

Formula One Study

UK–Japan Joint Study for Risk Factors of Lymph Node
Metastasis in Submucosal Invasive (pT1) Colorectal Cancer

Assessment criteria of
pathological parameters

Ver.2

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2. List of abbreviations

CRC	colorectal cancer
F1 study	UK–Japan Joint Study for Risk Factors of Lymph Node Metastasis in Submucosal Invasive (pT1) Colorectal Cancer
JSCCR	Japanese Society for Cancer of the Colon and Rectum
LNМ	lymph node metastasis
MUC	mucinous adenocarcinoma
NPG	nonpolypoid growth
PDC	poorly differentiated cluster
PG	polypoid growth
POR	poorly differentiated adenocarcinoma
SIG	signet-ring cell carcinoma
UK	United Kingdom

3. Assessment of the growth of tumours

3.1 Japanese endoscopic classification (Fig 1)

The endoscopic appearance of tumours (including adenoma’s component) are to be divided into protruded (polypoid) and superficial (tumours with or without minimal elevation or depression relative to the surrounding mucosa) tumour.¹

Protruded tumours are categorized as pedunculated (Ip), subpedunculated (Isp) or sessile (Is) type. Superficial tumours are categorized as slightly elevated tumour (IIa), tumours without elevation or depression (IIb) or slightly depressed tumours (IIc). If the tumour is composed of two different types, the type occupying the largest area is recorded first, followed by ‘+’ and the other type (e.g. IIa+IIc).

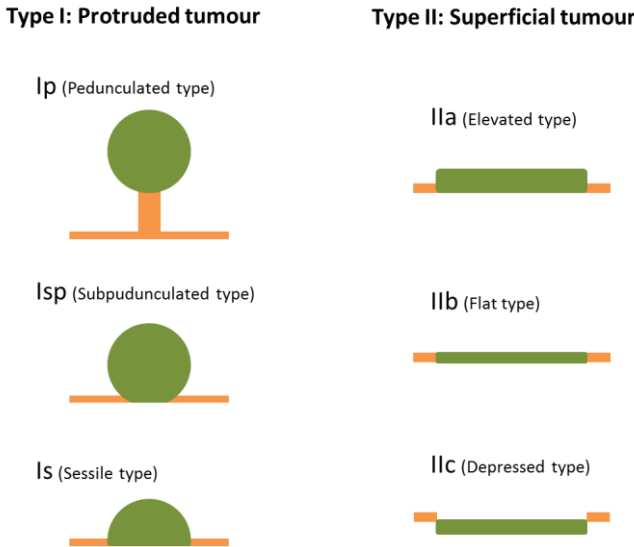


Fig 1. Japanese endoscopic classification of early invasive colorectal tumour

3.2 Pathological classification of the growth type of tumour (Fig 2)

Histologically, tumors are to be classified into two types: polypoid growth (PG) carcinoma from intramucosal proliferation of adenoma or carcinoma, and nonpolypoid (NPG) growth carcinoma without intramucosal protuberant growth.²

Furthermore, PG carcinomas are to be classified into pedunculated lesions and sessile or broad-based lesion.

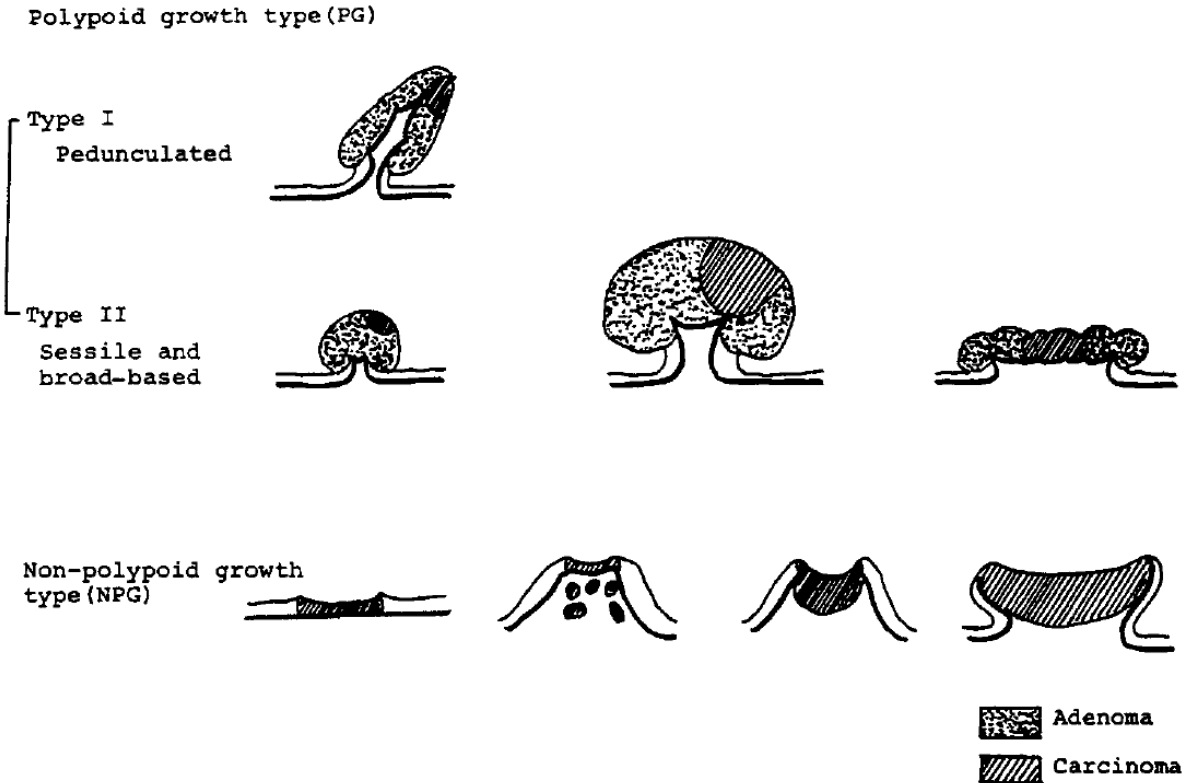


Fig 2. Pathological classification of the growth type of early invasive tumours

4. Assessment criteria for new pathological risk parameters

4.1 Lymphatic vessel invasion

Lymphatic vessel invasion is defined as the invasion of the tumor cells into the lymphatic vessels, and is judged as positive only when tumor cells were identified within endothelial-lined space³⁾. Lymphatic lacks muscular wall and red blood cells, which are features of blood vessels. In submucosa, normal lymphatic vessels spread along the muscularis mucosae. And tumor spreading through lymphatic vessel can be found in lymphatic vessels around the tumor (Fig 3. A, B).

4.2 Blood vessel invasion

Blood vessel invasion is defined as the invasion of the tumor cells into the blood vessels³⁾. Blood vessel invasion is assessed as positive when tumor cells were within the endothelial-lined space, often containing red blood cells, and/or were surrounded by a rim or of the smooth muscle (Fig 4. A)⁴⁾. The structure of blood vessels is often disrupted through the process of tumor's involvement and the vessel wall can be totally replaced by fibrous tissue (Fig 4. B)⁵⁾. Even though, as mentioned above, only definite lesions in H.E staining should be assigned as positive.

Note

Problem in the diagnosis of small vessel invasion

pT1 colorectal cancers often shows small vessel invasion. Definite differentiation between small blood vessel invasion and lymphatic vessel invasions can be difficult. Therefore, based on pathological findings in this instruction, participants in this study are required to discriminate lymphatic and blood invasion as far as possible. Only definite lesions should be assigned as positive.

