Best practice No 159

Examination of large intestine resection specimens

S H Burroughs, G T Williams

Abstract

Macroscopic examination of large intestinal resection specimens by the surgical pathologist provides important diagnostic and prognostic information. This review summarizes current recommended protocols and evidence based guidelines for gross description, dissection, and histological block selection in both neoplastic and non-neoplastic colorectal disease. Specific lesions discussed include colorectal cancer, polypectomies and polyposis syndromes, and inflammatory bowel disease. Microscopic examination is briefly described, with emphasis on certain pitfalls that might be encountered in routine practice. A section covering special techniques for the investigation of occult bleeding is included.

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Keywords: large intestine; colorectal cancer; inflammatory bowel disease

Large intestinal resections performed for neoplastic, vascular, and inflammatory disease form part of the everyday workload of many surgical histology laboratories. The importance of careful handling of colorectal cancer resections by the pathologist has received much attention recently. Identification of lymph node and resection margin involvement by adenocarcinoma is of paramount importance in determining whether patients will receive postoperative chemotherapy and/or radiotherapy. Macroscopic examination of the resection specimen also plays a major role in monitoring the quality of surgical practice.

Gross examination of the large intestine in non-neoplastic conditions can also yield valuable diagnostic information, particularly in the classification of inflammatory bowel disease. Other conditions, such as angiodysplasia, require special preparation of the macroscopic specimen if the lesions are to be identified on subsequent microscopy.

These guidelines aim to summarize current “good practice” recommendations for macroscopic examination and dissection of colorectal resections and polypectomy specimens, including the selection of appropriate blocks for histology. Details of microscopic examination and interpretation of biopsy specimens are beyond the remit of this paper; comprehensive guidelines for biopsy diagnosis of suspected inflammatory bowel disease have been published recently. However, we have included a few guidelines relating to particular pitfalls in the histological assessment of large bowel lesions.

Laboratory examination of large intestinal resections: general comments

Resection specimens should be received fresh, unfixed, and unopened for optimum anatomical orientation. If the specimen is received outside laboratory hours, it can be refrigerated at 4°C overnight without risk of appreciable autolysis. Routine diagnostic immunocytochemistry and many molecular biological techniques can be applied to formalin fixed, routinely processed tissue, but receipt of fresh specimens also permits sampling for electron microscopy and research. Where receipt of fresh specimens is completely impractical, the theatre staff should be encouraged to send unopened specimens in an adequate volume of fixative—at least 10 times greater than the tissue volume.

The laboratory should ensure that the specimen is accompanied by a properly completed request form before commencing examination. A minimum amount of patient information (full name, date of birth, and hospital number) should be present on both the form and the specimen container. The pathologist needs to be aware of the clinical diagnosis, the results of any previous relevant histological investigations, and whether treatment (radiotherapy and/or chemotherapy) has been given that could affect tissue morphology and histological interpretation. Whenever possible, the pathologist who performs the macroscopic examination should also report the microscopic appearances. Routine specimen photography has been endorsed by some authors; we have found that a clear gross description of the specimen and indication of the origin of tissue blocks, with a diagram if appropriate, provides a satisfactory record in most cases. However, photography can be invaluable for documentation of macroscopic lesions in resections performed for inflammatory bowel disease.
might be desirable. The unfixed specimen is opened anteriorly, apart from the area extending 1–2 cm above and below the tumour, which should be left intact. In our department, a paper towel “wick” is passed through the residual lumen at the tumour site to aid fixative permeation. The specimen is then pinned to a cork board and immersed in an adequate volume of formalin. Resections should be allowed to fix for at least 48 hours before further dissection and block taking; this not only facilitates obtaining thin transverse slices through the tumour, but also has been shown to increase lymph node yield. Specimens can be unpinched from the board after 24 hours and allowed to float free so as to avoid the risk of suboptimal fixation of tissue previously adjacent to the cork surface.

Once fixed, the section of bowel containing the tumour and attached mesentery is sliced transversely at 3–4 mm intervals. This protocol has been endorsed for rectal cancer specimens, but we suggest that all large bowel cancers can be appropriately dissected by this method.

