National Guidelines in Histopathology

Handling & reporting of Gastrointestinal Tract Tumours

Second edition
2021

Ministry of Health, Sri Lanka
and College of Pathologists of Sri Lanka

National Guidelines in Histopathology

Handling & Reporting of Gastrointestinal Tumours

Second edition, 2021

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Published online by Ministry of Health, Sri Lanka

ISBN 978-624-6147-01-3

First published: 2007

Message by the Director General, Health Services

Ministry of Health, Sri Lanka

Cancer is one of the leading non-communicable diseases in Sri Lanka. With the establishment of national cancer policy on cancer prevention and control, there has been a commendable improvement in the cancer services provided island-wide.

The pathologists play a major role in the diagnosis of cancer and it is of utmost importance to formulate new guidelines as well as to update existing guidelines to improve the quality of diagnosis and to predict the prognosis of the disease in cancer patients.

These guidelines on handling tumours of the gastrointestinal tract, breast and gynaecological region as well as the guidelines on specimen handling and transport appear to be comprehensive guides to the histopathologists practicing in Sri Lanka and hope that these guidelines will help to improve the quality and the consistency of the histopathology reports across the country.

I am very grateful to the College of Pathologists of Sri Lanka for having identified the need and have been able to accomplish this difficult task amidst many hardships faced during the Covid-19 pandemic. I wish to thank the editors, authours and the clinicians who have contributed to these guidelines for their commitment in formulating these guidelines.

As these guidelines will be available in a freely available, easy to use, electronic format and I hope that these will help to improve the quality and delivery of diagnostic services to cancer patients in Sri Lanka.

I wish the College of Pathologists of Sri Lanka all the success in their future endeavours to improve the quality of histopathology services in the country.

Dr. Asela Gunawardena
Director General of Health Services
Ministry of Health
Sri Lanka

Message by the Deputy Director General Laboratory Services

Ministry of Health, Sri Lanka

In the provision of health care services, the laboratory sector plays a vital role by providing timely and accurate test results enabling the clinicians in diagnosis and treatment. Cancer is one of the leading health issues in Sri Lanka which needs effective curative and preventive diagnostic services. In order to achieve this, the contribution of histopathologists is invaluable.

The Laboratory Services Unit, Ministry of Health works with a vision to achieve standards for medical laboratories set by the international organizations for standardization and a mission to provide timely, reliable, high-quality diagnostic services to relevant health care providers. These guidelines published by the College of Pathologists, Sri Lanka have given a valuable contribution to achieving our mission and improving the services provided by the histopathology laboratories to the public.

I am pleased to note that the availability of these guidelines in electronic format hence histopathologists working all over the country will be able to get the maximum use of it.

I wish the College of Histopathologists, Sri Lanka all the success in their future endeavors to provide a tremendous service to uplift the health of the citizens in Sri Lanka.

Dr. Sudath K. Dharmaratne
Deputy Director General-Laboratory Services
Ministry of Health
Sri Lanka

Message by the President

College of Pathologists of Sri Lanka

Since the first series of the National Guidelines in Histopathology were published in 2007, the necessity to revise these guidelines and formulate new guidelines was considered to keep pace with the rapid advancements occurring in the field of histopathology worldwide. The College of Pathologists has been able to complete and publish this new series of guidelines with the objective of improving the diagnostic services in histopathology and histopathology reporting across the country. I am extremely happy that we were able to accomplish this task during the Covid-19 pandemic, utilizing the lockdown periods effectively.

The guidelines have been formulated after extensive discussion by the members of the guideline committees and clinicians in the relevant fields, conforming to the latest, accepted international guidelines in histopathology reporting. These offer a comprehensive guide to the pathologists when handling tumours of the gastrointestinal tract, breast and genaealogical region as well as to specimen handling and transport.

The structure of the guidelines has been made similar to the first series wherever possible with X, Y and Z denoting the mandatory, desirable and optional recommendations respectively. (X; Mandatory; recommendations that can be carried out in most of the institutions in Sri Lanka, Y; Desirable; investigations that can be carried out in selected institutions in Sri Lanka including the private sector and Z; Optional; investigations that are not freely available in Sri Lanka which may be performed in the private sector or abroad).

The guidelines will be in the electronic format to allow maximum visibility to the histopathologists working across the country.

On behalf of the College of pathologists of Sri Lanka, I wish to acknowledge the contributions made by the series editors, content editors, authours, clinicians and all the members of the guideline committees and thank them for their commitment to formulate these guidelines to be on par with international guidelines.

I am also grateful to the Director General of Health Services Dr. Asela Gunawardena and the Deputy Director General Laboratory Services, Dr. Sudath Dharmaratne for facilitating the electronic publication process of these guidelines.

I hope that the histopathologists working across the country will make full use of these guidelines to improve the quality of diagnostic services and reporting in histopathology.

Prof. Dulani Beneragama
President, College of Pathologists of Sri Lanka, 2021.

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Chapter 12	**Polyps of the gastrointestinal tract**	Janaki Hewavisenthi Charisma Fernando **Illustrations**: Dulani Beneragama, Charisma Fernando

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Contents

OVERVIEW

National guidelines on reporting of common malignancies including those of the gastrointestinal tract (GIT) were formulated and published by the College of Pathologists of Sri Lanka under the patronage of Ministry of Health and Nutrition, funded by the Sri Lanka Health Sector Development project IDA/ World Bank for the first time in 2007. In 2017, after a lapse of 10 years, the College of Pathologists decided that it was timely to revise and update these guidelines with the emergence of new evidence regarding the prognostic significance of some of the histological parameters and the criteria for defining each of these. Most importantly, this would include the revision of the current classification and staging systems in use, namely the World Health Organization Classification of tumours and the TNM classification. The current guidelines are therefore the result of this decision to update and revise the reporting of some of these malignancies, including those of the GIT.

These guidelines were drawn up with the main objective of bringing about uniformity and completeness in reporting of malignancies thus enabling future research and comparison of data throughout the island. Since local data with regard to prognostic indices in malignancies, is very sparse, much of these guidelines have been based on those of the Royal College of Pathologists of UK, Royal College of Pathologists of Australasia and College of American Pathologists. The fact that many histopathology laboratories throughout the island possess only basic facilities has been taken into account and therefore three levels of recommendations have been introduced throughout this book. These are namely Grade X category – mandatory which are those that can be practiced in any of the institutions in all parts of the island, Grade Y category – desirable, being those which include investigations and procedures that are available only in few institutions in the country and in the private sector and Grade Z category – optional, which include investigations and procedures that are not freely available in the country and require samples being sent to the private sector or abroad such as electron microscopy, molecular techniques etc.

In this edition, unlike the first edition published in 2007, an entire book has been dedicated to the reporting of GIT neoplasms, mainly malignancies. Therefore, in addition to reporting of common GIT malignancies that were described in the previous edition, which included only the tumours of the oesophagus, stomach and colon / rectum, the revised version deals with tumours of the gastro-oesophageal junction, small intestine, appendix and the anal canal. Two additional chapters have been included on the reporting of neuroendocrine tumours (NET) and gastrointestinal stromal tumours (GIST) of the GIT highlighting the differences

between these tumours and the conventional adenocarcinomas with regard to handling and histopathological reporting.

Two chapters on handling small samples such as polypectomies and endoscopic resections, namely endoscopic submucosal resections/dissections, transanal endoscopic mucosal resections have also been included. It is envisaged that the latter surgical procedures, though not very commonly practiced in Sri Lanka at present, will become popular with the advent of time and with more and more younger surgeons being trained in these procedures.

The TNM 8th edition is used throughout this book, unlike the previous edition in which the 6th edition was used as it was the version in use at the time of publication. In each chapter, special notes have been added to emphasize controversial issues that have been constantly changing, for example tumour satellites in colorectal carcinoma.

A special feature is that the surgeon's contribution to optimizing reporting has been added as a separate chapter/section enabling easier referencing for our busy surgical colleagues.

A consensus decision has not been reached at a National level whether synoptic reporting should be adopted in this country. Currently, the decision to use free text reporting, synoptic reporting or both lies with the individual pathologist. Thus, proformas for the reporting of each tumour have been included in the relevant chapters envisaging the possibility that some degree of synoptic reporting may be required in the future.

Though some areas, such as tumour grading of conventional adenocarcinomas, assessment of the completeness of resection, and tumour regression grading is similar in some areas of the GIT, they have been included under each chapter as it was thought to be more useful for a pathologist dealing with a specific type of tumour to have all the relevant information in one chapter.

It is hoped that these guidelines would be useful in aiding the pathologists of this country to reach a standard of excellence and uniformity in reporting of GIT malignancies.

Prof. Janaki Hewavisenthi

Chairperson, Committee to formulate National Guidelines in Histopathology on Gastrointestinal Tract Tumours, 2nd edition.

CHAPTER 1

Clinician's role in optimizing reporting of gastrointestinal specimens

Tumours of the oesophagus

Clinical details

- A diagram indicating relevant information, type of surgery and important surgical margins should be provided whenever possible
- The tumour location by the surgeon should be mentioned in relation to the upper, middle and lower oesophagus.
- Details of imaging findings should be provided
- Neoadjuvant therapy, if given should be documented
- Specimen should be transported immediately to the laboratory with adequate clinical history

Special precautions to be taken in the theatre

- Measure the length of oesophagus in the fresh state whenever possible as it contracts during fixation and is less than one third of its normal length after fixation. **[Y]**
- If part of the stomach is included, left gastric artery should be marked with a suture.
- If part of the stomach is not included, the proximal and distal ends of the specimen should be orientated with sutures.
- If lymph node groups are submitted, they should be identified separately

Tumours of the gastro-oesophageal junction

Clinical details

- Details of previous surgeries, biopsies
- Presence of Barrett oesophagus
- Siewert classification / exact tumour location with regard to the gastro-oesophageal junction should be mentioned if neoadjuvant therapy has been given

Special precautions to be taken in the theatre

As specified in the oesophagectomy and gastrectomy specimens.

Tumours of the stomach

Clinical details

- Details of previous surgeries
- Previous histology of biopsies (where performed and case number if available)
- Tumour site - Identified by radiology and/or endoscopy
- Involvement of gastro-oesophageal junction
- Type of resection
- Histological type of tumour (if known)
- Neoadjuvant therapy - If given, should be documented
- Pre-operative disease stage

Special precautions to be taken in the theatre

- The specimens should ideally be received in the fresh state. **[Z]**
- If received in the fixed state, an incision should be made away from the tumour to drain gastric contents and then placed in a large volume of formalin.
- Stapling of both resection margins is not recommended
- Important structures / margins should be marked with sutures
- A diagram indicating relevant information, type of surgery and important surgical margins should be provided whenever possible

Tumours of the small Intestine

Clinical details

- Tumour site
- Previous biopsy diagnosis if available
- Imaging findings
- Tumour stage on imaging studies
- Pre surgical CEA level if available
- Personal or family history of familial GI malignancies (eg: familial adenomatous polyposis, Lynch syndrome, Peutz-Jegher syndrome)

Special precautions to be taken in the theatre

- The bowel may be opened longitudinally along the antimesenteric border away from the tumour up to 10-20 mm above and below the tumour if delay in transport is anticipated
- Surgeons are strongly advised not to cut through the tumour

Tumours of the appendix

Clinical details

- Intraoperative findings should be informed if suspicious
- Imaging findings

Tumours of the colorectum

Clinical details

- Preoperative radiological / clinical stage of the tumour
- Radiological staging (CT / MRI) to be indicated in rectal tumours
- Extramural vascular invasion to be included when known by MRI / CT.
- Family history of colorectal cancer and other cancers
- Other premalignant conditions: Inflammatory bowel disease (IBD), Familial adenomatous polyposis (FAP)
- Details of previous surgeries, biopsies
- Neoadjuvant therapy

Special precautions to be taken in the theatre

- Large specimens should be accompanied by a clear diagram
- In any cases of complete radiological regression, the original tumour site is to be indicated by a diagram and preferably marked on the specimen by a suture or tattoo (if available)

Tumours of the anal canal

Clinical details

- Tumour location
- Previous biopsy diagnosis if available
- Imaging findings
- Tumour stage on imaging studies
- History of solid organ transplantation, HIV / AIDS status, Human papillomavirus infection, Crohn disease
- Administration of neoadjuvant therapy

Special precautions to be taken in the theatre

Please refer colorectal guidelines.

Gastrointestinal stromal tumours

Clinical details

- All cases of GIST should be discussed at an appropriate multidisciplinary team (MDT) meeting
- Details of previous surgeries, biopsies
- Nature of the surgical resection
- Site of the tumour: Clinical and radiological
- Size of the tumour
- Family history of colorectal cancer and other tumours such as neurofibromas or paragangliomas, other premalignant conditions (IBD, FAP)
- Per-operative findings: Completeness of excision, liver metastases etc.
- Neoadjuvant therapy

Special precautions to be taken in the theatre

- Important structures / margins should be marked with sutures
- A diagram indicating type of surgery and important surgical margins should be provided whenever possible

Neuroendocrine tumours

Clinical details

- Tumour size
- Previous biopsy diagnosis with grading if available
- Imaging findings
- Tumour stage on imaging studies
- Clinical symptoms like flushing, diarrhea, bronchospasm etc.
- History suggestive of Zollinger Ellison Syndrome, Multiple endocrine neoplasia (MEN) type 1.

Endoscopic resections (ER)

Clinical details

The clinician or endoscopist should provide information regarding the following:

- Location of the lesion
- Size of lesion
- Possibility of submucosal invasion
- Ulcer scar or biopsy site

Special precautions to be taken in the theatre

- ER (either EMR or ESD) should be pinned out on a cork board or polystyrene or wax block and oriented by the endoscopist, before dispatch to the laboratory (Figure 1).
- The distal and proximal margins of the specimen may also be identified / oriented as oral and anal margin or by the 12 0' clock position only.
- The peripheral edges (lateral / horizontal margins) of the specimen need meticulous pinning
- The mucosal side should face upwards
- The specimen should be pinned by stretching the specimen out to an approximate the length taking care regarding the following;
  - Not to overstretch the margins so as to cause tissue damage and distortion.
  - To include the entire thickness of the specimen
  - To place the pins closely to prevent curling and retraction of the margins in between the pins during fixation.
  - Not to pin through the tumour.
- This pinned specimen should then be floated upside down (mucosal surface downwards) in 10% neutral buffered formal saline
- The specimen should be marked as to the presence of sharps.

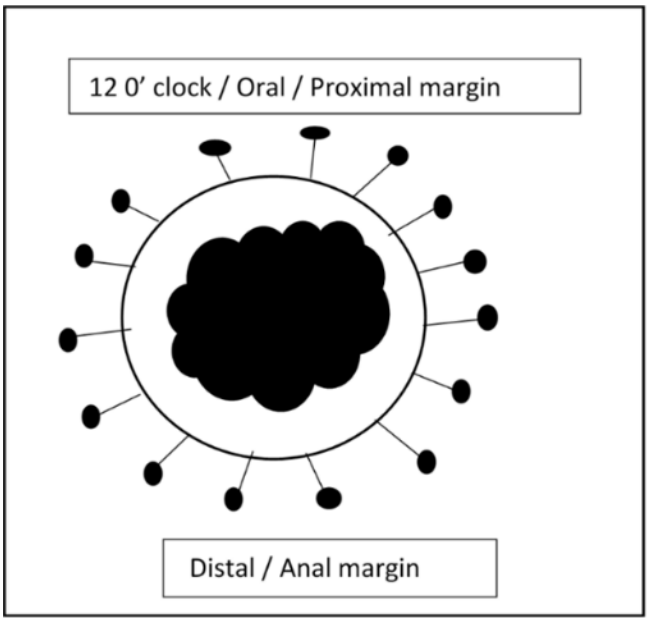

Figure 1. Pinning of the specimen

CHAPTER 2

Tumours of the oesophagus

Introduction and epidemiology

Oesophagectomy and oesophago-gastrectomy specimens are performed for all types of oesophageal carcinomas and carcinomas that straddle the junction of the oesophagus and the proximal stomach.

Oesophageal and gastro oesophageal junction (GOJ) carcinomas can be of either squamous or glandular type. The geographic distribution, epidemiology, aetiology and prognosis are different for these two histological subtypes.

Oesophageal cancer was reported as the eighth commonest cancer and the sixth leading cause of cancer death worldwide in 2018.

In Sri Lanka, oesophageal carcinoma was the seventh commonest gastrointestinal malignancy and the third commonest malignancy in males according to cancer incidence data of Sri Lanka 2014.

The commonest type of carcinoma of oesophagus in Sri Lanka is squamous cell carcinoma (not otherwise specified). The aetiology is multifactorial. The main risk factors are tobacco use, alcohol use and low socioeconomic status.

The most important risk factor for the oesophageal adenocarcinoma is Barrett oesophagus secondary to gastro-oesophageal reflux disease.

Adenocarcinomas of the GOJ share many characteristics with those of the distal oesophagus in countries where distal oesophageal adenocarcinomas are more common.

While using the same protocol for this entire group of malignancies, it is important to recognize that there are inherent biological differences within the group especially with regards to location and the type of cancer.

Specimen handling

Specimen collection and transport

Refer National Guidelines in Histopathology – Collection, Handling and Transport of Specimens (second edition, 2021).

Grossing procedure

- In addition to the request made to the surgeons to measure and record the length of the specimen in the fresh state, ideally the oesophagus should be pinned on a corkboard before fixation, so that the exact length can be assessed **[Y]**
- Paint the oesophageal circumferential margin with ink (Inspect for visible tumour or tumour perforations and note any surgical tears / defects prior to inking the entire circumferential margin).
- **Specimen dissection**
  There are two recommended methods of opening the oesophagus
  1. If the tumour is circumferential on inspection and by palpation - "bread-slice" the tumour transversely (Figure 1A). This gives better assessment of the circumferential margin.
  2. If the tumour is not circumferential on inspection and by palpation - open the oesophagus longitudinally with scissors taking care to avoid cutting into the tumour. This allows better assessment of the mucosal surface of the tumour and oesophagus (Figure 1B).

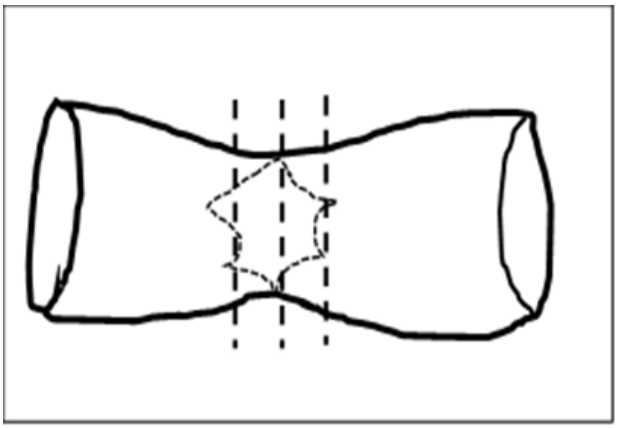

Figure 1A. "Bread-slice" the tumour transversely

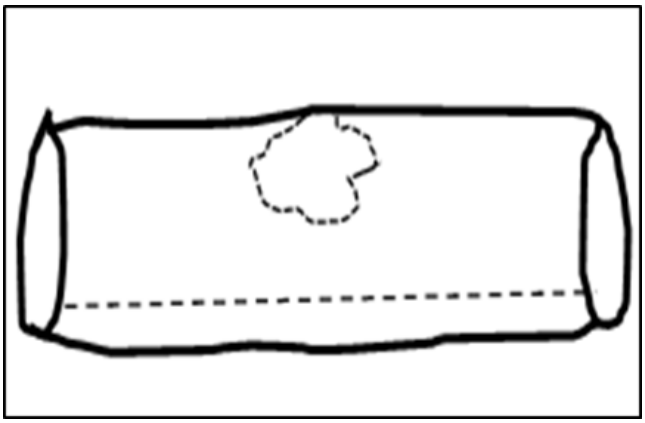

Figure 1B. Longitudinal incision for non-circumferential tumours

Macroscopy

- **Specimen type**
  - Oesophagectomy
  - Oesophago-gastrectomy
  - Others: Endoscopic mucosal resection (EMR)
- **Specimen size**
  - Measure the length of the tubular oesophagus and the stomach, along the greater curvature if present.
- **Inspection of specimen**
  - Note down visible tumour or tumour perforations
  - Designate and describe any synchronous tumour separately.
  - Describe the nature and extent of any peri-oesophageal tissue.
  - Note any surgical tears/defects.
- **Tumour site**

  Note:
  - It may not be possible to determine the exact tumour location within the oesophagus without proper information from the surgeon
  - Tumour site is important in the stage grouping of oesophageal squamous cell carcinoma
  - For tumours straddling gastro-oesophageal junction; record overall length of the tumour (Please refer chapter 3 for guidelines on gastro-oesophageal junction [GOJ] tumours)
- **Tumour size:** mm or cm - Greatest length of tumour
- **Maximum depth of invasion**
- **Tumour appearance:** Polypoid / ulcerated / thickening / others, specify
- **Distance to surgical margins:** Proximal / distal / circumferential
  - Measure distance from the proximal edge of the tumour to the proximal end of the specimen.
  - Measure distance between the distal edge of the tumour and the distal resection margin
  - Measure the distance from the tumour to the circumferential margin – Figure 2B.
  - Tumour involving the circumferential margin by gross examination is classified as R2 (Table 5)
- **Surrounding mucosa:** Normal / Barrett oesophagus (if present – measure the vertical length) / atrophic / thickened / ulcerated /erythematous / haemorrhagic
- **Involvement of adjacent structures:** Pleura, pericardium, diaphragm
- **Lymph nodes**
  - Describe site(s): regional / non-regional
  - Number retrieved

- **Doughnuts**
  - Received or not
  - Dimensions (mm)

Block selection

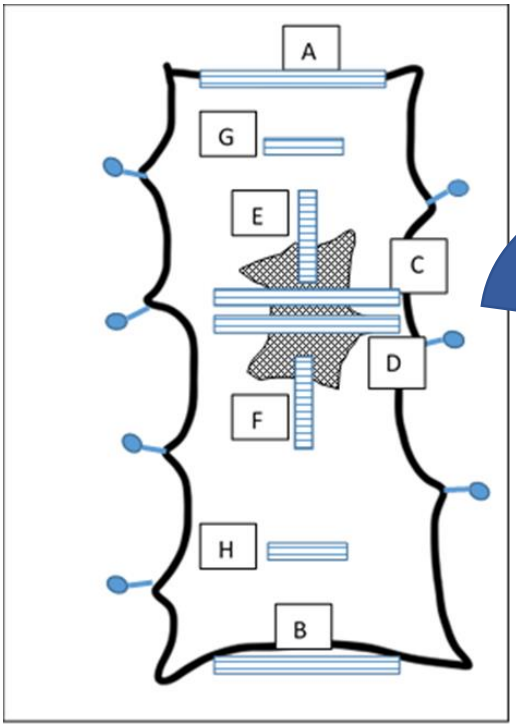

Figure 2A. Block selection - Oesophagectomy

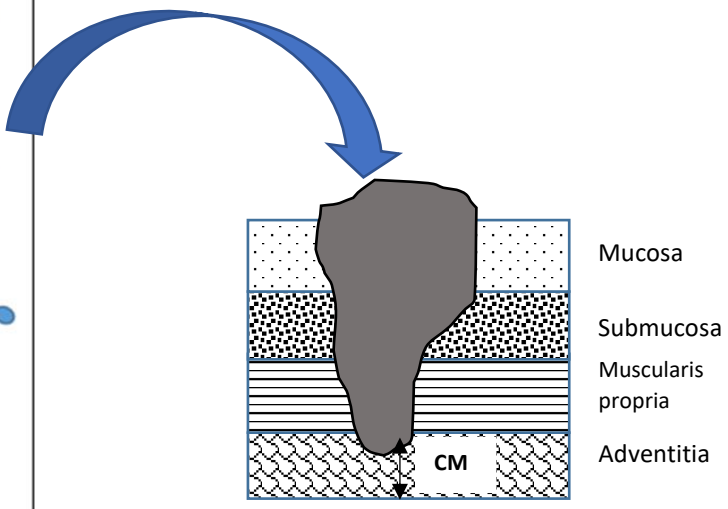

Figure 2B. Selection of blocks C and D. Deepest penetration with distance to circumferential margin (CM)

- The nature and the site of all blocks must be recorded. (Figure 2A, 2B)
- Proximal and distal resection margins parallel to the surgical resection. (Blocks A, B)
  (If the tumour is very close to margin, use perpendicular sections to aid microscopic measurement of tumour clearance)
- Multiple blocks from the central part of the tumour including the point of deepest penetration. (Blocks C, D)
  (If the sections are too large, composite blocks to the nearest circumferential margin should be taken.)
- Perpendicular blocks taken at proximal and distal tumour-normal mucosa interface. (Blocks E, F)
- Uninvolved proximal and distal parts of the oesophagus (Blocks G, H)
- Gastric serosa if tumour is close to it
- Squamo-columnar junction if included
- All macroscopically identified lymph nodes.
  (in the absence of macroscopically visible lymph nodes, peri-oesophageal fat should be sampled).

- **Note:**
  Following pre-operative chemoradiation, the lesion may become smaller or even invisible. On cut section, lesion may also appear as irregular white scars with fibrosis. Extensive sampling is recommended.

Microscopy and conclusion

Tumour type	Refer oesophageal tumour types in WHO Classification of tumours (5th edition of Digestive system tumours, currently in use in 2021 -Table 1)
Tumour grade	Table 2
Tumour size	Microscopically corrected tumour size mm / cm
Extent of invasion	Assessed according to the latest TNM staging system
Lymphovascular invasion	Present / Absent
Perineural invasion	Present / Absent
Degree of tumour regression after pre-op treatment	If applicable - Table 4
Other pathology	Barrett mucosa / squamous or glandular dysplasia / gastritis / eosinophilic oesophagitis / other
Completeness of resection (R status)	Table 5
Involvement of doughnut	Present / Absent
Regional lymph nodes	Number of lymph nodes examined Number of lymph nodes involved by tumour
Pathological tumour stage	Refer the TNM classification for staging oesophageal tumours (AJCC TNM 8th edition currently in use in 2021 - Table 2)
Ancillary tests	**Note:** Currently specific ancillary tests are not routinely recommended for oesophageal tumour classification

Annexure

Reporting proforma for oesophageal malignancy

Gross description		
Type of surgery	:	
Maximum length of specimen	:	_____ mm
Length of oesophagus	:	_____ mm
Length of stomach	:	_____ mm
Location of tumour	:	
Length of tumour	:	_____ mm
Tumour edge to nearest distal margin	:	. _____ mm
Tumour edge to nearest proximal margin	:	_____ mm
Tumour appearance	:	Polypoidal / Other (specify)
Microscopic description		
Type of tumour	:	Squamous / Adenocarcinoma / Other (specify) - Table 1
Differentiation by worst area	:	Well / moderately / poorly differentiated - Table 2
Depth of invasion	:	Table 3
Extent of invasion	:	Includes direct invasion to stomach
Involvement of margins		
▪ Proximal margin	:	Normal / dysplasia / involved by carcinoma
▪ Distal margin	:	Normal / dysplasia / Barrett / involved by carcinoma
▪ Circumferential margin	:	▪ Involved (< 1mm) ▪ Not involved - give distance of carcinoma to nearest circumferential margin in mm
Treatment effect	:	Tumour regression grade - Table 4
Lymphovascular invasion	:	Present / Absent
Perineural invasion	:	Present / Absent

Mucosal adjacent to tumour	:	Barrett metaplasia adjacent to tumour
Lymph nodes		
▪ Number examined	:	
▪ Number positive	:	
Completeness of resection (R status)	:	Table 5
Involvement of doughnut	:	Involved / Not involved
Pathological tumour stage	:	Refer the latest TNM classification -Table 2
Ancillary tests	:	**Note:** Currently specific ancillary tests are not routinely recommended for oesophageal tumour classification.

