is superior with a sensitivity which varies between 56% and 100% and a specificity between 58% and 93% depending on the coloration (modified Giemsa seems superior).[50]

Histopathology is certainly adequate for the diagnosis of other infections such as Giardia lamblia and strongyloides stercoralis.

A subtle increase of eosinophils in the duodenum may be associated with allergy and functional dyspepsia.[51]

Biopsy of the small intestine remains superior for the diagnosis of Whipple's disease and it is the gold standard for the diagnosis of celiac disease. Biopsies of the descending duodenum,

rather than the more distal intestine seem sufficient for the diagnosis of celiac disease. Jumbo forceps have no marked advantage over standard size biopsies.[52] Due to the patchy nature of villous changes, multiple biopsies are necessary. It has been suggested that at least four endoscopic biopsies must be taken.[53, 54] Ideally, the specimens are oriented properly in order to allow adequate assessment of villous height and crypt depth. The specimens can therefore be immersed in the fixative after being placed on a Millipore filter paper, luminal side upwards.

The recognition of the spectrum of histological changes in celiac disease as classified by Marsh or modifications of this classification has provided a major advantage in the diagnosis. The earliest lesions have still a normal villous architecture but show intraepithelial lymphocytosis (>30-40 per 100 epithelial cells).[55] An intraepithelial lymphocytosis is not, however specific for celiac disease and may be seen in infective enteropathies, Crohn's disease, non steroidal anti-inflammatory drug usage, giardiasis and other conditions. Furthermore, celiac disease is not the only possible cause of subtotal or total villous atrophy. Other possibilities such as autoimmune enteropathy must be considered, especially in neonates, but also in adults. Serology remains therefore and important diagnostic tool. Histopathology is also essential for the diagnosis of rare congenital disorders such as microvillous inclusion disease and "tufting enteropathy" (also called intestinal epithelial dysplasia, with the term dysplasia used in its ethymological meaning of "malformation" ; the pathology is due to defects in cell adhesion due to defects in the EpCam gene).

7.2. Neoplastic conditions

Refractory sprue is a condition that appears to consist of several diseases, including collagenous sprue and enteropathy-type T-cell lymphoma (ETL). Histology can help identify these.[56]

Duodenal biopsies are also indicated in patients presenting with duodenal polyps. Many of these, especially in the first duodenum, are benign lesions and represent inflammatory polyps or ectopic gastric tissue.

Malignant small bowel tumours constitute less than 5% of GI malignancies. Four major different histological types of malignant small bowel tumours can be distinguished : adenocarcinomas, endocrine tumours, lymphomas and soft tissue tumours. Adenocarcinoma is the most common type. As in the large bowel, most adenocarcinomas arise from pre-existing adenomas that occur sporadically or in the context of familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC) or variant syndromes. In patients with FAP "adenomas" are most commonly found in the duodenum. In a prospective study of 100 patients upper GI endoscopy revealed adenomatous polyps in the duodenum in 33. They occur mainly in the second part of the duodenum but may involve also the first and third part. A special staging system for duodenal polyposis has been designed whereby the lesions were subdivided in different stages according to the polyp number, size and histological type. The histological part of this system distinguishes the various types of polyps and grades of dysplasia. The types are : tubular/ hyperplastic/ inflammatory polyp = 1 point; tubulo-villous = 2 points; villous = 3 points; dysplasia is

graded into mild = 1 point, moderate = 2, severe = 3. [57] Other polyps that may occur in the duodenum or sporadic hamartomas or Peutz-Jeghers polyps and polyps observed in other non-adenomatous polyposis syndromes.

Endocrine tumours of the small intestine include well differentiated neuro-endocrine tumours and malignant large cell neuro-endocrine carcinomas. In the GI tract, most endocrine tumours occur in the small bowel (29% of total) with the highest frequency in the ileum. Endoscopic biopsies are often negative because of the superficial nature of the samples.

Lymphomatous infiltrates in the GI tract are frequently found as part of a disseminated disease. Primary GI lymphoma defined as an extra-nodal lymphoma arising in the GI tract with bulk of the lesion in this site, is a rare disorder. These lymphomas represent 5 to 10% of all Non Hodgkin lymphomas. Despite the fact that the small intestine is the preferential part of the gut where the mucosa associated lymphoid tissue (MALT) is localized, less than 25% of the GI lymphomas affect the small intestine.

