Pathology

1. Assessment of the completeness of tumour resection and of the pathological stage of rectal cancer are important for prognosis, choice of additional treatment, and control of the quality of the surgical resection. Standardisation of data, the application of well-defined criteria, and the acceptance of an identical and unique staging system allow integration and comparison of data. *Level of evidence IIb. Grade of recommendation B. Consensus 2A (in view of the obligatory medico-administrative data in Belgium).*

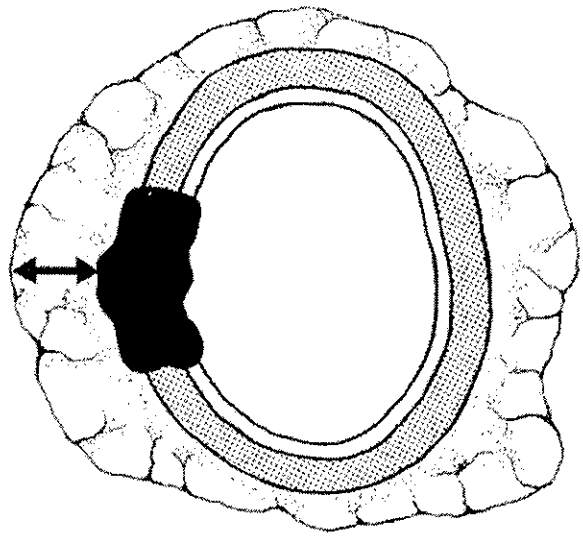
2. A rectal cancer resection specimen should be delivered to the pathologist fresh (within 2 to 3 hours), unopened, and unpinned (except for local excision specimen; cf. supra) *(level of evidence IV).* Administrative data, information on presence of a personal or family history of HNPCC-related cancer(s), cTNM staging, the type of surgery performed, and preoperative treatment modalities should be provided *(level of evidence III). Grade of recommendation C. Consensus 2A.*

3. Macroscopy and sampling. The resection specimen should be examined by the pathologist. It is mandatory to determine the exact topography of the tumour, also with reference to the serosal surface, i.e. above, at or below the peritoneal fold of Douglas. The quality of the mesorectal excision should be assessed, what is only possible on an unopened specimen. The mesorectal surface of a good resection should be smooth with no violation of the fat, good bulk to the mesorectum around the rectum. The distal margin should appear adequate with no coning near the tumour. No defect should be more than very superficial or 5 mm deep. The quality of the mesorectum can be graded (complete, nearly complete, incomplete). **Complete i.e. smooth, regular external surface of TME** : intact mesorectum with only minor irregularities of a smooth mesorectal surface. No defect is deeper than 5 mm, and there is no coning toward the distal margin of the specimen. There is a smooth circumferential resection margin on slicing. **Nearly complete i.e. mildly irregular external surface of TME** : moderate bulk to the mesorectum, but irregularity of the mesorectal surface. Moderate coning of the specimen is allowed. At no site is the muscularis propria visible, with the exception of the insertion of the levator muscles. **Incomplete i.e. severely irregular external surface of TME**: little bulk to mesorectum with defects down onto muscularis propria and/or very irregular circumferential resection margin. It is advisable to photograph the external surface of the TME to document the quality of the surgical specimen. **The description of the quality of the mesorectal surface is strictu senso limited to the description of the rectum above the sphincters.** The distance between the deepest point of extension of the tumour and the surgical circumferential surface is defined as the circumferential margin, which needs to be assessed with great care. After examination of the external surface, one should ink it before opening the specimen. The resection specimen should be sectioned in parallel cuts of 3-4 mm perpendicular to the length of the bowel allowing to assess the deepest point of invasion and to measure the distance to the nearest lateral surface. It is mandatory to photograph the anterior and the posterior surface of the mesorectum as well as the parallel cuts taken through the TME to document the quality of the surgical specimen and the extent of the disease. The deepest point of invasion should be sampled for microscopy, and the distance to the nearest circumferential surface should be measured and reported in mm. No distinction should be made between the various modes of involvement i.e. direct spread, involved lymph node, lymphatic or vascular spread. Measurement can be made by using a measurement device incorporated in the microscope itself (e.g. Vernier scale). Otherwise a sheet of graph paper that is photocopied onto a sheet of acetate and cut to size can be used. *Level of evidence IIb. Grade of recommendation B. Consensus 2A.*

4. Ideally, samples should be fixed in formol in order to allow additional molecular pathological examination. Freezing biopsy samples in liquid nitrogen with preservation in liquid nitrogen or in a freezer at – 80°C may be important especially if there are clinical arguments for HNPCC. (Checklist : additional samples) **Number of biopsy samples.** The number of blocks to be taken

from the tumour is 3 at minimum and 5 at maximum *(level of evidence IV)*. One block at least should include the transition from the surrounding 'normal' mucosa to the tumour and at least one other should include the deepest point of invasion *(level of evidence IV)*. **Proximal and distal section margins** do not have to be embedded if the tumour is situated at a distance of more than 3 cm from these margins. If the tumour is close to a margin, it is useful to sample this margin and to demonstrate the relationship to the tumour by perpendicular sections. Biopsies have to be taken to assess the **circumferential (radial, lateral) margin** *(level of evidence IIb)*.