Tables

Table 1. WHO classification of tumours of the oesophagus (WHO Classification of Tumours of the Digestive System, 5th edition)

▪ Adenocarcinoma NOS
▪ Adenoid cystic carcinoma
▪ Mucoepidermoid carcinoma
▪ Adenosquamous carcinoma
▪ Squamous cell carcinoma NOS o Verrucous squamous cell carcinoma o Squamous cell carcinoma, spindle cell o Basaloid squamous cell carcinoma
• Carcinoma, undifferentiated NOS o Lymphoepithelioma like carcinoma
• Neuroendocrine tumour NOS o Neuroendocrine tumour, Grade 1 o Neuroendocrine tumour, Grade 2 o Neuroendocrine tumour, Grade 3
• Neuroendocrine carcinoma NOS o Large cell neuroendocrine carcinoma o Small cell neuroendocrine carcinoma
▪ Mixed neuroendocrine – non-neuroendocrine neoplasm (MiNEN)
▪ Combined small cell-adenocarcinoma
▪ Combined small cell-squamous cell carcinoma

Table 2A. Histological grade of tumour based on the AJCC classification of adenocarcinoma (College of American Pathologists Protocols, February 2020)

Grade	Description
G1 (well-differentiated)	> 95% of the carcinoma composed of well-formed glands
G2 (moderately differentiated)	50% to 95% of carcinoma demonstrating gland formation
G3 (poorly differentiated)	< 50% of carcinoma demonstrating gland formation

Note:

- Glandular structures are absent in undifferentiated tumours
- Mucoepidermoid carcinoma and adenoid cystic carcinoma of the oesophagus are not graded.

Table 2B. Histological grading of squamous cell carcinoma

Grade	Description
Grade 1 **(Well-differentiated)**	▪ Enlarged squamous cells with abundant eosinophilic cytoplasm. ▪ Keratin pearl formation. ▪ Low mitotic figures. ▪ Cells well-ordered ▪ Invasive margin is pushing type.
Grade 2 **(Moderately differentiated)**	▪ Surface parakeratosis. ▪ Cytological atypia is evident. ▪ Keratin pearl formation is infrequent. ▪ Easily identifiable mitotic figures. ▪ Cells less orderly.
Grade 3 **(Poorly differentiated)**	▪ Basal-like squamous cells forming nests in a sheet-like arrangement with central necrosis ▪ Occasional parakeratosis or keratinizing cells.

Note: When a tumour shows different grades, the higher grade should determine the final categorization (CAP Guidelines, February 2020)

Table 3. Tumour stage- AJCC/ TNM 8th edition

Primary Tumour (pT)	
pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pTis	Carcinoma in situ / high-grade dysplasia
pT1	Tumour invades the lamina propria, muscularis mucosae or submucosa
pT1a	Tumour invades lamina propria or muscularis mucosae
pT1b	Tumour invades the submucosa
pT2	Tumour invades the muscularis propria
pT3	Tumour invades adventitia
pT4	Tumour invades adjacent structures
pT4a	Tumour invades the pleura, pericardium, azygos vein, diaphragm, or peritoneum
pT4b	Tumour invades other adjacent structures, such as aorta, vertebral body or trachea
Regional lymph nodes (pN)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in 1-2 regional lymph nodes
pN2	Metastasis in 3-6 regional lymph nodes
pN3	Metastasis in 7 or more regional lymph nodes
Distant metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis Specify site(s), if known

Note:

- Regional lymph nodes include supraclavicular nodes, paratracheal nodes, mediastinal nodes, aortopulmonary nodes, subcarinal nodes, pulmonary ligament nodes,

tracheobronchial nodes, diaphragmatic nodes, paracardial nodes, left gastric nodes, common hepatic nodes, splenic nodes and coeliac nodes.

- Other lymph node groups are non-regional nodes.
- A regional lymphadenectomy specimen ordinarily includes 7 or more lymph nodes.
- If lymph nodes are negative, classify as pN0 even if the ordinary number of nodes is not met.
- The peri-oesophageal soft tissue should be dissected thoroughly to maximize the lymph node yields.
- In patients who receive preoperative treatment, lymph nodes may become fibrotic / atrophic.
- Tumour nodule with smooth contour in a regional node area is classified as a positive node
  - A tumour nodule larger than 3 mm in diameter irrespective of its shape, is also classified as a regional lymph node metastasis.
  - A similar nodule on the peritoneal surface is considered as distant metastasis (M1)
  - Lymph nodes with acellular mucin lakes are not considered as positive lymph nodes.
  - Cytokeratin stains may aid identification of residual cancer cells in lymph nodes; however, they should be interpreted in conjunction with morphologic findings

Table 4. The Mandard system for assessing the tumour regression grade (TRG) of carcinoma after neoadjuvant therapy

Grade	Description
TRG 1	Absence of residual cancer, with fibrosis extending through the various layers of the oesophageal wall (Complete regression)
TRG 2	Rare residual cancer cells scattered through the fibrosis.
TRG 3	An increase in the number of residual cancer cells, but fibrosis still predominates
TRG 4	Residual cancer cells outgrowing fibrosis
TRG 5	Absence of regressive changes

Table 5. Completeness of resection of tumour (R status)

Category	Description
R0	Fully resected tumour with no involvement of any margins
R1	Macroscopically a clear resection but which proves on histological examination to have positive margins
R2	One in which margin involvement is obvious macroscopically.

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5. World Health Organization Classification of Tumours Pathology and Genetics of Tumours of the Digestive System, 2019, 5th edition. Tumours of the oesophagus and Gastro-oesophageal junction, Structured Reporting Protocol, 1st Ed, 2013.
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CHAPTER 3

Tumours of the gastro-oesophageal junction (GOJ)

Introduction and epidemiology

Carcinomas in the gastro-oesophageal junction region can be of either squamous or glandular type. Squamous cell carcinomas that occur in this region are considered carcinomas of the distal oesophagus, even if they cross the gastro-oesophageal junction. Adenocarcinomas that extend across the gastro-oesophageal junction, are designated as gastro-oesophageal junction (GOJ) tumours.

Cancer registry in the United States show an approximate 2.5-fold increase in the incidence of GOJ adenocarcinoma from 1973–1992, with rates stabilizing in the last two decades. The rates are significantly higher in males. Smoking, obesity, and gastroesophageal reflux disease are significant risk factors.

This chapter seeks to emphasize the importance of identification of the GOJ of the specimen and its relationship to the tumour epicenter. Further details regarding reporting, staging and grading are referred to, in the relevant sections.

Specimen handling

Grossing procedure

- Measurement of the specimen, external examination, inking should be done as specified in the guidelines for oesophageal carcinoma or for gastric carcinoma depending on the type of specimen received.
- Opening the specimen
  - For circumferential tumours: Open longitudinally to the edge of the tumour and insert a “wick” of formalin-soaked paper towel, then once fixed “bread-slice” * the specimen
  - For non-circumferential tumours: Open longitudinally, avoiding the tumour

Note:

- “Bread slicing” the tumour will allow to correlate the extent of infiltration of the tumour with radiological images and easy assessment of circumferential margin for tumour involving the oesophagus.
- Dissect the peri-oesophageal fat and look for lymph nodes

Macroscopy

- **Type of specimen**
  This depends on the location of tumour in relation to GOJ;
  - Trans-mediastinal oesophagectomy
  - Extended total gastrectomy
  - Extended total gastrectomy with trans-hiatal resection of the distal oesophagus
  - Limited resection of the GOJ and distal oesophagus (in patients receiving neoadjuvant chemotherapy or T1N0 tumours)
- **Orientation of the specimen**
- **Specimen integrity** (intact / opened / disrupted)
- **Tumour size:** Greatest dimension
- **Maximum depth of infiltration**
- **Tumour appearance:** Polypoid / ulcerative / thickened / other (specify)
- **Distance from tumour to the resection margins:** Proximal, distal, circumferential
- **Distance from GOJ to distal resection margin**
- **Barrett mucosa if present:** vertical length
- **Location of tumour in relation to GOJ**
  Note: Identification of GOJ
  In resected specimens, the level of the proximal extent of gastric mucosal rugal folds can be used as the landmark for GOJ. However, these can get obscured by extensive tumour involvement or a columnar-lined oesophagus. If so, the junction can be located at the highest point of the peritoneal reflection on the serosal surface.
- **Whether the tumour extends across the GOJ or not**
- **Location of tumour epicenter** (midpoint of the vertical length of the tumour) and its distance to GOJ
  - Whether it is above or below the GOJ
  - Distance from tumour epicenter to GOJ
- **Lymph node groups:** Number retrieved (from resected specimen and separately submitted)
- **Doughnuts:** Yes / No

Location of tumour epicentre in relation to GOJ and the extension of tumour across the GOJ determine whether oesophageal carcinoma protocol or gastric carcinoma protocol should be applied.

Oesophageal carcinoma protocol applies to,

1. Siewert type I tumours: Carcinomas with their tumour epicentre located 1–5 cm above the GOJ (Figure 1 A)
2. Siewert type II tumours: Carcinomas involving distal oesophagus, with the tumour epicenter 1 cm above the GOJ (Figure 1B).
3. Siewert type II tumours: Carcinomas involving distal oesophagus, and the tumour epicenter ≤2 cm below the GOJ (Figure 1C).

Gastric carcinoma protocol applies to,

1. Siewert type II tumours: Carcinomas of the cardia/proximal stomach with the tumour epicenter is ≤2 cm below the GOJ and no extension to oesophagus* (Figure 2A)
2. Siewert type III tumours: Carcinomas with the tumour epicenter located 2-5 cm below the GOJ, irrespective of the extension to oesophagus (Figure 2B)

* **Note:** If the tumour involves the oesophagus microscopically**,** even without obvious macroscopic invasion, the tumour is reported using the oesophageal cancer reporting format.

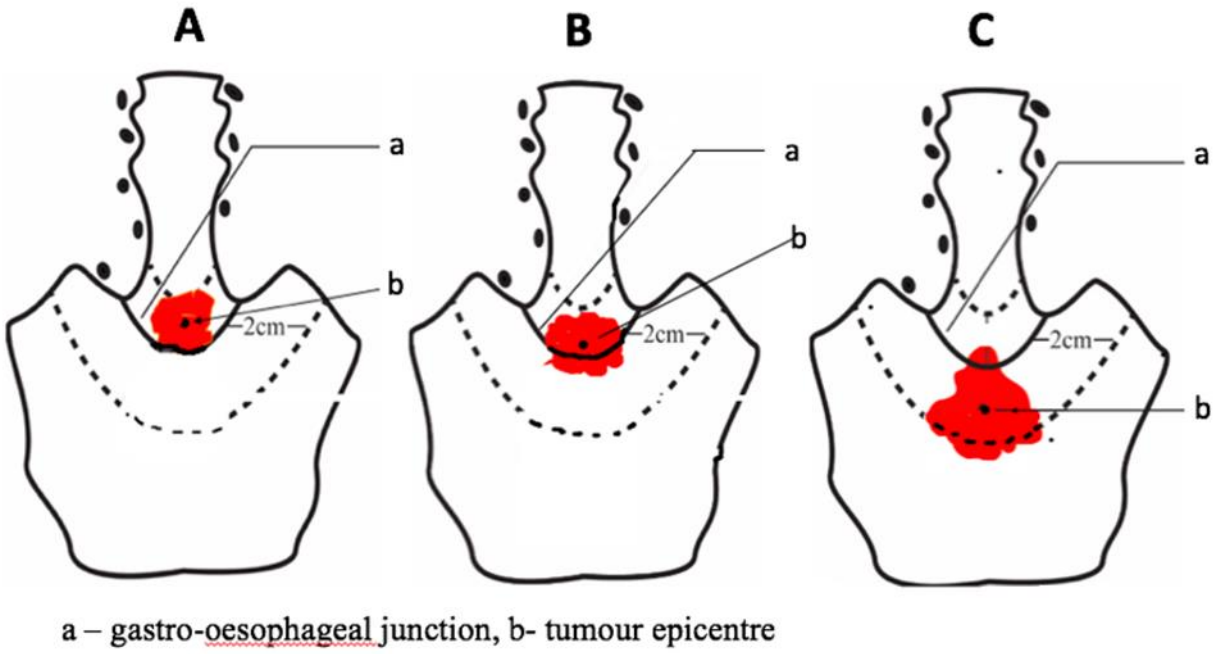

Figure 1. Siewert type I tumours (A), Siewert type II tumours with tumour involving GOJ and epicenter within 1 cm above GOJ (B), Siewert type II tumours with tumour involving GOJ the epicenter ≤2 cm into the proximal stomach / cardia (C) are reported using oesophageal carcinoma protocol

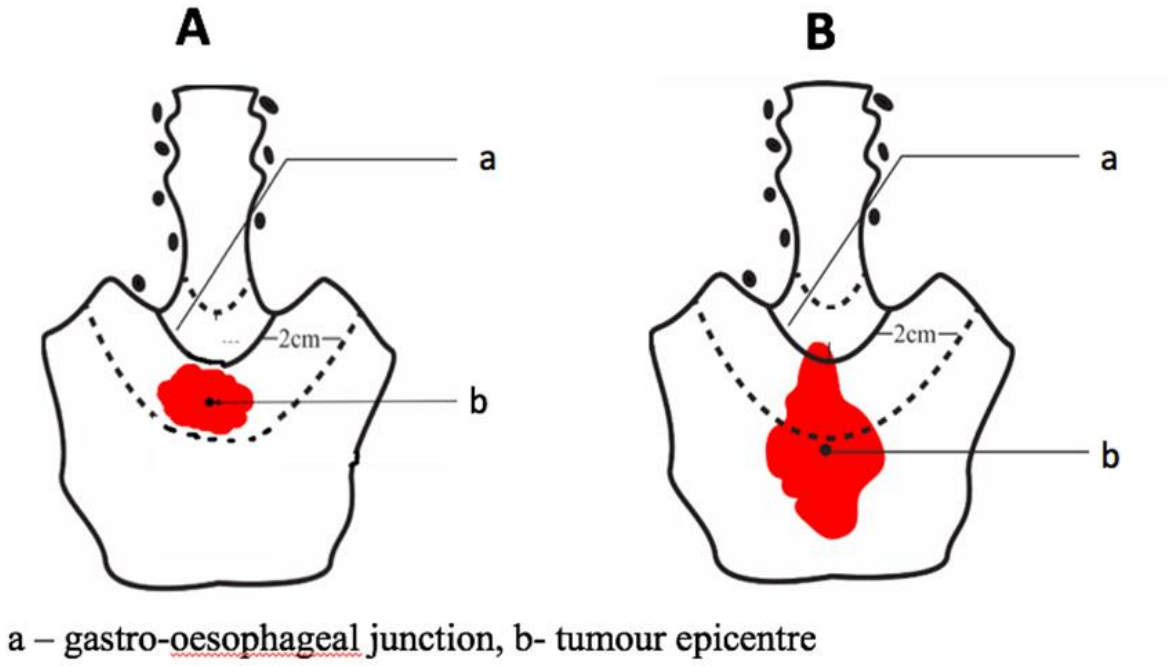

Figure 2. Siewert type II tumours with tumour confined to stomach and the epicenter ≤2 cm into the proximal stomach / cardia (A) and Siewert type III tumours are reported using gastric carcinoma protocol

Block selection

- Sectioning may be done vertically (Figure 3A) or horizontally (Figure 3B), to ensure optimum sampling to include required information.
  Note: Horizontal sections are more informative for assessing maximum depth of infiltration and distance to circumferential margins, especially for tumours involving the oesophagus. However, longitudinal sections best demonstrate the relationship of the tumour to the surrounding mucosa and are useful in assessing early neoplasia arising in Barrett oesophagus.

- Tumour, at least three blocks including:
  - Deepest point of invasion
  - Tumour to closest circumferential margin or non-peritonealised margin and gastric serosa if applicable
- Interface of tumour with adjacent mucosa
- Gastro-oesophageal junction / squamocolumnar junction, if not included in above sections
- Uninvolved stomach and oesophagus
- Proximal resection margin
- Distal resection margin
- Lymph nodes

Note: After taking blocks, pack the specimen in such a way so that it can be easily reconstructed if more blocks need to be taken.

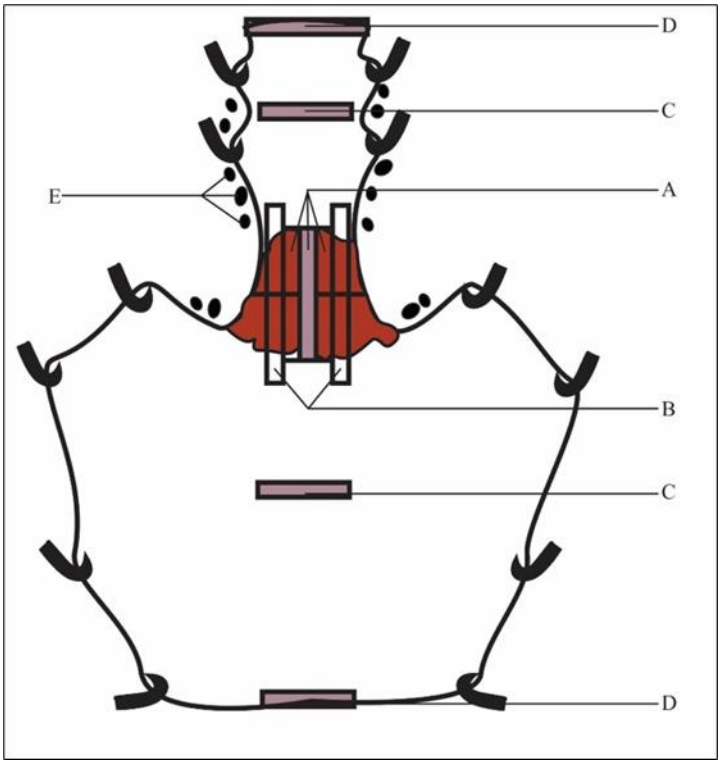

Figure 3A. Vertical sectioning of the tumour

A. At least three blocks from the tumour, including the deepest penetration and circumferential margin involvement, if applicable.
B. Normal mucosa and tumour interface
C. Non neoplastic mucosa
D. Proximal and distal resection margins
E. Lymph nodes

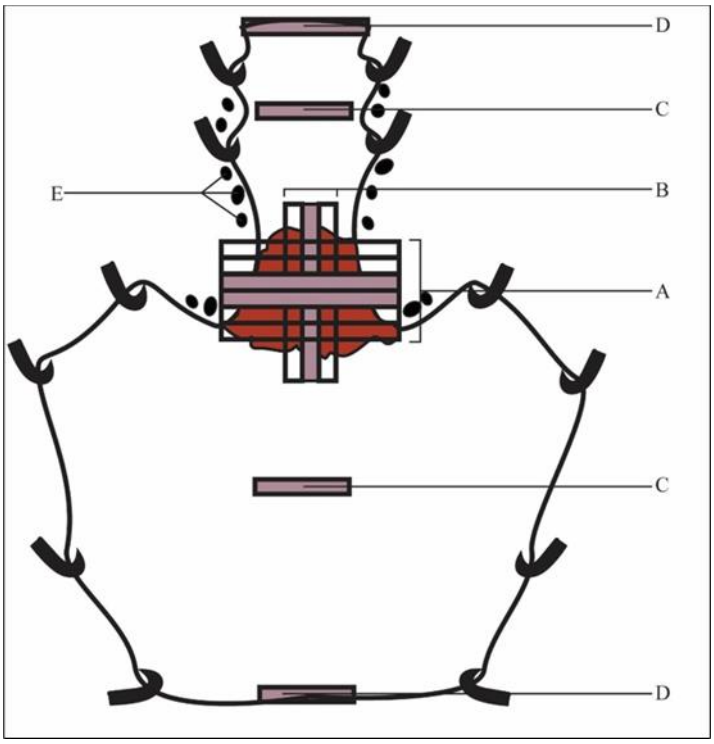

Figure 3B. Horizontal sectioning of the tumour

A. At least three blocks from the tumour, including the deepest penetration and circumferential margin involvement, if applicable.
B. Normal mucosa and tumour interface
C. Non neoplastic mucosa
D. Proximal and distal resection margins
E. Lymph nodes

Microscopy and conclusion

Tumour type, grade, extent of tumour spread, distance to resection margins, lymph node involvement, treatment effects, lymphovascular invasion, perineural invasion, status of non-neoplastic mucosa, completeness of resection as specified in oesophageal or gastric carcinoma protocols depending on the location of the tumour.	
Staging of tumour	Please see macroscopy above. ○ Staging of adenocarcinomas should be done using either oesophageal or gastric carcinoma reporting format depending on whether the tumour was defined macroscopically / microscopically as being of oesophagus or stomach (please see box above regarding tumour epicentre and Siewert classification). ○ Squamous cell carcinomas and mixed histologic types, such as adenosquamous carcinomas, are staged using the oesophageal carcinoma staging.
Ancillary investigations	Pathologists may be expected to report on HER2 status of GOJ tumours upon request by clinicians.

Annexure

Reporting proforma for GOJ tumours

Please follow the proforma for oesophageal carcinoma or gastric carcinoma according to the location of tumour as specified in macroscopy / microscopy.

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CHAPTER 4

Tumours of the stomach

Introduction and epidemiology

Gastric carcinoma is the third commonest cancer worldwide with cases estimated to amount to more than 1 million as reported in 2018. The highest incidence rates have been reported in central and eastern Asia, Central and South America and eastern Europe. In Sr Lanka, it is the 4th commonest cancer in males and 7th commonest in females. The peak incidence occurs in the 65-69 year age group in females and 55-59 year age group in males, according to cancer incidence data in Sri Lanka in 2014. The commonest histological type is adenocarcinoma (not otherwise specified), followed by squamous cell carcinoma and adenosquamous carcinoma, signet-ring cell carcinoma and adenocarcinoma.

There is sufficient evidence that Helicobacter pylori infection, rubber manufacturing industry, tobacco smoking and X-radiation are involved in the carcinogenesis of gastric carcinoma. The location of these tumours have shown a geographical variation with distal gastric carcinoma (involving antral-pyloric regions) commoner in Asia, Central and South America and eastern Europe with cancers involving the proximal stomach (cardia and fundus) being commoner in northern Europe and USA.

Specimen handling

Specimen collection and transport

Refer National Guidelines in Histopathology – Collection, Handling and Transport of Specimens (second edition, 2021).

Ideally the specimen should be sent in the fresh state. **[Z]**

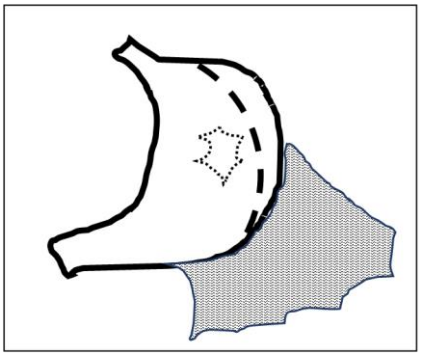
However, most specimens may be received un-opened and partially fixed.

Grossing procedure

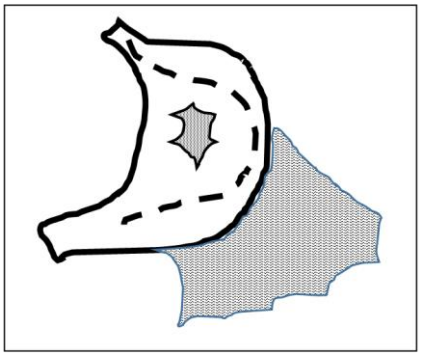
A. Opening of the specimen

- If the tumour arises at or close to the gastric cardia, the circumferential resection margin of the lower oesophagus needs to be inked. **[X]**
- Depending on the location of the tumour, the specimen should be opened by the following methods: **[Y]**

- If the tumour is located posteriorly, the specimen should be opened along the greater curve on the anterior surface (Figure 1A)
- If the tumour is located on the anterior aspect, the incision should go around the tumour avoiding the tumour (Figure 1B).
- For greater curvature tumours, it should be opened along the anterior surface of the lesser curve (Figure 1C).
- If there is a gastro jejunostomy-anastomosis, it should be avoided and the jejunal loop is opened longitudinally by a separate incision (Figure 1C).

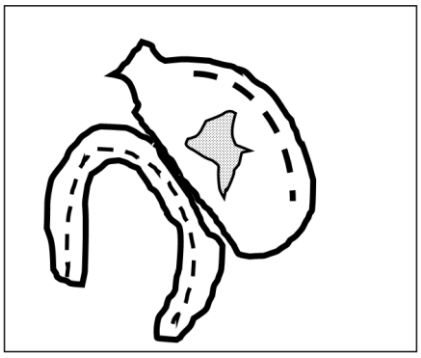


1A. Tumour on the posterior wall



1B. Tumour on the anterior wall

1C. Tumour involving greater curvature



1D. Subtotal gastrectomy with a gastro jejunostomy-anastomosis

Figure 1. Methods used for opening of a gastrectomy specimen

B. Fixation

It should then be ideally pinned flat onto a cork board and floated in a large volume of formalin for 24 –48 hours and then flipped to allow fixation of the serosal aspect. **[Z]**

Macroscopy

- **Tumour site** (Figure 2)
  - Fundus – Anterior / posterior wall
  - Body – Anterior / posterior / greater curvature / lesser curvature
  - Antrum – Anterior / posterior / greater curvature / lesser curvature
  - Pylorus – Anterior / posterior wall
  - Other – Specify

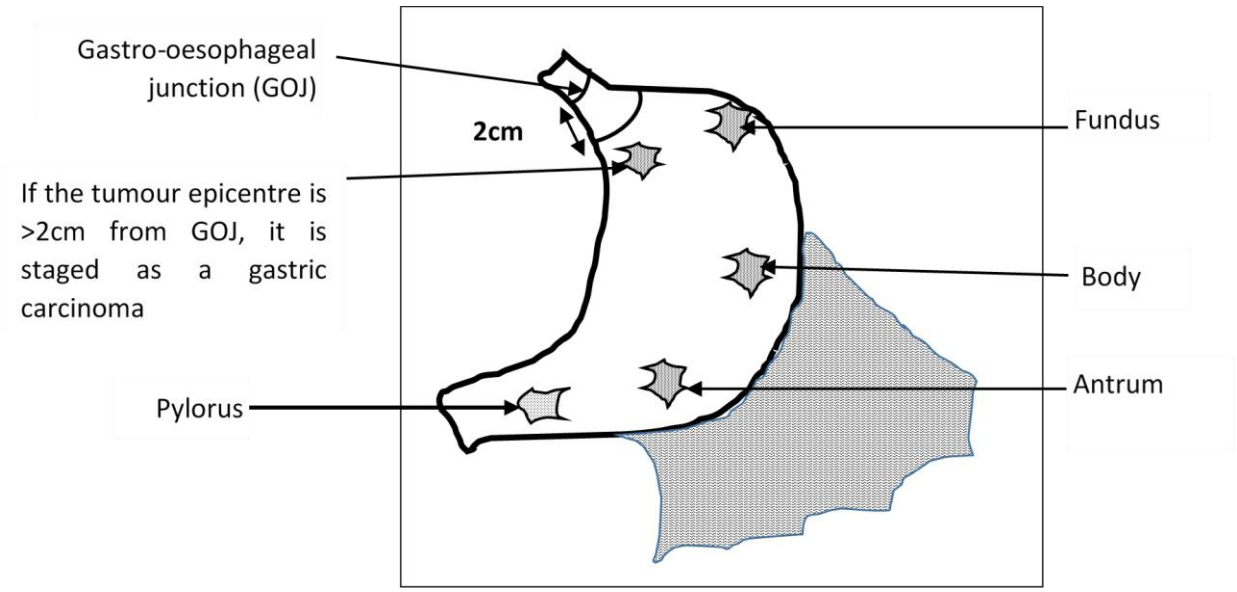


Figure 2. Description of tumour site

- **Tumour configuration** (Borrmann types)
  - Type 1 – Polypoid
  - Type 2 – Fungating
  - Type 3 – Ulcerated
  - Type 4 – Diffusely infiltrating
- **Tumour size**
  - Maximum dimension
- **Distance of the tumour to the resection margins**
  - Proximal margin
  - Distal margin
  - Omental (radial) margin - Greater omental margin / lesser omental margin
  - Mucosal and deep resection margin - For endoscopic mucosal resections
  - Other- Specify
- **Specimen type**
  - Partial Gastrectomy
  - Total Gastrectomy
  - Other- Specify

- **Specimen size:**
  - Along greater curvature
  - Along lesser curvature
  - Length of oesophagus
  - Length of duodenum
  - Omentum
- **Non neoplastic mucosa:** Normal / atrophic / thickened / ulcerated / erythematous / haemorrhagic
- **Lymph nodes**
  - Lymph node regions – Lesser curvature / greater curvature / cardiac / pyloric / perisplenic / omental

Block selection

The recommended blocks that should be taken are given below (Figure 3A and 3B).

- Proximal resection margin (Block A)
- Distal resection margin (Block B)
- Tumour (Block C) - At least four blocks to assess:
  - Deepest tumour penetration into / through the wall
  - Serosal involvement
  - Tumour proximity to the circumferential resection margin (if present)
  - Tumour with nearest margin (If the tumour is close to a margin- Block D)

  Note:

  - The proximal margin of the oesophagus should ideally be examined completely, regardless of distance from the tumour to exclude foci of discontinuous or intramural spread of the carcinoma in the proximal oesophagus. **[Z]**
  - If the tumour is in close proximity with any of the resection margins, a longitudinal section which includes the tumour edge and margin should be submitted. **[X]**
  - Sampling of the distal resection margin can be restricted to the part nearest to the tumour **[Y]**
- Background stomach (Block E), oesophagus and duodenum (if present)
- Spleen, omentum or other organs (if present).

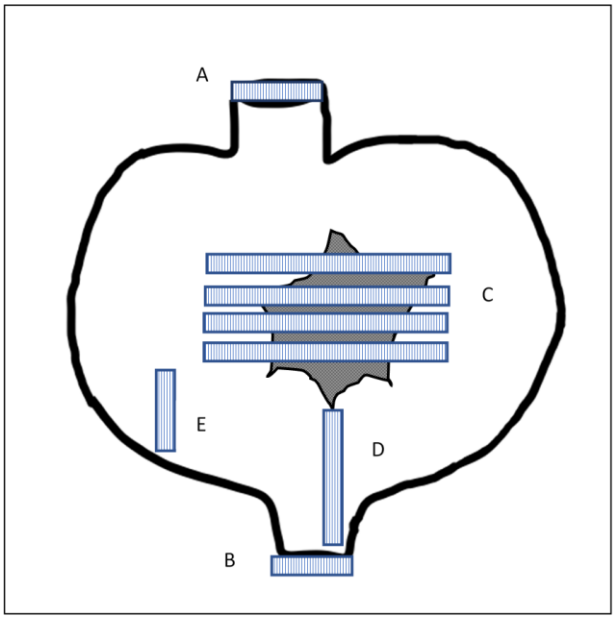


Figure 3A. Block selection - Total gastrectomy specimen

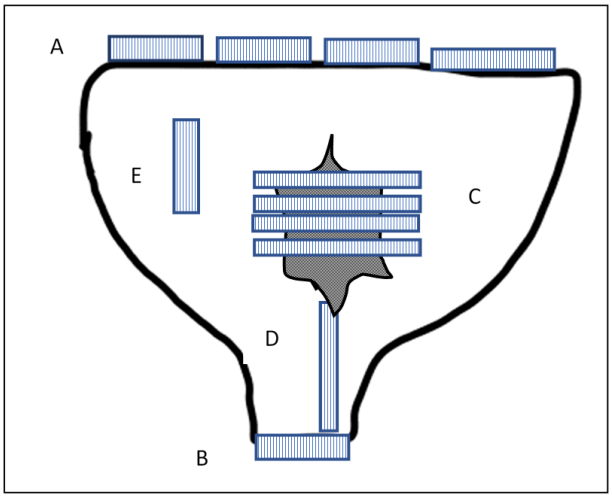


Figure 3B. Block selection - Partial /distal gastrectomy specimen

- Regional lymph nodes - All regional lymph nodes should be sampled **[X]**
  Note:
  These regional lymph node groups are shown in Figure 4 to facilitate optimal retrieval of lymph nodes.
  Regional lymph nodes in a gastrectomy specimen include:
  - Perigastric nodes
    - Along the greater curvature (including greater curvature, greater omental)
    - Along the lesser curvature (including lesser curvature, lesser omental)
  - Right and left paracardial (cardio-oesophageal)
  - Pyloric nodes
    - Suprapyloric (including gastroduodenal)
    - Infrapyloric (including gastroepiploic)
  - Left gastric artery nodes
  - Coeliac artery nodes
  - Common hepatic artery nodes
  - Hepatoduodenal (along the proper hepatic artery, including portal)
  - Splenic artery nodes

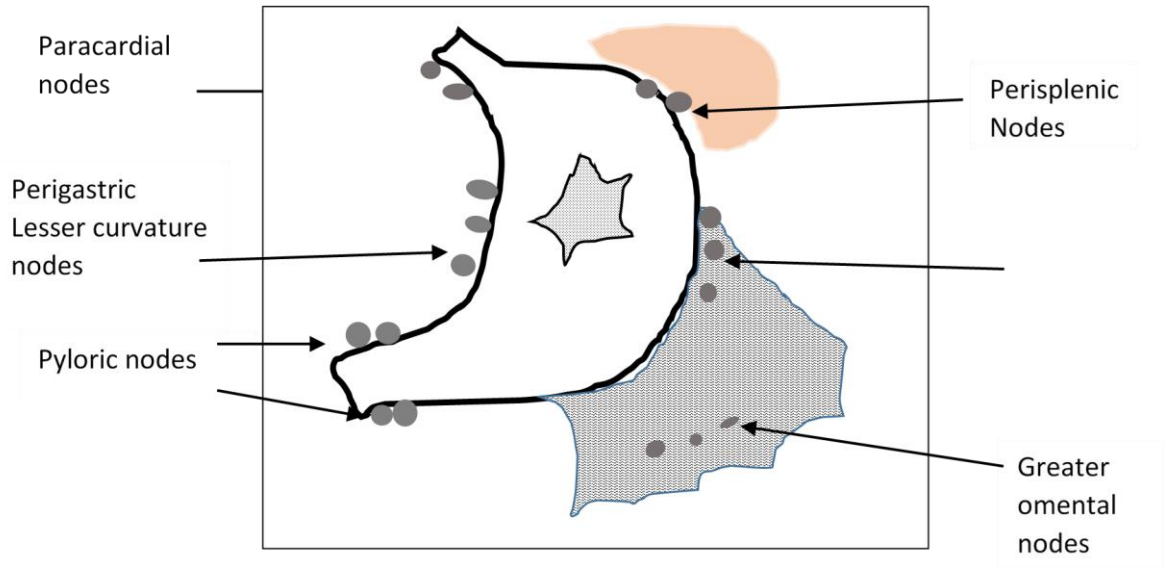


Figure 4. Regional lymph nodes in a gastrectomy specimen

Microscopy and conclusion

Tumour type	Refer types of tumours of the stomach in WHO Classification of Tumours of the Digestive System, 5th edition (currently in use in 2021) - Table 1
Tumour grade	Predominant grade and worst grade - Table 2 ▪ Low grade (This includes tumours previously classified as well differentiated and moderately differentiated) ▪ High grade (This includes tumours previously classified as poorly differentiated) **Note:** o This applies primarily to tubular and papillary adenocarcinomas of stomach and not to other gastric carcinoma subtypes. o Both the predominant grade and the worst grade should be mentioned as it is currently unclear which is of greater significance **[Y]**
Resection margin status	▪ Proximal margin involvement – Positive / Negative / Cannot be assessed ▪ Distal margin involvement – Positive / Negative / Cannot be assessed ▪ Circumferential margin at the lower oesophagus – Positive / Negative / Cannot be assessed

	Note: o A margin is considered positive in the following circumstances: – Direct extension of the primary tumour – Tumour present in lymphatic vessels or veins with adherence of the tumour cells to the endothelium at the resection margin – Tumour present in lymph nodes or soft tissue within equal or less than 1 mm of the resection margin o In tumours arising close to the margins, the pathologist should report whether the tumour is at the margin (0mm), < 1mm, or >1 mm from the resection margin. **[X]** o If the surgeons have removed lymph nodes from resection margins, the margins cannot be assessed reliably, and such cases should be reported as 'not assessable' **[Y]**
Lymph node status	▪ Number examined ▪ Number of positive nodes
Lymphovascular invasion	Present/Absent
Perineural invasion	Present/Absent
Completeness of resection	Table 3
Tumour stage (TNM)	Refer TNM classification for staging tumours of the stomach (AJCC TNM 8th edition currently in use in 2021 - Table 4) Maximum extent of invasion through wall - Figure 5. **Note:** Serosal perforation has been an independent prognostic marker and is associated with a cancer recurrence.
Non-neoplastic stomach	Presence of the following should be mentioned: ▪ Helicobacter pylori ▪ Intestinal metaplasia ▪ Glandular atrophy ▪ Dysplasia

Effects of neoadjuvant therapy	(Tumour regression grade – if applicable) – Table 6 (Modified Ryan / AJCC 2010 classification)
Molecular data (if applicable)	HER2 **[Z]**
Pathological tumour stage	Refer the TNM classification for staging oesophageal tumours (AJCC TNM 8th edition currently in use in 2021 - Table 2
Ancillary tests	**Note**: Currently specific ancillary tests are not routinely recommended for oesophageal tumour classification

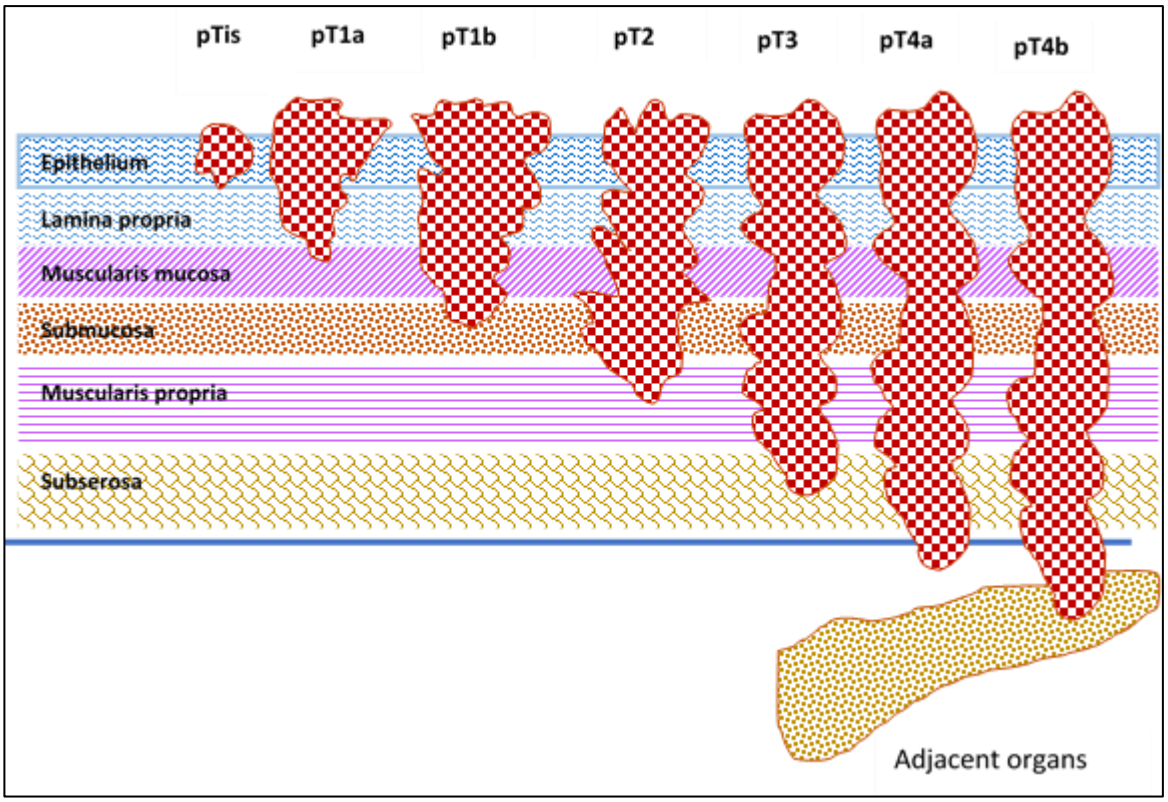


Figure 5. Depth of infiltration of primary tumour

Annexure

Reporting proforma – Gastrectomy for tumour

Macroscopy		
Type of specimen	:	Oesophago-gastrectomy / Distal gastrectomy / Total gastrectomy / Local resection
Macroscopic tumour type - Borrmann classification	:	Polypoid / ulcerating / fungating / diffusely infiltrating
Specimen dimensions	:	Length of stomach ▪ Greater curvature _____ mm ▪ Lesser curvature _____ mm Length of oesophagus / duodenum _____ mm
Site of tumour	:	Cardia / Body / Antrum / Pylorus
Tumour size	:	Maximum tumour diameter _____ mm
Distance of tumour to nearest margin (cut end)	:	_____ mm
Microscopy		
Tumour type	:	
Tumour grade	:	Two tier system
Involvement of margins	:	
Proximal margin involvement	:	Positive / Negative / Cannot be assessed
Distal margin involvement	:	Positive / Negative / Cannot be assessed
Circumferential margin involvement	:	Positive / Negative / Cannot be assessed
Lymphovascular invasion	:	Present / Absent
Perineural invasion	:	Present / Absent
Non neoplastic stomach	:	Atrophy / H. pylori / chronic gastritis
Completeness of resection	:	R0 / R1 / R2
Pathological tumour stage	:	
Treatment effect (if applicable)	:	Tumour regression grade

Tables

Table 1. WHO Classification of tumours of stomach, Digestive system tumours, 5th edition

▪ Adenocarcinoma NOS o Tubular adenocarcinoma o Parietal cell o Adenocarcinoma with mixed types o Papillary adenocarcinoma NOS o Micropapillary carcinoma NOS o Mucoepidermoid carcinoma o Mucinous adenocarcinoma o Poorly cohesive carcinoma o Signet-ring cell carcinoma o Medullary carcinoma with lymphoid stroma o Hepatoid adenocarcinoma o Paneth cell carcinoma
▪ Squamous cell carcinoma NOS
▪ Adenosquamous carcinoma
▪ Carcinoma undifferentiated NOS o Large cell carcinoma with rhabdoid phenotype o Pleomorphic carcinoma o Sarcomatoid carcinoma o Carcinoma with osteoclast-like giant cells
▪ Gastroblastoma
▪ Neuroendocrine tumours (NET) NOS
▪ Neuroendocrine carcinoma (NEC) NOS
▪ Mixed neuroendocrine-non-neuroendocrine carcinoma (MiNEN)

Table 2. Tumour grade (Two tier system) – For tubular and papillary adenocarcinomas (College of American Pathologists Protocols, February 2020)

Grade	Description
Low-grade (Well and moderately differentiated in previous grading systems)	Well-formed glands
High-grade (Poorly differentiated in previous grading systems)	Poorly formed glands Solid areas Individual cells

Note:

- This applies primarily to tubular and papillary adenocarcinomas of stomach and not to other gastric carcinoma subtypes.
- Squamous cell carcinomas should be graded similar to such tumours elsewhere.

Table 3. Completeness of resection

Category	Description
R0	No residual tumour
R1	Microscopic tumour at resection margins
R2	Macroscopic tumour at resection margins

Table 4. Tumour stage – AJCC/ TNM 8th edition

Primary tumour (pT)	
Tx	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in-situ Intraepithelial tumour without invasion of lamina propria High-grade dysplasia
T1	Tumour invades lamina propria, muscularis mucosae or submucosa
T1a	Tumour invades lamina propria or muscularis mucosae
T1b	Tumour invades submucosa
T2	Tumour invades muscularis propria
T3	Tumour invades subserosa
T4	Tumour perforates serosa (visceral peritoneum) or invades adjacent structures
T4a	Tumour perforates serosa (visceral peritoneum)
T4b	Tumour invades adjacent structures (see note * below)
Regional lymph nodes (pN)	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1 to 2 regional lymph nodes
N2	Metastasis in 3 to 6 regional lymph nodes
N3	Metastasis in 7 or more regional lymph nodes
N3a	Metastasis in 7 to 15 regional lymph nodes
N3b	Metastasis in 16 or more regional lymph nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis

pTis	In-situ carcinoma
pT1a	Lamina propria, muscularis mucosa invasion
pT1b	Submucosal invasion
pT2	Muscularis propria invasion
pT3	Subserosal invasion
pT4a	Serosal *perforation*
pT4b	Involvement of adjacent structures- Spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine and retroperitoneum

Note: Primary tumour – figure 5

- Adjacent structures * : Spleen, transverse colon, liver, diaphragm, pancreas, abdominal wall, adrenal gland, kidney, small intestine, and retroperitoneum.
- Intramural extension to the duodenum or oesophagus does not automatically categorize it as pT4 unless there is serosal perforation. Such tumours should be categorized based on greatest depth of invasion in any of these sites including stomach.
- Tumour extending into gastrocolic or gastro-hepatic ligaments or greater / lesser omentum, without perforation of visceral peritoneum, is classified as T3.
- Regional node metastasis
  - The regional lymph nodes of the stomach include perigastric nodes along the lesser and greater curvatures, the nodes along the left gastric, common hepatic, splenic, coeliac arteries, and the hepatoduodenal nodes.
  - Optimum number of lymph nodes to be sampled – 16 or more **[Z]**
  - If all examined lymph nodes are negative (regardless of the total number removed/ examined), tumour is classified as N0.
  - Involvement of other intra-abdominal lymph nodes such as retropancreatic, mesenteric, and para aortic is classified as distant metastasis (M1)
- Distant metastasis
  - Distant metastasis includes peritoneal seeding, positive peritoneal cytology and omental tumour not part of continuous extension
  - pM0 and pMx are not valid categories
  - Involvement of other intra-abdominal lymph nodes such as retropancreatic, mesenteric, and para aortic is classified as distant metastasis
  - Micrometastasis – Cases with micrometastasis (Metastasis less than 2mm) only – Staged as pN1 (mi)
  - Isolated tumour cell clusters ITCs (single tumour cells or small clusters of tumour cells not more than 0.2 mm in greatest dimension)

- Cases with only ITC staged as pN0 (i+) and ITC in distant sites are staged as pM0 (i+)

Table 5. Tumour Regression Grade - Modified Ryan grade

Description of tumour	Regression Grade
No viable cancer cells (Complete response)	0
Single cells or small groups of cancer cells (Near complete response)	1
Residual cancer outgrown by fibrosis (Minimal response)	2
Minimal or no tumor kill; extensive residual cancer (Poor response)	3

Note:

- The histological features following chemotherapy include mucosal oedema, ulceration, inflammation, foamy histocytes, haemorrhage, necrosis, vascular changes, acellular mucus, fibrosis, keratin and multinucleated giant cells.
- Large pools of acellular mucin following chemoradiation should not be interpreted as representing residual tumour.
- This protocol does not preclude the use of other systems for assessment of tumour response, such as the schemes reported by Memorial Sloan-Kettering Cancer Center investigators and others.

References

1. World Health Organization – Cancer Country Profiles, 2014
2. World Cancer Research Fund, American Cancer Research Fund
3. WHO Classification of tumours-Digestive System Tumours, 5th edition,
4. Dataset for histopathological reporting of oesophageal and gastric carcinoma Royal College of Pathologists, October 2019
5. Protocol for The Examination of Specimens from Patients with Carcinoma of The Stomach, February 2020, College of American Pathologists
6. National guidelines/ Common GI Malignancies College of Pathologists of Sri Lanka 2007

CHAPTER 5

Tumours of the small Intestine

Introduction and epidemiology

Malignant tumours of the small intestine are uncommon, with a global incidence of 1.1-2.5 per 100 000 population per year. The majority arise in the duodenum (55-75%) and most commonly around the ampulla of Vater. Approximately 15-25% arise in the jejunum and 10-15% in the ileum. The most common tumour types arising in the small intestine are adenocarcinomas followed by neuroendocrine tumours (NET), gastrointestinal stromal tumours (GIST) and lymphoma.

According to national cancer incidence data published by National Cancer Control programme in 2014, 75 cases of small intestinal malignancies have been reported in Sri Lanka.

Surgical resection is the first line treatment for small intestinal cancers. Usually these tumours present at an advanced stage and are often diagnosed as an emergency. This chapter describes the guidelines for specimen handling and reporting of small bowel resections for non-ampullary carcinomas. Specimen handling procedures for neuroendocrine tumours and gastrointestinal stromal tumours of the small intestine are more or less similar to epithelial malignancies. However, relevant staging and grading systems should be applied accordingly.

Specimen handling

Specimen collection and transport

Refer National Guidelines in Histopathology – Collection, Handling and Transport of Specimens (second edition, 2021).

Grossing procedure

- Take the relevant measurements and ink the mesenteric resection margin. **[X]**
- Specimen should be opened longitudinally along the antimesenteric border up to a segment extending 10-20 mm above and below the tumour.
- Washout the contents gently and fix the specimen preferably after pinning on to a cork board and immerse in adequate formalin. **[Y]**

Note: 24–48 hour fixation before further dissection facilitates subsequent slicing (3-4 mm) through the tumour and the identification of lymph nodes.

Macroscopy

- **Specimen type**
  - Segmental resection of duodenum/ jejunum/ ileum
  - Ileocolic resection
  - Pancreaticoduodenectomy (Handling of these type of specimens for peri ampullary/ampullary carcinomas are not included in this chapter)
  - Small intestine

- **Specimen size**
  - Length of the specimen
  - Depth of the attached mesentery
  - Size of Meckel's diverticulum (if present)

- **External inspection of the specimen**
  - Describe the serosal surface: fibrosis, peritonitis, adhesions, fat wrapping,
  - Identify tumour perforation (Tumour perforation may be obscured by puckering of the wall of the intestine or fat wrapping at the point of perforation)
  - For duodenal resection specimens, identify the area where serosa is lacking (retroperitoneal part).

  Note: For other parts of the small intestine (jejunum and ileum), the non-peritonealized, perimuscular area is the cut end of the mesentery.
    - Identify any other attached organs or structures
    - Presence/absence of macroscopic tumour deposits on the serosal surface or in any other structures
    - Gross involvement of lymph nodes by the tumour

- **Tumour site**
  - Site and relationship of tumour to the mesentery / retroperitoneal part in the duodenal resections
- **Tumour size**
  - Length of the tumour (The thickness of the tumour is not essential)
- **Tumour configuration**
  - Macroscopic appearance (Polypoid / Ulcerated / Thickened / Other, specify)
  - Luminal obstruction
- **Extent of tumour**
  - Level of invasion into the bowel wall
  - Involvement of serosa
  - Invasion into adjacent structures

- **Distance to each resection margin**
  - Record the distance of tumour to proximal, distal and mesenteric resection margins
  - Macroscopic tumour involvement of the non-peritonealized margins (antimesenteric margin or retroperitoneal margin for duodenal specimens) are classified as R2
- **Non- neoplastic mucosa**
  - Ischaemia/infarction (blue/black or haemorrhagic in appearance)
  - Adhesions
  - Thickening
  - Ulceration
  - Cobblestone appearance
  - Fistula
  - Anastomosis
  - Erythema
  - Fibrous exudate
  - Other, specify

Block selection

- The segment of bowel including the tumour together with the mesentery is sectioned transversely at 3–4 mm intervals with a sharp knife to produce slices that include the tumour and the mesenteric resection margin (Figure 1A and 1B).
- Slices could be displayed on a cutting board serially (e.g., proximal to distal end) to permit better macroscopic assessment.
- Photograph the serially laid out sections **[Y]** to enable a permanent record of the macroscopic appearance and to correlate blocks with subsequent microscopy.

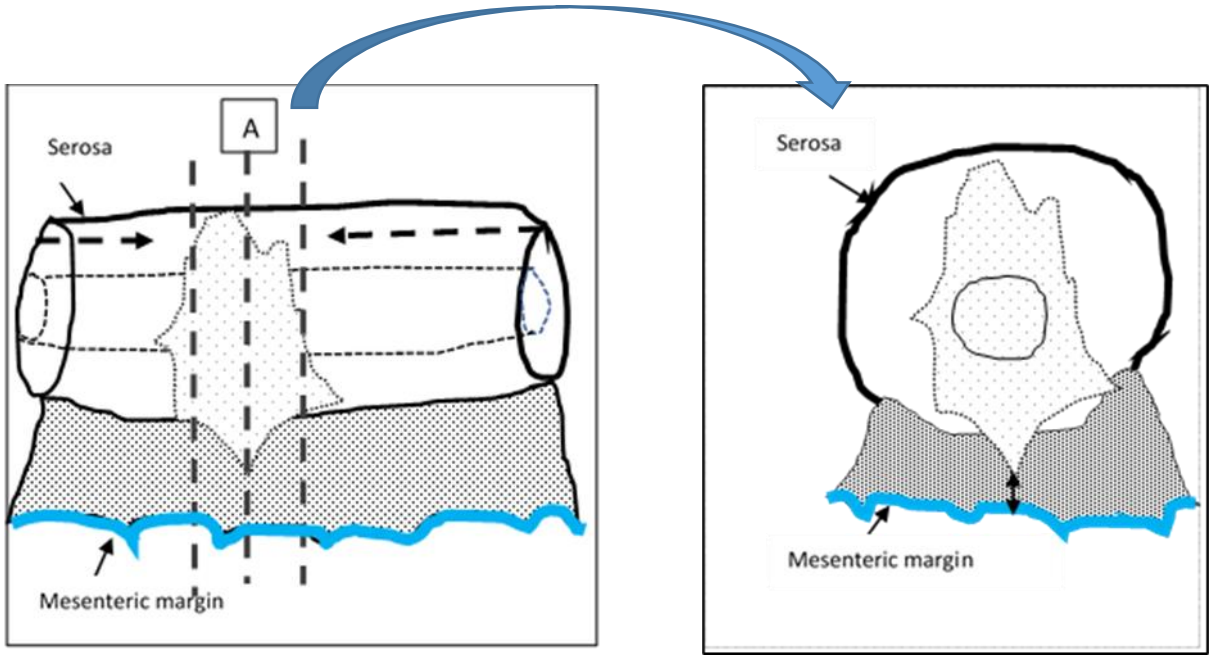


Figure 1A. Tumour with the mesenteric resection margin. Discontinuous arrow shows method of opening of the specimen; discontinuous lines show method of slicing the tumour

Figure 1B. Tumour with the mesenteric resection margin – Slice A - Across the point of deepest invasion to the mesenteric margin

- The following standard blocks of tissue are recommended;
  - At least five blocks of the tumour (more blocks for larger tumours) including the deepest tumour penetration into or through the bowel wall
  - Tumour with serosal surface and involvement of any adjacent organs
  - Tumour- macroscopically normal mucosa interface
  - Tumour with mesenteric/non-peritonealized resection margin
  - Tumour with longitudinal margin where relevant (to give a more accurate measurement if the lesion is <10mm from the margin)
  - Mesenteric tissue with blood vessels

- Lymph nodes
  - All lymph nodes should be sampled; grossly positive nodes can be submitted partly for microscopic confirmation.

Microscopy and conclusion

Tumour type	Refer types of small intestinal tumours in WHO Classification of Tumours of the Digestive System, 5th edition (currently in use in 2021) - Table 1
Tumour grade	Table 2
Microscopic extent of tumour	Tumour extension into the anatomical layers of the bowel wall should be assessed.
Tumour perforation	Specify whether tumour perforates the visceral peritoneum or directly invades other organs or structures (specify the organ or the structure). This can be other loops of small intestine, mesentery of adjacent loops of bowel, and the abdominal wall by way of serosa.
High-grade dysplasia (carcinoma in situ) / adenoma	Present / Absent
Margins	▪ Distance of invasive carcinoma from the proximal, distal and mesenteric margins should be stated. ▪ If the tumour is present within 0-1 mm from the mesenteric resection margin, it is considered as a positive margin (R1 resection) ▪ Distance to the closest longitudinal margin from carcinoma in situ (high-grade dysplasia) / adenoma should also be stated.
Lymphovascular invasion	Present / Absent
Perineural invasion	Present / Absent
Regional Lymph Nodes	▪ Number of lymph nodes involved ▪ Number of lymph nodes examined

Note:

- Minimum number of lymph nodes that predicts regional lymph node negativity has not been defined for small intestinal cancers yet and all lymph nodes are to be sampled.
- Lymph nodes especially in larger specimens should be categorized as regional and non-regional. (Table 3 - TNM staging of tumours)

Additional pathologic findings	Adenoma(s), Crohn disease, Coeliac disease, other polyps (mention the type)
Tumour stage (TNM)	Refer the TNM classification of staging for small intestinal tumours (AJCC TNM 8th edition currently in use in 2021 - Table 3)
Completeness of excision	R0 / R1 / R2

Annexure

Reporting proforma for small intestinal malignancies

Gross description		
Type of surgery	:	
Maximum length of specimen	:	_____ mm
Location of tumour	:	
Microscopic tumour perforation	:	Present / Absent
Size (length) of tumour	:	_____ mm
Tumour edge to proximal resection margin	:	_____ mm
Tumour edge to distal resection margin	:	_____ mm
Distance to mesenteric resection/non-peritonealized margin	:	_____ mm
Tumour appearance	:	Polypoidal / Other (specify)
Microscopic description		
Type of tumour	:	
Tumour differentiation/ grade	:	
Depth of invasion	:	
Invasion into adjacent structures	:	if present specify
Involvement of margins		
▪ Proximal longitudinal margin	:	Normal / dysplasia / carcinoma
▪ Distal longitudinal margin	:	Normal / dysplasia / Barrett / carcinoma
▪ Circumferential margin (<1 mm)	:	
Lymphovascular invasion	:	Present / Absent
Lymph nodes		
▪ Number examined	:	
▪ Number positive	:	
Distant metastases	:	
Completeness of excision	:	R0 / R1 / R2
Pathological staging	:	
Additional pathological findings	:	
Other comments	:	

Tables

Table 1. WHO Classification of tumours of the small intestine, Digestive system tumours, 5th edition

- Adenocarcinoma NOS
  - Mucinous adenocarcinoma
  - Signet-ring cell carcinoma
  - Medullary carcinoma NOS
  - Adenocarcinoma, intestinal type
  - Pancreaticobiliary – type carcinoma
  - Tubular adenocarcinoma
- Neuroendocrine tumour NOS
  - Neuroendocrine tumour, Grade 1
  - Neuroendocrine tumour, Grade 2
  - Neuroendocrine tumour, Grade 3
  - Gastrinoma NOS
  - Stomatostatinoma NOS
  - Enterochromaffin-cell carcinoid
  - Extra adrenal paraganglioma NOS
- Neuroendocrine carcinoma NOS
  - Large cell Neuroendocrine carcinoma
  - Small cell Neuroendocrine carcinoma
- Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN)

Table 2. Histological grade of adenocarcinoma (College of American Pathologists Protocols, February 2020)

Grade	Description
Grade X	Grade cannot be assessed
Grade 1	Well-differentiated (more than 95% of tumour composed of glands)
Grade 2	Moderately differentiated (50% to 95% of tumour composed of glands)
Grade 3	Poorly differentiated (less than 50% of tumour composed of glands)

Table 3. AJCC/ TNM 8th edition staging for small intestinal tumours

Primary Tumour (pT)	
pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pTis	High-grade dysplasia/carcinoma *in situ*
pT1	Tumour invades the lamina propria or submucosa
pT1a	Tumour invades the lamina propria
pT1b	Tumour invades the submucosa
pT2	Tumour invades the muscularis propria
pT3	Tumour invades through the muscularis propria into the subserosa or extends into the non-peritonealised perimuscular tissue (mesentery or retroperitoneum) without serosal penetration (see note below) *
pT4	Tumour perforates the visceral peritoneum or directly invades other organs or structures (e.g., other loops of small intestine, mesentery of adjacent loops of bowel, and abdominal wall by way of serosa; for duodenum only, invasion of pancreas or bile duct)
Regional lymph nodes (pN) (See note below) **	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in one or two regional lymph nodes
pN2	Metastasis in three or more regional lymph nodes
Distant metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis. Specify site(s), if known

Note:

- **Primary Tumour**
  - For jejunal / ileal pT3 tumours; the non-peritonealized, perimuscular tissue is part of the mesentery. *
  - For duodenal pT3 tumours in areas lacking serosa; the non-peritonealized perimuscular tissue is part of the interface with the pancreas. *

- **Regional lymph nodes**
  - Regional lymph nodes for the non-ampullary duodenum include the retropancreatic, hepatic artery, inferior pancreaticoduodenal and superior mesenteric nodes.
  - Regional lymph nodes for the jejunum or ileum include the mesenteric and superior mesenteric nodes; for the terminal ileum, the caecal and ileocolic nodes are also included. **
  - A regional lymphadenectomy specimen ordinarily includes 6 or more lymph nodes. If lymph nodes are negative but the above number is not met, this should still be classified as pN0
  - Metastasis to non-regional lymph nodes is classified as distant metastases and designated as M1

References

1. Cancer incidence data, National Cancer control programme, Sri Lanka 2014.
2. WHO Classification of Tumours Editorial Board. Digestive system tumours. Lyon (France): International Agency for Research on Cancer; 2019. (WHO classification of tumours series, 5th ed.; vol. 1).
3. Standards and datasets for reporting cancers Dataset for histopathological reporting of colorectal cancer, The Royal College of Pathologists September 2018
4. CAP guidelines: Protocol for the Examination of Specimens from Patients with Carcinoma of the Small Intestine, Version: Small Intestine 4.1.0.0

CHAPTER 6

Tumours of the appendix

Introduction and epidemiology

The appendix is commonly removed due to appendicitis but may also be resected in colectomies or other surgeries.

There is a slight female predominance for mucinous adenocarcinoma (53-57%) but a slight male predominance for non-mucinous adenocarcinoma (55%).

While tumours of the appendix are uncommon, neuroendocrine (carcinoid) tumours and adenocarcinomas do occur, and can be incidental findings in cases of appendicitis found in 0.1 to 0.6 per cent of appendectomies. In these cases, more extensive sampling of the appendix is required.

Specimen handling

Specimen collection and transport

Refer National Guidelines in Histopathology – Collection, Handling and Transport of Specimens (second edition, 2021).

Grossing procedure

Routinely, the appendix is sectioned longitudinally at the distal third (tip) of the appendix and transversely up to the base.

The grossing of the specimen should be modified depending on the following situations;

- Radiologically detected tumours/ tumours detected on external examination (Figure 1A)
- Tumours detected on sectioning (Figure 1B)
- Tumours detected on microscopy (Figure 1C)

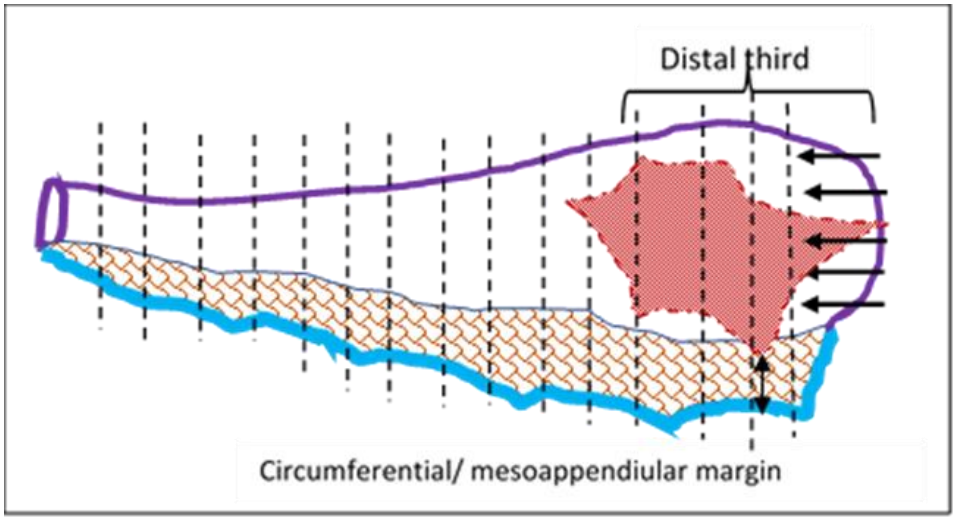


Figure 1A. Grossing technique – For radiologically detected tumours/ tumours detected on external examination – Distance to circumferential /mesenteric margin is shown by the two-way arrow; serosal margin-purple and mesoappendiular margin/non peritonealized margin (blue). Distal slice of the tip with the tumour is sectioned transversely to include the serosal margin.

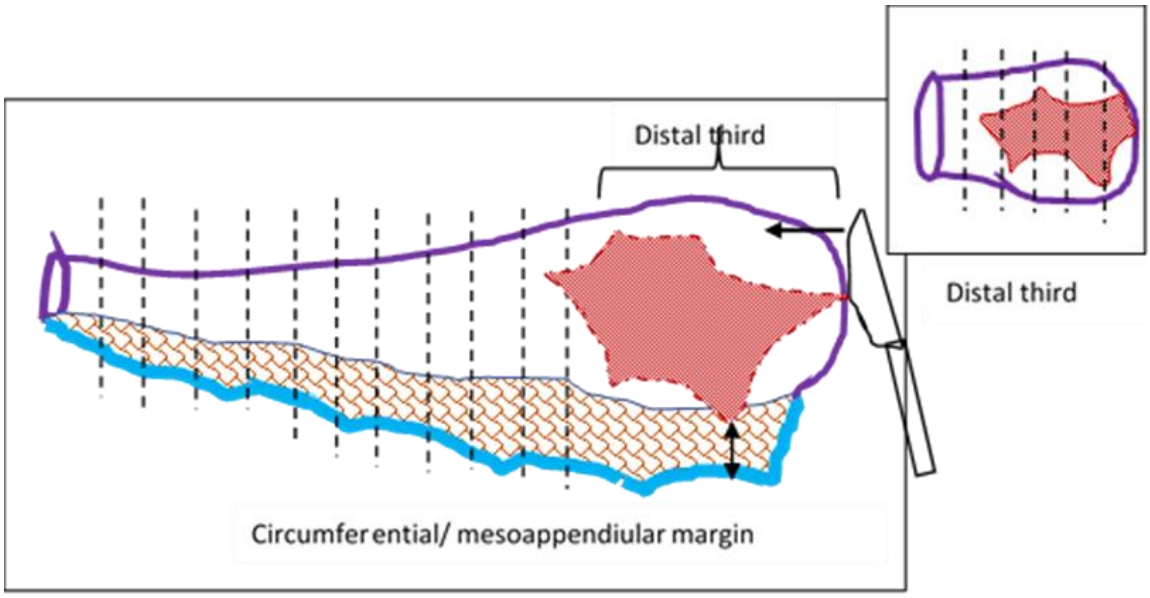


Figure 1B. Grossing technique – For tumours detected on sectioning – Distance to circumferential /mesenteric margin is shown by the two-way arrow; serosal margin-purple and mesoappendiular margin/non peritonealized margin(blue). Each half of the distal third with tumour is then sectioned transversely (Inset).

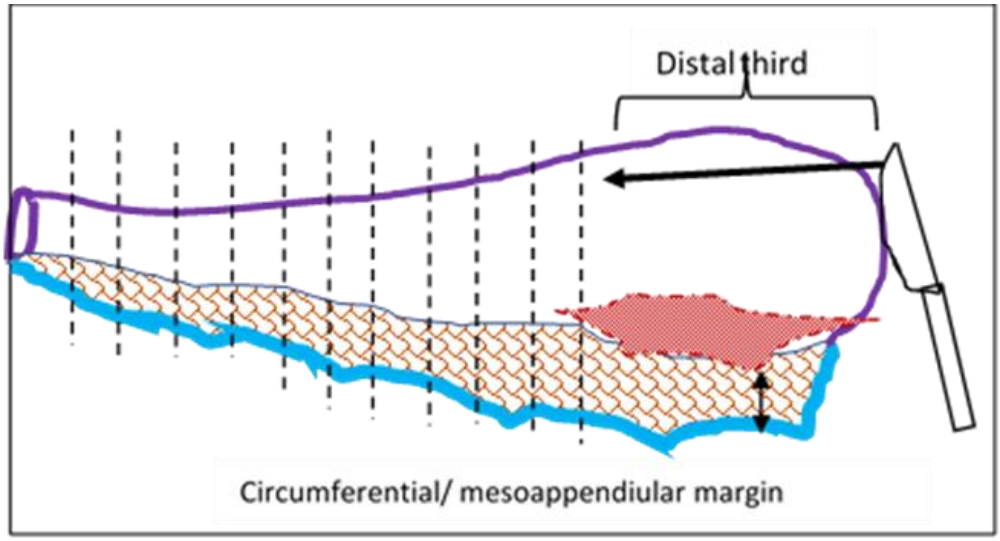


Figure 1C. Grossing technique - Tumours detected on microscopy- The remaining half of the distal third should be sectioned transversely and examined. The entire appendix should also be submitted for histological assessment.

Macroscopy

- **Type of specimen**
  - Appendicectomy
  - Right hemicolectomy with appendicectomy (see colorectal tumour guidelines)
  - Other (Specify)

  Note: Ideally photographs should be taken **[Y]**
- **Anatomical components and their dimensions** (mm/cm)
  - Appendix, in two dimensions - length and diameter
  - Mesoappendix, two dimensions
  - Other, describe and measure according to the relevant tissue protocol.
- **Specimen integrity**
  - Intact
  - Disrupted/piecemeal - Number of pieces
- **Shape of appendix** – Normal / distended / mucocele
- **Outer surface**
  - Unremarkable
  - Haemorrhagic
  - Perforation and its location
  - Adhesions
  - Fibrotic
  - Purulent
  - Mucin
  - Tumour present or absent

- **Internal inspection**
  - Describe the mucosal surface and the contents of the lumen.
  - Note any thickening or narrowing of the mucosa or lumen; presence of faecoliths, foreign bodies, fibrous obliteration, mucin and parasites and presence or absence of any lesions such as tumours.
- **Tumour description**
  - Absent
  - Present – Mention number; if more than one tumour, designate and describe each tumour separately.
- **Tumour site**
  - Proximal half of appendix.
  - Base of appendix involved by tumour.
  - Base of appendix uninvolved by tumour.
  - Involvement of base of appendix cannot be assessed.
  - Distal half of appendix.
  - Diffusely involving appendix.
  - Appendix not otherwise specified.
  - Other (Specify)
- **Tumour size**
  - Greatest dimension (mm)
  - Additional dimension (mm)
  - Cannot be determined (explain)
  - Unremarkable / congested / fibrinous
- Tumour margins
  - In a routine appendicectomy specimen the margins include:
    - Base of appendix
    - Mesenteric/radial margin
  - In right hemicolectomy specimens the margins include:
    - Proximal margin is the ileal end resection margin
    - Distal margin is the distal end resection margin of the colon
    - Mesenteric/radial margin

Note:

- The mesenteric margin of the appendix is considered circumferential margin as the appendix is completely peritonealised.
- In a right hemicolectomy, proximal and distal margins are ileum and colon respectively; these are considered negative if they are more than 5 cm from the tumour.

Block selection (Figure 1A – 1C)

- The entire appendix should be submitted for histology.
- Blocks should include,
  - En face block of the base of the appendix
  - In a hemicolectomy – the proximal and distal end resection margins
  - Tumour with serosal surface
  - Tumour with mesenteric/radial margin

Microscopy and conclusion

Tumour size	Confirmed by microscopy
Tumour Type	Refer types of tumours of the appendix in WHO Classification of Tumours of the Digestive System, 5th edition (currently in use in 2021) - Table 1
Tumour grade	Table 2
Tumour extent	Table 4
Margins	Involved / uninvolved by invasive carcinoma Distance of invasive carcinoma from closest margin (mm/cm) In a routine appendicectomy specimen: o Base of appendix o Mesenteric/radial margin In right hemicolectomy specimen: o Proximal margin is the ileal end resection margin o Distal margin is the distal end resection margin of the colon o Mesenteric/radial margin
Lymphovascular Invasion	Present / Absent
Mesenteric tumour deposits	If present specify the number of deposits.
Perineural Invasion	Present / Absent
Regional lymph nodes	Present / Absent Number of lymph nodes submitted or found Number of positive nodes
Completeness of resection	R0 / R1 / R2 - table 3

Pathologic stage classification	Refer TNM classification for staging of tumours of the appendix (AJCC TNM 8th Edition currently in use in 2021) – Table 4
Additional pathologic findings	None identified / Appendicitis / Perforation, not at tumour / Ulcerative colitis / Crohn disease / Diverticulosis / Other (specify)

Annexure

Reporting proforma for appendiceal malignancy

Gross description		
Type of surgery	:	
Anatomical components included and dimensions	:	_____ mm
Specimen integrity	:	
Shape of appendix	:	
Outer surface	:	
Internal inspection	:	
Tumour specimen description	:	
Tumour site	:	
Tumour size	:	_____ mm
Tumour margins	:	_____ mm
Microscopic description		
Type of tumour	:	
Tumour size	:	_____ mm
Tumour grade	:	
Tumour extension	:	
Margins	:	
Lymphovascular invasion	:	
Tumor deposits	:	
Peri neural invasion	:	
Regional lymph nodes	:	
Pathological stage	:	
Ancillary Studies	:	Immunohistochemistry where necessary o Carcinoma – CK7, CK20 o Neuroendocrine tumour – Synaptophysin, Chromogranin, Ki67

Tables

Table 1. Tumour types: (WHO Classification of Tumours of the Digestive System, 5th edition)

- Low grade appendiceal mucinous neoplasm – Refer table 2A & 2B
- High grade appendiceal mucinous neoplasm – Refer table 2A & 2B
- Adenocarcinoma, NOS
  - Mucinous adenocarcinoma
  - Signet-ring cell carcinoma
- Carcinoma undifferentiated NOS
- Goblet cell adenocarcinoma
- Neuroendocrine tumour NOS
  - Neuroendocrine tumour, Grade1
  - Neuroendocrine tumour, Grade2
  - Neuroendocrine tumour, Grade3
  - L-cell tumour
  - Glucagon-like peptide producing tumour
  - PP/PYY producing tumour
  - Enterochromaffin-cell carcinoid
- Neuroendocrine carcinoma NOS
  - Large cell Neuroendocrine carcinoma
  - Small cell Neuroendocrine carcinoma
- Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN)

Note:

- Mucinous adenocarcinoma is considered when a tumour is composed of >50% extracellular mucin; it may contain < 50% signet ring cells
- Signet ring carcinoma adenocarcinoma is considered when a tumour is composed> 50% signet ring cells.

Table 2A. Histological grading of appendiceal mucinous neoplasms, adenocarcinomas and their peritoneal metastases (College of American Pathologists Protocols, February 2020)

Tumour grade	Histological criteria	
	In the appendiceal primary tumour	**In the peritoneal metastasis**
1	Low-grade cytology with a pushing margin (Low-grade appendiceal mucinous neoplasm)	Hypocellular mucinous deposits Neoplastic epithelial cells have low-grade cytology No infiltrative type invasion
2	High-grade cytology with a pushing margin (High – grade appendiceal mucinous neoplasm) Invasive mucinous adeno-carcinoma without signet ring cell component	Hypocellular mucinous deposits as judged at x20 magnification Neoplastic epithelial cells have High-grade cytological features Infiltrative type invasion characterized by jagged or angulated glands in a desmoplastic stroma, or a small mucin poll pattern with numerous mucin pools containing clusters of tumour cells
3	Signet ring cell adenocarcinoma with numerous signet ring cells in mucin pools or infiltrating tissue	Mucinous tumour cells with signet-ring cells

Note:

- If the grades of the appendiceal and peritoneal tumour deposits are discordant, each grade should be separately reported.
- In some cases of signet ring cell adenocarcinoma, the primary appendiceal tumour may be a goblet cell adenocarcinoma.
- Non-mucinous adenocarcinoma – grading is similar to conventional colorectal carcinoma and graded using a two-tier grading system, similar to that for colorectal carcinoma.

Table 2B. Three-tiered grading system for goblet cell adenocarcinoma

Tumour grade	Histological criteria	
	Tubular or clustered growth (Low-grade pattern)	**Loss of tubular or clustered growth (Any combination of high-grade pattern)**
1	> 75%	<25%
2	50-75%	25-50%
3	<50%	>50%

Table 3. Completeness of resection

Description	Completeness of resection
No residual tumour	R0
Microscopic tumour at resection margins	R1
Macroscopic tumour at resection margins	R2

Table 4. AJCC/ TNM 8th edition staging for tumours of the appendix

Primary Tumour (pT)	
pTx	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pTis	Carcinoma in situ-Intraepithelial or invasion of lamina propria
pTis (LAMN)	Low-grade appendiceal mucinous neoplasm confined to the appendix (defined as involvement by acellular mucin or mucinous epithelium that may extend into muscularis propria).
pT1	Tumour invades submucosa
pT2	Tumour invades the muscularis propria
pT3	Tumour invades subserosa or mesoappendix.
pT4	Tumour perforates the visceral peritoneum, including mucinous peritoneal tumour or acellular mucin on the serosa of appendix or mesoappendix and/or directly invades other organs or structures.
pT4a	Tumour perforates visceral peritoneum, including mucinous peritoneal tumour or acellular mucin on the serosa of appendix or mesoappendix.
pT4b	Tumour directly invades other organs or structures

Regional lymph nodes (pN) (see note below)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in 1 to 3 regional lymph node
pN1a	Metastasis in 1 regional lymph node
pN1b	Metastasis in 2 to 3 regional lymph node
pN1c	Tumour deposits i.e satellites, in the subserosa or in non-peritonealized pericolic or perirectal soft tissue without regional lymph node metastasis
pN2	Metastasis in 4 or more regional lymph nodes
Distant metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis
pM1 a	Intraperitoneal acellular mucin, without identifiable tumour cells in the disseminated peritoneal mucinous deposits
pM1 b	Intraperitoneal metastasis only, including mucinous epithelium
pM1 c	Non-peritoneal metastasis

Note:

- **Primary Tumour**
  - Tis include cancer cells confined within the glandular basement membrane (Intra epithelial) or lamina propria (Intra mucosal) with no extension through muscularis mucosa into submucosa.
  - Direct invasion in T4 includes invasion of other intestinal segments by way of the serosa. e.g., invasion of ileum.
  - Tumour adherent to other organs or structures, macroscopically, is classified cT4b. However, if no tumour is present in the adhesion, microscopically, classification should be pT1, 2 or 3.
  - Low grade appendecial mucinous neoplasms (LAMN) with involvement of the subserosa or the serosal surface (visceral peritoneum) should be classified as T3 or T4a respectively.
- **Regional node deposits**
  - Tumour deposits (satellites) are discrete macroscopic or microscopic nodules of cancer in the lymph drainage area of a primary carcinoma of the pericolic adipose tissue that are discontinuous from the primary and without histological evidence of residual lymph node/s or identifiable vascular or neural structures.
  - If a vessel wall is identifiable on H&E, elastic or other stains, it should be classified as venous invasion (V1/2) or lymphatic invasion (L1).
  - If neural structures are identifiable, it is classified as perineural invasion (Pn1)

- **Distant metastasis**
  - pM1- Distant metastasis microscopically confirmed.
  - pM0 and pMX are not valid categories.

References

1. WHO Classification of Tumours Editorial Board. Digestive system tumours. Lyon (France): International Agency for Research on Cancer; 2019. (WHO classification of tumours series, 5th ed.; vol. 1).
2. Standards and datasets for reporting cancers Dataset for histopathological reporting of colorectal cancer, The Royal College of Pathologists September 2018
3. CAP guidelines: Protocol for the Examination of Specimens From Patients With Carcinoma of the Appendix 2016

CHAPTER 7

Tumours of the colon and rectum

Introduction and epidemiology

Tumours of the colon and rectum are considered the second most common cancer in females and third most common cancer in males with estimated cases to be more than 1.8 million recorded worldwide, as reported in 2018. In Sri Lanka, according to the National Epidemiology Cancer Registry (2014), colorectal malignancy is the fourth commonest malignancy in males and fifth commonest in females. According to this data, the incidence of rectal cancers appears to be more than the colon in both genders.

The highest incidence rates are seen in high income countries where dietary habits like consumption of processed and red meat, alcohol and sedentary habits with excess body fat have been established as risk factors. The other established risk factors include genetic predisposition and chronic inflammatory bowel diseases.

Specimen handling

Grossing procedure

- The specimen should be ideally received fresh and pinned to a cork board. **[Y]**
- The segment of bowel is however generally received fixed in formalin and should be measured and then inspected exteriorly to locate the tumour (refer Chapter 1 Section F on fixation, in National Guidelines in Histopathology - Collection, Handling and Transport of Surgical Specimens, second edition, 2021).
- The circumferential resection margin should be inked. The circumferential resection margin is identified as follows:
  - Low rectal tumours (below peritoneal reflection) – complete circumference
  - Upper rectal tumours (above peritoneal reflection) – posterior and lateral margins
  - Transverse and proximal sigmoid colon – narrow, non-peritonealized margin /mesenteric margin
  - Ascending and descending colon – posterior margins
  - Caecum - posterior margin – variable (none / small / large non-peritonealized area)
- Once the circumferential margin is inked, the specimen is then opened anteriorly up to 2 cm above and below the tumour. For further details on opening the specimen for fixation, please refer Chapter 1 Section F on fixation,

in National Guidelines in Histopathology - Collection, Handling and Transport of Surgical Specimens, second edition, 2021.

- A piece of gauze or an absorbent paper is inserted through the lumen at the tumour site to assist in fixation.
- The specimen is fixed for 24-48 hours.
- It is then examined for all the macroscopic data items and recorded.
- The tumour is sliced transversely at 3-4 mm intervals, laid out sequentially and photographed if possible. **[Y]**
- The slices should include the tumour, serosal and circumferential margins, and the adjacent lymph nodes.
- It should be examined to identify the point of deepest invasion.
- The lymph node identification should start with the highest lymph node (apical / high tie) which is the first lymph node identified after sectioning serially and distally from the sutured vascular margin.

Note:

- The rectal tumour resection usually has one 'high tie'. The colonic resections, however, may have the tumour located between two major arteries, and hence it will be appropriate to record both high tie nodes. These nodes should be blocked separately.
- The lymph nodes in the rest of the mesocolon are identified by slicing the fat thinly.
- The entire lymph node is blocked if nodes are <4mm in size. If larger, a single block is taken through the longest axis.
- Although the minimum number of lymph nodes is set at 12, the pathologists should endeavor to retrieve as many lymph nodes as possible and submit for histology.

Macroscopy

- **Type of specimen**
  - Right hemicolectomy
  - Left hemicolectomy
  - Transverse colectomy
  - Sigmoid colectomy
  - Low anterior resection
  - Abdominoperineal resection (APR)
  - Total colectomy

- **Specimen dimensions**
  - Length of the bowel and measurement of the attached mesocolon.

- **Site of the tumour**
  - Caecum
  - Ascending colon
  - Transverse colon
  - Descending colon
  - Sigmoid colon
  - Rectum

Note:

- Any discrepancy with the stated site on the request form and the examined specimen should be queried immediately with the surgeon and corrected if necessary.
- If the tumour straddles two sites, the site where the tumour is extensively involved is recorded.
- If however, the tumour involvement of both sites are more or less equal then the tumour should be considered an overlapping lesion.

FOR RECTAL TUMOURS ONLY

- Tumour location: **Relationship to the peritoneal reflection** (Figure 1)

Rectal tumours are classified as below, above or at the peritoneal reflection. It is important to note that the tumours below the peritoneal reflection have the highest rates of local recurrence.

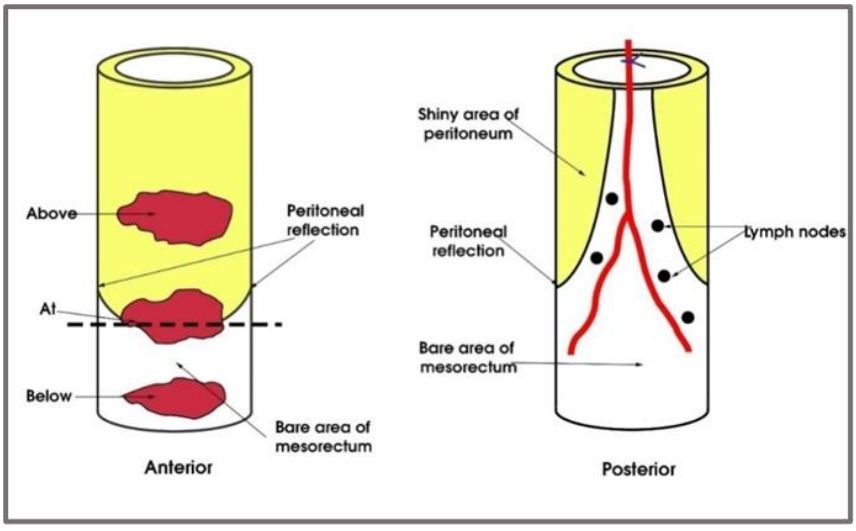


Figure 1. Diagrammatic representation of anterior resection / abdomino perineal resection specimens with the different sites of tumour in relation to the peritoneal reflection

- Plane of mesorectal excision

The three planes of excision are as follows:

- Mesorectal / complete excision
- Intramesorectal / nearly complete excision
- Muscularis propria / incomplete excision

Mesorectal / complete excision: In this plane of excision, the mesorectum is smooth, good bulk with intact fascial covering. There should be no coning in the distal margin. (figure 2)

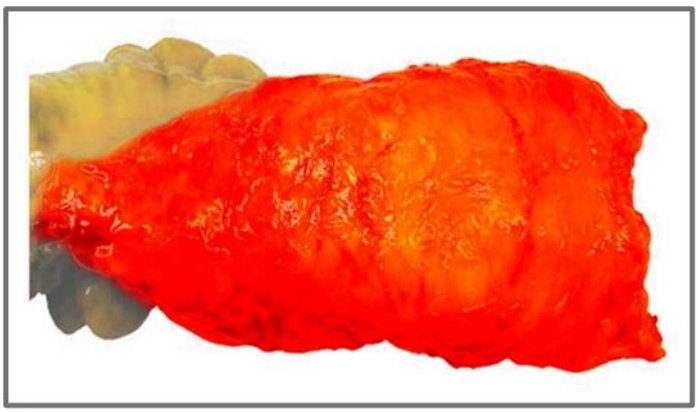


Figure 2. Mesorectal plane of excision in an anterior resection specimen. Circumferential margin is inked red.

Intramesorectal / nearly complete excision: In this plane of excision, there is a moderate bulk to the mesorectum, with minor irregularity of the mesorectal surface. The distal margin shows moderate degree of coning. The muscularis propria should not be visible except at the insertion of the levator muscles at the very distal aspect.(figure 3)

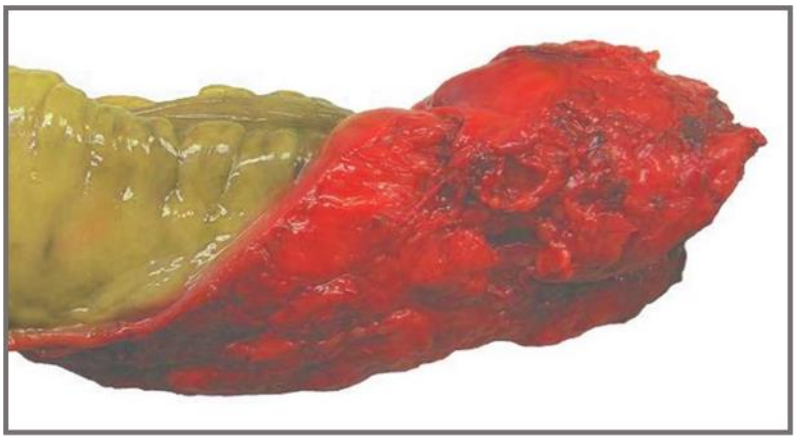


Figure 3. Intramesorectal plane of excision in an anterior resection specimen. Circumferential margin is inked red.

Muscularis propria (incomplete excision): In this plane of excision, there are significant mesorectal defects which expose extensive areas of muscularis propria. (figure 4)

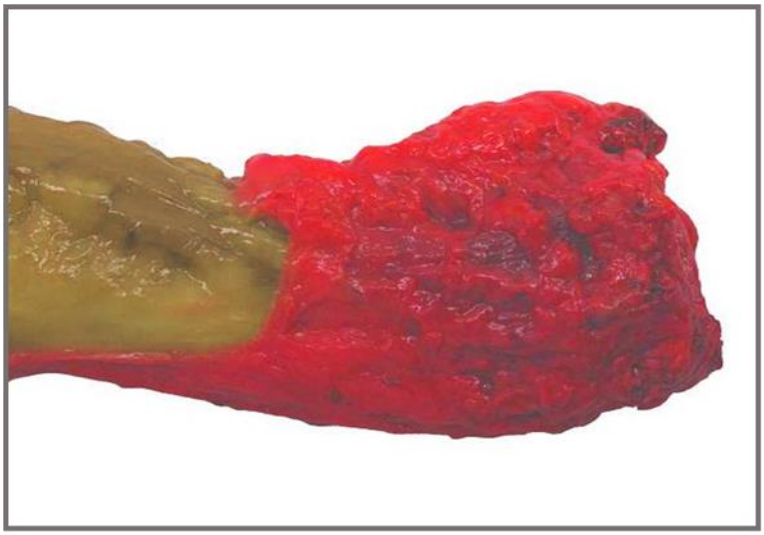


Figure 4. Muscularis propria plane of excision in an anterior resection specimen. Circumferential margin is inked red.

For **abdomino-perineal specimens**, in addition to this, there is a separate assessment of the plane of surgical dissection in the levator/sphincter area around the anal canal and below the mesorectum.

This plane of excision of the levators/sphincters are as follows:

Extralevator: The excision is external to the levator ani muscle (figure 5)

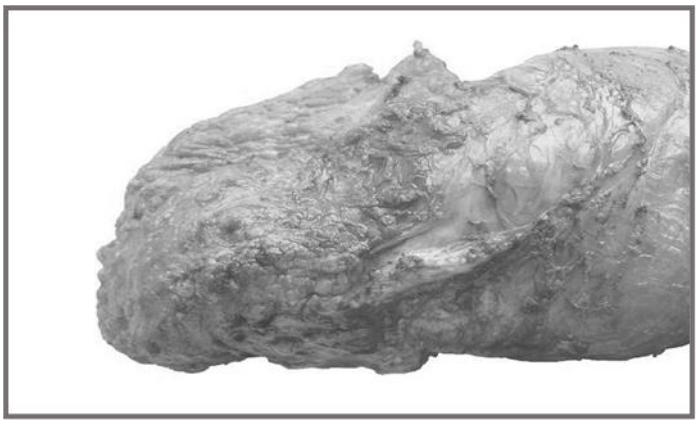


Figure 5: Extralevator plane of excision in an abdominoperineal resection specimen

Sphincteric: In this plane of excision, there is the classical surgical waist. There is no levator wrap, and if at all, a small cuff of levator is seen. There is no entry into the sphincteric muscle. (figure 6)

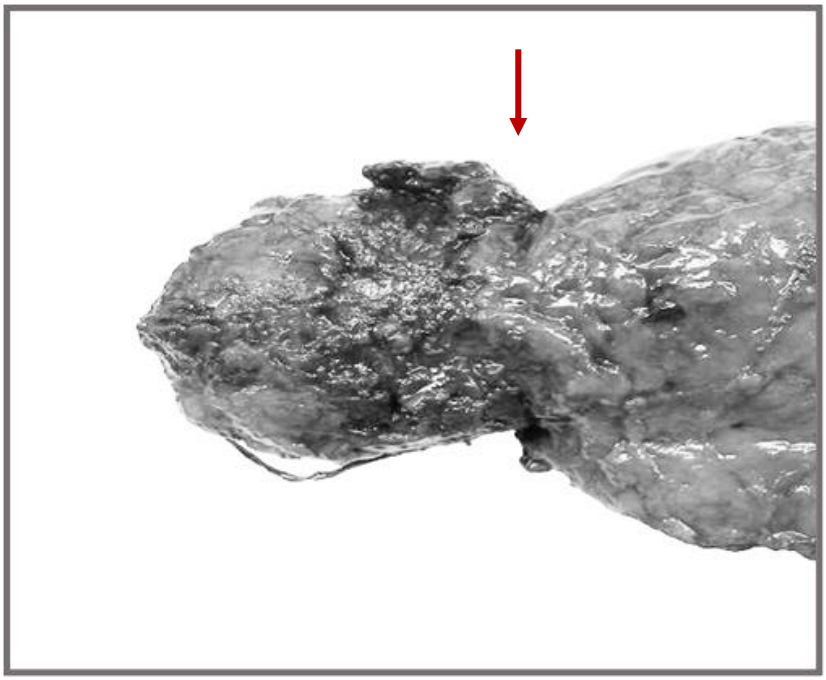


Figure 6. Sphincteric plane of excision in an abdominoperineal resection specimen. Arrow indicates the surgical waist.

Intrasphincteric / submucosal perforation: In this plane there is a definite entry into the sphincter muscle or even deeper into the submucosa. Any perforations of the specimen below the peritoneal reflection also should be classified in this group. (figure 7)

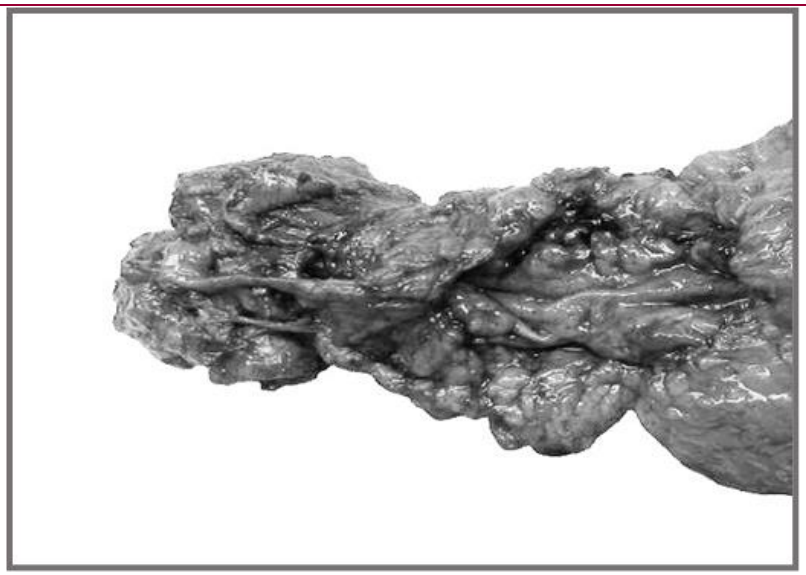


Figure 7. Intrasphincteric/submucosal plane of excision in an abdominoperineal resection specimen

- Distance from the dentate line

  This is assessed for the low rectal tumours in abdominoperineal resection specimen to indicate the relationship of the tumour to the internal sphincter. (figure 8)

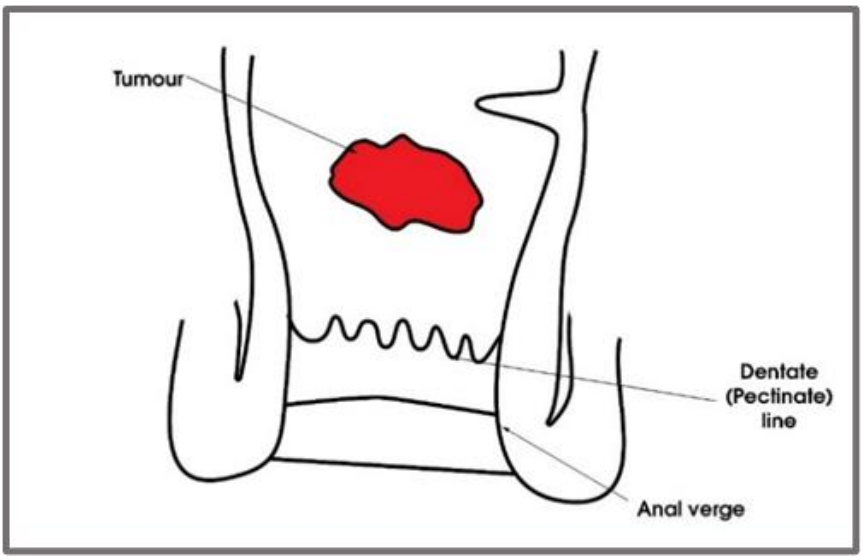


Figure 8. Diagrammatic representation of tumour in relation to the dentate line

- **Gross appearance of the tumour**

  Polypoid, fungating, ulcerating, infiltrating, obstructing, or mixed patterns

- **Maximum tumour size**

  The length of the tumour is measured.

- **Macroscopic depth of tumour penetration/ distance from the circumferential resection margin**
- **Macroscopic tumour perforation:**

  This is a macroscopically visible defect through the tumour. Tumour involvement of the serosal margin should also be suspected if the serosal surface shows induration, puckering or lack of normal lustre.

- **Distance of the tumour from the nearest longitudinal (end-resection) margin**

- **Non-neoplastic mucosa:**
  - Dilatation or narrowing of the bowel
  - Presence of polyps
  - Associated bowel pathology
  - Satellite nodules away from the tumour.

  Note: If polyps or satellite nodules are present, these should be measured.

- Lymph nodes:
  - The minimum number of lymph nodes is set at 12 but all lymph nodes that are retrieved should be submitted for histology.
  - The size of the largest lymph node should be noted.

Block selection

- Tumour blocks should be taken to demonstrate the following:
  - Maximum depth of penetration through the bowel wall.
  - Involvement of the serosal surface
  - Any areas of perforation.
  - Areas suspicious for extramural venous invasion.
  - Relationship to the closest circumferential margin (either a separate extramural deposit or tumour in a lymph node or in continuity with the main tumour mass whichever is closest).
- Tumour with adjacent mucosa
- Longitudinal/end resection margins (Proximal and distal)
- Polyps and other pathology evident macroscopically
- Non neoplastic/normal mucosa
- High tie node/s
- All lymph nodes identified (Block in entirety if <4mm and a block through the longest axis for larger nodes)

Microscopy and conclusion

Tumour type	Refer tumours of the colon and rectum in WHO Classification of Tumours of the Digestive System, 5th edition (currently in use in 2021) - Table 1
Tumour grade	Table 2
Depth of Invasion	Figure 9

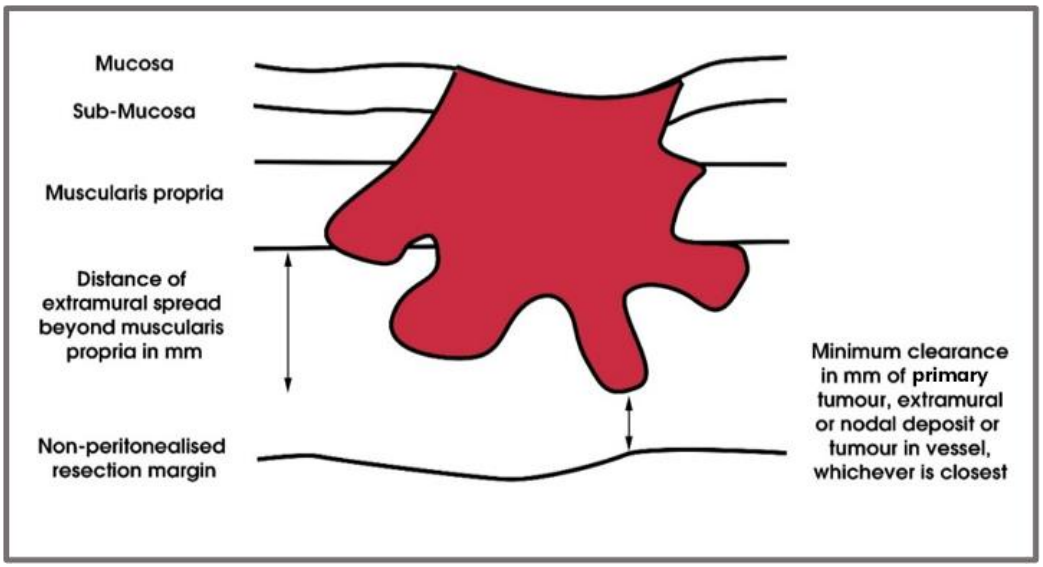


Figure 9. Measurement of extramural spread and tumour clearance from the circumferential margin

Involvement of serosa/peritoneal surface	This is defined by the following conditions, as explained in the Royal College of Pathologists UK dataset 2018: o Tumour breaching the serosa with presence of tumour cells on the peritoneal surface. o Tumour breaching the serosa with presence of tumour cells free in the peritoneal cavity. o Visible presence of tumour cells separated from the peritoneal surface by inflammatory cells only. o For tumours that are □1 mm from the serosal surface or in tumours with equivocal serosal invasion, multiple levels and additional sampling is advised. o Ancillary tests such as elastic stain to confirm the internal elastic lamina of the serosa or CK7 immunohistochemistry to confirm mesothelial cells may be performed. **[Y]** o If serosal surface involvement cannot be demonstrated, the tumour should be considered pT3. o pT4a staging should not be used in non-peritonealized portions of the colorectum (eg. posterior aspects of ascending and descending colon, lower rectum etc).

Resection margins	If the distance to the circumferential margin is < 1mm, it is regarded as involved. This involvement could be by the tumour in continuity with the main tumour or discontinuity (tumour deposit), involvement of a lymph node, vein or a lymphatic.
Venous invasion	Level of venous spread – extramural or intramural (intramuscular or submucosal)
Lymphatic invasion	Present / Absent
Response to neoadjuvant therapy	Table 3
Lymph node status	Table 4
Background abnormalities and non-neoplastic mucosa	○ Inflammatory bowel disease ○ Polyposis syndromes ○ Diverticulosis ○ Polyps including their size, number and type (adenomatous, hyperplastic, serrated, hamartomatous etc).
Perineural invasion	Defined by involvement of at least one third of the circumference of the nerve. It can be present in any of the three layers namely, epineurium, perineurium and endoneurium.
Tumour budding [Y]	Tumour budding should be noted as present or absent. However, grading of tumour budding is recommended whenever possible (Table 5)
Tumour borders)	Pushing / rounded / infiltrative in type
Immune response (formerly known as host lymphocytic response)	This indicates the presence of intratumoral lymphocytes or a Crohn-like reaction. Both these features are associated with MSI and indicates a better outcome.
Pathological tumour stage	Refer TNM classification for staging of tumours of the colon and rectum (AJCC TNM 8th edition currently in use in 2021) - Table 4
Ancillary studies	○ KRAS mutation **[Y]** ○ BRAF V600E mutation **[Z]** ○ Mismatch repair gene status (MLH1, MSH2, MSH6, PMS2) **[Z]**

Annexure

Reporting proforma for colorectal carcinoma

Tumour type
Tumour site
Maximum tumour dimension
Tumour grade
Tumour depth
Lymph node involvement
Treatment effect (Modified Ryan Classification)
Lymphovascular invasion (Lymphatic / Venous)
Immune response
Tumour budding
Completeness of resection (R0 / R1 / R2)
For rectal tumours
Relationship of tumour to peritoneal reflection
Plane of mesorectal excision (Anterior resection and Abdominoperineal resection)
Distance to the dentate line (Abdominoperineal resection only)
Pathological tumour stage; pTNM (Table 4)
Ancillary studies
KRAS mutation **[Y]**
BRAF V600E mutation **[Z]**
Mismatch repair gene status (MLH1, MSH2, MSH6, PMS2) **[Z]**

Tables

Table 1. WHO Classification of Tumours of the Digestive System, 5th edition

- Adenocarcinoma
  - Serrated adenocarcinoma
  - Adenoma-like adenocarcinoma
  - Micropapillary adenocarcinoma
  - Mucinous adenocarcinoma
  - Poorly cohesive carcinoma
  - Signet-ring cell carcinoma
  - Medullary adenocarcinoma
  - Adenosquamous carcinoma
  - Carcinoma, undifferentiated, NOS
  - Carcinoma with sarcomatoid component

- Neuroendocrine tumours (NET) NOS
  - Neuroendocrine tumour, grade 1
  - Neuroendocrine tumour, grade 2
  - Neuroendocrine tumour, grade 3
  - L-cell tumour
  - Glucagon-like peptide producing tumour
  - PP/PYY-producing tumour
  - Enterochromaffin-cell carcinoid
  - Serotonin-producing tumour

- Neuroendocrine carcinoma NOS
  - Large cell neuroendocrine carcinoma
  - Small cell neuroendocrine carcinoma

- Mixed neuroendocrine-non-neuroendocrine carcinoma (MiNEN)

Table 2. Tumour grade – based on WHO Classification of Tumours of the Digestive System, 5th edition.

Grade	Description
Low-grade	Well-differentiated in previous grading systems; (Well-formed glands > 95%)
	Moderately differentiated in previous grading systems (50% to 95% of tumour composed of glands)
High-grade	Poorly differentiated in previous grading systems (49% or less of tumour composed of glands

Note:

- Grading is based on the least- differentiated component.
- Whilst grading the tumour, the invasive front where the poorly differentiated clusters or tumour budding occurs as a sign of epithelial-mesenchymal transition, should not be taken into account but instead reported separately.

Table 3. Tumour Regression Grade - Modified Ryan scheme

Description of tumour	Regression Grade
No viable cancer cells (Complete response)	0
Single cells or small groups of cancer cells (Near complete response)	1
Residual cancer outgrown by fibrosis (Minimal response)	2
Minimal or no tumor kill; extensive residual cancer (Poor response)	3

Table 4. Pathological staging TNM /AJCC 8th edition

Primary tumour (pT)	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ, Intramucosal carcinoma (involvement of lamina propria with no extension through muscularis mucosae)

T1		Tumour invades submucosa (through the muscularis mucosa but not into the muscularis propria)
T2		Tumour invades muscularis propria
T3		Tumour invades through the muscularis propria into the pericolorectal tissue
T4	**T4a**	Tumour invades through the visceral peritoneum (including gross perforation of the bowel through tumour and continuous invasion of tumour through areas of inflammation to the surface of the visceral peritoneum)
	T4b	Tumour directly invades or adheres to other adjacent organs or structures
Regional lymph nodes (pN)		
NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1		Metastasis in 1 - 3 regional lymph nodes
N1a		Metastasis in 1 regional lymph node
N1b		Metastasis in 2 - 3 regional lymph nodes
N1c		No regional lymph nodes are positive but there are tumour deposits in the sub-serosa, mesentery or non-peritonealized pericolic or perirectal / mesorectal tissues
N2		Metastasis in 4 or more regional lymph nodes
N2a		Metastasis in 4 - 6 regional lymph nodes
N2b		Metastasis in 7 or more regional lymph nodes
Distant metastasis (pM)		
M0		No distant metastasis by imaging; no evidence of tumour in other sites or organs (this category is NOT assigned by pathologists)
M1		Distant metastasis
M1a		Metastasis confined to 1 organ or site without peritoneal metastasis
M1b		Metastasis to 2 or more sites or organs is identified without peritoneal metastasis
M1c		Metastasis to the peritoneal surface is identified alone or with other site or organ metastases

Note:

- **Primary Tumour**

  Tis Includes cancer cells confined within the lamina propria (Intramucosal). There is no extension through the muscularis mucosa into the submucosa.

  T4 T4 has been separated into two categories (T4a and T4b) based on the different outcomes in expanded datasets.

  T4a Includes tumours which directly invade the serosal surface (visceral peritoneum).

  T4b This includes the following:

  - Tumours which invade other organs or segments of the colorectum by way of serosa as confirmed on microscopic examination (This involves direct invasion of other organs or structures by virtue of extension beyond the muscularis propria.)
  - Tumours in a retroperitoneal or subperitoneal location.
  - Tumours that are adherent to other organs or structures macroscopically is classified as cT4b. However, if no tumour is present in the adhesion microscopically, the classification should be pT1-3 depending on the anatomical depth of the wall invasion.

- **Regional lymph nodes**
  - Histological examination of a regional lymphadenectomy specimen will ordinarily include 12 or more lymph nodes. If the lymph nodes are negative, but if this number is not met, classify as pN0.
  - Tumour deposits (satellites) are discrete macroscopic or microscopic nodules of cancer in the lymph drainage area of a primary tumour in the pericolorectal adipose tissue that are discontinuous from the primary and without histological evidence of residual lymph node or identifiable vascular or neural structures.
  - If a vessel wall is identifiable on H&E, elastic or other stains, it should be classified as venous invasion (V1/2) or lymphatic invasion (L1).
  - Similarly, if neural structures are identifiable, the lesion should be classified as perineural invasion (Pn1).
  - The presence of tumour deposits does not change the primary tumour (T category) but changes the nodal status (N category) to pN1c if all regional lymph nodes are negative on pathological examination.

Table 5. Tumour budding **[Y]**: Based on the three-tiered scoring system proposed by the International tumour budding consensus conference (ITBCC) per 20 objectives (0.785 mm^2).

Budding Type	Number of buds
Low budding (Bd1)	0 – 4 buds
Intermediate budding (Bd2)	5 – 9 buds
High budding (Bd3)	10 or more buds

Note:

- This is determined in a hotspot at the invasive front on H&E staining.
- It is defined by the presence of either single cells or clusters of as many as 4 tumour cells at the invasive front.
- The International tumour budding consensus conference (ITBCC) proposed a three-tiered scoring system based on a single worst (hotspot) per 20 objective (0.785 mm^2)

References

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7. Lugli, A., Kirsch, R., Ajioka, Y. et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Mod Pathol 30, 1299–1311 (2017)
8. Standards and datasets for reporting cancers: Dataset for histopathological reporting of colorectal cancer. Royal College of Pathologists UK. September 2018.https://www.rcpath.org/uploads/assets/c8b61ba0-ae3f-43f185ffd3ab9f17cfe6/G049-Dataset-for-histopathological-reporting-of-colorectal-cancer.pdf

CHAPTER 8

Tumours of the anal canal

Introduction and epidemiology

The anal canal is the most terminal part of the large intestine, which lies between the anal verge (anal orifice, anus) in the perineum below and the rectum above. According to national cancer incidence data published by National Cancer Control programme 2014 in Sri Lanka, 54 cases of anal malignancies have been reported and most of the cases were squamous cell carcinomas (SCC). The incidence of anal SCC ranges from 0.2 cases per 100 000 person-years in Asia to 1.7 cases per 100 000 person- years in North America and Northern Europe. High risk HPV infection is a well-established risk factor for developing anal SCC. The other risk factors include immunodeficiency, anoreceptive intercourse, coinfection with other sexually transmitted infection and cigarette smoking.

Most anal canal carcinomas are managed without surgery, using combination of chemotherapy and radiotherapy. Resection of anal tumours is performed primarily for small anal tumours, after failure of other treatment modalities or if the cancer recurs.

This chapter describes the guidelines for specimen handling and reporting of tumours involving the anal canal.

Specimen handling

Grossing procedure

- Anal tumours are surgically resected as abdomino-perianal resections (APR) or excision specimens: Polypectomy, perianal skin resection/ local excision /transanal resection.
- (Polypectomy and local resection/transanal resections are performed for small early-stage anal cancers.)
- Macroscopic handling and block selection in APR specimens for anal tumours is almost similar to colorectal carcinoma protocol and the chapter on APR specimen handling for colorectal carcinoma could be followed.
- Excision biopsy (Polypectomy and local resection/transanal resection) specimen handling is discussed in chapters 10 and 11.
- Perianal skin resection specimens are handled similar to wide local specimens for skin malignancies.

- Therefore, only features relevant for reporting anal malignancies will be highlighted in this chapter.

Macroscopy

- **Specimen type / procedure**
  - APR
  - Perianal skin
  - Trans anal resection
  - Polypectomy
- **Tumour site / location in anal carcinomas**
  - Specimen site should be identified accurately and specified as perianal skin, anal canal, anorectal junction rectum (Figure 1).
  - During grossing procedure, tumour location should be identified with the help of clinical notes provided by the clinician.
    Note: Tumours that are localized in the perineal region and not obviously arising from anus or vulva, should be classified as "favour perianal" or "favour vulvar" based on clinical assessment

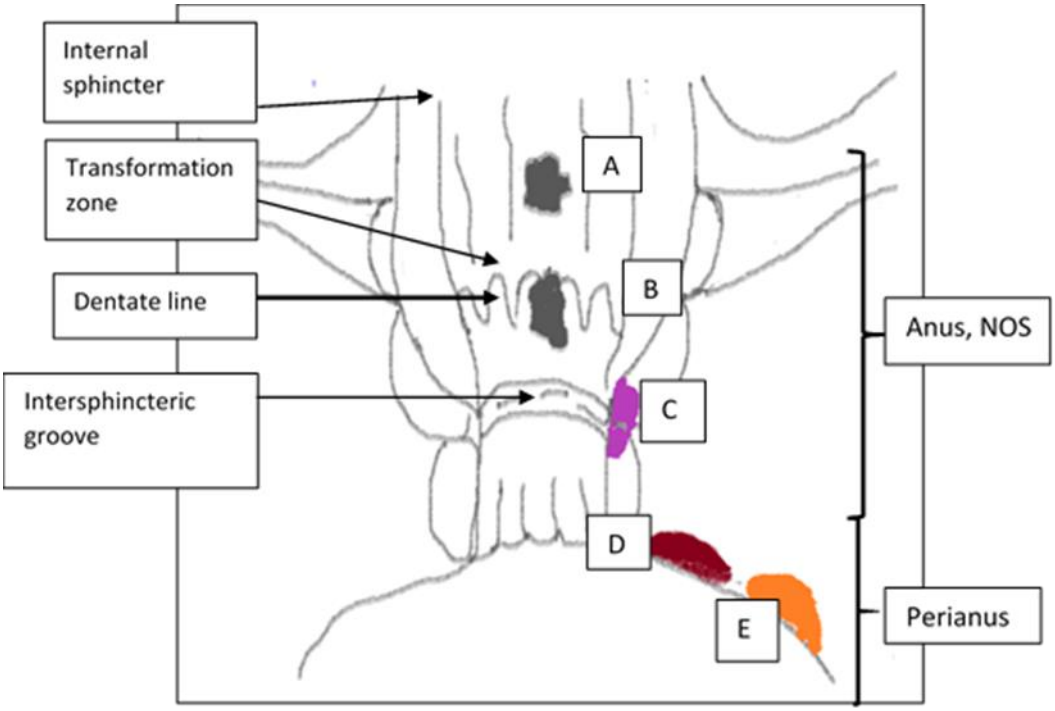

Figure 1. Tumour location in relation to dentate line
A, B, C – Anal cancer: Tumours arising in the anal canal including glandular, anal transformation zonal and squamous mucosa that cannot be entirely visualized by gentle traction placed on the buttocks. **D** – Perianal cancers: Tumours arising < 5cm from anus. These tumours arise in the skin distal to the mucocutaneous junction and can be entirely visualized with gentle traction placed on the buttocks. **E** – Skin cancer: Tumours arising > 5cm from anus

- **Macroscopic appearance** - Polypoid / Ulcerated / Thickened / Others, specify

- **Extent of tumour/ depth of tumour invasion**

- **Distance to resection margins**
  - Measure the distance from proximal and distal end resection margin and the circumferential margin
  - For perianal tumours very close to perianal skin resection margin (Figure 1; tumour D): Take longitudinal/cruciate section/s to assess the accurate distance from the tumour to perianal skin resection on microscopic examination.
  - Measure the distance to deep and mucosal/peripheral margins (for polypectomy and local excision specimens)

- **Involvement of adjacent structures** e.g., vagina, bladder

- **Surrounding mucosa**

Block selection

- For local excision specimen (trans anal resection, polypectomy) - Chapters 10 and 11
- For APR specimens - Chapter 6

Note: Post-neoadjuvant therapy specimens should be sampled extensively to look for treatment response and the presence or absence of residual tumour cells.

Microscopy and conclusion

Tumour size	Confirmed by microscopy
Tumour Type	Refer types of tumours of the anal canal in WHO Classification of Tumours of the Digestive System, 5th edition (currently in use in 2021) - Table 1
Tumour grade	Tables 2 and 3 **Note:** It is important to differentiate primary anal carcinoma from a metastatic deposit/ direct tumour spread of a carcinoma from an adjacent organ - Table 5
Tumour extent	Specify: ▪ Tumour invades lamina propria, muscularis mucosae or submucosa

	▪ Tumour invades into but not through anal sphincter muscle ▪ Tumour invades into but not through muscularis propria of rectum ▪ Tumour invades through anal sphincter muscle into perianal or perirectal soft tissue without involvement of adjacent structures ▪ Tumour directly invades adjacent structures ▪ Tumour invades perianal skin
Margins	**Abdomino-perineal resection (APR)** o Proximal margin: Normal / High-grade intraepithelial neoplasia / involved by carcinoma o Distal margin: Normal / High-grade intraepithelial neoplasia / involved by carcinoma o Circumferential margin: – Involved (< 1 mm) – Not involved; distance of carcinoma to nearest circumferential margin in mm) **Local resection specimens**: Deep and mucosal / peripheral margins

Note:

Measure the accurate microscopic distance from tumour to resection margins when relevant

If local resection specimens (perianal skin resection and transrectal resection) are oriented by the surgeon, specify location of invasive carcinoma (e.g., o'clock position) and location of high-grade dysplasia (eg, o'clock position).

Lymphovascular Invasion	Present / Absent
Perineural Invasion	Present / Absent
Regional lymph nodes	Number of lymph nodes examined Number of involved nodes

Note:

- Histologic examination of a regional perirectal / pelvic lymphadenectomy specimen will ordinarily include 12 or more lymph nodes. Histologic examination of an inguinal lymphadenectomy specimen will ordinary include 6 or more lymph nodes. If the lymph nodes are negative, but the number ordinarily examined is not met, classify as pN0

- Mesorectal, inguinal (superficial and deep), superior rectal (haemorrhoidal), external iliac and internal iliac (hypogastric) lymph nodes are considered as regional lymph nodes for reporting anal malignancy (N category).
- All other nodal groups represent sites of distant metastasis (M category).

Response to neo-adjuvant therapy	Table 6
Completeness of excision	
Additional pathological features	o Crohn disease o Condyloma acuminatum o Anal fistula o Squamous intraepithelial lesions o Associated rectal carcinoma (Paget disease)
Pathologic stage	Refer TNM classification for staging of tumours of the anal canal (AJCC TNM 8th Edition currently in use in 2021) – Table 7
Ancillary Studies [Y]	o To differentiate poorly differentiated squamous from poorly differentiated anal adenocarcinoma: Immunohistochemical panel CK5/6. CK 7, CK20, P53, P63 and mucin stains. o To distinguish primary anal Paget disease from secondary Paget disease of the perianal area and primary anal adenocarcinoma from metastatic adenocarcinoma - Table 5

Annexure

Reporting proforma for anal malignancies

Gross description		
Type of surgery	:	
Specimen size	:	_____ mm
Location of tumour	:	
Macroscopic tumour extension	:	
Size of tumour in cm/mm	:	
Tumour edge to the nearest longitudinal resection margin, skin resection margin or deep and mucosal resection margins	:	
Tumour appearance	:	Polypoid / Other
Microscopic description		
Type of tumour	:	
Tumour size	:	_____ mm
Tumour grade	:	
Margins		
Proximal margin	:	Normal / High grade intraepithelial neoplasia / Involved by carcinoma
Distal margin	:	Normal / High grade intraepithelial neoplasia / Involved by carcinoma
Circumferential margin		Involved (<1 mm) Not involved – mention distance of carcinoma to nearest circumferential margin in mm
Deep and mucosal / peripheral margins	:	For local excision specimens
Tumour regression grade	:	If applicable
Lymphovascular invasion	:	Present / Absent
Peri neural invasion	:	Present / Absent
Lymph nodes		
Number examined	:	
Number positive	:	
Distant metastases	:	
Completeness of excision	:	
Ancillary Studies	:	
Pathological staging (TNM stage)	:	
Any other comments	:	

Tables

Table 1. WHO Classification of tumours of the anal canal (WHO Classification of Tumours of the Digestive System, 5th edition)

▪ Squamous cell carcinoma NOS o Verrucous squamous cell carcinoma
▪ Adenocarcinoma NOS
▪ Neuroendocrine Tumour NOS o Neuroendocrine Tumour Grade 1 o Neuroendocrine Tumour Grade 2 o Neuroendocrine Tumour Grade 3
▪ Neuroendocrine carcinoma NOS o Large cell neuroendocrine carcinoma o Small cell neuroendocrine carcinoma
▪ Mixed Neuroendocrine-non-neuroendocrine neoplasms (MiNEN)

Table 2. Tumour Grade: anal canal squamous carcinoma (College of American Pathologists Protocols, February 2020)

Grade	Description
Grade X	Grade cannot be assessed
Grade 1	Well-differentiated
Grade 2	Moderately differentiated
Grade 3	Poorly differentiated

Note: If there are variations in the differentiation within the tumour, the highest (least favorable) grade is recorded as the overall grade.

Table 3: Non-invasive squamous lesions of the anal canal

These lesions are categorized based on a two-tiered nomenclature recommended by the Lower Anogenital Squamous Terminology (LAST) project.

LSIL - Low-grade squamous intraepithelial lesion	This includes ▪ lesions previously classified as mild dysplasia, anal intraepithelial neoplasia I (AIN I)) and squamous intra epithelial lesion I ▪ Condyloma acuminatum
HSIL - High-grade squamous intraepithelial lesion	This includes ▪ lesions previously classified as moderate and high-grade dysplasia, anal intraepithelial neoplasia II and III (AIN II & AIN III) ▪ Carcinoma in situ ▪ Bowen disease ▪ Bowenoid papulosis

Table 4. Tumour grades for primary anal adenocarcinomas (College of American Pathologists Protocols, February 2020)

Grade	Description
Grade X	Grade cannot be assessed
Grade 1	Well-differentiated (more than 95% of tumour composed of glands)
Grade 2	Moderately differentiated (50% to 95% of tumour composed of glands)
Grade 3	Poorly differentiated (less than 50% of tumour composed of glands)

Note: Colorectal cancers which extend to the anal canal, should be graded using the two-tiered system as recommended under colorectal cancer guidelines.

Table 5. Classification scheme for adenocarcinoma involving the anal canal. (CRC, colorectal carcinoma, IHC, Immunohistochemistry)

Anal adenocarcinoma		Histologic type	Mucin production	Typical IHC
Primary anal adeno-carcinoma	Mucosal Origin	Intestinal	Either mucinous or non-mucinous	CK7+/- CK20+ CDX2+ IHC panel may not be helpful
	Extra mucosal origin	Anal gland	Typically non-mucinous	
		Fistula associated (can be intestinal or anal gland)	Typically mucinous	
		Non anal gland non fistula associated	Either mucinous or non-mucinous	
Metastatic adeno-carcinoma	Colorectal carcinoma metastasis (can seed anal fistula	Intestinal	Either mucinous or non-mucinous	CK7+/- CK20+ CDX2+
	True Paget disease	Apocrine like	Typically non mucinous	CK7+ GCDFP -15 + CK20 – CDX2 -
	Skin adnexal (non-Paget type)	Skin adnexal	Typically non mucinous	CK7+ CK20 – CDX2 –
	Non CRC Metastasis	Variable	Variable	Variable

Table 6. Tumour Regression Grade - Modified Ryan scheme

Description of tumour	Regression Grade
No viable cancer cells (Complete response)	0
Single cells or small groups of cancer cells (Near complete response)	1
Residual cancer outgrown by fibrosis (Minimal response)	2
Minimal or no tumor kill; extensive residual cancer (Poor response)	3

Table 7. Pathologic stage for anal carcinomas (TNM/AJCC 8th Edition)

Primary Tumour (pT)	
pTX	Primary tumour not assessed
pT0	No evidence of primary tumour
pTis	Carcinoma in situ, Bowen disease, high-grade squamous intraepithelial lesion (HSIL), anal intraepithelial neoplasia II-III (AIN II-III)
pT1	Tumour 2cm or less in greatest dimension
pT2	Tumour more than 2cm but not more than 5 cm in greatest dimension
pT3	Tumour more than 5cm in greatest dimension
pT4	Tumour of any size invading adjacent organ(s) such as the vagina, urethra, or bladder *
Regional Lymph Nodes (pN)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Metastasis in regional lymph node/s
pN1a	Metastasis in inguinal, mesorectal, and /or internal iliac lymph nodes
pN1b	Metastasis in external iliac lymph nodes
pN1c	Metastasis in external iliac and in inguinal, mesorectal and/or internal iliac nodes
Distant Metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis

Note:

- This staging system applies to all epithelial carcinomas arising in the anal canal, including carcinomas that arise within anorectal fistulae and anal glands BUT excludes melanomas, low-grade neuroendocrine tumours (carcinoid tumours), and sarcomas
- Direct invasion of the rectal wall, perianal skin, subcutaneous tissue or the sphincter muscle/s alone is not classified as a T4 *
- Regional lymph nodes (N) - see above

References

1. Cancer incidence data, National Cancer control programme, Sri Lanka 2014.
2. World Health Organization Classification of Tumours. Pathology and Genetics. Tumours of the Digestive System, 5th edition. Lyon, France: IARC Press; 2019
3. Standards and datasets for reporting cancers. Dataset for histopathological reporting of for colorectal carcinoma. The Royal College of Pathologists, 2020.
4. Protocol for the Examination of Resection Specimens from Patients with Carcinoma of the Anus 4.1.0.0 Protocol, February 2020

CHAPTER 9

Gastrointestinal stromal tumours (GIST)

Introduction and epidemiology

Gastrointestinal stromal tumour (GIST) is a mesenchymal neoplasm characterized by differentiation towards the interstitial cells of Cajal. It is a tumour with a variable behaviour and can occur anywhere in the gastrointestinal tract as well as in other locations. The common anatomic areas include, stomach (54%), small intestine (30%), duodenum, jejunum, ileum, colon and rectum (5%) and oesophagus (1%). Extra gastrointestinal sites include omentum, mesentery and retroperitoneum where they may represent an undetected primary from the gastrointestinal tract. The incidence is 15 per million population per annum. In the stomach, GIST account for 2.2% of all malignant gastric tumours. Although most GISTs are sporadic, 5-10% of tumours are associated with a variety of syndromes. The peak age is around 50-60years with an equal incidence among males and females. Smaller GISTs are detected incidentally during endoscopy and imaging while patients with larger tumours present with vague abdominal symptoms and acute and chronic bleeding due to mucosal ulceration. Although there are other mesenchymal tumours of the gastrointestinal tract which include true smooth muscle tumours (eg: schwannomas, intra-abdominal fibromatosis, gastric glomus tumours, synovial sarcomas, inflammatory myofibroblastic tumours, plexiform fibromyxomas, calcifying fibrous tumours and melanomas)only the reporting guidelines of GISTs are discussed in this chapter.

Specimen handling

Grossing procedure

- Grossing technique is similar to grossing of tumours in oesophagus, stomach, small intestine, appendix and colorectum – Please refer the relevant section depending on the location of the GIST.
- If the tumour is very large, it can be serially sliced to facilitate fixation.
- The tumour should be serially sectioned at 5–10 mm intervals along the length of specimen.
- Areas of necrosis, haemorrhage or myxoid change should be noted.

  Note:
  - Areas of unusual gross appearance may also be seen following neoadjuvant therapy, including calcification and myxoid change.

- The slices should be laid out and examined.
- Ideally, the specimen should be photographed for histological correlation **[Y]**

Macroscopy

- **Specimen type**
  - Oesophagectomy
  - Gastro-oesophagectomy
  - Partial or total gastrectomy
  - Small intestine resection
  - Left or right hemicolectomy
  - Anterior resection
  - Abdominoperineal resection
  - Other - Specify
- **Tumour site**
  - Gastrointestinal
    - Oesophagus
    - Stomach
    - Small intestine
    - Colo-rectum
    - Other
  - Extra-gastrointestinal
    - Omentum
    - Mesentery
    - Pelvis
    - Retroperitoneum
- **Tumour size**
  - Maximum tumour diameter mm / cm
- **Macroscopic extension**
  - Mucosal ulceration
  - Depth of invasion
- **Tumour perforation**

  Note: This is an important prognostic factor associated with a high risk of recurrence intra-abdominally and is independent of size and mitotic count in predicting survival.
- **Distance to resection margins**
  - Proximal resection margin
  - Distal resection margin
  - Circumferential resection margin - where relevant

Block selection

Blocks to be submitted include:

- Resection margins: Longitudinal/end resection margin and circumferential resection margins
- Tumour
  - Adequate blocks to ensure sampling of all macroscopically different areas (e.g. areas of haemorrhage or myxoid change).
  - The number of blocks will depend on tumour size and presence of different macroscopically variable areas.
  - The recommended number of blocks is one block per centimetre of tumour diameter. **[Y]**

  Tumour blocks should include,

  – Suspected mucosal infiltration/ulceration
  – Possible blood vessel invasion
  – The closest circumferential margin
  – Involvement of any adjacent organs.
- A block containing tumour and adjacent mucosa/muscularis propria to be used as an internal control for immunohistochemistry. **[X]**
- A tumour block should be designated for molecular genetic analysis with a high number of tumour cells **[Z]**
- Normal /non-neoplastic tissue (If syndromic GIST is suspected, more blocks should be submitted)
- Any identifiable lymph nodes
  Note: Lymph node involvement is unusual except in paediatric cases and GISTs associated with Carney syndromes
- Blocks from any other macroscopic abnormality.
- Blocks from suspected synchronous metastases resected-e.g., liver or peritoneum.

Microscopy and conclusion

Tumour subtype	Table 1
Tumour grade	Refer grading of GIST in WHO Classification of Tumours of the Digestive System, 5th edition (currently in use in 2021) - Table 2
Mitotic count per 5 mm²	The total area to be counted should amount to 5mm². **Note:** ▪ This area should be established by the individual user for their microscope: – In older microscopes, 5mm² may be equivalent to 50 high-power fields (HPF). – In more modern microscopes, 5mm² may be equivalent to 20–25 high-power fields ▪ The Ki67 proliferation marker is useful to assess proliferation rate. **[Y]**
Mucosal invasion	Present / Absent
Haemorrhage	Present / Absent
Necrosis	Present / Absent
Mucosal ulceration	Present / Absent
Lymphovascular space invasion	Present / Absent
Resection margins	○ Involvement of proximal resection margin ○ Involvement of distal resection margin ○ Involvement of circumferential resection margin - where relevant **Note:** ▪ The mainstay of treatment for GISTs is surgery with wide local resection to include a margin of 10–20 mm for most tumours. ▪ Involvement of surgical margins is associated with a higher local recurrence. ▪ Radical resection with lymphadenectomy is usually not performed.

Response to treatment	**Note:** Changes associated with tumours treated with neoadjuvant therapy include loss of cellularity, with the formation of a loose myxoid stroma and a reduction in mitotic activity or Ki67 proliferation index with rare rhabdomyoblastic transformation.
Metastatic spread	
Immunohistochemistry	
▪ **CD117 (KIT) [X]**	Approximately 95% of GISTs are immunoreactive for KIT (CD117). This is usually strong and diffuse but can be focal in unusual cases. The stain is considered positive in the presence of diffuse cytoplasmic, membrane staining or dot-like perinuclear staining (Figure 1A.1B, 1C). In <5% of GISTs, it is negative (especially in GISTs with PDGFRA mutations). In these cases, DOG1 immunohistochemistry should be performed.

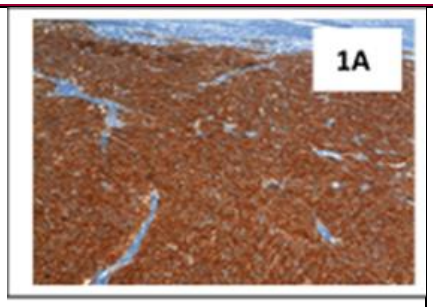

Figure 1A. CD117+ : Strong and diffuse cytoplasmic staining pattern

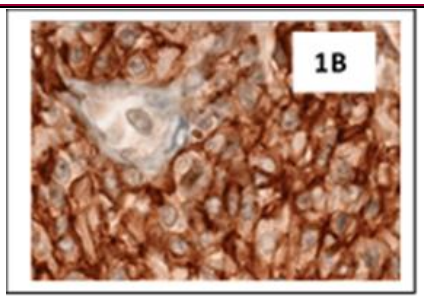

Figure 1B. CD117+ : Strong and diffuse membrane staining pattern

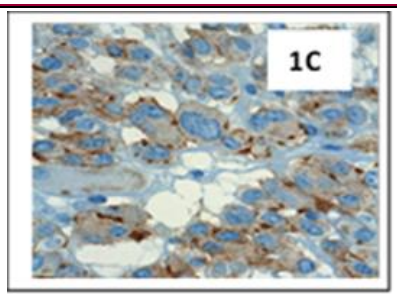

Figure 1C. CD117+ : Strong and diffuse perinuclear dot-like staining pattern

▪ **DOG1 [X – if CD117 is negative]**	Approximately 95% of GISTs are immunoreactive for DOG 1 Approximately 50% of KIT negative tumours are positive for DOG1
▪ **Other markers [Y]**	○ **CD34** – Spindle cell GISTS are usually positive ○ **Smooth muscle actin (SMA)** – 30% to 40% GISTS are positive ○ **S100** – 5% GISTS are positive (usually focal) ○ **Desmin** – 5% GISTS are positive (usually focal)

	○ **Keratin** – 1% to 2% GISTS are positive (weak/focal). **Note:** Other markers should be performed in the presence of CD117 and DOG1 negativity as it is important to distinguish GIST from leiomyoma, leiomyosarcoma, schwannoma, desmoid/ fibromatosis due to the therapeutic implications.
▪ **Mutational analysis - KIT and PDGFRA [Z]**	This should be done ideally in ○ All resected moderate and high-risk GISTs of any site ○ All biopsies diagnostic of GIST prior to neoadjuvant treatment ○ All biopsies from unresectable/widely metastatic GIST **Note:** Most KIT-negative GISTs are gastric or extra-visceral GISTs that are positive for the platelet-derived growth factor receptor A (PDGFRA) mutation.
▪ **Additional pathologic findings**	Specify
▪ **Pathologic staging**	Refer TNM staging of GIST (AJCC 8th TNM currently in use in 2021) - Table 3
▪ **Prediction of tumour behaviour / risk category**	Table 4 (Risk category - Armed Forces Institute of Pathology [AFIP] Lasota / Miettinen classification)

Annexure

Reporting proforma for gastrointestinal stromal tumours (GIST)

Gross description	
Specimen type	:
Site of tumour	:
Maximum tumour dimension	: _____ mm
Distance of tumour to nearest longitudinal resection margin	: _____ mm
Distance of tumour to closest circumferential resection margin	: _____ mm
Serosal perforation	: Present / Not identified
Microscopic description	
Tumour type	: Spindle / Epithelioid /Mixed cell type
Mitotic count	: _____ per 5 mm^2
Mucosal invasion	: Not applicable / Present / Not identified
Involvement of longitudinal margins	: Yes / No
Involvement of circumferential margins	: Yes / No
Features indicating a response to treatment	: Not applicable / Present / Not identified If present to be specified
Metastatic spread	: Yes / No
Lymph node involvement	
Number of lymph nodes present	:
Number of lymph nodes involved	:
Peritoneal metastasis	: Present / Not identified
Liver metastasis	: Present / Not identified
Other metastasis	: Specify
Immunohistochemistry	
CD117	: Positive / Negative / Not tested
DOG1	: Positive / Negative / Not tested
Risk category	: None / Very Low / Low / Moderate / High
Mutational analysis	: Positive / Negative / Not tested

Tables

Table 1. Tumour subtypes of GIST – RCPath UK dataset for histopathological reporting of gastrointestinal stromal tumours, January 2020

Spindle cell subtype (70%)
Epithelioid cell subtype (20%)
Mixed cell subtype (10%)

Table 2. Histological grading of GIST – WHO Classification of Tumours of the Digestive System, 5th edition

Tumour grade	Description
Low mitotic rate	Mitotic rate < 5 / 50 HPF
High mitotic rate	Mitotic rate > 5 / 50 HPF

Note: The mitotic rate of GIST is best expressed as the number of mitoses per 50 high power fields (HPF) using the 40x objective (total area 5mm^2 in 50 fields)

This area should be established by the individual user for their microscope (In older microscopes, 50 HPF is equivalent to 5 mm^2. In most modern microscopes with wider 40x lenses/fields, area equivalent to 5 mm^2 may include fewer fields and the field of view should be established for individual microscopes).

Table 3. Pathological stage for GIST - AJCC/TNM 8th edition

Primary Tumour (pT)	
pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pT1	Tumour 2 cm or less
pT2	Tumour more than 2 cm but not more than 5 cm
pT3	Tumour more than 5 cm but not more than 10 cm
pT4	Tumour more than 10 cm in greatest dimension
Regional lymph nodes (pN)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Regional node metastases
Distant metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis

Note:

- pM0 and pMx are not valid categories
- Metastasis to lymph nodes is rare therefore lymphadenectomy is performed in rare instances when an enlarged lymph node is encountered.
- If lymph nodes are not assessed clinically or pathologically, it should be categorized as N0 and not as Nx or pNx.
- Distant metastasis is predominantly to the liver or to the peritoneal surfaces.
- Lung and bone metastases are rare and is associated with very advanced disease.

Table 4. Relationship of mitotic count and tumour size to prognosis of gastrointestinal stromal tumour (GIST) based on United States Armed Forces Institute of Pathology (AFIP) data from large, long term follow up studies.

Category	Mitotic rate Per 50 high-power fields (HPF)	Size (cm)	Gastric	Small bowel
1	≤5	≤2	0%	0%
2		>2 - ≤5	1.9%	4.3%
3a		>5 - ≤10	3.6%	24%
3b		>10	12%	52%
4	>5	≤2	0	50%
5		>2 - ≤5	16%	73%
6a		>5 - ≤10	55%	85
6b		>10	86%	90%

Note: Prognostic assessment of GISTs of all non-gastric sites can follow the criteria for small bowel GISTs.

References

1. WHO Classification of Tumours of the Digestive System, 5th edition.
2. Royal College of Pathologists UK Standards and datasets for reporting cancers. Dataset for histopathological reporting of Gastrointestinal Stromal Tumours, January 2020
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4. Ricardo González-Cámpora, Mario Díaz Delgado, Alicia Hernández Amate, Sofía Pereira Gallardo, María Sánchez León Antonio López Beltrán. Old and New Immunohistochemical Markers for the Diagnosis of Gastrointestinal Stromal Tumors. Analytical and Quantitative Cytology and Histology, February 2011: volume 33 (1); pages 1-11

CHAPTER 10

Neuroendocrine neoplasms

Introduction and epidemiology

Neuroendocrine neoplasms (NENs) constitute a heterogenous group of tumours arising from neuro-endocrine cells which are thought to have either an epithelial or neuroectodermal origin. Gastrointestinal NENs (GI NENs) are mainly of epithelial origin.

NENs arising in the tubular gastrointestinal tract and pancreas are relatively rare. The annual incidence in the United States is approximately 3.56 per 100,000 population and the incidence has been rising over time, owing to increased detection. The distribution patterns of NENs in the GI tract is different between Eastern and Western populations. The most common location of NENs in the GI tract among patients in the United States is the small intestine (38% of GI NENs), followed by the rectum (34%). However, in Korea, the rectum (48%) is the most frequent location of NENs followed by the stomach (15%).

The classification and nomenclature of neuroendocrine neoplasms is complex and has undergone major changes over the last few decades. Currently, there are two widely used systems (ENET and WHO systems) for GI NEN classification and grading. There are only minor differences between these two systems.

The ENET staging system and WHO/UICC staging system are comparable for stomach, duodenum, jejunum, ileum, colon, rectum and pancreas, but not for the appendix. Differences are present for the T stage of appendiceal NENs. Other minor differences include the N status for the jejunum and ileum, which is subdivided by UICC/TNM 8 into N1 and N2 taking into consideration both the number of lymph node metastases as well as the presence and size of a mesenteric mass. There are also minor differences in the M category since the UICC staging subdivides this into M1a, M1b and M1c depending on the presence of metastases in the liver and extrahepatic sites.

The **WHO 2019 classification** includes the following changes (Table 1):

- **NENs** - These are separated into two main categories:
  1. Neuroendocrine tumour (NETs): A well-differentiated epithelial neoplasm with morphological and immunohistochemical features of neuroendocrine differentiation. NETs can be low-grade (G1), intermediate-grade (G2) or high-grade (G3) tumours (Table 2).

2. Neuroendocrine carcinomas (NECs): NECs are a poorly differentiated epithelial neoplasms with morphological and immunohistochemical features of neuroendocrine differentiation. NEC is, by definition, high-grade (for this reason, the WHO 2019 classification proposed not to assign a grade to NECs, whereas previously all NECs were graded G3). NEC is not defined by local vascular invasion or metastasis, but by tumour histology. (Mitoses >20 per 2 mm2 and/or Ki-67 >20%). NECS are subtyped as small cell NEC (SCNEC) and large cell NEC (LCNEC) (Table 2).

- **MiNEN** - This replaces the term MANEC (for mixed tumours) which was previously used in the WHO 2010 classification.
  Mixed neuroendocrine-non neuroendocrine neoplasms (MiNEN) is a mixed epithelial neoplasm in which a neuroendocrine component is combined with a non-neuroendocrine component, each of which is morphologically and immunohistochemically recognisable as a discrete component and constitutes ≥30% of the neoplasm. MiNENs may have a non-endocrine component other than adenocarcinoma (e.g. squamous cell carcinoma, acinar cell carcinoma or any other definable tumour category). Hence the use of the term MiNEN is recommended rather than the term MANEC, which indicates the presence of only an adenocarcinoma component. Different types of MiNENs arise in different sites throughout the gastroenteropancreatic (GEP) system (Table 3).

- **Goblet cell adenocarcinoma**
  According to the 5th Edition of WHO classification of tumours of the digestive system, the goblet cell tumours (previously called “goblet cell carcinoids”) of the appendix have been re-named “Goblet cell adenocarcinoma”.
  The staging of appendiceal goblet cell adenocarcinoma is identical to that of appendiceal adenocarcinoma.

WHO classification and grading of NENs 2019 and the UICC/TNM staging system is recommended in this guideline to standardize and maintain the uniformity of reporting NENs in Sri Lanka.

The clinical presentation and the behaviour of GI NENs varies with the anatomic site of origin. There are also site-specific histological, immunological and functional features of these tumours (Table 4a & 4b).

Specimen handling procedures for NENs of the gastro-intestinal tract are more or less similar to epithelial malignancies. Therefore, it is recommended that the the guidelines for specimen handing of the relevant sites (chapters) should be followed when dealing with a neuroendocrine neoplasm of the GI Tract. This

chapter highlights only the special points of specimen handing and reporting of NENs of the GI tract and excludes PanNENs (Pancreatic NENs).

Specimen handling

Grossing procedure

- Specimen handling for a GI NEN i.e stomach, small intestine including duodenum, jejunum, ileum, appendix and colorectum are as for carcinomas of these respective organs.
- The presence of multiple tumours should be recorded with their dimension.

Macroscopy

- Surgical procedures to resect NENs of GI tract are similar to that of epithelial malignancies in each organ.
- Therefore, it is advised to follow site-specific macroscopic description for the relevant surgical resection specimen. (Refer relevant sections)
- Small NENs of the stomach, duodenum, colon and rectum may be treated initially by polypectomy, endoscopic mucosal resection, endoscopic submucosal dissection or transanal endoscopic microsurgical excision (Chapter 10).
- Less commonly, more advanced tumours may undergo palliative local excision in debilitated patients and the specimen should be handled accordingly.

Block selection

The following blocks of tissue are recommended as minimum sampling:

- Blocks of the tumour to demonstrate
  - the deepest tumour penetration into or through the organ wall
  - involvement of the serosal surface
  - vascular invasion if suspected
  - involvement of adjacent organs.
- Tumour with any non-peritonealized resection margin, e.g. mesenteric margin (either in continuity with the main tumour mass or a separate extramural deposit of tumour in a lymph node, whichever is closest)
- To show the closest approximation of the tumour to the proximal or distal end resection margin.
- For endoscopic resection specimen handling - Chapter 10

- In an appendectomy specimen, appendicular and mesoappendicular margins should be sampled - Chapter 5
- A block to include tumour and the adjacent mucosa.
- Blocks from normal-appearing mucosa to include antral and corpus mucosa in case of gastric NENs.
- All lymph nodes identified should be submitted
- Sampling of any other macroscopic abnormalities
- Sampling of any additional organs in the resection

Microscopy and conclusion

Tumour Type

Tumour should be categorized using WHO 2019 GI-NEN classification (Table 6)

- Immunohistochemistry and other ancillary techniques are recommended in confirming the diagnosis of neuroendocrine tumours. **[X]**
- Specific markers that may be used to establish/confirm neuroendocrine differentiation include chromogranin A, synaptophysin, CD56 and NSE.
- It is recommended to use at least two neuro-endocrine markers before excluding the diagnosis of morphologically suspected case of NEN.

Tumour grade

Tables 2

Mitotic count and Ki 67 proliferation index should be evaluated for accurate grading of the tumour.

Mitotic rate

- It is recommended to assess the mitotic rate by evaluating at least 10 mm2 in the most mitotically active part of the tumour (divide the resulting number of mitoses by 5 to determine the number of mitoses per 2 mm2)
- Only clearly identifiable mitotic figures should be counted.
- Because of variations in field size in different microscopes, the number of high-power fields to be counted (40X magnification) for 10 mm2 must be determined for each microscope.

Ki 67 index %

- Ki-67 index is reported as percent positive tumour cells in areas of highest nuclear labeling ("hot spot")
- Usually counting is done by "eyeballing". It is recommended to count 2000 cells and give the value as a percentage.
- However, for tumours with Ki-67 index close to grade cut-offs, it is recommended to perform the manual count on the print of camera-captured image of the hot spot. **[Z]**
- Usually, the grade assigned based on Ki-67 index is higher than that grade based on mitotic count. In such instance, the case is assigned to the higher grade.
- It is recommended to do Ki 67 proliferation index in all cases of a NEN except if mitotic rate is very high / > 20/2mm2 on H & E sections.

Maximum extent of local invasion	
Serosal involvement	
Margin involvement	
Lymph node status	Number present, number involved
Lymphovascular invasion	
Perineural invasion	
Tumour necrosis	Present / Absent

Histologically confirmed distant metastases and site	
Background abnormalities	e.g., o Stomach: Atrophic gastritis, Intestinal metaplasia of gastric mucosa, Glandular dysplasia of gastric mucosa, Endocrine cell hyperplasia, Absence of parietal cells o Appendix: Acute appendicitis
Completeness of resection	
Stage	TNM 8th edition stage: Table 6

Annexure

Reporting proforma for GI NENs

Gross description		
Type of specimen	:	
Site of tumour	:	
Specimen dimensions	:	_____ mm
Tumour perforation	:	
Number of tumours	:	Solitary or multiple
Maximum tumour dimension	:	_____ mm
Resection margin status	:	End resection margins and non-peritonealised margins
Relation of the tumour to the peritoneal reflection	:	Rectal tumours only
Distance of the tumour from the dentate line	:	For abdominoperineal excisions only. _____ mm
Tumour adhesions:	:	
Microscopic description		
Tumour type	:	
Histological grade	:	including the mitotic rate and/or proliferation index
Maximum extent of local invasion	:	
Serosal involvement	:	
Margin involvement	:	
Lymph node status	:	Number present, number involved
Lymphovascular invasion	:	Yes / No
Perineural invasion	:	Yes / No
Histologically confirmed distant metastases and sites	:	
Background abnormalities	:	e.g., Enterochromaffin-like (ECL) cell or G cell hyperplasia in stomach
Pathological staging	:	TNM 8th edition

Tables

Table 1: Comparison of the WHO 2010 GEP (Gastroenteropancreatic) -NEN classification with WHO 2019 GI-NEN classification

WHO 2010 GEP-NEN classification	WHO 2019 GI-NEN classification
Well-differentiated NETs: • NET G1 · NET G2	**Well-differentiated NETs:** • NET G1 · NET G2 · NET G3
Poorly differentiated NECs: • NEC G3 (large cell or small cell NEC)	**Poorly differentiated NECs:** • NEC (large cell or small cell NEC)
MANEC	**MiNEN**
Hyperplastic and pre neoplastic lesion	**Abolished, but recognised in gastric NENs**

Note: The 2010 WHO classification used the terms 'high-grade' and 'poorly-differentiated' interchangeably for tumors in the G3 category. Recent studies have challenged this by demonstrating that the G3 category is in fact heterogeneous, comprising of two different subgroups: ie: well-differentiated NETs with a high proliferative rate and true poorly differentiated NECs (small-cell or large-cell types). A lower response rate for platinum based systemic chemotherapy but a longer median overall survival in patients in the former group established the heterogeneity of the G3 category leading to this change in classification.

Table 2: WHO Classification and grading criteria for of neuroendocrine neoplasms of the GI Tract in WHO Classification of Tumours of the Digestive System, 5th edition

Terminology	Differentiation	Grade	Mitotic rate (mitoses/2mm$^{2)}$	Ki-67 index
NET, G1	Well Differentiated	Low	<2	<3%
NET, G2		Intermediate	2-20	3-20%
NET, G3		High	>20	>20%
NEC, small cell type (SCNEC)	Poorly Differentiated	High	>20	>20%
NEC, small cell type (SCNEC)			>20	>20%
MiNEN	Well or poorly differentiated	Variable	Variable	Variable

Table 3: Specific subtypes of mixed neuroendocrine-non neuroendocrine neoplasms (MiNENs) of the GI Tract - WHO Classification of Tumours of the Digestive System, 5th edition

Oesophagus and oesophagogastric junction ▪ Mixed *SCC-NEC (SCNEC or LCNEC) ▪ Mixed adenocarcinoma -NEC (SCNEC or LCNEC) ▪ Mixed adenocarcinoma –NET
Stomach, appendix, colon and rectum ▪ Mixed adenocarcinoma -NEC (SCNEC or LCNEC) ▪ Mixed adenocarcinoma - NET
Small intestine and ampulla ▪ Mixed adenocarcinoma -NEC (SCNEC or LCNEC)
Anal Canal ▪ Mixed SCC-NEC (SCNEC or LCNEC)

SCC = Squamous cell carcinoma

Table 4 (a and b). Site specific feature of GI NENS

Table 4a. Gastric well-differentiated neuroendocrine tumours.

Gastric well-differentiated NENs are divided into 3 types:

	Type 1	**Type 2**	**Type 3**
Demographic Profile	70-80% are females in their 50s and 60s	Equally in males and females. Mean age 50 years	More common in males, mean age 55 y
Frequency	80-90% of cases	5-7%	10-15%
Multiplicity	Multifocal (small nodules or polyps)	Multifocal	Solitary
Location	Corpus	Corpus	Anywhere in stomach
Size	0.5-1.0cm	1.5cm or less	Variable; one third are larger than 2cm
Hypergastrinaemia	Present	Present	Absent

Acid secretion	Low or absent	High	Normal
Association	Chronic atrophic gastritis, often autoimmune	Multiple neuroendocrine type 1 (MEN-1) with Zollinger-Ellison syndrome	Sporadic
Background gastric mucosa	Enterochromaffin like (ECL) cell hyperplasia, partial or complete loss of parietal cells, intestinal metaplasia	Parietal cell hyperplasia; ECL cell hyperplasia	Usually normal
Grading	G1 G2 (rare) G3 (exceptional)	G1 G2 (rare)	G1 (rare) G2 G3 (rare)
Clinical behaviour	Usually indolent: may regress, ~100% 5-year survival Lymph node metastasis very rare	10-30% metastasize	71% of tumours >2cm with muscularis propria and vascular invasion have lymph node metastases

In addition to the above 3 types, the WHO 5th Edition has included 3 rare variants:

1. Serotonin-producing enterochromaffin (EC)-cell neuroendocrine tumours, which have morphologic features similar to those of ileal EC cell neuroendocrine tumours.
2. Gastrin-producing G-cell neuroendocrine tumours and gastrinomas
3. Somatostatin-producing D-cell neuroendocrine tumours.

Note:

- Benign/preneoplastic neuroendocrine proliferations are identified and described only for gastric NENs. **[Y]**

- **ECL-cell hyperplasia**
  - Simple (diffuse): defined as an increased number (more than two times greater than normal values) of endocrine cells, otherwise retaining their normal distribution
  - Linear or chain forming: defined as linear sequences of at least five cells along the basement membrane and at least two chains per millimeter length of mucosa
  - Micronodular: defined as clusters of five or more cells (size 30–150 μm), either within glands or the deep aspect of the lamina propria, and at least one micronodule per millimeter length of mucosa
  - Adenomatoid: defined as at least five adjacent micro nodules with intervening basal membrane in the lamina propria.

- **ECL-cell dysplasia**
  - This is defined as large confluent micro nodules of ECL-cells lying deep in the mucosa, ranging from 150 to 500 μm in size.

Table 4b. Well-differentiated neuroendocrine tumours of the GI tract other than the stomach

	Duodenum including ampulla	**Jejunal and ileal**	**Appendix**	**Colorectal**
Epidemiology	4% of all NENs of GI tract	Most common site for GI NENs.	5th most common GI neoplasm. Highest incidence before 40years. Slight female predomi-nance	Prevalence higher in Asians. Often in the 6th – 7th decade of life

Localization	>95% are in the D1 and D2 Somatostatin expressing NET and paraganglio-cytic para-ganglioma are located exclusively in the ampullary region.	Most common site in the GI tract is the ileum.	Tip of the appendix	Most NETs are common in the rectum.
Clinical features	Most are asymptomatic. Non-functional. Present as Tumour – mass effects (e.g. jaundice). *or* Functional effects – see below	Intestinal obstruction, bowel ischemia, intermittent abdominal pain.	Incidental In 1.86% of the appendicect omies	Clinically silent or associated with bleeding and pain. Rectal NENs may present as polyps.
Associations	Gastrinomas are associated with Zollinger – Ellison Syndrome MEN -1	Carcinoid syndrome (About 50% have liver metastasis)	-	Carcinoid syndrome (In serotonin producing EC cell NETs)
Size	Usually small <2cm	-	<2cm	Average – 4.9cm (Larger than intestinal NETs)
Multiplicity	-	25- 40% cases	-	-
Histology / Subtypes	See Table 6			

Specific IHC findings / other	Somatostatin expressing NENs; less likely to express chromogranin	Ileal serotonin producing EC cell NETs: CDX2 positive	Diffusely and strongly positive for Synato-physin, Chromo-granin A S100 positivity in spindle cells surrounding cell nests	**EC cell NET** – Diffusely, strongly positive for Synatophysin, Chromo-granin A, CDX2 and Serotonin. **L-cell NET** – Positive for Synapto-physin. Only focally positive for Chromograni n A **Rectal NET** – Positive for Prostatic acid phosphatase (PAP)

Table 5. Special / rare variants of neuroendocrine tumours in the GI tract

Duodenum	The rare functional NETs of the duodenum are as follows: ▪ **Duodenal gastrin-secreting neuroendocrine tumours / gastrinomas** o Most common functional tumour o Associated with Zollinger-Ellison syndrome and multiple endocrine neoplasia type 1 (MEN1). o Sporadic tumours also occur. ▪ **Duodenal somatostatin-producing tumours (Somatostatinomas)** o Less common o Seldom associated with the functional syndrome of mild diabetes mellitus, cholelithiasis and steatorrhoea. o These tumours often have a pure glandular growth pattern with scattered psammoma bodies. o Associated with neurofibromatosis type 1. ▪ **Duodenal Gangliocytic paraganglioma** o Rare neuroendocrine tumour with a distinctive histology composed of 3 components: S-100-positive spindle cells, ganglion cells, and paraganglioma.
Appendix	▪ **L-cell neuroendocrine tumours of the appendix** o A distinctive growth pattern of tear-drop-shaped tubules embedded in a fibrous stroma is seen and are sometimes called tubular neuroendocrine tumours. o It should be noted that these tumours are negative for chromogranin A. o Tubular neuroendocrine tumours are usually small lesions confined to the appendix.

Table 6. WHO Classification of neuroendocrine neoplasms of the digestive system, WHO Classification of Tumours of the Digestive System, 5th edition

Oesophagus

- Neuroendocrine tumour NOS
  - Neuroendocrine tumour - Grade 1
  - Neuroendocrine tumour - Grade 2
  - Neuroendocrine tumour - Grade 3
- Neuroendocrine carcinoma NOS
  - Large cell neuroendocrine carcinoma
  - Small cell neuroendocrine carcinoma
- Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN)
  - Combine small cell-adenocarcinoma
  - Combined small cell-squamous cell carcinoma

Stomach

- Neuroendocrine tumours NOS
  - Neuroendocrine tumour- Grade 1
  - Neuroendocrine tumour- Grade 2
  - Neuroendocrine tumour- Grade 3
  - Gastrinoma NOS
  - Somatostatinoma NOS
  - Enterochromaffin –cell carcinoid
  - ECL-cell carcinoid, malignant
- Neuroendocrine carcinoma NOS
  - Large cell neuroendocrine carcinoma
  - Small cell neuroendocrine carcinoma
- Mixed neuroendocrine-non-neuroendocrine carcinoma (MiNEN)

Small Intestine

- Neuroendocrine tumours NOS
  - Neuroendocrine tumour- Grade 1
  - Neuroendocrine tumour- Grade 2
  - Neuroendocrine tumour- Grade 3
  - Gastrinoma NOS
  - Somatostatinoma NOS
  - Enterochromaffin –cell carcinoid
  - Extra-adrenal paraganglioma NOS
- Neuroendocrine carcinoma NOS
  - Large cell neuroendocrine carcinoma
  - Small cell neuroendocrine carcinoma
- Mixed neuroendocrine-non-neuroendocrine carcinoma (MiNEN)

Appendix

- Neuroendocrine tumour NOS
  - Neuroendocrine tumour- Grade1
  - Neuroendocrine tumour, Grade2
  - Neuroendocrine tumour, Grade3
  - L-cell tumour
  - Glucagon-like peptide producing tumour
  - PP/PYY producing tumour
  - Enterochromaffin-cell carcinoid
  - Serotonin- producing carcinoid
- Neuroendocrine carcinoma NOS
  - Large cell Neuroendocrine carcinoma
  - Small cell Neuroendocrine carcinoma
- Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN)

Colon and Rectum

- Neuroendocrine tumour NOS
  - Neuroendocrine tumour, Grade1
  - Neuroendocrine tumour, Grade2
  - Neuroendocrine tumour, Grade3
  - L-cell tumour
  - Glucagon-like peptide producing tumour
  - PP/PYY producing tumour
  - Enterochromaffin-cell carcinoid
  - Serotonin- producing carcinoid
- Neuroendocrine carcinoma NOS
  - Large cell neuroendocrine carcinoma
  - Small cell neuroendocrine carcinoma
- Mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN)

Table 7. TNM staging for well – differentiated neuroendocrine tumours (G1, G2 and G3) of the gastrointestinal tract - gastric, jejunum / ileum, appendix, colonic and rectal (TNM 8th edition)

GASTRIC TUMOUR	
Primary Tumour (pT)	
pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pT1	Tumour invades the mucosa or submucosa and 1cm or less in greatest dimension
pT2	Tumour invades the muscularis propria or greater than 1 cm in size
pT3	Tumour invades subserosa
pT4	Tumour perforates visceral peritoneum (serosa) or other organs or adjacent structures
Regional Lymph Nodes (pN)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Regional lymph node metastasis
Distant Metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis
pM1a	Hepatic metastasis only
pM1b	Extrahepatic metastasis only
pM1c	Both hepatic and extra hepatic metastases

DUODENAL AND AMPULLARY TUMOURS	
Primary Tumour (pT)	
pTX	Primary tumour cannot be assessed
T0	No evidence of primary tumours
pT1	Duodenal: tumour invades the mucosa or submucosa only and 1cm or less in greatest dimension Ampullary: tumour ≤1 cm and confined within the sphincter of Oddi
pT2	Duodenal: Tumour invades the muscularis propria or >1 cm in greatest Dimension Ampullary: Tumour invades through sphincter into duodenal submucosa or muscularis propria, or >1 cm in greatest dimension
pT3	Tumour invades the pancreas or peripancreatic adipose tissue
pT4	Tumour perforates the visceral peritoneum (serosa) or invades other organs

Regional lymph nodes (pN)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node involvement
pN1	Regional lymph node involvement
Distant Metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis
pM1a	Hepatic metastasis only
pM1b	Extrahepatic metastasis only
pM1c	Hepatic and extrahepatic metastases

JEJUNAL AND ILEAL TUMOURS

Primary Tumour (pT)	
pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pT1	Tumour invades the mucosa or submucosa and 1cm or less in greatest dimension.
pT2	Tumour invades the muscularis propria or greater than 1 cm in size
pT3	Tumour invades through the muscularis propria into subserosal tissue without penetration of the overlying serosa (jejunal or ileal)
pT4	Tumour perforates visceral peritoneum (serosa) or other organs or adjacent structures
Regional lymph nodes (pN)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Less than 12 regional lymph nodes metastasis without mesenteric mass(es) greater than 2cm in size
pN2	12 or more regional lymph nodes and /or mesenteric mass(es) greater than 2cm in maximum dimension
Distant Metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis
pM1a	Hepatic metastasis only
pM1b	Extrahepatic metastasis only
pM1c	Hepatic and extrahepatic metastases

TUMOURS OF THE APPENDIX

Primary Tumour (pT) *	
pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pT1	Tumour 2 cm or less in greatest dimension
pT2	Tumour more than 2 cm but not more than 4cm in greatest dimension
pT3	Tumour more than 4 cm or with subserosal invasion or involvement of the mesoappendix
pT4	Tumour perforates the peritoneum or directly invades other adjacent organs or structures other than direct mural extension to adjacent subserosa of adjacent bowel, eg, abdominal wall and skeletal muscle **

Regional Lymph Nodes (pN)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastasis
pN1	Regional lymph node metastasis

Distant Metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis
pM1a	Hepatic metastasis only
pM1b	Extrahepatic metastasis only
pM1c	Both hepatic and extra hepatic metastases

Note:

- High grade neuroendocrine carcinoma, mixed adenoneuroendocrine carcinomas and goblet cell carcinoid are excluded and should be classified according to criteria for classifying carcinomas. *
- Tumours that is adherent to other organs or structures macroscopically, is classified T4. However, if no tumour is present in the adhesion microscopically, the tumour should be classified as pT1-3 as appropriate. **

COLORECTAL TUMOURS

Primary Tumour (pT)	
pTX	Primary tumour cannot be assessed
pT0	No evidence of primary tumour
pT1	Tumour invades the lamina propria or submucosa or is no greater than 2cm in size
pT1a	Tumour less the 1cm in size
pT1b	Tumour 1 to 2cm in size
pT2	Tumour invades the muscularis propria or is >2 cm in size
pT3	Tumour invades the subserosa or non peritionealized pericolic or perirectal tissue.
T4	Tumour perforates the visceral peritoneum (serosa) or invades other organs

Regional Lymph Nodes (pN)	
pNX	Regional lymph nodes cannot be assessed
pN0	No regional lymph node involvement
pN1	Regional lymph node involvement

Distant Metastasis (pM)	
pM0	No distant metastasis
pM1	Distant metastasis
pM1a	Hepatic metastasis only
pM1b	Extrahepatic metastasis only
pM1c	Both hepatic and extrahepatic metastases

Note:

- Staging system only applies to well-differentiated neuroendocrine tumors of the GI tract (NET G1, G2 and G3).
- For any T add (m) for multiple tumours
- Poorly differentiated neuroendocrine carcinomas; Small cell (SCNEC) and large cell neuroendocrine carcinoma (LCNEC)) and tumors with mixed glandular/neuroendocrine differentiation (MiNEC) should be staged using the staging system of epithelial tumours of the relevant organs.
- As well-differentiated neuroendocrine tumours of the oesophagus and the anus are so rare, a separate staging system is not warranted.

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CHAPTER 11

Endoscopic resections (ER)

Introduction

Transanal endoscopic microsurgery (TEM) was introduced in 1983 to treat rectal lesions including benign tumours, early rectal carcinoma, rectal fistula and strictures. This, minimally invasive technique, offered the advantages of superior visualization and easier access to the lower margins of the lesion with lower morbidity, fewer long-term recurrences and a faster cure.

The increasing use of gastrointestinal endoscopy, coupled with the advent of technological advances such as chromo-endoscopy and narrow band imaging, has increased the detection of early neoplastic lesions. Although these lesions are precancerous in most cases, the possibility of early invasion needs exclusion. It has been proven that endoscopic biopsies are not entirely suitable for this, as there is a substantial rate of histological upstaging from biopsies to adequately resected specimens. Thus, endoscopic resections (ER) was pioneered in Japan in 2003. ER has also been shown to be an adequate treatment modality for patients with early gastrointestinal cancers with little or no submucosal involvement and no additional risk factors.

Most superficial gastrointestinal neoplasias may be treated by means of endoscopic mucosal resection (EMR). However, EMR is unsuitable for en bloc resection of lesions larger than 20mm or for non-lifting lesions. To overcome these limitations, endoscopic submucosal dissection (ESD) was developed. ESD enables the operator to achieve an en bloc resection regardless of the tumour size. However, ESD is technically demanding and associated with a higher risk of adverse events.

ERs have thus become an approved treatment for effective removal of non-invasive lesions and mucosal or superficial submucosal invasive lesions of the oesophagus, stomach, duodenum and colon. In addition, some submucosal lesions, such as neuroendocrine tumours can also be treated by ER.

Although lymph nodes are not included in ERs, there are important pathological risk factors predictive of lymph node metastasis that could be assessed in ERs. For invasive lesions, poor differentiation, lymphovascular invasion and deep invasion are factors associated with a higher risk of lymph node metastasis in early neoplastic lesions. Margin involvement is associated with residual disease/recurrences. Therefore, these factors determine the curability of ERs.

An important component to achieve optimum results and outcomes in endoscopically resected lesions is the accurate pathological assessment because this has a significant impact on therapeutic decisions, follow-up and survival. Applying a standard protocol for handling, grossing, staging and reporting of these ER specimens is therefore critical to provide a consistent and accurate diagnosis and follow-up of the patient by the clinician.

Specimen handling

Please refer section on clinician's contribution to optimizing reporting of gastrointestinal (GI) tract tumours on endoscopic resections in this book and National guidelines on Collection, Transport and Handling of Surgical Specimens 2021; chapter 2A.

Grossing procedure

- ER (either EMR or ESD) should be pinned out on a cork board or polystyrene or wax block and oriented by the endoscopist, before dispatch to the laboratory (Fig. 1).
- The distal and proximal margins of the specimen may also be identified / oriented as oral and anal margin or by the 12 0' clock position only.
- The peripheral edges (lateral / horizontal margins) of the specimen needs meticulous pinning
- The mucosal side should face upwards
- The specimen should be pinned out by stretching the specimen out to an approximate the length taking care regarding the following;
    - Not to overstretch the margins so as to cause tissue damage and distortion.
    - To include the entire thickness of the specimen
    - To place the pins closely to prevent curling and retraction of the margins in between the pins during fixation.
    - Not to pin through the tumour.
- This pinned specimen should then be floated upside down (mucosal surface downwards) in 10% neutral buffered formol saline
    - The specimen should be marked as to the presence of sharps

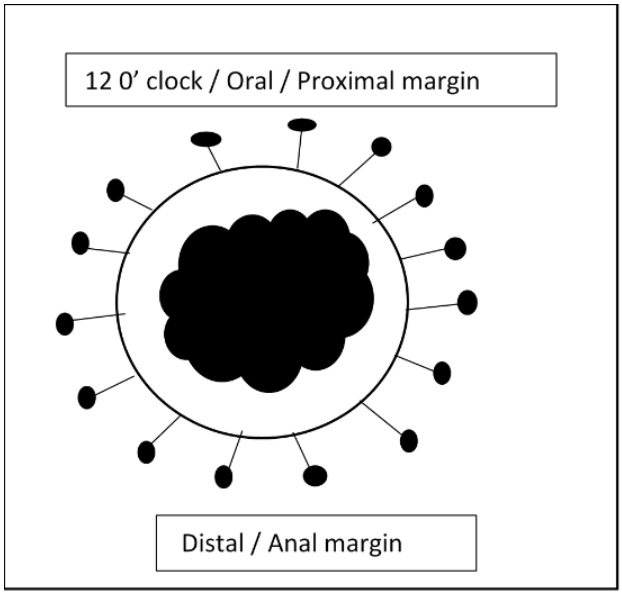

Figure 1. Pinning of the specimen

- The clinician or endoscopist should provide information regarding the following.
  - Location of the lesion
  - Size of lesion
  - Possibility of submucosal invasion
  - Ulcer scar or biopsy site.
- If the specimen is not oriented or fixed as given above, the pathologist should attempt to pin it on a cork board as soon as possible following the steps outlined above. The guidance of the endoscopist should be sought in orienting the specimen if possible.
  - Sometimes the specimen is received piece meal in which case such orientation is not possible.
  - The specimen should be fixed for 12- 72 hours.

Macroscopy

- Measurement should be carried out optimally in the fresh state. **[Y]**
- The specimen should be measured in three dimensions.
  - If several pieces are received all pieces should be measured and processed separately.
- Any visible lesions on the surface of the resection specimen should be described as polypoid, elevated, flat or depressed.
- The distance to the nearest lateral / peripheral / horizontal margin should be measured.
- The margins of the specimen should be inked as follows:

- For unoriented specimens - One colour of ink should be applied to the lateral and deep margins.
- For oriented specimens - Ink is applied at the peripheral / lateral / horizontal margins differentially. (Fig. 2A)

Block selection

- The specimen should then be cut at 2 -3mm intervals parallel to the direction of the closest margin. (Fig. 2a).