MACROSCOPIC DESCRIPTION

The gross description should include the specimen type (as indicated by the surgical operative description on the request form), site of tumour, and distance from closest resection margin. Tumour length and width are more difficult to measure when the specimen is dissected as described above, but these dimensions have no independent prognostic value. The position of rectal tumours in relation to the peritoneal reflection (entirely above, entirely below, or astride) and the distance from the tumour to the dentate (pectinate) line should be recorded. These data provide independent verification of the site of the tumour in the rectum and of the fact that the anal sphincter has been sacrificed, both of which are important in auditing the quality of surgery. It has been suggested that the pathologist can also assess the quality of surgical dissection of the pelvis in total mesorectal excision specimens by examining the contour of the resection for its bulk, surgical defects, and the degree of “coning” in the distal portion. The presence of perforation must be recorded because it is a poor prognostic feature, denoting stage pT4 tumour. Any background abnormalities should also be described.

BLOCKS TO BE TAKEN

After transverse sectioning, the tumour slices are laid out and blocks selected for histology to include greatest depth of tumour invasion and any peritoneal (serosal) or retroperitoneal/circumferential margin involvement; at least four blocks of tumour are recommended. We have observed that serosal involvement is especially prone to occur at or adjacent to peritoneal reflections (fig 1A), and should be suspected in any areas of serosa that appear dulled, fibrotic, or haemorrhagic. The Royal College of Pathologists’ minimum data set for rectal tumours requires histological measure-
Table 1 Minimum pathological dataset for colorectal cancer reporting

<table>
<thead>
<tr>
<th>Patient details</th>
<th>Gross description</th>
<th>Site of tumour</th>
<th>Maximum tumour diameter</th>
<th>Distance of tumour to nearer margin (cut end)</th>
<th>Presence of tumour perforation (pT4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name, date of birth, hospital, hospital number, histology number, surgeon</td>
<td>Site of tumour</td>
<td>Maximum tumour diameter</td>
<td>Distance of tumour to nearer margin (cut end)</td>
<td>Presence of tumour perforation (pT4)</td>
<td></td>
</tr>
</tbody>
</table>

For rectal tumours

Tumour position in relation to peritoneal reflection

Histology

Type (adenocarcinoma or other)

Differentiation (well/moderate or poor)

Local invasion (submucosa pT1)

(muscularis propria pT2)

(beyond muscularis propria pT3)

(peritoneum breached or invasion of adjacent organs pT4)

Margin involvement (if applicable)

Doughnut

Margin (cut end)

For rectal tumours

Circumferential margin involvement

Histological measurement from tumour to circumferential margin (in mm)

Metastatic spread

Number of lymph nodes examined

Number of positive lymph nodes (pN1: 1–3 nodes, pN2: 4+ nodes involved)

Apical node positive (Duke’s C2)

Extramural vascular invasion

Background abnormalities

Adenoma(s)

Synchronous carcinoma(s)—these require a separate minimum dataset to be completed

Ulcerative colitis

Crohn’s disease

Familial adenomatous polyposis

Other

Pathological staging

Complete resection at all margins

TNM

Duke’s

Histologically confirmed liver metastases


diagnosis of vascular invasion is made when tumour is identified within an extramural endothelium lined space that is either surrounded by a rim of muscle or contains red blood cells, and should be demonstrable on routine haematoxylin and eosin stains.

SPECIAL TECHNIQUES

The role of multiple section levels and immunocytochemistry in the detection of lymph node micrometastases is a subject for ongoing scrutiny; it is not currently our

ment of the minimum distance from the tumour to the circumferential resection margin, and an appropriate block should be taken for this purpose. We recommend a similar approach with regard to any non-peritonealised margin (see above) in colonic tumours. Lymph nodes identified in the tumour containing slices can now be harvested. If the tumour is > 30 mm from the cut end of the main specimen it is not always necessary to examine the margin (or any corresponding doughnuts) microscopically, although the closest cut margin can be used to provide the required block of background mucosa. However, cut margins should subsequently be examined histologically if the initial tissue sections reveal the presence of extensive infiltrative tumour growth or lymphovascular invasion, and in tumours with poor prognosis histology (signet ring, small cell, or undifferentiated carcinomas). After block selection from the tumour, a careful search for regional lymph nodes is performed, starting with the highest node. This is the first node identified by sectioning serially and distally from the sutured vascular margin, regardless of the actual distance between node and tie; it should be identified and blocked separately. Whereas only one vascular “high tie” is present in rectal resections, several vessels might drain colonic resections; if the tumour lies between two major arteries it might be appropriate to examine both high tie nodes for assessment of Duke’s C stage. The TNM staging system requires the total number of positive nodes to be identified (pN1 = 1–3 nodes, pN2 = 4 or more nodes). The pN3 category, relating to nodes lying above the bifurcations of the major colorectal vessels, has been dropped from the 1997 TNM revision because it does not add any further prognostic data to pN2; therefore, separate identification of these nodes does not appear to be necessary.

There is no consensus view regarding the “minimum” number of lymph nodes necessary to ensure correct pathological staging. We strongly advise that the pathologist should submit as many nodes as possible for histology, regardless of site or size. It is possible that the median number of lymph nodes harvested from colorectal resections will be used as a future measure of the quality of pathological reporting. It is our experience, supported by other published evidence, that careful serial sectioning of adequately fixed specimens provides a satisfactory number of lymph nodes in most cases. Fat clearance of the mesentery might be of use when very few nodes have been harvested from an apparent Duke’s B tumour. It is uncertain as to whether multiple sections and complete embedding of lymph nodes is more effective than partial embedding or single node slices. Our practice is to embed all small nodes (< 5 mm in diameter) completely and embed half of each larger node. Lymph nodes lying close to the circumferential (non-peritoneal) resection margin should be sampled in continuity with that margin; any tumour deposit, including entirely intranodal and intravascular tumour, found within 1 mm of the surgical margin indicates margin involvement.

MICROSCOPIC DESCRIPTION

The minimum data set for colorectal cancer published by the Royal College of Pathologists is shown in table 1; it can be downloaded from the college website (http://www.rcpath.org). The use of a proforma report to ensure completeness of data recording in every case is strongly encouraged. Tumour should be graded by predominant area: small foci of poor differentiation at the advancing edge of tumours are insufficient to classify the carcinoma as poorly differentiated. Peritoneal involvement should only be diagnosed if tumour is present on the serosal surface or has ulcerated the serosa; the presence of tumour close to an inflated area of peritoneum is insufficient evidence of stage T4 (serosal involvement). Infiltration of extramural veins (but not submucosal or intramuscular veins) is associated with an increased incidence of distant metastases and death from local recurrence. The diagnosis of vascular invasion is made when tumour is identified within an extramural endothelium lined space that is either surrounded by a rim of muscle or contains red blood cells, and should be demonstrable on routine haematoxylin and eosin stains.

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SPECIAL TECHNIQUES

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Preservation of tissue for electron microscopy in the detection of vascular invasion. Necessity for the routine use of immunocytochemistry in the diagnosis of vascular invasion. Preservation of tissue for electron microscopy is probably only necessary in suspected stromal tumours or in otherwise unusual rare lesions.

Polypectomy specimens

Local excision of polyps
Small polyps can be embedded whole. Biopsy section through the stalk is only recommended for lesions less than 1.0 cm in largest diameter. For lesions over 1.0 cm, the edges should be trimmed to leave a central section containing the intact stalk, and all fragments submitted for histology.