Problem of the space around tumor nests

Shrinkage artefacts and mucous lake often exhibit tissue space around the tumor nests. On the other hand, these lesions must be discriminated from lymphatic or blood vessel invasion. Note that nuclei of endothelial cells protrude into the vascular space which often contains lymphatic fluid or red blood cells^{6,7)}. In colorectal cancers, shrinkage artefacts are often seen around tumor area with budding, poorly differentiated clusters, or micropapillary component. Shrinkage seemed to be developed as a result during processing. This space tends to occur along one margin of tumor nest, and counter of the surrounding stroma is much similar with that of tumor nests. Spicula of slender connections often bridge the space formed by shrinkage (Fig 5. A). Nuclei of stromal cells may be left at the margin of surrounding stroma of shrinkage artefact. They have slender nuclei that do not protrude into the surrounding stroma (Fig 5. A, arrow, and compare with Fig 3. B, arrow)⁷⁾. Mucous lake is not lined by endothelial cells (Fig 5. B).

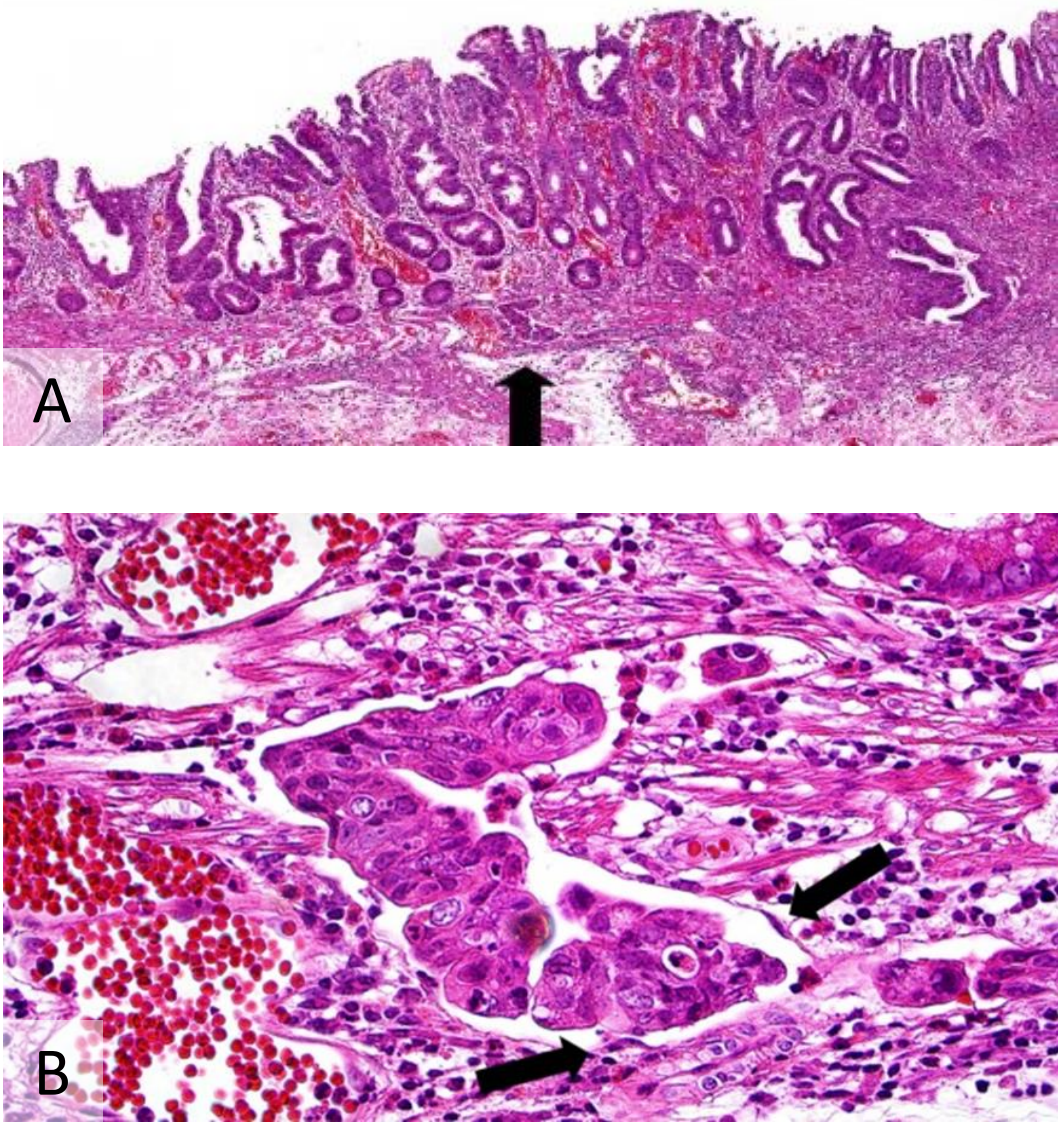


Fig 3. Lymphatic vessel invasion

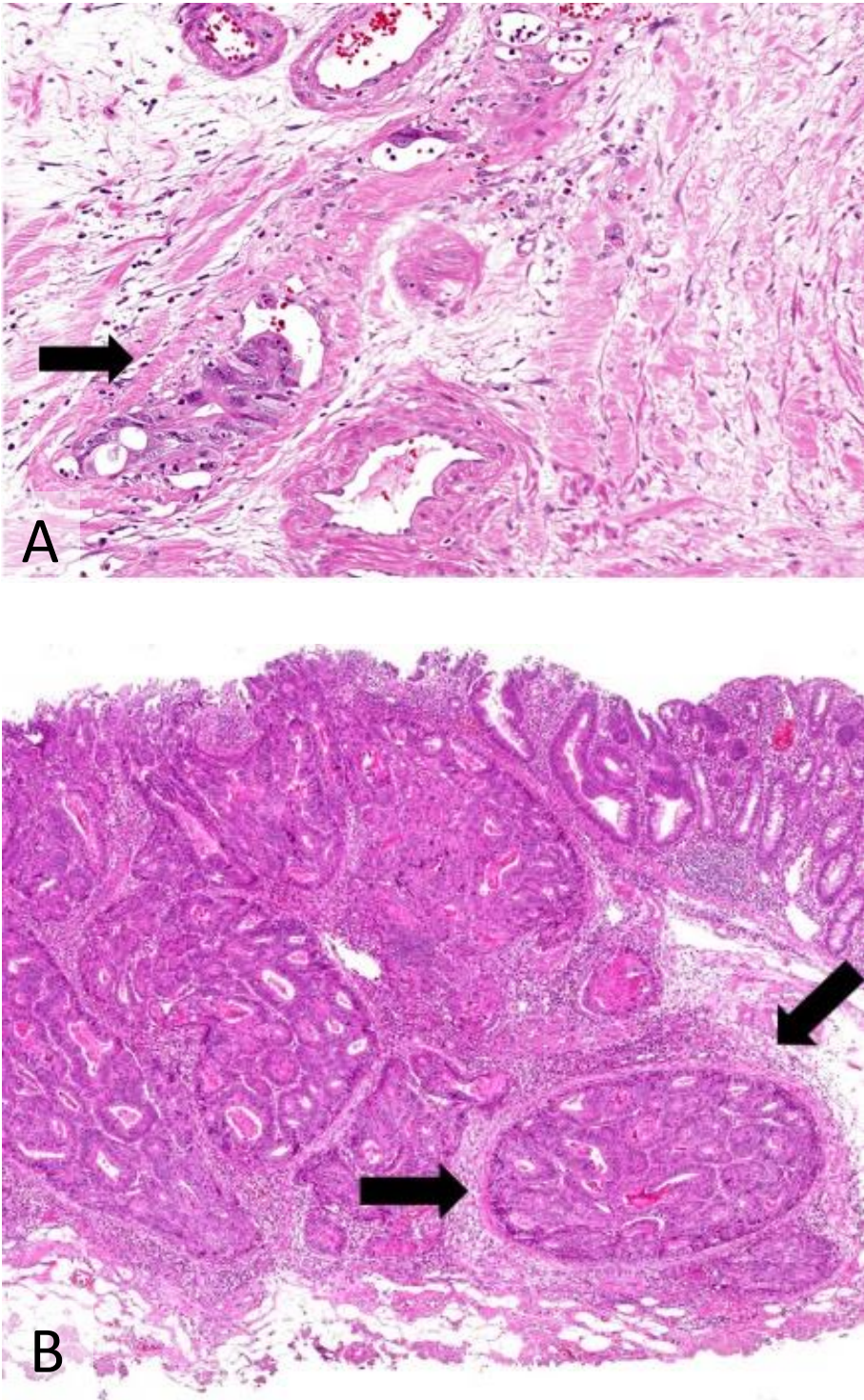


Fig 4. Blood vessel invasion

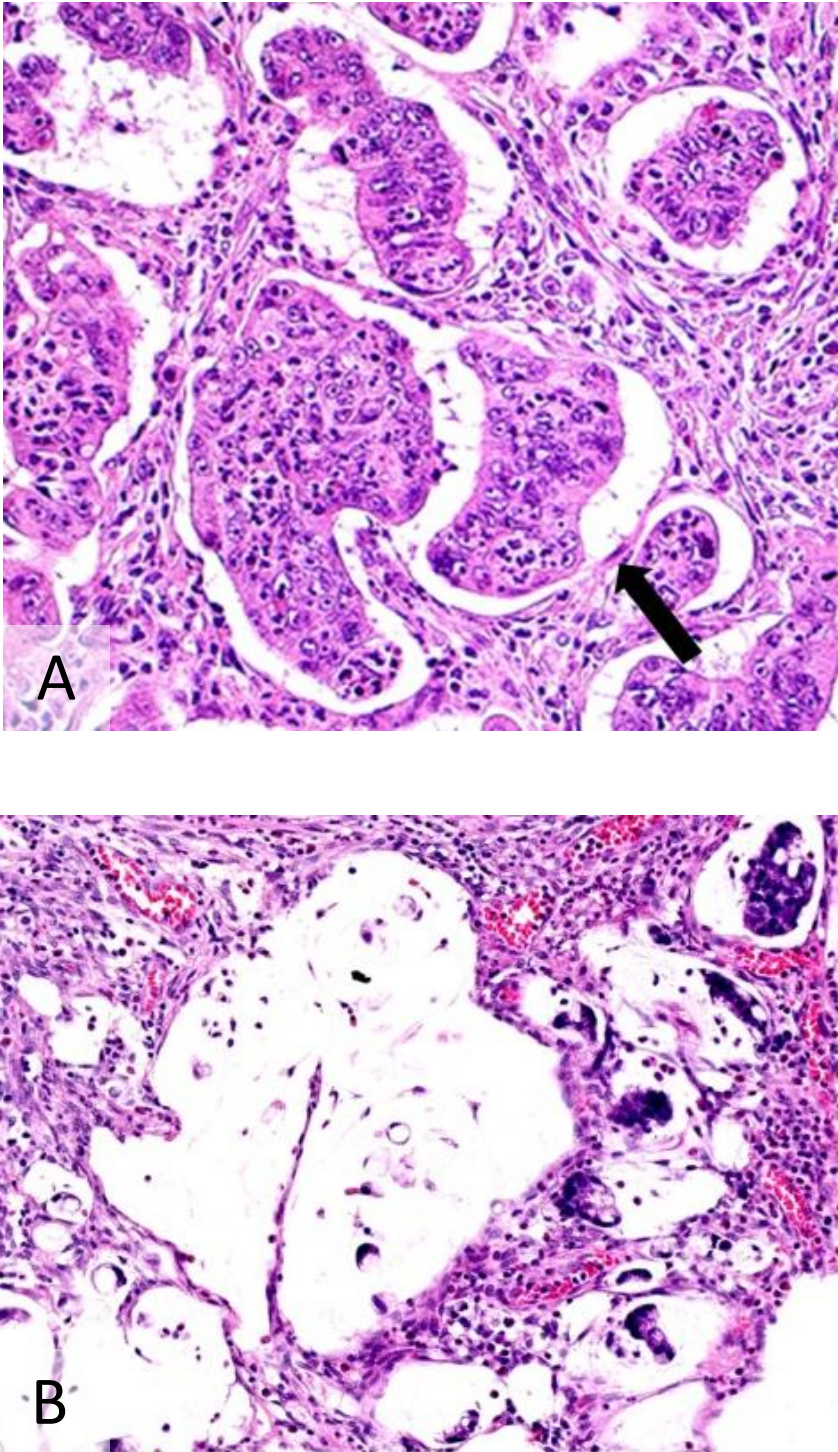


Fig 5. space around tumor nests

5. Assessment criteria for new pathological risk parameters

5.1 Tumour budding

The assessment of tumour budding is basically performed according to the recommendations of the International Tumour Budding Consensus Conference (ITBCC) 2016.⁸⁾

Tumour budding is defined as a cancer cell nest consisting of 1 or <5 cells that have infiltrated the interstitium at the invasive margin of the cancer (Fig 6).⁹⁻¹²⁾