The duodenum is also the site of the papilla of Vater where the extra-hepatic bile and pancreatic ducts end. Tissue histopathology may be obtained during endoscopic retrograde cholangiopancreatography (ERCP) by brushing, biopsy, bile aspiration or a combination of these. Biopsies of the bile ducts have a specificity between 90% and 100% with a sensitivity between 43% and 81% for the diagnosis of cholangiocarcinoma. Brush cytology has a similar high specificity of nearly 100% but sensitivity is lower ranging from 18% - 60%. The low sensitivity is linked to low cellularity of many of these tumours. Repeated brushing may increase the yield. During ERCP, miniature cholangioscopes can be used and with these endoscopes, directed tissue biopsies can be obtained. The biopsies are usually smaller than standard forceps biopsies of the GI tract and may be inadequate in up to 28% of the samples. [58] However, with more modern equipment adequate tissue for examination can be obtained.[59]

8. The terminal ileum and colon

8.1. Inflammatory conditions

Ileocolonoscopy is an important tool for the diagnosis of diarrhoea and colitis. Several studies show that colonoscopy and biopsy is useful in the investigation of patients with chronic diarrhoea yielding a histological diagnosis in 22 –31% of patients who had a macroscopically normal colon at colonoscopy.[60-63] Histological diagnosis includes a variety of conditions such as spirochetosis, pseudomelanosis coli, collagenous colitis and lymphocytic colitis and variant forms.(Fig 7) The correct diagnosis of collagenous colitis implies multiple biopsies from different segments because thickening of the collagen layer can be discontinuous.[64] Histopathology can also help to identify amyloidosis and rare metabolic lysosomal or storage disorders such as Tangier disease and systemic diseases such as mastocytosis.[65]

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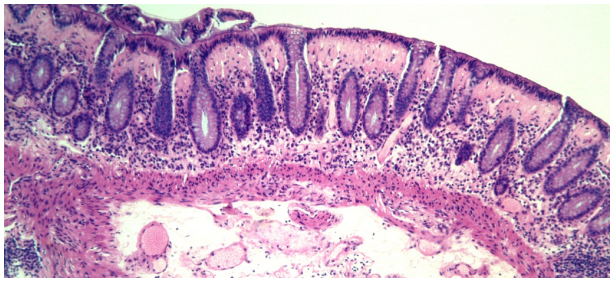

Figure 7. Biopsy from the colon showing thickening of the subepithelial collagen table in collagenous colitis

In inflammatory diarrhoea, a precise diagnosis and differential diagnosis between infections and IBD and between ulcerative colitis and Crohn's disease is important for therapy and follow up. Histopathology can identify a variety of pathogens such as amoeba, schistosoma and Cytomegalovirus. In transplant patients a diagnosis of Graft versus host disease or Cord Colitis can be confirmed and graded.[66] A correct diagnosis of ulcerative colitis can be made by the pathologist without clinical information in 64% of the cases with rectal biopsies only and in 74% of the cases when multiple biopsies from different segments of the colon, including the ileum are available.[10, 67] With clinical information a correct diagnosis is reached in more than 90% of the cases. A diagnosis of Crohn's disease on endoscopic samples of the colon relies particularly on the analysis of multiple biopsies from different segments of the colon including the ileum.[68] Analysis of multiple biopsies yields a positive diagnosis of Crohn's disease in 64% of the cases compared to 24% for one single rectal biopsy.[64, 69] Biopsies of the terminal ileum are mainly useful in patients with inflammatory diarrhoea.[70, 71] The differential diagnosis between infections and IBD relies on the distribution of the inflammatory infiltrate in the lamina propria and the presence of architectural changes. Focal or diffuse basal plasmacytosis is a strong predictor for the diagnosis of IBD, especially ulcerative colitis (occurring in over 70% of the patients). It is only rarely observed in infectious colitis (+/- 3% of the patients). Structural epithelial changes include the presence of an irregular surface, sometimes called pseudovillous or villiform surface and a disturbed crypt architecture.[72-74]

Atypical presentations such as ulcerative colitis with left-sided colitis and peri-appendicular inflammation or caecal patch are occasionally observed. However, the major clinical conditions where endoscopic and histological lesions may not be characteristic include initial onset of the disease, inflammatory diarrhoea in children, patients with liver disease and IBD, patients under treatment and patients presenting with severe, fulminant disease. Colonic biopsies from children between 1 and 10 years of age, presenting with new-onset ulcerative colitis show significantly less crypt branching, plasma cells in the lamina propria, cryptitis, crypt abscesses, and epithelial injury when compared with samples from adults. In 4 to 8% of cases the initial biopsy samples are completely normal. Rectal sparing has been well documented.[75, 76] Rectal sparing and patchy and focal inflammation are also more common in patients with

primary sclerosing cholangitis (PSC) without clinically overt colitis, when compared to patients with ulcerative colitis without PSC.[77, 78]

When the differential diagnosis between ulcerative colitis and Crohn's disease can not be solved with endoscopic biopsies the patient should be categorized as "IBD unclassified".[79] Clinical and histo-pathological follow up will eventually solve the diagnosis in most cases.