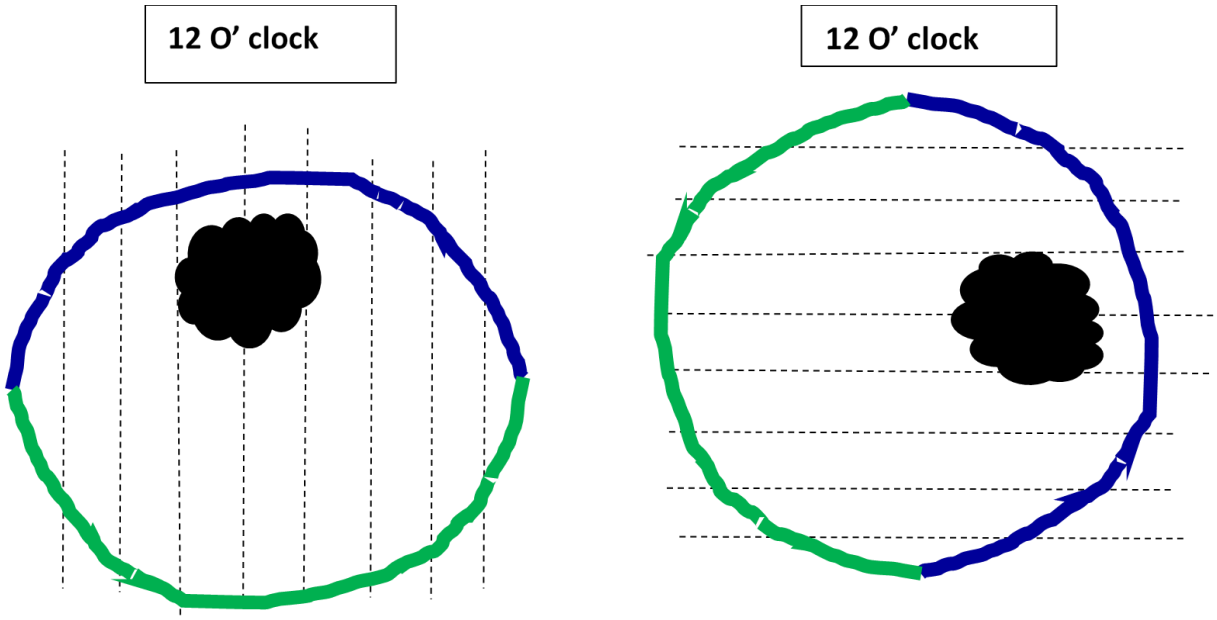

Figure 2a. When the tumour is asymmetric the specimen should be sectioned at 2-3 mm intervals parallel to the direction of the closest margin.

- The specimen should be sectioned along the long axis (Figure 2b) in the following cases
  - Negative margins are obvious and approximately similar
  - No gross lesion is identified
  - Lesion appears to involve all the margins
- Perpendicular sections of the first and last slices are then taken to ensure complete evaluation of the margins (Note: if the sample is small this may not be practical.

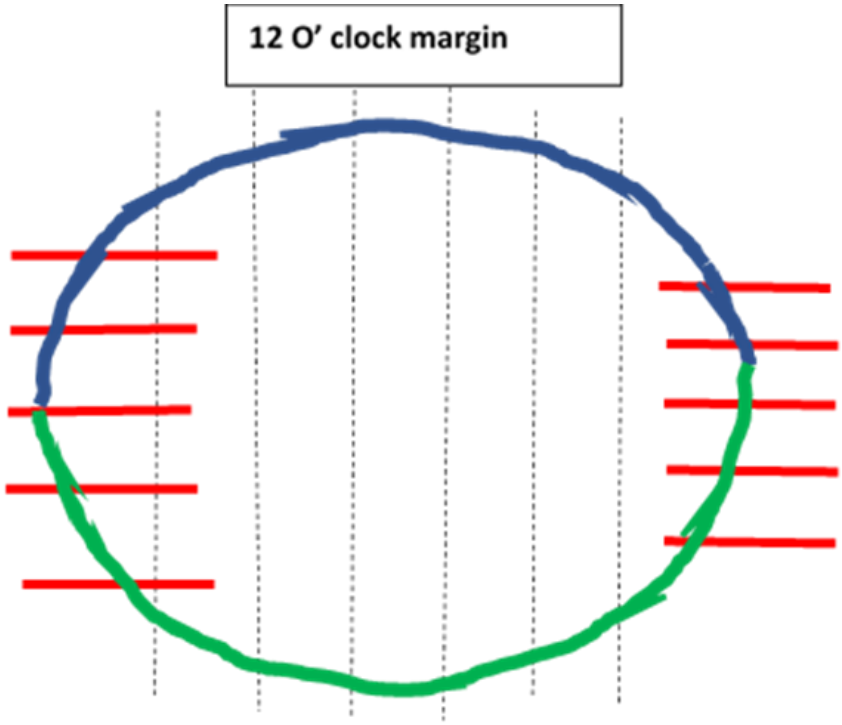

Figure 2b. Sectioning of the specimen when the margins are negative, obvious and approximately similar, no gross lesion is identified, lesion appears to involve all the margins.

Note:

- The entire sample is submitted for histology
- Each of these slices are submitted in cassettes sequentially and placed en face into the cassette.
- The slices are placed into the cassettes starting with slice 1.
- Ideally, more than three slices should not be placed into one cassette.
- The first sliced piece is inverted so the exterior margin side will be cut first in the block.
- The maintenance of orientation is crucial between the histopathologist and histotechnologist.
- Detailed photographs or diagrams with associated block key will help map out the lesion and involved margins accurately.
- Larger tissue slices should be divided taking care that the main lesion or a portion suspected of having an invasive component into the submucosa, or a site of an ulcer scar is not transected by such a division.

Microscopy and conclusion

Histological tumour subtype	See the relevant sections for the histological types.
Tumour grade	See the relevant sections for histological grading.
Size of the tumour	The size of the tumour needs to be confirmed histologically since the macroscopy may under- estimate the size. **Note:** It is the size of the invasive portion of the tumour that is important in determining whether the patient needs further therapy.
Depth of invasion	Depth of invasion should be recorded in microns, in association with a descriptor to the deepest layer of the wall involved (i.e. mucosa / lamina propria or muscularis mucosae / submucosa) regardless of the site. Tumour depth is stated as follows in gastric and oesophageal resections. **pT1a** - Invasion of the lamina propria and muscularis mucosae **pT1b** - Invasion of the submucosa

Oesophageal specimens

- Two subclassifications for the depth of intramucosal invasion have been proposed for oesophageal adenocarcinomas that divides the mucosa into three (M1/M2/M3) or four levels (M1/M2/M3/M4).
- The second of these classifications takes the duplicated muscularis mucosae into account. This is important because the latter forms a rich lymphovascular network contributing to the possibility of lymph node metastases.
- The depth of invasion below the deepest point of muscularis mucosae is used to determine the SM stage.
- The SM1, SM2, SM3 staging is mostly used for oesophageal adenocarcinomas.
  - SM1: > 0 – 500 µm
  - SM2: > 500 – 1000 µm
  - SM3: > 1000 µm
- A cut-off of 200 µm for SM1 has been suggested for squamous cell carcinomas, reflecting the more biologically aggressive nature of this tumour

Gastric endoscopic resections

- pT1b is classified into two categories:
  - pT1b1 - tumour depth of less than 0.5 mm from the lower edge of muscularis mucosae
  - pT1b2 - tumour depth of 0.5 mm or more from the lower edge of muscularis mucosae

  (This is because the risk of lymph node metastases is significantly higher in the pT1b2 category.)
- When the muscularis mucosae is obscured it is recommended to identify the muscularis mucosae by using immunohistochemistry for desmin **[Y]**
- The depth of invasion is only determined in cases with a negative vertical margin.
- In cases with a positive vertical margin, the findings should be described, for example, as follows: "at least pT1b2 / SM2 : 1200 micrometers from the muscularis mucosae".
- The depth of submucosal invasion may be assessed by SM1, SM2 and SM3 staging as given above.

Colorectal specimens

- There are differences in the evaluation of submucosal invasion with regard to pedunculated lesions and non-pedunculated lesions.
- When it is possible to identify the muscularis mucosae, the depth of submucosal invasion is the distance from the deeper edge of the muscularis mucosae to the deepest invasive portion.
- When the muscularis mucosae cannot be identified,
  - In non – pedunculated lesions - the depth of submucosal invasion is the distance between the surface of the tumour and the deepest invasive portion.
  - In pedunculated tumours, the depth of submucosal invasion is the distance between the deepest invasive site and the reference line, defined as the boundary between the tumour head and the pedicle.
  -

Evaluation of the surgical margins	**Horizontal margin (peripheral / lateral margin)** ▪ In the case of a negative horizontal margin, the distance (mm) to the closest margin should be recorded. ▪ in the case of an un-oriented specimen, it is recommended that the number of sections with positive margins should be described.

- In an oriented specimen the location of the positive margin should be recorded.
  Note: In cases with diathermy artefact, it is sometimes difficult to judge the horizontal margin and immunohistochemical staining of p53 and Ki-67 may be useful. **[Y]**

Vertical / deep margin

- When the deep margin is negative, the distance of the tumour cells to the closest vertical / deep margin should be recorded.
- In cases with positive vertical margins both the positive location within the wall (i.e. lamina propria or submucosa) and the distance from the lower edge of the muscularis mucosae to the positive margin should be recorded. (see above – depth of invasion)

Vessel invasion	Record the presence or absence of lymphatic permeation and/or vascular invasion **Note:** Since lymphatic invasion can be present even in pT1a tumours, careful histological assessment is mandatory.
Additional features to be reported	**Oesophageal resection specimens** ▪ Tumour margins (expansive or infiltrative) ▪ Barrett mucosa **Gastric resection specimens** ▪ Ulceration ▪ Chronic gastritis, intestinal metaplasia or identification of H pylori. **Colorectal resection specimens** ▪ Tumour budding or sprouting (Follow the same guidelines given in the chapter of colorectal carcinoma) ▪ Presence of precursor adenomas. ▪ Any other features with increased risk of dysplasia or malignancy, e.g. IBD **Note:** The identification of the muscularis propria should be notified to the endoscopist / clinician immediately as it implies a risk of perforation.

Ancillary investigations	**Cytokeratin** ▪ To identify subtle infiltrating cells such as those in poorly differentiated adenocarcinoma e.g. signet ring cells ▪ To differentiate poorly differentiated adenocarcinoma from squamous cell carcinoma in the oesophagus. ▪ To identify distorted tumour cells at the cauterized margins. **Smooth muscle markers (Desmin)** ▪ To highlight the vessel wall in cases of suspected vascular invasion. ▪ To assess the depth of invasion when the muscularis mucosae is obscured due to fibroblastic proliferation. **Vascular markers** ▪ To demonstrate vascular and lymphatic invasion (eg: CD34 or CD31 and D2-40) **Neuroendocrine markers** ▪ To confirm or exclude neuroendocrine differentiation in poorly differentiated carcinomas

Annexure

Reporting proforma for endoscopic resections

Gross description	
Type of specimen	: Endoscopic mucosal resection (EMR) / Endoscopi submucosal dissection (ESD)
Specimen site	: Oesophagus / Stomach / Duodenum / Colorectum
Specimen integrity	: Intact or piecemeal
Specimen orientation	: Oriented / Unoriented
Mounted prior to fixation	: Yes / No
Specimen size	: _____ mm
Lesion visible or not	: Visible / Not visible If visible: ▪ Tumour configuration ▪ Measurements of the lesion ▪ Measurement to the closest horizontal margin
Distance of the tumour from the dentate line	: For abdominoperineal excisions only.
Tumour adhesions	:
Microscopic description	
Histological tumour type	:
Histological grade	:
Size of the tumour	: Greatest dimension of the Invasive portion of the tumour if applicable.
Lymphovascular invasion	: Present / Absent
Depth of invasion	: Given in µm : Lamina propria/ muscularis mucosa / submucosa
Involvement of margins	: **Vertical / deep margin** – Negative / Positive If negative - specify distance from point of deepest invasion to the deep margin If positive - specify distance from the lower edge of the muscularis mucosae **Lateral margins** – Negative / Positive If negative - specify distance to the closest margin (specify the margin if oriented)

	If positive – specify location of positive margin (in an oriented specimen) or number of positive slices (in an unoriented specimen)
Tumour stage	:
Additional features	:

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CHAPTER 12

Polyps of the Gastrointestinal Tract

Introduction

Gastrointestinal (GI) polyps are defined as any discrete tissue masses protruding above the colonic mucosa into the gut lumina. They can occur anywhere in the GI tract but most commonly in the colorectum. They can be pedunculated, sessile protuberant, slightly elevated, flat or even depressed on macroscopy. The endoscopic classification of polyps is shown in Fig. 1. They are further divided into non-neoplastic and neoplastic groups. Histological subtypes are given in table 1.

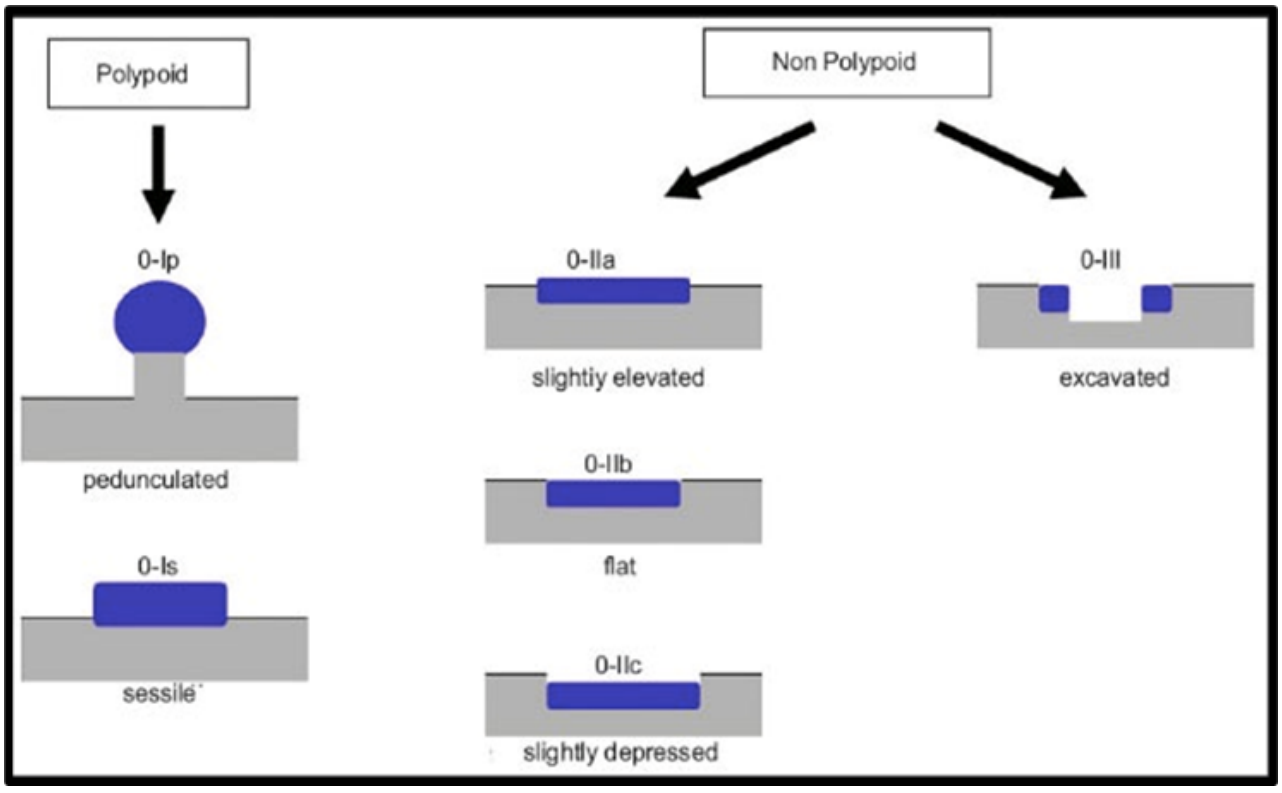

Figure 1. Paris endoscopic classification of superficial neoplastic lesions

Specimen handling

Grossing procedure – Polypectomy

- The surgical resection margin should be inked.
  Note: Ideally the base of the polyp should be marked with a hypodermic needle or ink by the endoscopist.

- **Small polyps < 10mm** (Figure 2A)
  - These should either be blocked as a whole or bisected through the base.

- **Polyps > 10mm** (Figure 2B)
  - These specimens should be sectioned in a plane which passes through the polyp base and demonstrates the full length of the polyp stalk (where present).
  - The polyp is sliced serially and embedded from edge to edge in strict sequence.

- Each block should be examined at a minimum of 3 levels **[Y]**

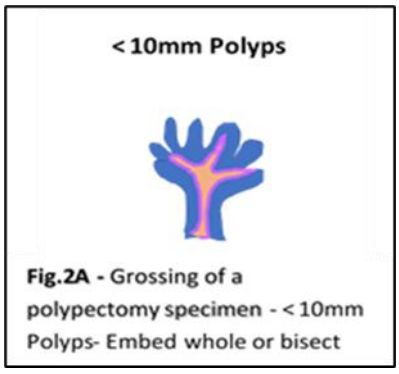

Fig.2A - Grossing of a polypectomy specimen - <10mm Polyps- Embed whole or bisect

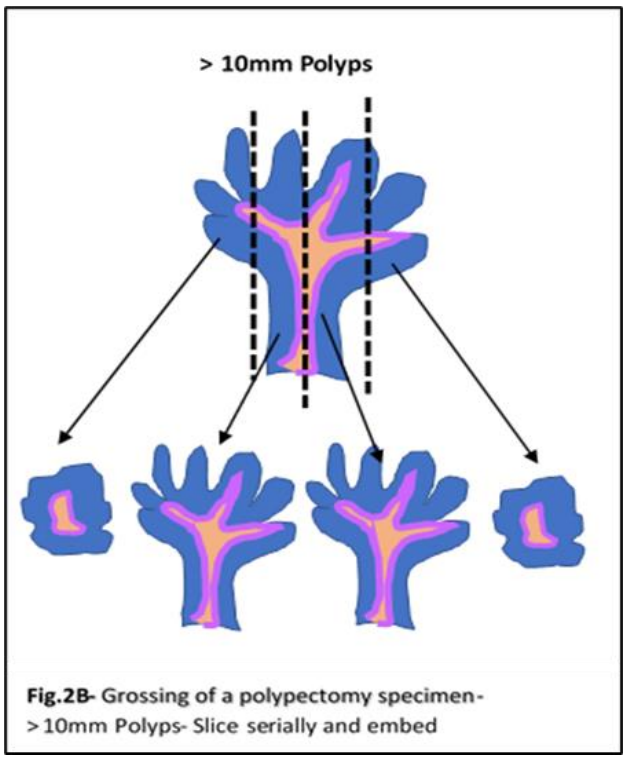

Fig.2B- Grossing of a polypectomy specimen- >10mm Polyps- Slice serially and embed

Figure 2. Grossing of a polypectomy specimen

Macroscopy

- **Type of specimen:** Polypectomy
- **Number of polyps submitted**
- **Configuration:** Describe the colour, appearance (smooth, lobulated, fronded etc.), whether pedunculated or sessile.
- **Tumour size**
  - If pedunculated, measure the head of the polyp separately
  - If sent piecemeal, the diameter of the largest fragment should be measured separately, and the rest should be measured in aggregate.

Block selection

- The entire specimen should be sampled in all cases of polypectomy.

Microscopy and conclusion

Histological tumour type	Table 1
Degree / Grade of dysplasia	Low or high grade
Presence of an Invasive malignancy	Carcinoma in a polyp is classified according to pT definitions used for colorectal carcinoma; i.e. invasion into lamina propria or incomplete invasion into muscularis mucosa is considered pTis. A tumour that has invaded the submucosa of the head or stalk of the polyp is considered pT1.
Resection margins	
Lymphovascular invasion	Lymphovascular invasion has been associated with the possibility of lymph node metastasis and therefore the need for further surgery.
Depth of invasion	Maximum depth of invasion beneath the muscularis mucosa is of prognostic importance. If the muscularis mucosa is destroyed or not identifiable, the thickness of the invasive tumour should be assessed.
Tumour stage	Figures 3 and 4: Haggitt level / Kikuchi level **Note:** When a fragmented polyp is received, the maximum dimension of invasive carcinoma present in any given fragment should be measured.
Ancillary investigations	CD34 and D-2 40 in the presence of equivocal vascular or lymphatic invasion.

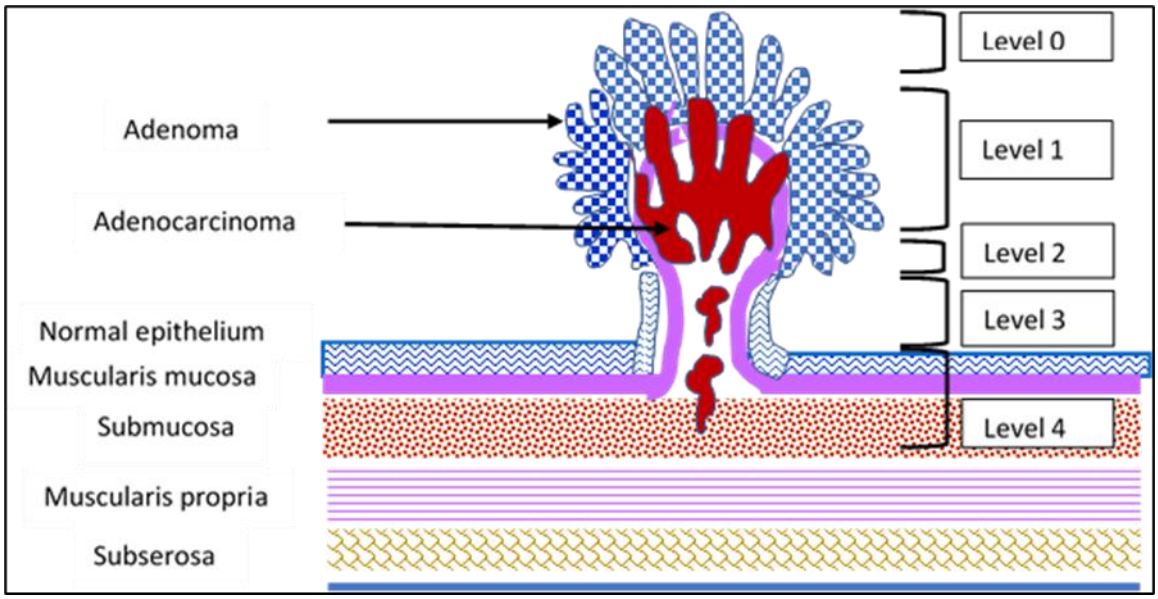

Figure 3. Haggitt classification of malignant pedunculated polyps

Level 0	-	Non-invasive (severe dysplasia)
Level 1	-	Invading through the muscularis mucosa but limited to the head of a pedunculated polyp
Level 2	-	Invading the neck of a pedunculated polyp
Level 3	-	Invading the stalk of a pedunculated polyp
Level 4	-	Invading into the submucosa below the stalk of a pedunculated polyp

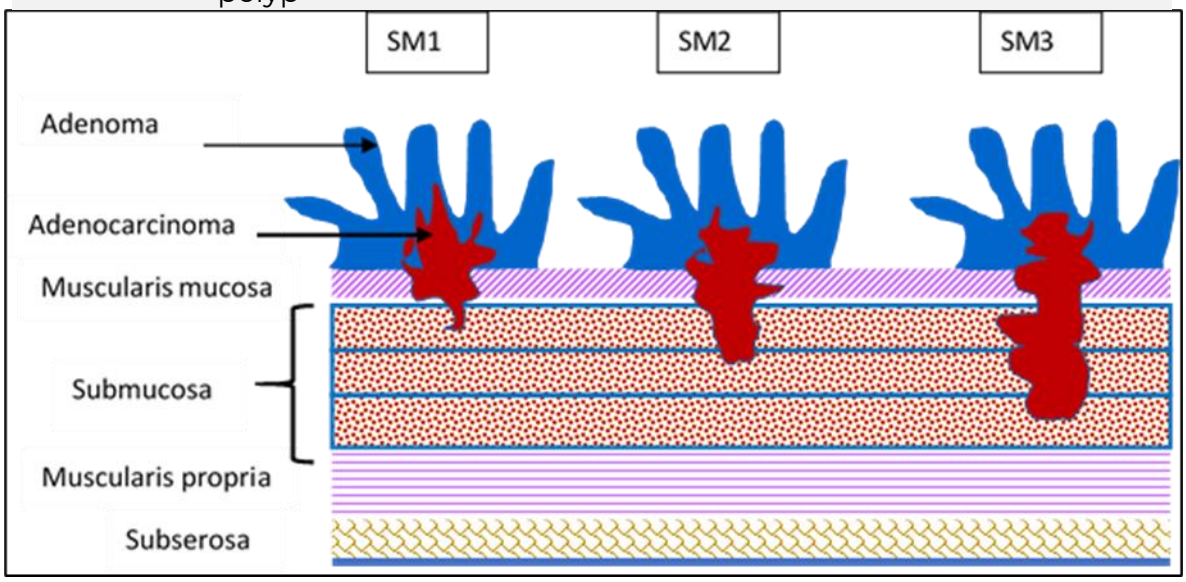

Figure 4. Kikuchi classification of malignant sessile polyps

Depth of invasion into submucosa:		
Sm1	-	Non-invasive (severe dysplasia)
Sm2	-	Invasion into upper one-third of submucosa
Sm3	-	Invasion into upper two-thirds of submucosa
Level 4	-	Invasion into lower one-third of submucosa

Annexure

Reporting proforma

Site of polyp	:	
Type of polyp	:	
Number of polyps	:	
Polyp size	:	_____ mm
Dysplasia	:	Present / absent. If present, mention the grade of dysplasia – low / high
Invasive component	:	Present or absent If present, maximum depth of invasion beneath the muscularis mucosa or the thickness of the invasive tumour.
Lymphovascular invasion	:	Present or absent
Tumour stage	:	Haggitt or Kikuchi classification (Figures 3 and 4)
Completeness of resection	:	

Tables

Table 1. Histological types of polyps – WHO Classification of Tumours of the Digestive System, 5th edition

▪ Conventional adenoma (low grade dysplasia or high grade dysplasia) o Tubular o Tubulovillous o Villous o Rare subtypes – Paneth cell rich subtype
▪ Hyperplastic polyp o Microvesicular hyperplastic polyp o Goblet cell rich hyperplastic polyp
▪ Serrated adenoma o Traditional serrated adenoma (TSA) o Sessile serrated lesion (SSL) o Sessile serrated lesion with dysplasia (SSLD)
▪ Mixed polyp (specify components)
▪ Carcinoma
▪ Neuroendocrine tumour
▪ Hamartomatous polyp
▪ Inflammatory polyp
▪ Juvenile type polyp
▪ Mesenchymal polyp o Fibroblastic polyp (Perineurioma) o Schwannoma o Neurofibroma o Ganglioneuroma o Leiomyoma o Lipoma o Granular Cell Tumour o Inflammatory Fibroid Polyp o Gastrointestinal Stromal Tumour
▪ Mucosal prolapse syndrome
▪ Other (specify)

- Advanced adenoma - a term used for international comparison. This group includes adenomas with:
  - Size >10mm
  - Tubulovillous or villous architecture
  - High grade dysplasia or intramucosal carcinoma

  These adenomas have a very significant effect on cancer prevention programmes as they are associated with a high risk of both synchronous and metachronous adenomas which are indications for a complete colonoscopic examinations or more stringent surveillance.

References

1. Rodriguez-Justo M, Novelli M. Polyps in the Bowel Cancer Screening Programme. Cut up Protocols. University College London Hospitals NHS Foundation Trust, London, 2016.
2. Loughrey MB, Quirke P, Shepherd N. Dataset for Colorectal Cancer Histopathology reports. The Royal College of Pathologists, London, 2014.
3. Kakar S et al. Protocol for the examination of specimens from patients with primary carcinoma of the colon and rectum. College of American Pathologists, 2017.
4. WHO Classification of Tumours of the Digestive System, 5th edition, 2019.