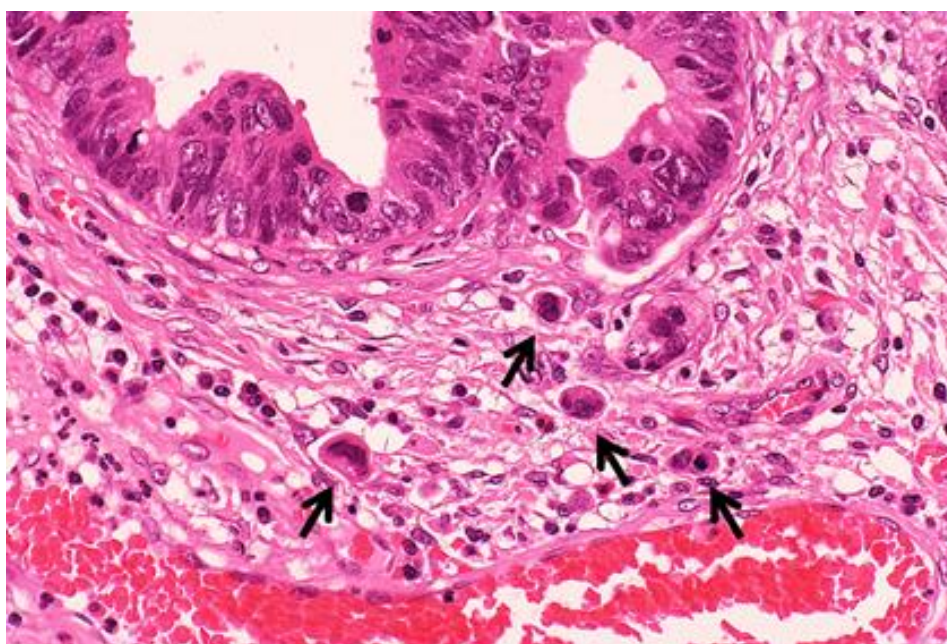


Fig 6. Tumour budding

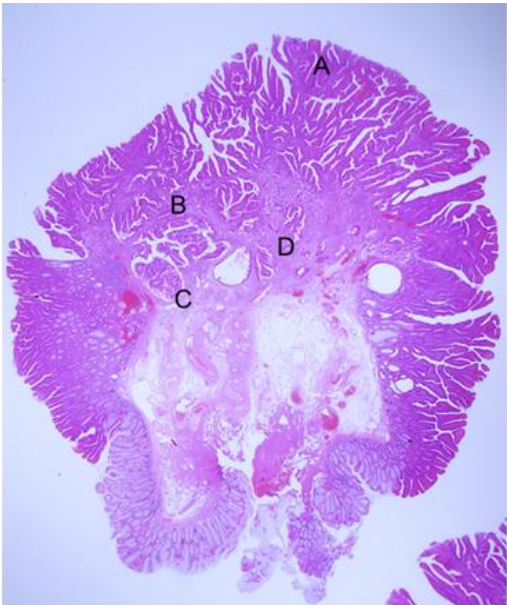
For grading of budding, the number of buddings is counted in a field observed through a 20× objective lens (WHK 10× ocular lens; 0.785 mm²) after selecting one field wherein the number of buddings is the greatest (Fig 7).¹⁰⁻¹²⁾ Depending on the number of buddings, the grade of budding is defined as follows:

Grade 0: 0

Grade 1: 1 to 4

Grade 2: 5 to 9

Grade 3: ≥10



Grade 0 : 0
Grade 1 : 1–4
Grade 2 : 5–9
Grade 3 : ≥10

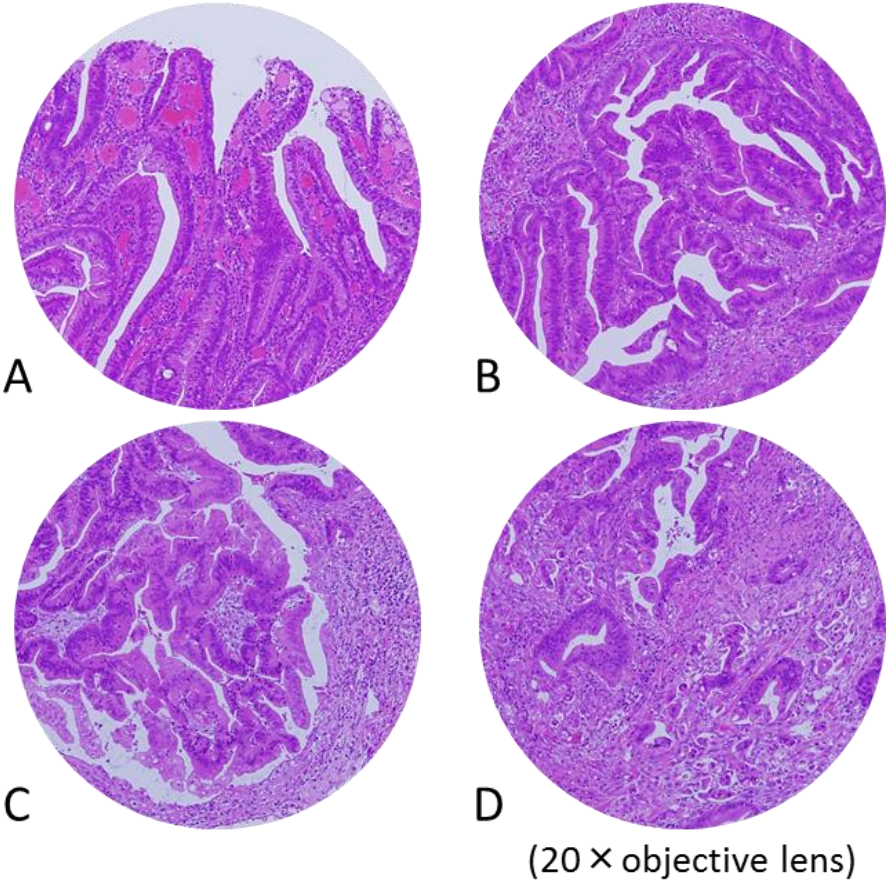


Fig 7. Grading of tumour budding

After meticulous investigation to choose a field where budding is most intensive (D), the number of budding focus is counted for grading of budding.

5.2 Poorly differentiated clusters (PDCs)

Cancer cell clusters located in the stroma that are composed of ≥ 5 cancer cells and lack a glandular cavity are classified as PDCs regardless of whether they are located inside or outside the vascular space (Fig 8).¹³⁻¹⁵ Fragmented cancer components encased by necrotic degenerative and inflammatory cells or cancer clusters floating within a mucinous pool are not classified as PDCs.

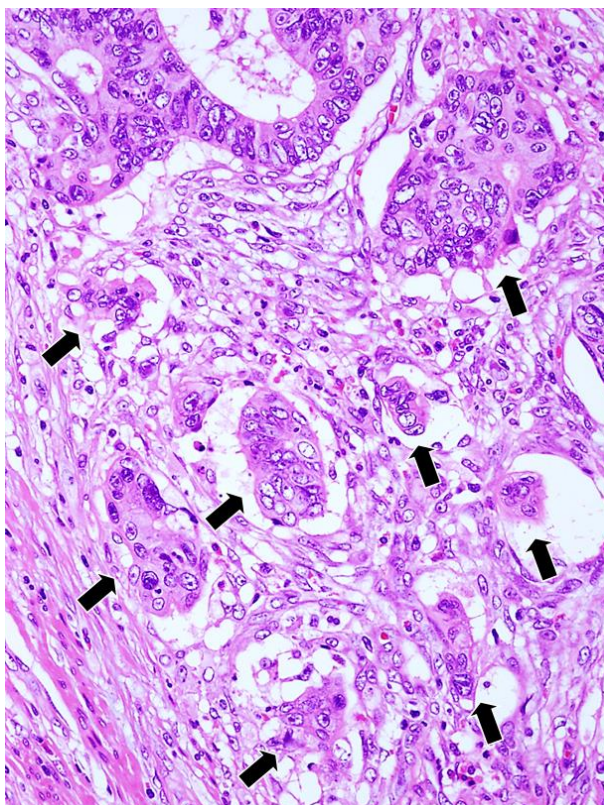


Fig 8. PDCs

PDCs include cancer cell clusters that do not show glandular cavity formation or show minimal glandular cavity formation, e.g. intracytoplasmic lumina (Fig 9. A). Cancer clusters showing evident glandular cavity formation are not classified as PDCs.

PDCs do not include parts of cancerous glands that are fragmented by necrotic and degenerative changes or by inflammatory cells (Fig 9. B).

With regard to the assessment of PDCs in mucinous carcinoma, cancer cell clusters within a large mucin pool (the so-called mucinous lake) (Fig 9. C) are not classified as PDCs. On the

other hand, cancer cell clusters without glandular cavity formation infiltrating the stroma with minimal extracellular mucin formation (Fig 9. D) are classified as PDCs. In particular, the ratio of the size of the cancer cluster to that of the mucinous area surrounding the cluster should be considered before making the decision. Cancer cell clusters accompanied by a mucinous area with size comparable to that of the cancer cell clusters can be classified as PDCs.