During follow up of IBD, histopathology can identify persistent active inflammation in ulcerative colitis more reliably than endoscopy.[80] Persistent microscopic inflammation may be important in the pathogenesis of dysplasia in IBD.

A complication of Kock pouch and ileal pouch anal anastomosis (IPAA) is the development of a primary inflammation within the pouch which is associated with a clinical syndrome termed "pouchitis". This condition is common after surgery for ulcerative colitis, but can occur also after surgery for other indications. Pouch biopsy specimens from well functioning pouches can show mild villous shortening and chronic inflammation. The most consistent finding in pouchitis is ulceration. Grading of pouchitis depends on clinical features, endoscopic findings and histology. The degree of polymorphonuclear infiltration and the proportion of ulcerated area are items of the score. There are no guidelines for the number and location of biopsies from a pouch but there is some evidence that a biopsy, taken 5 cm above the ileoanal anastomosis from the posterior and anterior wall may be the most sensitive for a diagnosis of pouchitis. Pouchitis must be distinguished from "cuffitis" or "short-strip pouchitis", which is inflammation in the columnar cuff mucosa distal to the pouch. The top end of the anal canal is lined by columnar mucosa like that of the rectum. In a hand sewn pouch-anal anastomosis, this mucosa is stripped, albeit often incompletely since the junction between columnar epithelium and squamous or transitional epithelium is difficult to distinguish. Islands of columnar mucosa may be left behind.

Histology is also important for the differential diagnosis of eosinophilic disorders of the gastrointestinal tract. Eosinophils are constitutively present in the gastrointestinal mucosa outside the oesophagus and the precise normal numbers have not been defined. In the colon geographical and seasonal differences in numbers have been observed. In humans, appendix, caecum and ascending colon contain the highest numbers Therefore a diagnosis of eosinophilic (gastro-)enteritis is difficult. An intraepithelial position of eosinophils may be the most reliable marker of disease. Eosinophilic disorders can be separated into primary (idiopathic) and secondary diseases, primary having no known cause, and secondary due to other illnesses associated with eosinophilia such a infections, celiac disease, IBD and drug related pathology. A third situation is observed in the hypereosinophilic syndrome, a heterogeneous group of rare diseases defined by persistent blood eosinophilia for more than 6 months with evidence of organ involvement (blood eosinophilia > 1500/mm^3).

Primary eosinophilic enteritis has been called allergic gastro-enteropathy, because a subset of patients have an associated allergic component. Although considered idiopathic, an allergic mechanism may be involved as most patients exhibit increased food-specific IgE levels.

8.2. Neoplastic conditions

Crohn's disease and ulcerative colitis carry an increased cancer risk. A pathway of "colitis – dysplasia – cancer" has been identified and this allows surveillance of patients with an increased risk (longstanding disease; extensive colitis; ulcerative colitis with primary sclerosing cholangitis…).(Fig. 8) It has been estimated that 33 to 64 biopsies are required to detect dysplasia with 90% and 95% probabilities respectively. Yet, with 20-40 biopsies less than 0.1% of the colorectal mucosa is covered.[81, 82] Current practice guidelines recommend that 4 biopsy specimens be taken from every 10 cm (0.05 % of the entire area of the colon) of diseased bowel in addition to macroscopically atypical lesions.[83] However, the detection rate of IBD-related dysplasia can substantially be improved with targeted biopsies obtained with the newly developed endoscopic techniques and this procedure should replace the random biopsy guidelines in the future.[84] Dysplasia in IBD can appear as polypoid lesions or as flat lesions. Polypoïd lesions can occur in a mucosa with signs of colitis, or in a mucosa with flat dysplasia. Therefore, biopsies should be obtained from the elevated lesion and from the surrounding tissue. The microscopic diagnosis of "dysplasia" is based on the presence of cytological and architectural abnormalities showing "unequivocal, non-invasive (confined within the basement membrane), neoplastic transformation of the epithelium excluding all reactive changes".[85] Biopsies positive for dysplasia can be subdivided into low-grade and high-grade. The grade of dysplasia is determined by the features of the most dysplastic portion. The two grade classification appears to be reproducible, although in general the agreement is better for high-grade dysplasia. Because of the diagnostic problems related to dysplasia ancillary techniques such as staining for p53 and AMACR can be applied on the tissue samples in order to improve the diagnosis. P53/AMACR coexpression seems to be of potential value for predicting neoplastic progression in ulcerative colitis patients with flat low grade dysplasia or indefinite lesions.[86]