Submucosal polypectomies and full thickness local excision specimens
Submucosal excision is usually used for large carpet-like adenomas in the rectum. Transanal full thickness local excision specimens may contain adenomas and early carcinomas, or might be performed as a palliative procedure in patients who are unsuitable for more radical surgery. Both types of specimen tend to consist of the lesion with a narrow rim of surrounding normal tissue. For satisfactory examination, the fresh specimen needs to be pinned to cork, using dressmaker’s pins around the entire circumference, and fixed for at least 24 hours before dissection. Fixation of the specimen without pinning out can cause dramatic tissue shrinkage that renders anatomical orientation and assessment of resection margins very difficult. After fixation, the specimen margins should be identified with appropriate coloured markers (inks or gelatin). The whole of the specimen is transversely sectioned into 3 mm slices and submitted for histology in sequentially labelled cassettes. In cases where the margin of normal tissue is less than 3 mm, a 1 cm slice containing the relevant margin should be made and further sectioned at right angles as shown in fig 2. The multiple fragments from the margin section can be embedded in agar to maintain orientation.

Microscopic description
We recommend examination of three section levels from all neoplastic polypectomy specimens. The pathology report should indicate polyp size, architecture, degree of dysplasia, and completeness or otherwise of excision. Adenomas are classified as “tubular” or “villous” if the lesion contains at least 80% of the respective architectural component. Tubulovillous adenomas are composed of tubular and villous structures, each contributing to more than 20% of the tumour mass. Invasive malignancy is only diagnosed if there is infiltration beyond the muscularis mucosa. Lambina propria invasion or partial muscularis mucosa infiltration only does not indicate metastatic potential, and should be reported as adenoma with severe dysplasia. Radical surgery is indicated for invasive malignancy arising in a polyp or localised resection specimen only if there is doubt regarding the completeness of excision, evidence of muscularis propria (not mucosae) invasion, or if the carcinoma is poorly differentiated; these parameters must be recorded if present. The importance of lymphatic or vascular invasion in the polyp stalk and submucosa is more controversial, although most would regard it as an indication for further surgery, and it should also be included in the report.

Sampling of multiple polyps and polyposis syndromes
Multiple adenomatous and metaplastic polyps might be present in the background of colorectal resections performed for both neoplastic and non-neoplastic lesions, in addition to known polyposis syndromes. Invasive adenocarcinoma in colonoscopically removed adenomas is very uncommon in those measuring < 1 cm, but is present in 5.5% of lesions between 1 and 2 cm, and 26% of adenomas exceeding 2 cm. These figures support the guideline that although all polyps under 1 cm in a colectomy specimen do not necessarily need to be sampled, all suspected adenomas above 1 cm in diameter should be submitted for histology. However, sufficient numbers of polyps should always be sampled to ensure that histological sections are representative and to allow recognition of rare mixed polyposis syndromes.

Non-neoplastic colonic resections
Surgical resections for non-neoplastic colorectal disease may show a variety of pathologies including complicated diverticular disease, ulcerative colitis, Crohn’s disease, ischaemic colitis, volvulus, angiodysplasia, motility disorders, and radiation colitis. Specimen dissection and block selection need to be tailored to the specific pathology. Severe acute colitis (including toxic megacolon) is often a manifestation of inflammatory bowel disease, but other potential causes include infection, ischaemia, and obstruction. The main role of the pathologist in...
such cases is to determine the underlying disease process, although this might not always be possible.\textsuperscript{27} In cases of known inflammatory bowel disease, the role of pathological examination is to confirm the diagnosis, document disease extent, and identify clinically relevant complications, including precancerous lesions.

**SPECIMEN PRESERVATION**

Fresh specimens received unopened should be examined over the serosal surface for lesions, especially fistulae or perforations that might be missed after the specimen has been incised. We prefer to open the specimen throughout its length, along the antimesenteric wall, including any areas of stricture, and remove any faeces, blood, or mucus by gentle washing in cold running water. The resection is then pinned to a cork board and fixed, for a minimum of 48 hours; the specimen can be unpinned and floated free in the fixative after the initial 24 hours. An alternative method for strictured specimens is to wash the bowel luminal content away by inserting a gently flowing water hose at one cut end, and then to tie off one resection margin, inflate the specimen with formalin, secure the opposite cut margin, and float in fixative.\textsuperscript{3}

**MACROSCOPIC DESCRIPTION**

The distribution, extent, and nature of macroscopic changes in inflammatory bowel disease are frequently important diagnostic features. The following guidelines primarily relate to resections performed for Crohn’s disease and ulcerative colitis. The specimen nature (extent of surgical resection) should be recorded. Care should be taken to identify and document the distribution of serosal fat wrapping, severe dilatation, mural thickening or stricture formation, ulcers, perforation, pseudopolyps, and mucosal cobblestoning. Both ulcerative colitis and Crohn’s disease are associated with an increased risk of colorectal cancer, and specimens should be examined carefully for any suspicious lesions. Thorough macroscopic descriptions have a tendency to become lengthy, and specimen photography can prove extremely useful in this situation, providing an invaluable visual record for clinicopathological meetings. The appearance of fixed specimens can be improved by immersing in 70% ethyl alcohol for colour restoration before being photographed.\textsuperscript{4}

**BLOCKS TO BE TAKEN**

The selection of blocks obviously depends on the lesions present. For inflammatory bowel disease, we recommend sections to be taken at each 10 cm distance from the proximal to distal resection margin, in addition to sampling of any other focal lesions. The site of the block should be recorded by stating the distance of the section from a specified resection margin.

In ulcerative colitis, the resection margins need to be examined histologically for the presence of dysplasia. There is no evidence to suggest that microscopic involvement of the resection margin by Crohn’s disease increases the risk of disease recurrence,\textsuperscript{28} and blocking of the cut margins or doughnuts might not be necessary unless there are obvious lesions present. Any strictures should be sampled adequately to exclude the possibility of malignancy. Lymph nodes should be sampled routinely, although it does not appear necessary to embed all nodes as suggested for colorectal cancer.

**MICROSCOPIC DESCRIPTION**

Histological features of non-neoplastic colorectal disease are well described in standard reference works,\textsuperscript{5, 29} and detailed guidelines for biopsy reporting in inflammatory bowel disease have been published recently.\textsuperscript{1} Pathological mimicry of chronic inflammatory bowel disease by other forms of colitis is well recognised.\textsuperscript{30, 31} The presence of granulomatous inflammation is not synonymous with Crohn’s disease, and the accompanying macroscopic and microscopic features must be evaluated critically before making this diagnosis. Pitfalls in the diagnosis of ulcerative colitis include rectal sparing after local steroid treatment and “skip lesion” involvement of the caecum or appendix with coexistent distal proctitis but normal intervening colon.\textsuperscript{32, 33} The histological assessment of colonic tissue after diversion of the faecal stream requires special care, with review of any previous tissue sections.\textsuperscript{34} Correlation of clinical, radiological, and pathological findings in all suspected cases of inflammatory bowel disease is strongly recommended.

**Investigation of occult intestinal bleeding**

Suspected cases of colonic angiodysplasia require special handling if the ectatic vessels are to be demonstrated histologically. Close cooperation between surgeon, radiologist, and pathologist is necessary to identify and appropriately prepare the macroscopic specimen. Pounder and colleagues\textsuperscript{35} have described a technique of barium/gelatin vascular injection, which is cheap, reliable, and practical for routine surgical pathology laboratories. During surgery, the artery and vein supplying the affected segment of bowel are cannulated and flushed with heparinised saline. Contrast medium (5 g gelatin and 50 g barium sulphate in 100 ml water, maintained at 40°C) is injected under fluoroscopic control into first the arterial tree and then the venous system, with x rays being taken after both injections. The unopened bowel is then carefully washed out and inflated with formalin so that it is fixed in the normal distended state. After more than 24 hours flotation in formalin, the bowel is opened lengthwise and examined with a hand lens; it is not unusual for the mucosal surface to appear entirely normal, and the radiographs will be required to guide block selection. If angiodysplasia is present, the subsequent histological sections will show ectatic mucosal blood vessels filled with barium/gelatin. An alternative dissection method that does not require intravascular injection has also been described.\textsuperscript{36}

Thanks to Dr B Warren for guidelines regarding the handling of transanal local excision specimens.

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