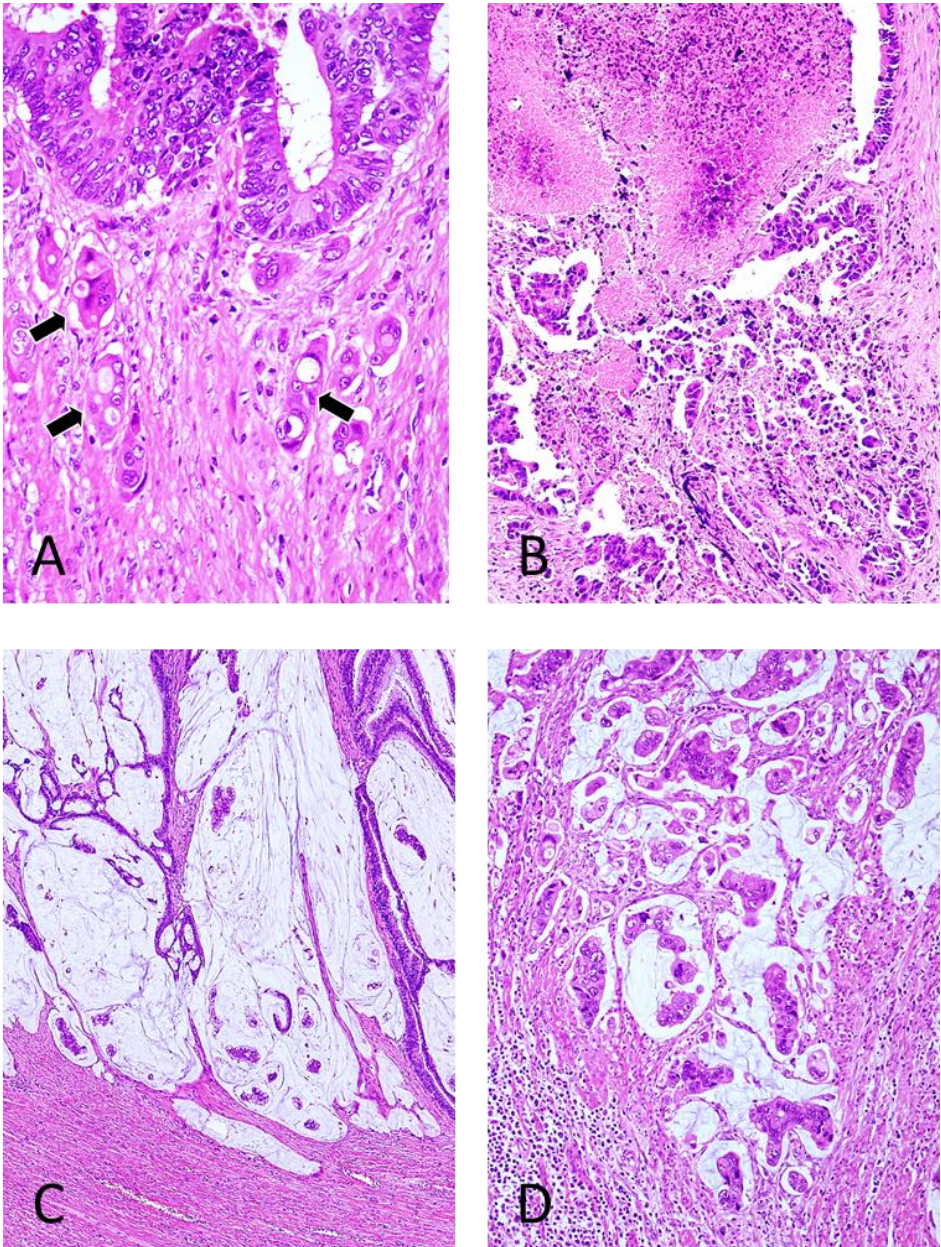


Fig 9. Criteria for judging PDCs

For grading of PDCs, the number of PDCs is counted in a field observed through a 20× objective lens (WHK 10× ocular lens; 0.785 mm²) after selecting one field wherein the number of PDCs is the greatest (Fig 10). Depending on the number of PDCs, the grade of PDCs is defined as follows:

Grade 0: 0

Grade 1: 1 to 4

Grade 2: 5 to 9

Grade 3: ≥10

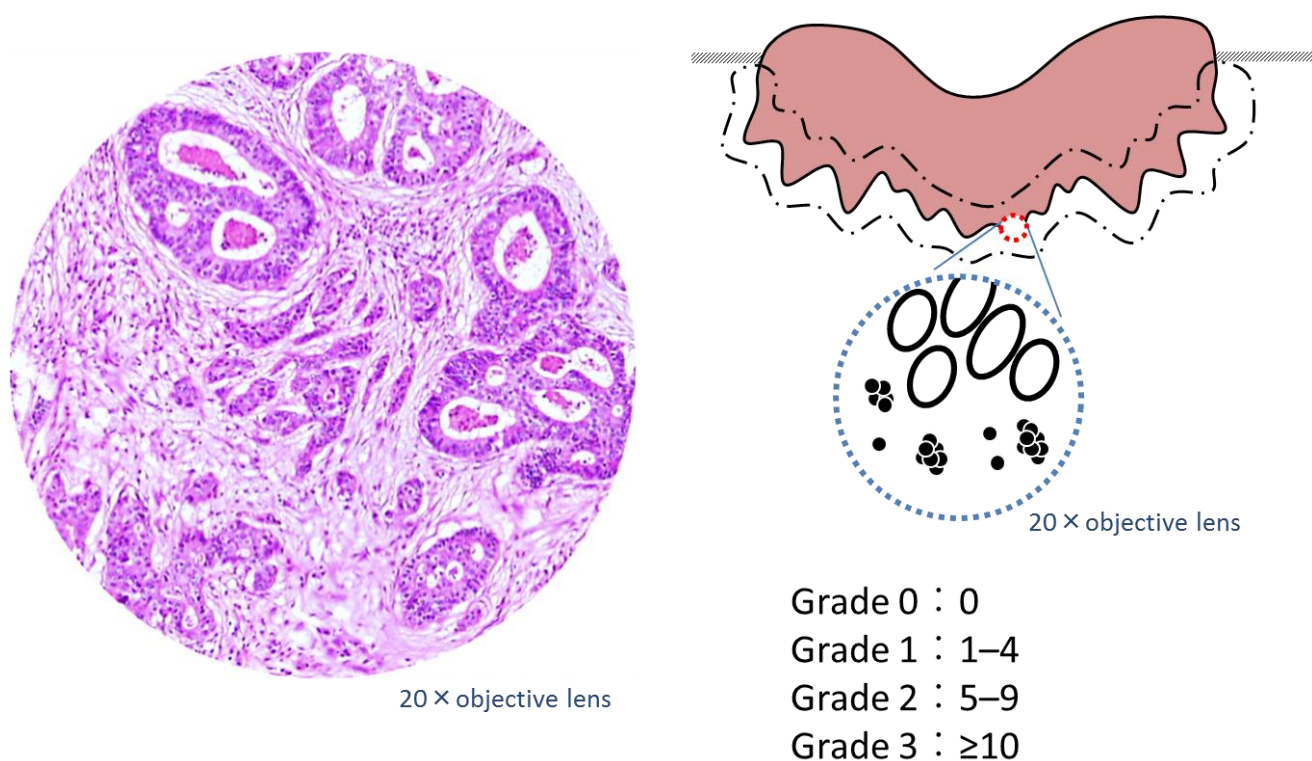


Fig 10. Grading of PDCs

After meticulous investigation to choose a field where PDCs are most intensive, the number of PDCs in a field observed through a 20× objective lens is counted for grading.