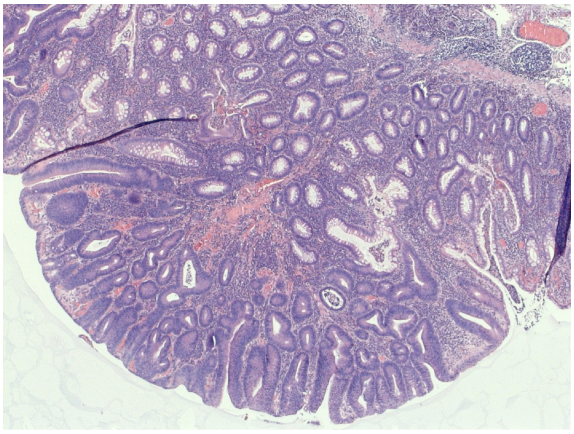

Figure 8. Raised polypoid lesion in a biopsy from a patient with ulcerative colitis showing microscopic features of dysplasia : DALM

Sporadic adenomas and polypoid "dysplasia" in IBD can be managed with endoscopic techniques and complete local excision appears to be adequate. Endoscopic resection specimens of IBD-related neoplasia should be handled properly, like all polypectomy specimens. They should be removed entirely if possible. Sporadic small polyps can be handled with a cold or hot biopsy forceps. While the latter can induce coagulation artefacts, the damage usually does not prevent adequate histological interpretation. Larger polyps should be oriented. The pathologist will identify the origin of the lesion, epithelial or not and the nature : neoplastic or not.

In recent years it has become clear that hyperplastic polyps are a heterogeneous group of lesions, now reported as "serrated lesions". They include benign polyps, so called (traditional) hyperplastic polyps which can be subdivided in several types (microvesicular type, goblet-cell-rich type and mucin-poor type) and lesions with a neoplastic potential. The distinction between the hyperplastic subtypes has a high inter-observer variation and therefore routine distinction of these subtypes is not necessary.[87] Among the lesions with a neoplastic potential, traditional serrated adenomas, with cytological dysplasia and sessile serrated adenomas or polyps have been identified. Both these lesions have a neoplastic potential through the serrated neoplastic pathway. In sessile serrated polyps, the epithelial cells show however some atypia or features of dysplasia.[88] Therefore a distinction is made between sessile serrated adenomas with and without dysplasia. A proper diagnosis of sessile serrated adenomas implies orientation of the endoscopic biopsy samples. The lesion is indeed characterized by dilatation of the crypts from top to bottom. Epithelial serration and dilatation are usually more prominent in the basal part of the crypts and this can not be evaluated properly on tangentially sectioned samples.

Histopathology allows grading of dysplasia in polyps and determination of the tubular or villous nature of the lesion. Tubular adenomas are by definition dysplastic and hence at least low-grade dysplastic lesions. Identification of high-grade dysplasia and intramucosal carcinoma is important. Endoscopic surveillance of patients with so-called "advanced adenoma" may need to be different from that performed in patients without advanced adenomas. In polyps, the occurrence of invasive cancer, must be differentiated from high-grade dysplasia, intramucosal cancer and entrapped (pseudo-invasive) mucosa. Only when cancer invades the submucosa, it is considered to have the potential to metastasize, although lymphangiogenesis can occur in the mucosa as shown in ulcerative colitis.[89] The established histopathological criteria that determine the treatment options of polypectomy versus subsequent surgical resection because of the risk of residual tumour are the status of the resection margin, the histological grade, lympho-vascular invasion, budding of cells and invasion into the submucosa below the stalk of the polyps but above the muscularis propria. Various staging systems have been proposed for this purpose.[7, 90]

As in the stomach and the small intestine, lymphomas and mesenchymal tumours can also occur in the colon and biopsies are suitable for a correct diagnosis.

9. Conclusions

Histopathology plays a critical role in GI practice. Endoscopic biopsies are important in order to establish, confirm or exclude a diagnosis suspected clinically or endoscopically, both in the absence and presence of endoscopic abnormalities. Biopsy diagnosis is greatly facilitated when the endoscopist provides adequate samples and understands the criteria used for histological diagnosis. Histopathology plays also a major role in the design of therapeutic strategy. A close collaboration between the endoscopist and the pathologist is therefore highly useful.

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