Recommendations for pathological examination and reporting for colorectal cancer
Belgian Consensus

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Introduction

For over years, numerous studies have demonstrated the utmost importance of pathological examination in assessing prognosis for cancer patients. For colorectal cancer, it has been shown that the depth of invasion, the presence of lymph node metastases, their number and localisation, the presence of tumour at the surgical margins and the presence of vascular invasion need to be assessed. These data are indicative in the choice for additional treatment. Therefore standardisation of pathological examination is important for the quality of care of the patient. The Belgian Club for Digestive Pathology has attempted to reach a National consensus on pathological examination and reporting. To start with J-P. Bogers and C. Sempoux analysed the literature and compared American, English and French data concerning standardisation for colorectal cancer (1,2,3,4,5). The authors concluded that unanimity exist for certain features to be included whilst others are still under debate (6). Therefore a group of Belgian pathologists* met to debate on these data and issues and to present a check-list that would help pathologists

– to include all information important for treatment and prognosis of the patient
– to specify data in a systematic way to facilitate usage by the clinician
– to standardise the approach and thus improve the quality of retrospective studies
– and finally to facilitate communication within one hospital and between different hospitals.

This Belgian document consists of two parts. The first part includes the recommendations concerning the macroscopic and microscopic examinations of resection specimen indicating all information that has to be included in the final report. The second part consists of a check-list of one page that incorporates all this information.

1. *Reception of the specimen :*

a. Administrative data : The classical administrative data include : specimen number ; name, first name, date of birth of the patient ; type of surgical intervention.
b. Description of the specimen : It is important to mention if the specimen was received fresh or fixed, pinned out on support or not. Fixation will induce shrinkage of the specimen especially if it was not pinned out. If the specimen is not fixed, it should be delivered in the laboratory within 2 to 3 hours at maximum. Optimal fixation lasts for 12 hours. Considering the amount of perirectal tissue in a complete total mesorectal excision (TME), the fact that the TME should be fixed unopened and the necessity to slice the specimen, fixation time of a TME should be at least 48 hrs. Resection specimen should ideally be fixed in formol in order to allow additional molecular pathological examination. The external surface of the resection specimen (circumferential margin – lateral section margin with or without peritoneal lining) should be inked.

2. *Macroscopic examination :*

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 anatomical structures, the presence or absence of peritoneal lining (rectum, caecum), 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

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oriented. If not, the section margins are described as the closest and most distant margin. One checklist should by used per tumour.

ii. The maximal diameter of the tumour should be recorded. The macroscopic appearance of the lesion should be included e.g. 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 discussion the case e.g. comparison with radiology.

iii. The presence of perforation at the tumour site should be reported since it will worsen prognosis as well as the presence of peritoneal deposits.

c. Associated lesions : Other lesions in the remainder of the organ should be reported such as synchronic cancers, polyps (solitary, FAP, ..) and chronic idiopathic inflammatory bowel disease (Crohn's disease, ulcerative colitis)

3. *Number of biopsy samples :* The number of blocks to be taken from the tumoral lesions is 3 at minimum and 5 at maximum. One block at least should include the transition form the surrounding "normal" mucosa to the tumour and at least one should include the deepest point of invasion. Freezing biopsy samples in liquid nitrogen with preservation in liquid nitrogen or in a freezer at –80°C may be important especially when more cases of cancer have occurred within one family and also in patients below age 35. 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 really 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) margin. Furthermore associated lesions (polyps, IBD, …) have to be sampled. 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. (Addendum 1) Distinction between paratumoral nodes and others i.e. local vs. regional lymph nodes is not requested anymore. Important is the number of lymph nodes analysed. Ideally, at least 12 lymph nodes should be found and embedded according to the fifth edition of the TNM. The numbers of lymph nodes retrieved depends mainly on the effort of the pathologist. 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 significantly decreased. (7) However it may be difficult to find numerous lymph nodes in limited resections (palliative surgery), after preoperative radio-chemotherapy and in rectum resections. Decisions concerning adjuvant therapy may be inadequate if insufficient lymph nodes were retrieved. Although pathologists need to go into great lengths to find as many lymph nodes as possible, there is insufficient scientific evidence to recommend micro-dissection techniques or fat clearance. Extra-regional lymph nodes are classified as metastases and should be embedded and described separately.