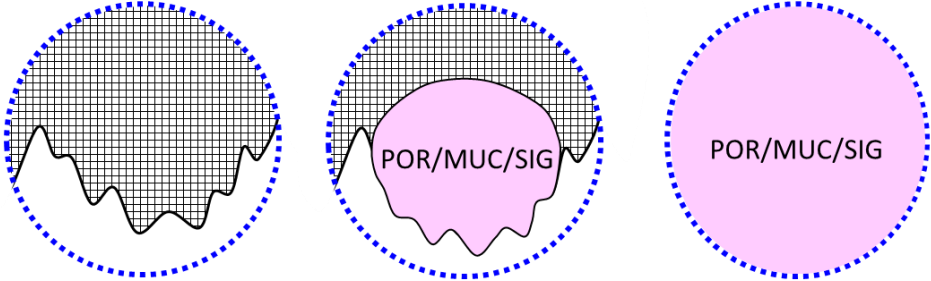
5.3 Tumour grade at the invasive front

Tumour grade at the invasive front is determined on the basis of semi-quantitative assessment of the poorly differentiated adenocarcinoma (POR), mucinous adenocarcinoma (MUC), and signet-ring cell carcinoma (SIG) using a microscopic field and a 40× objective lens (Fig 11).¹³

Pathological component of tumour to be evaluated

- poorly differentiated adenocarcinoma (POR)
- mucinous adenocarcinoma (MUC)
- signet-ring cell carcinoma (SIG)

Microscopic field with a 40× objective lens



None of POR/MUC/SIG component is observed

POR/MUC/SIG is not extensive enough to fully fill the field with the 40× objective lens

The field with the 40× objective lens is filled with POR/MUC/SIG

Tumour grade [invasive front]

Grade 1

Grade 2

Grade 3

Fig 11. Tumour grade at the invasive front

More specifically, the largest area of POR, MUC or SIG is determined first using a low-power microscopic field. The designation of grade 1 is applied to tumours with no POR/MUC/SIG; grade 2 is applied to tumours with POR/MUC/SIG that do not fill the field of a 40× objective lens and grade 3 is applied to tumours having POR/MUC/SIG components that fully fill the microscopic field of a 40× objective lens (Fig 12, Fig 13).

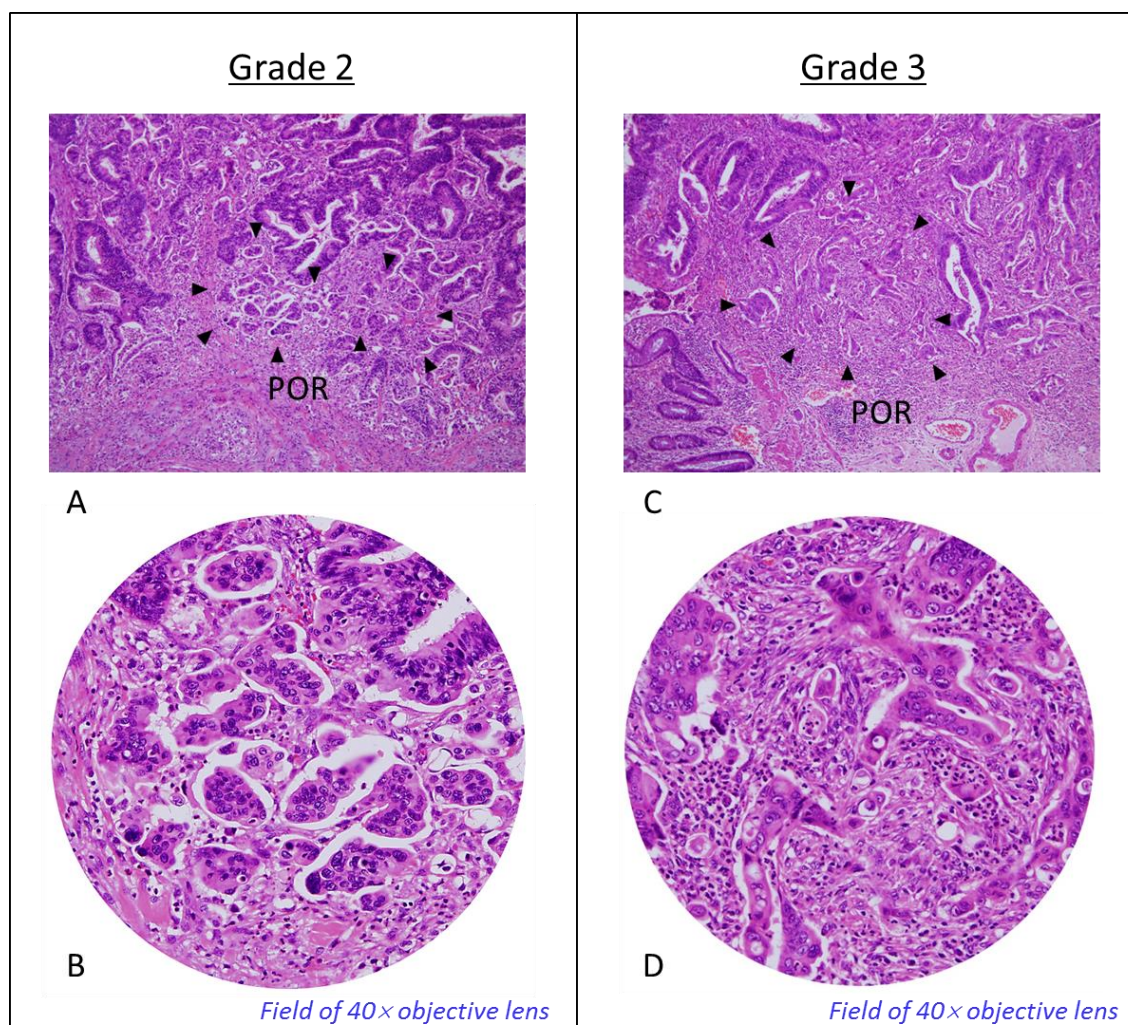


Fig 12. The poorly differentiated component (POR) in colorectal cancer

Figures **A, B** (case 1), grade 2 POR. Figures **C, D** (case 2), grade 3 POR.

A, The POR area existed as the least differentiated component of the tumour's leading front in case 1 (arrows; original magnification of the objective lens: 10x). **B**, Microscopic field with a 40x objective lens of the POR area in **A**. **C**, The POR area existed at the tumor's leading front in case 2 (arrows; original magnification of the objective lens: 10x). **D**, Microscopic field with a 40x objective lens of the POR area in **C**.

Note that the POR in case 1 is not extensive enough to fully fill the microscopic field with the 40x objective lens, and the differentiated cancer lesion is intermingled with the POR area in the microscopic field with the 40x objective lens. On the other hand, the microscopic field with the 40x objective lens is filled with the POR in case 2.