The distal margin



The circumferential or lateral margin

Furthermore, associated lesions (polyps, IBD, ...) have to be sampled *(level of evidence IIb)*. In polyposis cases, a reasonable number of biopsies should be taken as well as the (proximal and distal) section margins. Proximal and distal section margins should be embedded in IBD cases too. All **lymph nodes** included in a resection specimen are considered to be regional. Distinction between paratumoral nodes and others i.e. local vs. regional lymph nodes is not requested anymore. The regional nodes of the rectum are: perirectal, sigmoid mesenteric, inferior mesenteric, lateral sacral presacral, internal iliac, sacral promontory (Gerota's), internal iliac, superior rectal (haemorrhoidal), middle rectal (haemorrhoidal), inferior rectal (haemorrhoidal). The number of lymph nodes analysed is important. At least 12 lymph nodes should be found and embedded. The numbers of lymph nodes retrieved depends mainly on the effort of the pathologist *(level of evidence IIb)*. The number of positive lymph nodes relates to the number

investigated; when less than 8 lymph nodes have been analysed , the proportion of cancers with lymph node involvement is underestimated *(level of evidence IV)*. However, it may be difficult to find numerous lymph nodes in rectum resections, in particular after preoperative radio-chemotherapy *(level of evidence IV)*. Decisions concerning adjuvant therapy may be inadequate if insufficient lymph nodes were retrieved. Although pathologists need to go into great pain to find as many lymph nodes as possible, there is insufficient scientific evidence to recommend micro-dissection techniques or fat clearance *(level of evidence IIb)*. Extra-regional lymph nodes are classified as metastases and should be embedded and described separately.
Grade of recommendation B. Consensus 2A.

5. The pathology report should be standardised, providing all important macroscopic (cf. sub 6. *Level of evidence IIb*) and microscopic data (cf. sub 7. *Level of evidence IIb, although two items – marked with * still are a matter of non-uniform consensus*). One check-list should by used per tumour (cf. addendum. *Level of evidence IV*). *Grade of recommendation B. Consensus 2A.*

6. Macroscopic data.
    a. The report should include the **measurements** of the resection specimen, including those of adjacent structures and organs.
    b. Concerning the **tumour** it is necessary to specify :
        i. The localisation of the tumour in relationship to the peritoneal lining, the proximal, distal and lateral (circumferential, radial) section margins. The proximal and distal section margins are defined respectively as the margin situated at the oral end and the anal end. These terms are used when the specimen can be oriented. If not, the section margins are described as the closest and most distant margin.
        ii. The maximal diameter of the tumour. The macroscopic appearance of the lesion should be described as protruding/exophytic, ulcerating, infiltrating, flat. However, both features, the size and the macroscopic appearance, have been shown to have no prognostic significance. The description may be useful in discussing the case e.g. comparison with radiology.
        iii. The presence of perforation at the tumour site should be reported since it will worsen prognosis. The same applies for the presence of peritoneal deposits.
    c. Associated lesions. The presence of synchronic cancers, polyps (solitary, FAP, ...) and chronic idiopathic inflammatory bowel disease (Crohn's disease, ulcerative colitis) should be mentioned.

7. Microscopic data.
    a. Histologic type according to the WHO classification :
        i. Adenocarcinoma: the histological **grade** should be mentioned either in a four or three-tiers system as well (G1), moderately(G2), poorly differentiated (G3) and undifferentiated (G4), or in a two-tiers system as low (G1,G2) grade and high (G3, G4) grade. The high grade corresponds to less than 50% of glandular structures of the surface analysed.
        ii. Mucinous carcinoma (colloid carcinoma): a tumour composed of at least 50% of this type of proliferation. It is considered as poorly differentiated adenocarcinoma.

iii. Signet ring cell carcinoma: a tumour composed of at least 50% of this type of proliferation. It is also considered as poorly differentiated adenocarcinoma.
iv. Adenosquamous or squamous carcinoma.
v. Small cell carcinoma.
vi. Medullary carcinoma: is considered as undifferentiated carcinoma
vii. Undifferentiated carcinoma (G4): corresponds to less than 5% of glandular structures of the surface analysed.

b. The depth of invasion should be described in function of the anatomical structures i.e. mucosa, submucosa, muscularis propria, subserosa, serosa and translated into the new TNM classification.
   i. Tx and To: primary tumour cannot be assessed (Tx). No evidence of primary tumour (T0).
   ii. Tis: carcinoma in situ includes cancer cells confined within the glandular basement membrane (intraepithelial) or lamina propria (intramucosal) with no extension through the muscularis mucosae into the submucosa. The term 'high grade dysplasia' and 'severe dysplasia' may be used as synonyms for intraepithelial (in situ) carcinoma. The extent of mucosal cancer can be expressed in depth of invasion relative to the thickness of the mucosa : i.e. superficial third m1, middle third m2 and deepest third m3.
   iii. T1: tumour invades submucosa. The extent of submucosal cancer can be assessed absolutely (sm1=less than 0.5; sm2=0.5–1; sm3=over 1 mm) or relatively (sm1=superficial third; sm2=middle third; sm3=invasion reaching the deepest third).
   iv. T2: tumour invades muscularis propria without breaching
   v. T3: tumour invades through the muscularis propria into the subserosa, or into the non-peritonealised pericolic and perirectal tissues. The subserosa corresponds to the adipous connective tissue situated in between the outer surface of the muscularis propria and the mesothelial lining.
   vi. T4: tumour directly invades other organs or structures, and/or perforates the visceral peritoneum. "Direct invasion" in T4 includes invasion of other segments of the colorectum by way of the serosa. Tumour that is adherent to other organs or structures, macroscopically, is classified cT4. However if no tumour is present in the adhesion, microscopically, the classification should be pT3.

c. Grading systems are being developed to describe and to quantify regression of colorectal cancer after irradiation (ypTNM). After preoperative radiotherapy partial regression i.e. downstaging of the tumour may occur whilst complete regression of tumour has been reported in roughly one fifth of the patients. Pathological examination is required to assess the effects of preoperative radiotherapy according to Dworak e.a.. The following are characteristics of each grade:
   i. GR0, no regression;
   ii. GR1, dominant tumor mass with obvious fibrosis and/or vasculopathy [i.e. minor regression (dominant tumor mass with obvious fibrosis in 25% or less of the tumor mass)];
   iii. GR2, dominantly fibrotic changes with few tumor cells or groups (easy to find) [i.e. moderate regression (dominant tumor mass with obvious fibrosis in 26% to 50% of the tumor mass)];
   iv. GR3, very few tumor cells (difficult to find microscopically) in fibrotic tissue with or without mucous substance [i.e. good regression (dominant fibrosis outgrowing the tumor mass; ie, more than 50% tumor regression)];
   v. GR4, no tumor cells [total regression (no viable tumor cells, only fibrotic mass)].

Problems relating to the difficulty in finding lymph nodes and the occasional finding of mucin pools with and especially without neoplastic epithelium are described. Tumour related mucin pools represent areas throughout the bowel wall that were previously occupied by tumour and could still be depending on sampling.

d. Resection margins. Margins histologically involved (microscopic tumour remains after resection) should be reported (R1). The circumferential margin or lateral section margin refers to the distance between the deepest point of invasion and the external surface of the resection specimen. A tumour-free lateral margin of <1 mm is considered positive. Also, a tumour-free lateral margin of >1 mm but <2 mm was found to be related to an increased local recurrence rate (cf. supra *).
e. Involvement of regional lymph nodes. The number of lymph nodes analysed is mentioned. One microscopic section should be taken through each lymph node. The analysis should be performed on hematoxylin-eosin stained sections. There is insufficient scientific evidence to mandate semi-serial sectioning of lymph nodes or the performance of immunohistochemical stains. The report should include a statement on the number of positive lymph nodes and on the total number examined. The TNM is a follows:
    i. Nx: regional lymph nodes cannot be assessed
    ii. N0: no regional lymph node metastasis
    iii. N1: metastasis in 1 to 3 perirectal lymph nodes
    iv. N2: metastasis in 4 or more perirectal lymph nodes

    Classification of tumour deposits in the adipose tissue remains controversial (*). For this project, we use the 5th edition of the AJCC Cancer Staging Manual. Extramural deposits of tumour that are not obviously within lymph nodes are regarded as discontinuous extensions of the main tumour if they measure <3 mm in diameter, but as lymph node involvement if they measure >3 mm in diameter.
f. The presence of vascular invasion into extramural veins should be described. Presence of perineural and/or lymphatic invasion may be mentioned. The V and L substaging can be used to identify the presence of vascular or lymphatic invasion.
g. Distant metastasis. The report should mention M1 if microscopic examination of a sample confirms the presence of a metastasis. This finding can relate to a liver biopsy or non-regional lymph nodes or peritoneal carcinomatous deposits. Cytological examination of peritoneal fluid revealing tumour cells equals M1. If the existence of distant metastasis can not be assessed, one should indicate pMx.
h. Associated lesions. These lesions (polyps, IBD, diverticulosis, ...) should be described separately.

8. The results of the pathology report should be discussed in a multidisciplinary meeting (e.g. MOC) involving the pathologist, surgeon, radiotherapist, oncologist and gastroenterologists in order to determine further treatment. *Level of evidence IV. Grade of recommendation C. Consensus 2A.*

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