4. *Microscopic examination :* An adequate report should include the following information :

a. Histologic type according to the WHO classification :

i. Adenocarcinoma : the histological grade should be mentioned either in a four or three-tiers system (well (G1), moderately(G2), poorly differentiated (G3)) or in two-tiers system (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) : defined as a tumour composed of at least 50% of this type of proliferation.

iii. Signet ring cell carcinoma : defined as a tumour composed of at least 50% of this type of proliferation. Mucinous and signet ring cell carcinomas are considered as poorly differentiated adenocarcinomas.

iv. Adenosquamous or squamous carcinoma.

v. Small cell carcinoma.

vi. Medullary carcinoma : are considered as undifferentiated carcinomas.

vii. Undifferentiated carcinomas (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 (8) :

i. Tx and To

ii. Tis : carcinoma in situ : intraepithelial or invasion in the lamina propria. Tis 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 in situ carcinoma. These case should be assigned pTis.

iii. T1 : tumour invades submucosa.

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 between the outer surface of the muscularis propria and the mesothelial lining.

vi. T4 : tumour directly invades other organs or structures, and/or perforates 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 T4. However if no tumour is present in the adhesion, microscopically, the classification should be T3.

Grading systems are being developed to describe and to quantify regression of colorectal cancer after irradiation (yTNM) (Addendum 2).

c. Resection margins : Margins histologically involved (microscopic tumour remains after resection) should be reported. 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. By analogy to rectal cancer, according to some authors, a circumferential margin of less than 2 mm can be considered as positive for colic cancer (9). The importance of the circumferential margin or lateral section margin should be stressed especially in surgical specimen for rectal cancer because of the retroperitoneal position of this segment of the large bowel as well as the difficult surgical approach (surrounding bony structures). Addendum 3 relates specifically to rectal cancer.

d. 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 on the total examined. The TNM is a follows :

i. NX : regional lymph nodes cannot be assessed or insufficient lymph nodes have been assessed (< 12). According to the sixth edition of the TNM classification, a pN0 determination may be assessed even though fewer than the recommended number of nodes have been analysed. However the Belgian working group advises to keep on using the previous TNM classification on assessing lymph nodes and to use pNx when insufficient lymph nodes have been assessed (< 12).
ii. N0 : no regional lymph node metastasis
iii. N1 : metastasis in 1- to 3 regional lymph nodes
iv. N2 : metastasis in 4 or more regional lymph nodes

Classification of tumour deposits in the adipose tissue remains controversial. Comparing the fifth and sixth TNM classification, different features have been proposed in order to address this problem such as a diameter of 3 mm, the form and the contour of the deposit. According to the Working group, these metastatic deposits are more likely to have developed from invaded lymph nodes and should therefore be interpreted as such.

e. 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.

f. 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 received simultaneously or peritoneal carcinomatosis. Cytological examination of peritoneal fluid revealing tumour cells equals M1. If the existence of distant metastasis can not be assessed, one should indicate MX.

g. Associated lesions : These lesions (polyps, IBD, diverticulosis, …) should be described separately.

Conclusions

Studies have shown for years how important it is to assess pathological stage of cancer for prognosis and choice of additional treatment. Standardisation of data, the application of strict criteria such as the macroscopic evaluation of the mesorectal surface and the distance between the deepest point of invasion and the circumferential surface i.e. circumferential margin, the acceptance of an identical and unique staging system should allow better correlation of data and integration into clinical trials. Furthermore, this consensus will improve communication between hospitals and medical disciplines.

Addendum 1 : Regional lymph nodes

Caecum : pericolic, anterior caecal, posterior caecal, ileocolic, right colic

Ascending colon : pericolic, ileocolic, right colic, middle colic

Hepatic flexure : pericolic, middle colic, right colic

Transverse colon : pericolic, middle colic

Splenic flexure : pericolic, middle colic, left colic, inferior mesenteric

Descending colon : pericolic, left colic, inferior mesenteric, sigmoid

Sigmoid colon : pericolic, inferior mesenteric, superior rectal (hemorrhoidal), sigmoidal, sigmoid mesenteric

Rectosigmoid : pericolic, perirectal, left colic, sigmoid mesenteric, sigmoidal, inferior mesenteric, superior rectal (hemorrhoidal), middle rectal (hemorrhoidal)

Rectum : perirectal, sigmoid mesenteric, inferior mesenteric, lateral sacral presacral, internal iliac, sacral promontory (Gerota's), internal iliac, superior rectal (hemorrhoidal), middle rectal (hemorrhoidal), inferior rectal (hemorrhoidal).

Addendum 2 : Quantification of histologic regression of colorectal cancer after irradiation (yTNM)

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. A grading system (Tumour Regression Grade) for assessing tumour response after preoperative radio-chemotherapy in oesophageal cancer has been proposed and applied "by analogy" to rectal cancer (10). However, this measurement consisted of five poorly distinct categories, which introduces the problem of subjectivity. More recently a grading system (Rectal Cancer Regression Grade (RCRG) 1 to 3) has been proposed for rectal cancer (11). RCRG 1 indicates "good" radioresponsiveness where the tumour is either sterilized or only microscopic foci of adenocarcinoma remain. RCRG 2 reflects marked fibrosis but with macroscopic tumour still present, and RCRG 3 indicates a "poor" response with little or no fibrosis in the presence of abundant macroscopic tumour. 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. The use of this Rectal Cancer Regression Grading system in the future will have to prove its validity.