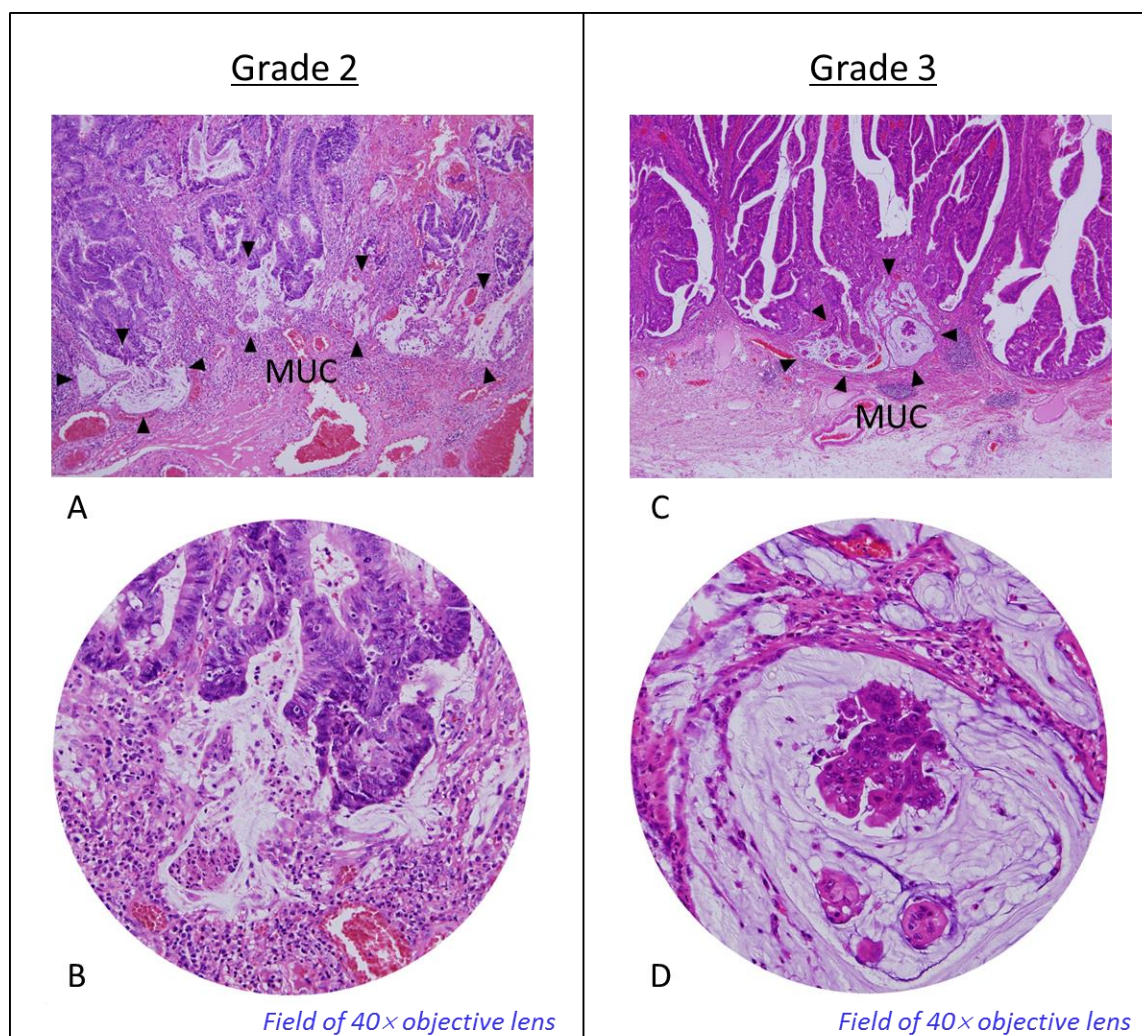


Fig 13. The mucinous component (MUC) in colorectal cancer

Figures **A, B** (case 3), grade 2 MUC. Figure **C, D** (case 4), grade 3 MUC.

A, MUC area observed in the tumor invasive front region of case 3 (arrows; original magnification of the objective lens: 4x). **B**, Microscopic field with a 40x objective lens of the MUC area in **A**. **C**, MUC area observed in the tumor invasive front region of case 4 (arrows; original magnification of the objective lens: 10x). **D**, Microscopic field with a 40x objective lens of the MUC area in **C**.

Note that the largest area of pools of extracellular mucin in the tumor fully occupied the microscopic field with the 40x objective lens in case 4, which is classified in grade 3, but not in case 3, which is classified in grade 2.

5.4 Depth of submucosal invasion (JSCCR method) (Fig 14)^{1,16}

When it is possible to identify the muscularis mucosae, the depth of submucosal invasion is the distance between the deeper edge of the muscularis mucosae and the deepest invasion.

When it is not possible to identify the muscularis mucosae, the depth of submucosal invasion is the distance between the surface of the tumour and the deepest invasion.

In polypoid tumour (Ip) with disrupted muscularis mucosae, the depth of submucosal invasion is the distance between the deepest invasion and the reference line defined as the boundary between the tumour head and pedicle. When cancer does not invade beyond the reference line, it is defined as head invasion.

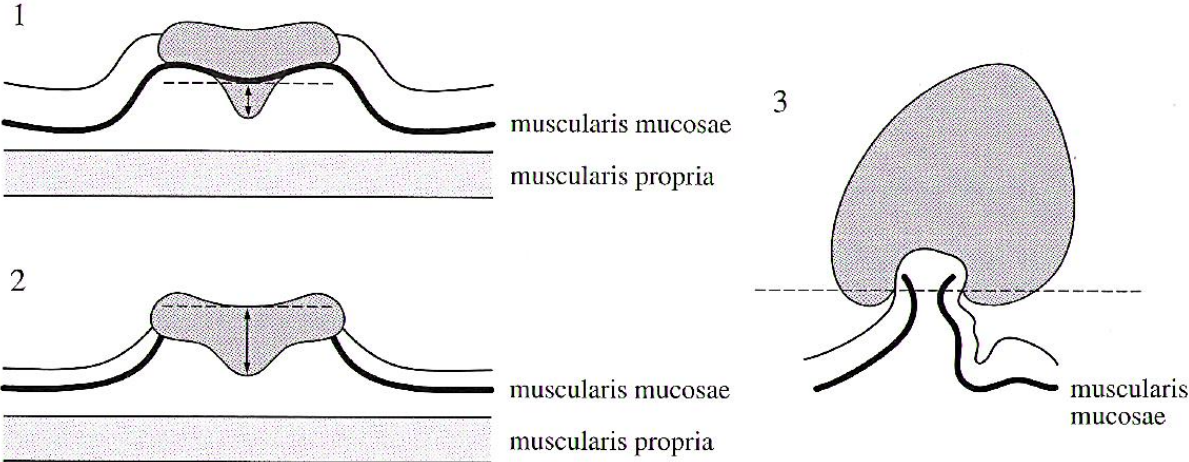


Fig 14. Depth of submucosal invasion according to the JSCCR method

5.5 Quantitative factors¹⁷

1. Maximum width of lesion (a lesion is defined as the summation of the adenoma and carcinoma component) (Fig 15)
2. Maximum width of carcinoma (Fig 15)
3. Maximum vertical depth of carcinoma from luminal surface (Fig 16)
4. Maximum vertical depth of carcinoma from muscularis mucosae (Fig 16)
5. Total area of submucosal invasion by carcinoma (Fig 17)

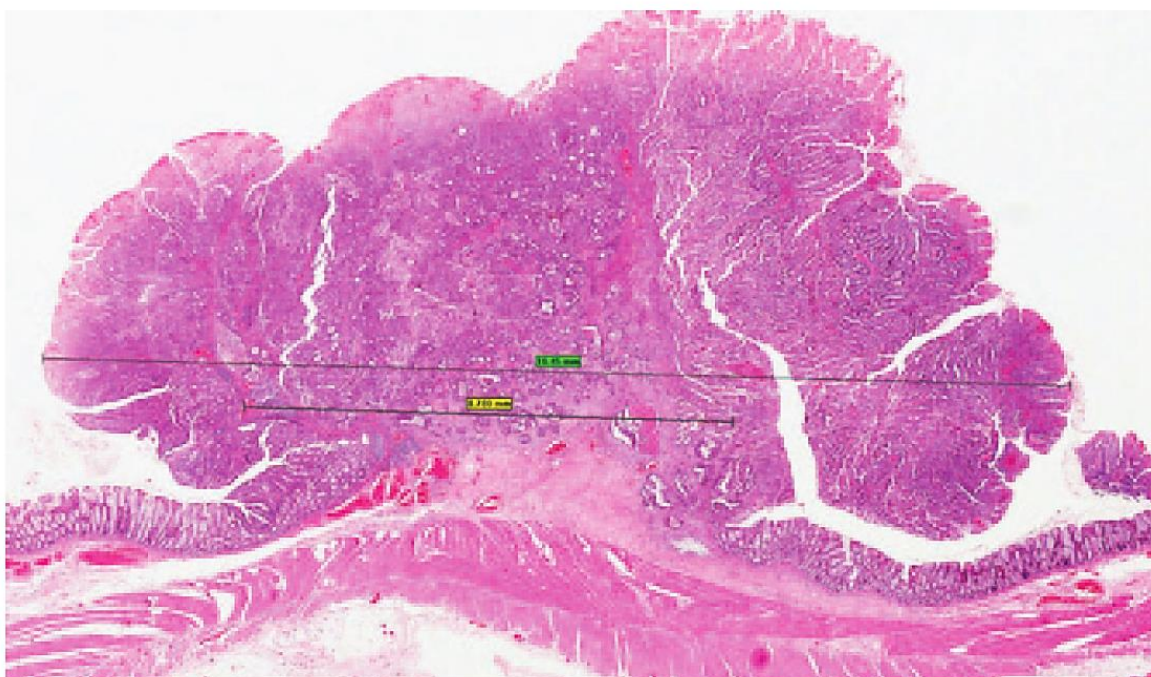


Fig 15. Width of lesion (green label)/carcinoma (yellow label).

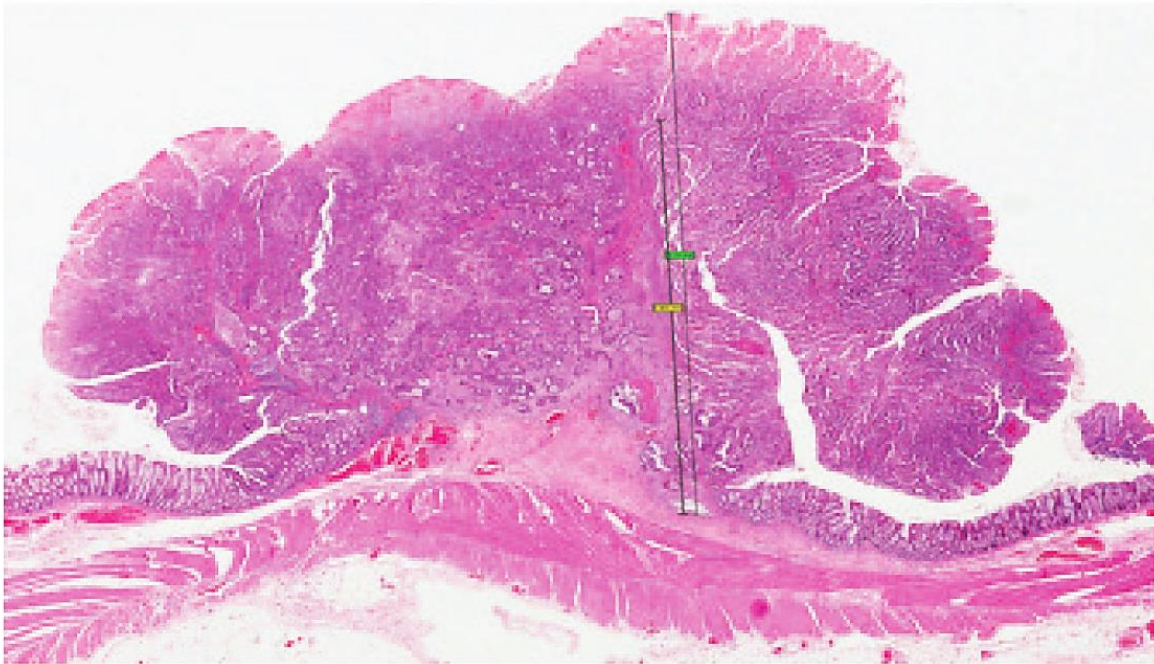


Fig 16. Depth of invasion from the luminal surface (green label) and muscularis mucosae (yellow label)

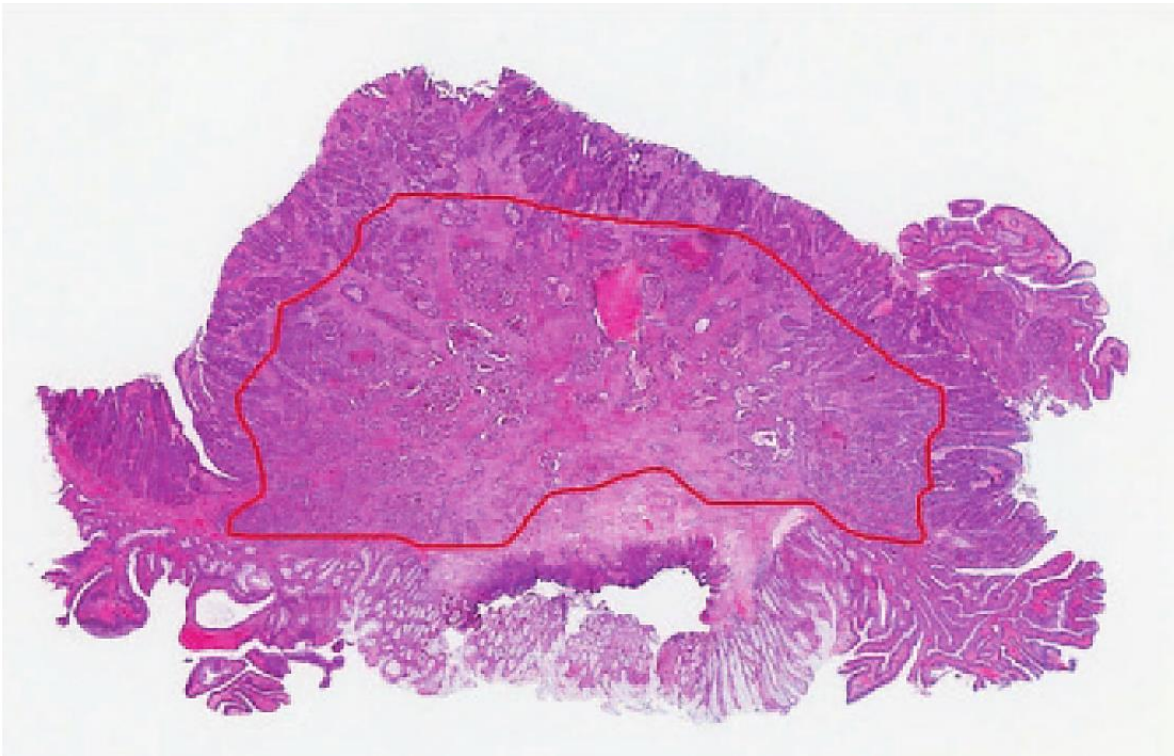


Fig 17. The estimated area of submucosal invasion (contained within the red area). The site of the destroyed muscularis mucosae has been estimated.

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