Addendum 3 : rectal carcinomas

The approach of a resection specimen for rectal cancer, the macroscopic and microscopic examinations are comparable to those of colic carcinomas. Some comments are however mandatory since they may interfere with adjuvant therapy and follow-up of patients.

The quality of the local excision and the presence or absence of residual tumour after excision of the mesorectum will define the frequency of loco-regional recurrences. Numerous authors have stressed the importance of completeness of resection (12-15).

Furthermore it is mandatory to indicate the exact topography of the tumour compared to the serosal surface or adventitial surface i.e. above or underneath the mesenteric fold of Douglas. Since the rectum is mainly retroperitoneally situated, the mesorectum corresponds to perirectal fat that covers the lateral and posterior surfaces of the rectum. At this level the circumferential or radial margin is not lined by peritoneum. The quality of the mesorectal resection should be assessed. Adequate assessment of this feature 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 all 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, moderate, incomplete).

The distance between the deepest point of extension of the tumour and the surgical circumferential surface is defined as circumferential margin. Numerous studies have confirmed the importance of this distance (16-20). Therefore we need to assess the distance with great care. In order to investigate this parameter, one should ink the external surface of the resection specimen before opening it. The resection specimen should be sectioned in parallel cuts of 5 mm perpendicular to the length of the bowel allowing to assess the deepest point of invasion and to measure the distance to the nearest circumferential surface. The deepest point of invasion should be sampled for microscopy, the distance to the nearest circumferential surface should be measured. A distance of 2 mm or less relates to a local recurrence rate of 16% compared to 5.8 % if the distance exceeds 2 mm (17). A distance of 1 mm or less equals a threefold increase in recurrence rate (37,6% vs 12.7% if the distance exceeds 1 mm) (17). One should consider a circumferential margin of less than 2 mm as incompletely excised (R1). No distinction is currently made between the various modes of involvement e.g. direct spread, lymph node spread, vascular etc. since all have shown an increased local recurrence rate. 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 sheet of acetate and cut to size can be used.

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CHECKLIST COLORECTAL CANCER
Pathological report

Patient's name : …… Registration number : ……

Given name : …… Hospital/Laboratory : ……..

Date of birth : ……/……/…….. Preoperative treatment:

TYPE OF INTERVENTION

❑ right colectomy ❑ transverse ❑ left ❑ sigmoidectomy
❑ rectal anterior resection ❑ rectal abdomino-perineal amputation
❑ total colectomy ❑ TME (Total Mesorectal Excision)

MACROSCOPIC EXAMINATION

❑ specimen not fixed ❑ fixed

- Tumour location :
❑ caecum ❑ right colon ❑ transverse col
❑ left colon ❑ sigmoid colon ❑ rectum
❑ multifocal * If 2nd location, please use separate sheet

- Length of resected specimen : …….. cm
- Tumour size (maximum diameter) : …….. cm
- Distance tumour- resection margins:
proximal : …..…… cm distal : …… cm
or between tumour and closest resection margin :…… cm

- Features :
❑ protruding ❑ ulcerating
❑ infiltrating ❑ flat

- Tumour perforation : ……..
- Associated lesions :

	Yes	No
Polyp(s)	❑	❑
Synchronic cancer(s)	❑	❑
Ulcerative colitis	❑	❑
Crohn's disease	❑	❑
Familial polyposis	❑	❑

HISTOLOGIC EXAMINATION

❑ Adenocarcinoma
❑ well ❑ low grade
❑ moderately ❑ high grade
❑ poorly differentiated
❑ Other : ……

Depth of invasion

❑ intramucosal or intraepithelial (not beyond muscularis mucosae) (Tis)
❑ limited to submucosa (T1)
❑ limited to muscularis (T2)
❑ subserosal invasion (T3)
❑ visceral peritoneal invasion or adjacent organs (T4)

Surgical resection

Proximal and distal longitudinal margins
❑ invaded ❑ free

Circumferential margin : …… mm remote from tumour

Extension

- Number of lymph nodes examined : ……
- Number of invade lymph nodes: …..
- Extramural vascular embolisms:……

- Metastases (liver, peritoneal dissemination,…)
❑ yes ❑ no ❑ impossible to determine

CONCLUSIONS

Stage pTNM

❑ Tis ❑ T1 ❑ T2 ❑ T3 ❑ T4
❑ Nx ❑ N0 ❑ N1 ❑ N2
❑ Mx ❑ M1

Signature :

……..

Date : ……/……/……

N.B. Samples of tumour frozen: