A Standardized Pathology Report for Gastric Cancer
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The Journal of Pathology and Translational Medicine is an open venue for the rapid publication of major achievements in various fields of pathology, cytopathology, and biomedical and translational research. The Journal aims to share new insights into the molecular and cellular mechanisms of human diseases and to report major advances in both experimental and clinical medicine, with a particular emphasis on translational research. The investigations of human cells and tissues using high-dimensional biology techniques such as genomics and proteomics will be given a high priority. Articles on stem cell biology are also welcome. The categories of manuscript include original articles, review and perspective articles, case studies, viewpoint reports, and letters to the editor.

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A standardized pathology report for gastric cancer: 2nd edition

Young Soo Park1,*, Myeong-Cherl Kook2,*, Baek-hui Kim3,*, Hye Seung Lee4,*, Dong-Wook Kang4, Mi-Jin Gu6, Ok Ran Shin5, Younghee Choi5, Wonae Lee6, Hyunki Kim6, In Hye Song7, Kyoungh-Mee Kim11, Hee Sung Kim12, Gyuhyun Kang13, Do Yun Park14, So-Young Jin15, Joon Mee Kim16, Yoon Jung Choi17, Hee Kyung Chang18, Soomin Ahn19, Mee Soo Chang19, Song-Hee Han19, Yoonjin Kwak1, An Na Seo21, Sung Hak Lee22, Mee-Yoon Cho23,

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The first edition of ‘A Standardized Pathology Report for Gastric Cancer’ was initiated by the Gastrointestinal Pathology Study Group of the Korean Society of Pathologists and published 17 years ago. Since then, significant advances have been made in the pathologic diagnosis, molecular genetics, and management of gastric cancer (GC). To reflect those changes, a committee for publishing a second edition of the report was formed within the Gastrointestinal Pathology Study Group of the Korean Society of Pathologists. This second edition consists of two parts: standard data elements and conditional data elements. The standard data elements contain the basic pathologic findings and items necessary to predict the prognosis of GC patients, and they are adequate for routine surgical pathology service. Other diagnostic and prognostic factors relevant to adjuvant therapy, including molecular biomarkers, are classified as conditional data elements to allow each pathologist to selectively choose items appropriate to the environment in their institution. We trust that the standardized pathology report will be helpful for GC diagnosis and facilitate large-scale multidisciplinary collaborative studies.

Key Words: Stomach neoplasms; Gastrorectomy; Endoscopic resection; Molecular pathology; Pathology report; Standardization

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Gastric cancer (GC) is the fifth most commonly diagnosed cancer and has the fourth-highest mortality rate worldwide [1]. Although the incidence and mortality rates of GC have decreased markedly during the past 50 years, Korean cancer registry data show that GC was still the most diagnosed cancer in 2018 [2]. The Gastrointestinal Pathology Study Group (GIPSG) of the Korean Society of Pathologists developed the first edition of ‘A Standardized Pathology Report for Gastric Cancer’ in 2005 to give pathologists a standard reporting format for GC diagnosis in daily practice [3].

Considerable changes in the pathology of GC have happened since then, such as the development of the histopathological classification for carcinoma and several pathologic features for prognostication [4,5]. In addition, molecular pathology tests for GC have become essential as treatment strategies for GC have developed rapidly, including advances in targeted therapy and immunotherapy [6,7]. Therefore, it is necessary to provide a second edition of the standardization report that reflects those changes.

In March 2022, a committee for revision of the report was formed within the GIPSG of the Korean Society of Pathologists. The committee consisted of subcommittees to discuss four topics: (1) radical resection specimens, (2) endoscopic resection specimens, (3) histologic classification, and (4) molecular markers for GC. This second edition of ‘A Standardized Pathology Report for Gastric Cancer’ was developed after several meetings of the subcommittees and entire committee.

The purpose of this report form is to standardize pathologic diagnosis of GC and enhance treatment capacity by facilitating communication between clinicians and pathologists. The basic pathologic findings for prognostication of GC are described in the “Standard data elements” section of the form, and other factors related to diagnosis and adjuvant therapy, including molecular biomarkers, are documented in the “Conditional data elements” section. A Korean version as well as an English version is also provided to enable Korean pathologists to use this report (Supplementary Material S1).

**APPLICATION OF STANDARD PATHOLOGY REPORT**

This standard pathology report is for use with primary gastric carcinomas. Neuroendocrine tumors, lymphomas, gastrointestinal stromal tumors, and other sarcomas are excluded. Carcinomas involving the esophagogastric junction (EGJ) with a center ≤ 2 cm into the proximal stomach are considered to be distal esophageal carcinoma and excluded, as defined in the American Joint Committee on Cancer (AJCC), 8th edition [8]. This pathology report is also used for residual (post-chemotherapy or post-endoscopic resection) carcinomas. The report forms for pathologic diagnosis from radical resection and endoscopic resection specimens are shown in Tables 1 and 2, respectively.

**Radical resection specimens**

**Gastrectomy (specimen) type**

The type of surgical procedure should be mentioned in the surgical record.

**Gross type**

The gross type of each lesion should be recorded individually. The classification of early gastric cancer (EGC) uses the Japanese guideline (subclassification of type 0) [9], and classification of advanced gastric cancer (AGC) uses the Borrmann classification. The unclassifiable type is Borrmann type 5, according to the Japanese guideline [9]. The gross type is determined by macroscopic examination. If there is discrepancy between the macroscopic and microscopic findings, i.e., EGC on macroscopic examination but tumor invades the proper muscle microscopically (AGC), the macroscopic type should remain as the gross finding and not be corrected according to the microscopic finding. In such cases, the following descriptions are recommended: AGC, mimicking EGC type X or EGC, mimicking Borrmann type X. If the lesion is AGC grossly, at least four representative sections should be submitted for microscopic examination, including the deepest invasion, and ink should be applied at the serosal surface nearest the tumor. If the lesion is EGC grossly, grid mapping should be performed at 4 to 5 mm width.

**Previous treatment**

Any treatment before surgical resection should be recorded when applicable. If there are residual tumor foci, it should be mentioned that these are residual tumors. In post-chemotherapy gastrectomy situations, representative sections are sufficient if the lesion is large and obvious. However, the entire tumor bed must be microscopically examined when the representative sections contain no residual cancer cells or the residual lesion is small or inconspicuous grossly. For post-endoscopic resection specimens, the entire tumor bed should be submitted for microscopic evaluation.

**Tumor focality**

Tumor focality should record whether it is a single lesion or
### Table 1. Report form for pathologic diagnosis using radical resection specimens

<table>
<thead>
<tr>
<th>Standard and Conditional data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastrectomy (specimen) type</strong></td>
</tr>
<tr>
<td>- Total gastrectomy</td>
</tr>
<tr>
<td>- Distal (subtotal) gastrectomy</td>
</tr>
<tr>
<td>- Proximal gastrectomy</td>
</tr>
<tr>
<td>- Wedge resection</td>
</tr>
<tr>
<td>- Others (__________)</td>
</tr>
<tr>
<td><strong>Gross type</strong></td>
</tr>
<tr>
<td>- EGC type</td>
</tr>
<tr>
<td>- Mixed EGC type (__________)</td>
</tr>
<tr>
<td>- AGC type</td>
</tr>
<tr>
<td>- Borrmann type 1/2/3/4/unclassifiable</td>
</tr>
<tr>
<td>- Others (__________)</td>
</tr>
<tr>
<td><strong>Residual with previous treatment</strong> (when applicable)</td>
</tr>
<tr>
<td>- Residual</td>
</tr>
<tr>
<td>- Previous treatment</td>
</tr>
<tr>
<td>- Chemotherapy</td>
</tr>
<tr>
<td>- Chemoradiotherapy</td>
</tr>
<tr>
<td>- Endoscopic mucosal resection</td>
</tr>
<tr>
<td>- Endoscopic submucosal dissection</td>
</tr>
<tr>
<td>- Unknown</td>
</tr>
<tr>
<td>- Others (__________)</td>
</tr>
<tr>
<td><strong>Tumor focality</strong></td>
</tr>
<tr>
<td>- Single</td>
</tr>
<tr>
<td>- Multiple</td>
</tr>
<tr>
<td><strong>Tumor location</strong></td>
</tr>
<tr>
<td>- Involvement</td>
</tr>
<tr>
<td>- Esophagus/Upper/Middle/Lower third of the stomach/Duodenum</td>
</tr>
<tr>
<td>- Cardia/Fundus/Antrum/Pylorus</td>
</tr>
<tr>
<td>- Lesser curvature/Greater curvature/Anterior wall/Posterior wall</td>
</tr>
<tr>
<td>- Others (__________)</td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
</tr>
<tr>
<td>One largest dimension</td>
</tr>
<tr>
<td>- ______ cm</td>
</tr>
<tr>
<td><strong>Tumor size</strong></td>
</tr>
<tr>
<td>Secondary or tertiary tumor dimensions</td>
</tr>
<tr>
<td>- ______ x ______ cm</td>
</tr>
<tr>
<td>- ______ x ______ x ______ cm</td>
</tr>
<tr>
<td><strong>Histologic type</strong></td>
</tr>
<tr>
<td>According to the principles described in “Histologic classification” section</td>
</tr>
<tr>
<td>- WHO</td>
</tr>
<tr>
<td>- Lauren</td>
</tr>
<tr>
<td><strong>Tumor regression grade</strong> (when applicable)</td>
</tr>
<tr>
<td>- Grade 0: Complete response (no viable cancer cells)</td>
</tr>
<tr>
<td>- Grade 1: Near complete response (single cells or rare small groups of cancer cells)</td>
</tr>
<tr>
<td>- Grade 2: Partial response (residual cancer with evident tumor regression, but more than single cells or rare small groups of cancer cells)</td>
</tr>
<tr>
<td>- Grade 3: Poor or no response (extensive residual cancer with no evident tumor regression)</td>
</tr>
<tr>
<td><strong>Lymph node tumor regression</strong> (when applicable)</td>
</tr>
<tr>
<td>- Not identified</td>
</tr>
<tr>
<td>- Present</td>
</tr>
<tr>
<td><strong>Depth of invasion (pT)</strong></td>
</tr>
<tr>
<td>- Invades lamina propria (pT1a)</td>
</tr>
<tr>
<td>- Invades muscularis mucosae (pT1a)</td>
</tr>
<tr>
<td>- Invades submucosa (sm1/sm2/sm3) (pT1b)</td>
</tr>
<tr>
<td>- Invades proper muscle (pT2)</td>
</tr>
<tr>
<td>- Invades subserosa (pT3)</td>
</tr>
<tr>
<td>- Invades serosa (visceral peritoneum) (pT4a)</td>
</tr>
<tr>
<td>- Directly invades adjacent structure (pT4b)</td>
</tr>
<tr>
<td>- Specify (__________)</td>
</tr>
<tr>
<td><strong>Resection margin</strong></td>
</tr>
<tr>
<td>- Proximal margin</td>
</tr>
<tr>
<td>- Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>- Involved by carcinoma</td>
</tr>
</tbody>
</table>

(Continued to the next page)
multiple lesions. Multiple lesions should be evaluated individually both macroscopically and microscopically in descending order from the tumor with the deepest level of invasion. However, regional lymph node metastasis, associated findings, and separate lesions are listed only for the deepest lesion.

Tumor location

The description of the tumor location is recorded in two parts: involvement and center. The involvement of the tumor uses up to three portions from the esophagus to duodenum beginning with the most involved area. The delineation of the upper, middle, and lower thirds of the stomach follows the Japanese guideline [9].

Tumor size

The tumor size is recorded using the largest dimension of the tumor [11]. Secondary or tertiary dimensions can be measured as conditional data elements. However, the tumor size is not used in the current staging of GC [8], and it is sometimes very difficult to

Table 1. Continued

<table>
<thead>
<tr>
<th>Standard and Conditional data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal margin</td>
</tr>
<tr>
<td>Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>Involved by carcinoma</td>
</tr>
<tr>
<td>Circumferential resection margin*</td>
</tr>
<tr>
<td>Applied in EGJ or cardia cancer</td>
</tr>
<tr>
<td>Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>Involved by carcinoma</td>
</tr>
<tr>
<td>Regional lymph node metastasis*</td>
</tr>
<tr>
<td>At least 16 regional lymph nodes should be assessed</td>
</tr>
<tr>
<td>no metastasis in ____ regional lymph nodes</td>
</tr>
<tr>
<td>metastasis in ___ out of ____ regional lymph nodes</td>
</tr>
<tr>
<td>Extranodal tumor extension*</td>
</tr>
<tr>
<td>Not identified</td>
</tr>
<tr>
<td>Present</td>
</tr>
<tr>
<td>Isolated tumor cell clusters*</td>
</tr>
<tr>
<td>Applied in incidentally identified tumor cell cluster less than 0.2 mm in greatest dimension with no other regional lymph node metastasis (pN0)</td>
</tr>
<tr>
<td>Not identified</td>
</tr>
<tr>
<td>Present</td>
</tr>
<tr>
<td>Lymphovascular invasion*</td>
</tr>
<tr>
<td>Not identified</td>
</tr>
<tr>
<td>Present</td>
</tr>
<tr>
<td>Venous invasion*</td>
</tr>
<tr>
<td>Applied when identified in large vessels with an identifiable smooth muscle layer or elastic lamina</td>
</tr>
<tr>
<td>Not identified</td>
</tr>
<tr>
<td>Present</td>
</tr>
<tr>
<td>Perineural invasion*</td>
</tr>
<tr>
<td>Not identified</td>
</tr>
<tr>
<td>Present</td>
</tr>
<tr>
<td>Pre-existing adenoma* (when present)</td>
</tr>
<tr>
<td>Used if the carcinoma is within the adenoma</td>
</tr>
<tr>
<td>Tubular/Tubulovillous/Villous adenoma</td>
</tr>
<tr>
<td>Low grade dysplasia/High grade dysplasia</td>
</tr>
<tr>
<td>Associated findings* (when present)</td>
</tr>
<tr>
<td>Tumor perforation</td>
</tr>
<tr>
<td>Serosal (peritoneal, mesenteric) seeding</td>
</tr>
<tr>
<td>Distant metastasis</td>
</tr>
<tr>
<td>Other organ, specify: ________________</td>
</tr>
<tr>
<td>Distant lymph node</td>
</tr>
<tr>
<td>Separate lesions* (when present)</td>
</tr>
<tr>
<td>Peptic ulcer</td>
</tr>
<tr>
<td>Adenoma</td>
</tr>
<tr>
<td>GIST</td>
</tr>
<tr>
<td>Others ( ____________ )</td>
</tr>
</tbody>
</table>

EGC, early gastric cancer; AGC, advanced gastric cancer; WHO, World Health Organization; EGJ, esophagogastric junction.

*Standard data elements; *Conditional data elements.
Table 2. Report form for pathologic diagnosis using endoscopic resection specimens

<table>
<thead>
<tr>
<th>Standard and Conditional data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen size*</td>
</tr>
<tr>
<td>[ ] [ ] x ___ cm</td>
</tr>
<tr>
<td>Gross type of tumor*</td>
</tr>
<tr>
<td>Same as method of surgical specimen</td>
</tr>
<tr>
<td>Tumor size*</td>
</tr>
<tr>
<td>One largest dimension</td>
</tr>
<tr>
<td>[ ] ___ cm</td>
</tr>
<tr>
<td>Histologic type*</td>
</tr>
<tr>
<td>According to the principles described in “Histologic classification” section</td>
</tr>
<tr>
<td>[ ] WHO</td>
</tr>
<tr>
<td>[ ] Lauren</td>
</tr>
<tr>
<td>Histologic components*</td>
</tr>
<tr>
<td>All morphologic components of tumor cell may be described</td>
</tr>
<tr>
<td>Depth of invasion (pT)</td>
</tr>
<tr>
<td>[ ] Invades lamina propria (pT1a)</td>
</tr>
<tr>
<td>[ ] Invades muscularis mucosae (pT1a)</td>
</tr>
<tr>
<td>[ ] Invades submucosa (submucosal depth: ___ mm or µm)</td>
</tr>
<tr>
<td>[ ] Invades proper muscle (pT2)</td>
</tr>
<tr>
<td>Depth of invasion (pT)*</td>
</tr>
<tr>
<td>In case of submucosa invasion, the invasion width can be additionally described</td>
</tr>
<tr>
<td>[ ] Invades submucosa (submucosal depth: ___ mm or µm)</td>
</tr>
<tr>
<td>[ ] [ ] submucosal width: ___ mm</td>
</tr>
<tr>
<td>Resection margin*</td>
</tr>
<tr>
<td>[ ] Lateral margin</td>
</tr>
<tr>
<td>[ ] Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>[ ] Involved by carcinoma</td>
</tr>
<tr>
<td>[ ] Deep margin</td>
</tr>
<tr>
<td>[ ] Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>[ ] Involved by carcinoma</td>
</tr>
<tr>
<td>Resection margin*</td>
</tr>
<tr>
<td>[ ] Proximal margin</td>
</tr>
<tr>
<td>[ ] Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>[ ] Involved by carcinoma</td>
</tr>
<tr>
<td>[ ] Distal margin</td>
</tr>
<tr>
<td>[ ] Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>[ ] Involved by carcinoma</td>
</tr>
<tr>
<td>[ ] Anterior margin</td>
</tr>
<tr>
<td>[ ] Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>[ ] Involved by carcinoma</td>
</tr>
<tr>
<td>[ ] Posterior margin</td>
</tr>
<tr>
<td>[ ] Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>[ ] Involved by carcinoma</td>
</tr>
<tr>
<td>[ ] Deep margin</td>
</tr>
<tr>
<td>[ ] Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>[ ] Involved by carcinoma</td>
</tr>
<tr>
<td>Ulceration*</td>
</tr>
<tr>
<td>[ ] Absent</td>
</tr>
<tr>
<td>[ ] Present</td>
</tr>
<tr>
<td>Ulceration*</td>
</tr>
<tr>
<td>[ ] Absent</td>
</tr>
<tr>
<td>[ ] Non-significant (≤ 4 mm)</td>
</tr>
<tr>
<td>[ ] Significant (&gt; 4 mm)</td>
</tr>
<tr>
<td>Cases with adenoma components*</td>
</tr>
<tr>
<td>[ ] Absent</td>
</tr>
<tr>
<td>[ ] Present</td>
</tr>
<tr>
<td>[ ] Specify: ________________________</td>
</tr>
<tr>
<td>En bloc resection*</td>
</tr>
<tr>
<td>[ ] Yes</td>
</tr>
<tr>
<td>[ ] No (piecemeal/tearing)</td>
</tr>
<tr>
<td>Lymphatic invasion*</td>
</tr>
<tr>
<td>[ ] Not identified</td>
</tr>
<tr>
<td>[ ] Present</td>
</tr>
<tr>
<td>Venous invasion*</td>
</tr>
<tr>
<td>[ ] Not identified</td>
</tr>
<tr>
<td>[ ] Present</td>
</tr>
</tbody>
</table>

WHO, World Health Organization.
*Standard data elements; **Conditional data elements.

measure accurately, such as in Borrmann type 4 cancer. For scattered residual tumor foci following previous treatment, it is recommended to measure the maximum diameter that includes all foci [12].

Tumor regression grade

Although preoperative chemotherapy has not been established as a standard treatment for patients in Korea [5], studies have shown survival benefits in local AGC in European [13], Asian [14], and Korean patients [15]. Therefore, the need to adequately evaluate the tumor response to chemotherapy is increasing [16]. Various tumor regression grading (TRG) methods are available for gastrointestinal cancers [17,18]. The Becker system [19] is one that has been proposed for GC. The previous edition of “A Standardized Pathology Report for Gastric Cancer” [3] used the Japanese guideline [9]. The Becker and Japanese systems both estimate the proportion of residual tumor and use it as a cutoff value between TRGs. However, because some tumors have more abundant fibrosis than tumor cells (before chemotherapy), estimation of the residual tumor proportion could show low concordance between observers [20,21]. Therefore, we suggest a new TRG system: the modified Ryan system currently recommended in the College of American Pathologists (CAP) guideline [11] and the second edition of the standardized pathology report for colorectal cancer in Korea [22]. It is a descriptive four-tier system that evaluates residual cancer rather than fibrosis as none, single cells or rare small groups, more than single cells but evident tumor response, and extensive residual cancer cells. Acellular mucin pools and necrotic or degenerative cells are not considered to be residual cancer [8]. Only the primary tumor is evaluated in this TRG, but tumor regression of the regional lymph nodes [16,23] can be reported as a conditional data element when there is evidence of partial (viable cancer cells with regressive changes) or complete tumor regression (only fibrosis, mucin pool, or foam cells without viable cells) in the regional lymph nodes.

Evidence suggests that the presence of tumor regression in the lymph nodes is associated with better clinical outcomes [24,25].

Depth of invasion

The depth of the tumor invasion follows the AJCC 8th edition [8] and Japanese guidelines [9]. Notably, the Japanese guideline does not accept carcinoma in situ (pTis). In the AJCC 8th edition, pTis is defined as an intraepithelial tumor without invasion of the lamina propria, which is equivalent to high-grade dysplasia. pT1b is subdivided into sm1, sm2, and sm3. If cancer cells are present below an imaginary line dividing the submucosa and...
proper muscle, the case is considered pT2 even if the cancer cells are not actually within the muscle fibers. If there is no proper muscle layer due to ulceration, and the cancer cells are below the imaginary line drawn at the lower border of the proper muscle, the case is considered pT3. Invasion of the omentum and perigastric fat is considered pT3. Ink should be applied at the serosal surface nearest the tumor during gross examination to properly evaluate serosal (visceral peritoneum) invasion. The case is considered pT4a if the cancer cells are adherent to or exposed beyond mesothelial cells. Because the mesocolon and gastric serosa (including the greater and lesser omentum) have different embryological origins, invasion of the mesocolon should be classified as pT4b. However, some areas are tightly fused, such as the posterior wall of the antrum, the gastric serosa, and the anterior side of the transverse mesocolon. Therefore, the Japanese guideline indicates that invasion of the transverse mesocolon is not pT4b unless it extends to the colic vessels or penetrates the posterior surface of the mesocolon [9]. Some cases can be either pT4a or pT4b, depending on the site of the tumor. Invasion of the pancreas capsule is considered pT4b. Direct duodenal or esophageal invasion is not considered pT4b. Any involvement of other organs, such as the liver, pancreas, colon, spleen, diaphragm, or kidney, should be recorded. Cancer cells within lymphatic or vascular spaces are not considered in the determination of invasion depth [8]. The presence of lymphatic or vascular invasion should be recorded separately in parentheses (e.g., tumor invades proper muscle [involvement of subserosa by lymphatic emboli]).

Resection margin

The distance from the proximal or distal resection margin is the length from the edge of the carcinoma to the nearest resection margin. It is important to locate the true resection margin in the gross specimen, especially when the stomach is opened along the lesser curvature or obliquely along the anterior or posterior wall. In some cases, cancer cells approach the resection margin much more closely than can be observed grossly (cancer spreading underneathe the mucosa). Therefore, the resection margin is finalized in a microscopic evaluation. The circumferential and radial resection margin statuses can be reported as conditional data elements. Determination of the circumferential margin is often required if the tumor is located near the EGJ.

Regional lymph node metastasis

The presence of lymph node metastasis is one of the most important prognostic factors, even post-chemotherapy [26,27]. Both the total number of evaluated lymph nodes and the number of metastatic lymph nodes are reported. Although pathological evaluation of more than 30 regional lymph nodes is desirable according to the AJCC 8th edition [8], a minimum of 16 regional lymph nodes is acceptable per the CAP guideline [11] because the definition of pN3b is 16 or more metastases. Therefore, if fewer than 16 lymph nodes were initially retrieved for evaluation, additional effort to recover more lymph nodes should be made and reported. This does not apply in cases of previous partial gastrectomy, preoperative chemotherapy, or radiation therapy. Microscopic evaluation should be performed on the largest plane of each lymph node. In general, if the size of the metastasis observed in the lymph node is ≤0.2 mm, the metastasis is called isolated tumor cells (ITCs); if the size is more than 0.2 mm but not greater than 2 mm, it is a micrometastasis. Because micrometastases are not reported separately in GC, they are considered to be positive lymph nodes [8]. According to the AJCC 8th edition, ITCs should not be reflected in the pN stage and should be reported as pN0 (i+) in the absence of another lymph node metastasis. However, it is hard to ignore ITCs, which are readily seen on hematoxylin and eosin (H&E) slides. Therefore, in most practices, all metastatic tumor cell clusters in the lymph nodes are reflected in the pN stage regardless of size, and only ITCs incidentally detected by cytokeratin immunohistochemistry (IHC) are excluded from the pN stage. The stations of the lymph nodes are not reported unless they are separately submitted with corresponding labels. Tumor deposit (TD) is defined as discrete tumor nodule not greater than 2 mm, the metastasis is called isolated tumor cells (ITCs); if the size is more than 0.2 mm but not greater than 2 mm, it is a micrometastasis. Because micrometastases are not reported separately in GC, they are considered to be positive lymph nodes [8]. According to the AJCC 8th edition, ITCs should not be reflected in the pN stage and should be reported as pN0 (i+) in the absence of another lymph node metastasis. However, it is hard to ignore ITCs, which are readily seen on hematoxylin and eosin (H&E) slides. Therefore, in most practices, all metastatic tumor cell clusters in the lymph nodes are reflected in the pN stage regardless of size, and only ITCs incidentally detected by cytokeratin immunohistochemistry (IHC) are excluded from the pN stage. The stations of the lymph nodes are not reported unless they are separately submitted with corresponding labels. Tumor deposit (TD) is defined as discrete tumor nodule 

Fig. 1. An example of a tumor deposit. It usually has irregular outlines without identifiable lymph node tissue or identifiable vascular or neural structures.
GC and are thus reflected in the pN stage. TD and serosal (peritoneal) seeding nodules should be distinguished because peritoneal seeding is graded as pM1. Metastasis to a distant lymph node is pM1 and should not be considered in the pN stage. The definition of distant lymph nodes is different in the AJCC 8th edition than in the Japanese guideline, and we recommend following the AJCC 8th edition, in which superior mesenteric lymph node metastasis is pM1 [8].

Extranodal tumor extension

If the cancer cells show infiltration of the extranodal adipose tissue beyond the capsule of the lymph node, extranodal tumor extension (ENE) can be reported. ENE is associated with poor prognosis in GC [28-30].

Lymphovascular invasion

Lymphovascular invasion includes both lymphatic and vascular invasion. Discrimination of lymphatics from blood vessels on H&E slides is often difficult, especially when they are small (Fig. 2A, B). Although IHC for D2-40 or CD31 can be used, the prognostic differences between lymphatic and blood vessel invasion have not been sufficiently evaluated in GC [12]; therefore, we recommend using 'lymphovascular invasion.' However, when tumor invasion or emboli are observed in large vessels with an identifiable smooth muscle layer or elastic lamina, it is called venous invasion and can be reported as a conditional data element (Fig. 2C). Venous invasion has been reported as a risk factor for recurrence in both early [31,32] and advanced GCs [33].

Perineural invasion

Perineural invasion is reported when cancer cells are observed within or around the nerve [34].

Pre-existing adenoma

Pre-existing adenoma is reported when carcinoma is observed within an adenoma. If the adenoma is discrete from the carcinoma, it is reported as a separate lesion.

Associated findings

Tumor perforation, serosal (peritoneal, mesenteric) seeding, and distant metastasis (including specific site) are reported when present.

Separate lesions

Peptic ulcers, adenomas, gastrointestinal stromal tumors, and other separate lesions are reported when present.

Endoscopic resection specimens

Description of the specimen

The size of the specimen is expressed as the length of the longest axis and the length perpendicular to the longest axis. The size of the tumor is indicated only by the length of the largest axis. The gross type of the tumor is described in the same way as for a surgical specimen.

Sectioning of the specimen

Apply ink to the entire deep margin and lateral margins of the specimen so that it can be viewed under a microscope. Prepare paraffin blocks by sequential parallel sectioning of the entire specimen at 2 mm intervals. Among the lateral margins of the four directions, the closest margin and the tumor should be included together in the sectioning direction.

For gastrointestinal specimens, the distal part is generally placed at the 9 o’clock position in a gross photograph. If the distances from each of the lateral margins are similar, serial sectioning of the specimen is commonly performed in the same direction. When visual observation indicates that the closest lateral margin is not included in this general sectioning direction, however, the direction of the sample or mapping frame should be turned so that the closest lateral margin and the tumor appear together on the section (Fig. 3).

Fig. 2. Histologic features of lymphovascular invasion in sections of gastric cancer. An example of lymphovascular invasion on hematoxylin and eosin examination (A) and stained for D2-40 (B). Tumors involving vessels with an identifiable smooth muscle layer are considered to have venous invasion (C).
Histologic type and components

The histologic type of the tumor is described in the same way as for a surgical specimen. For the criteria and description of each type, refer to the information in the “Histologic classification” section below. The histologic type of the tumor should be described; the histologic diversity of tumor cells may be described separately as histologic components. If various morphologic components are observed within the tumor, all are described according to the histologic type. In such a case, the description should signify the quantitative majority of the tumor components. The description method can be selected according to institutional preferences. For example, record in order: well differentiated (WD)–moderately differentiated (MD) > poorly differentiated (PD) > signet ring cell (SRC) carcinoma; interval variable: WD-MD > 50%, PD < 50%, SRC < 10%; and continuous variable: WD-MD 65%, PD 30%, SRC 5%. Many studies have reported that tumors with a mixture of differentiated-type and undifferentiated-type components have a higher risk of lymph node metastasis than tumors with only one component [35-40]. Within the undifferentiated type, SRC has a lower lymph node metastasis frequency, which is reported to be at a level similar to that of the differentiated type [41-43]. In addition, some reports indicate that the lymph node metastasis frequency is lower in pure SRC cases than in SRC cases mixed with other component types [44-47]. However, only the histologic type is applied for determining whether an endoscopic resection is curative, and because differences in histologic components are not applied, they are reflected as conditional elements rather than a standard element. A pathological study of the criteria for determining whether an endoscopic resection is curative is currently underway by the GIPSG of the Korean Society of Pathology as a research project of the National Evidence-based Healthcare Collaborating Agency. If important results are obtained from that study, they should be reflected in the elements of this guideline.

Fig. 3. Sectioning of an endoscopically resected specimen. When the direction of the photograph matches the direction of the closest lateral margin (A). If the direction of the photograph does not match, turn the specimen toward the closest lateral margin for mapping (B).
Tumor size

Only the length of the largest axis of a histologically confirmed tumor is recorded.

Depth of invasion

The method for describing the depth of invasion is basically the same as for a surgical specimen. The difference is that the invasion depth in the submucosal layer is measured and described in cases of submucosal invasion, and it is measured in mm or μm. The measurement is the length from the lowest surface of the muscularis mucosa to the most deeply invaded point. In some cases, the muscularis mucosa are modified by tumor invasion (hypertrophied, displaced, completely disappeared). In these cases, depth is measured using an imaginary line extending from adjacent muscularis mucosa in the normal area not deformed by the tumor (Fig. 4A). Always ensure that the lowest surface of the original, unmodified muscularis mucosa is used as the reference point. If the progressing course of the adjacent muscularis mucosa forms a curve, the virtual line is set as a matching curve (Fig. 4B).

No definitive description or research results indicate how to measure the depth of invasion when muscularis mucosa are modified. In the Japanese guideline, an explanation first appeared in the 14th edition from 2010: “if the muscularis mucosa are obscure due to ulcerative changes, the length should be measured on the virtual line based on the adjacent normal layer” [9]. In the 15th edition from 2017, it changed to recommend measuring from the surface of the tumor [48]. When muscularis mucosa are modified, some Korean pathologists measure from the lowest muscle fiber of the modified layer, and some measure from the imaginary line of the adjacent normal area. Two Korean studies reported that it is appropriate to measure from the imaginary line of the adjacent normal area in all modified situations [49,50]; accordingly, we use that recommendation as the standard measurement method in this guideline.

In cases of submucosal invasion, studies have shown that not only the invasion depth, but also the invasion width are significant risk factors for lymph node metastasis [50,51]. However, because few multicenter studies have been done and it has not yet been applied to the curative resection criteria, the invasion width is a conditional data element. This point is being addressed in the ongoing GIPSG pathological study on the criteria for determining whether an endoscopic resection is curative. The method for measuring the invasion width is as follows (Fig. 5): if submucosal invasion is observed on only one section, write the actual size measured on the slide of that section. If submucosal invasion is observed across two or more slices, write the larger of the following two values: (1) the actual size measured on the slide with the largest invasion width, or (2) the number of slices spanned by the invasion × 2 mm (thickness of slice).

Fig. 4. Method to measure submucosal invasion depth. Always use the lowest surface of the original, unmodified muscularis mucosa as the reference point (A). When the progressing course of the adjacent muscularis mucosa forms a curve, the virtual line is set as a matching curve (B).
Resection margin

The resection margin is described for the nearest lateral margin and deep margin. If the lateral margin is close (≤0.2 cm) or is involved in the tumor, the corresponding directions should be written together. If multiple margins are involved, all should be written. This is the information needed by the gastroenterologist to decide whether to perform additional procedures (endoscopic resection, argon plasma coagulation, follow-up biopsy). As a conditional element, the distance in all four directions of the lateral margin can be described.

The degree of invasion of the lateral resection margin and the probability of residual cancer are related. A high risk of residual cancer was reported when two or more of the four lateral margins were involved (multiple involvement) or when the length of involvement was large (more than 4 mm or 6 mm). However, it has not been determined whether additional treatment can be decided according to the degree of margin involvement because the risk is low but present in the group with a small degree of margin involvement.

Ulcer

Ulceration is defined as a full-thickness disruption of muscularis mucosae, both active and scarring, and determined by histological findings, not endoscopic findings [5,9,52]. The presence or absence of an ulcer is an important criterion for judging whether an endoscopic resection is curative in mucosal cancer [5], so it must be described in the pathology report for mucosal cancer. Because ulcers are included in the indications for an endoscopic resection, the presence of ulcers is determined by endoscopic findings. Ultimately, however, it must be confirmed by pathological examination findings of the resected specimen. Endoscopic diagnosis is difficult in the absence of a mucosal break [53], and ulcer-negative endoscopy findings with ulcer-positive pathology findings were reported in 4.6%–5.5% of cases [54,55].

Another problem that occurs in practice is a lack of clarity in the criteria for differentiating original small ulcers from biopsy-induced changes after endoscopic biopsy in a case that did not originally have ulcers. Due to the low accuracy of ulcer determination in endoscopic findings, a finding of no ulcer during endoscopy cannot guarantee a biopsy-induced change. Diagnostic criteria for this have been suggested by Shimoda et al. [56], and the Japanese gastric cancer treatment guidelines describe this as follows: “A biopsy-derived scar is usually observed histologically as fibrosis restricted to small areas just beneath the muscularis mucosae. If it cannot be discriminated from the ulcer scar, it should be classified as UL1.” [57]. According to JCOG1009/1010, a clinical study on undifferentiated-type EGC: “UL was judged as present if the muscularis propria was completely disrupted and if fibrosis in the submucosal layer was observed to be wider than the range of disrupted muscularis propria.” [58]. In our study group, ulcer size was measured in the ongoing GIPSG study on the criteria for curative resection, and the possibility of offering differentiation criteria for this problem was investigated. We found that the risk of lymph node metastasis with an ulcer of 4 mm or less was the same as in cases with no ulcer. Using that criterion, very small ulcers can be excluded from the risk factors for lymph node metastasis, which removes the need to differentiate them from biopsy-induced changes. The grading of ulcer size is reflected as a conditional element. The method for measuring the size of an ulcer (Fig. 6) is similar to that used to measure the submucosal invasion width. If an ulcer (full-thickness disruption of the muscularis mucosae) is observed on only one section, write the
actual size measured on the slide. If it is observed across two or more slices, write the larger of the following two values: (1) the actual size measured on the slide with the largest disruption size or (2) the number of slices spanned by the disruption × 2 mm (thickness of slice). The ulcer size is measured only within the tumor. If the ulcer spans the tumor and surrounding mucosa, measure the ulcer size only within the tumor area.

**Cases with adenoma components**

The adenoma component should be described only when the histological findings of adenoma are clear, and the intratumoral region is distinct from the adenocarcinoma component.

In diagnosis, only the adenocarcinoma contents should be described, and adenomas should be described separately as an additional item. For the size of the tumor, the size of the adenocarcinoma is described first, followed by the size of the total tumor. The distance from the resection margin describes the closest distance to any tumor component. If the resection margin is involved in a tumor or is less than 0.2 cm, the component should be described.

Unlike colorectal cancer, GC occurs in the adenoma-adenocarcinoma pathway in only a small number of cases, and adenomas of very small size are common. In addition, in many cases of WD adenocarcinoma, structural abnormalities are not severe, so areas that are difficult to differentiate from adenoma can be mixed in the tumor. Therefore, a background adenoma is identified only when the histological findings are clear and the area within the tumor is distinct from the adenocarcinoma component. If it is difficult to distinguish the mixed components, the entire lesion is treated as an adenocarcinoma. For example, if one component corresponds to adenocarcinoma and another component is severely dysplastic but difficult to determine as adenocarcinoma, the whole is treated as an adenocarcinoma component. For an adenoma, only the presence of the adenoma component is briefly described in a separate section.

**En bloc resection**

Piecemeal resection or full-thickness tearing should be confirmed and documented in the histological examination. Even if the specimen is resected into several pieces, it is not piecemeal if the tumor is intact within one piece.

**Lymphatic/venous invasion**

Unlike surgical specimens, lymphatic and venous invasions are recorded separately in endoscopic resection specimens because of the differing risks of lymph node metastasis. Both lymphatic invasion and venous invasion are criteria for determining a non-curative resection. However, the risk of lymph node metastasis posed by lymphatic invasion is times higher than that from venous invasion, and a higher score is assigned in the risk prediction model [59]. This information is helpful when clinicians decide whether or not to perform gastrectomy; thus, it is recommended to report them separately. The standard method for differentiating lymphatic and venous invasion is H&E staining with the following criteria: it is determined as a lymphatic vessel when there is a thin wall or lymphatic fluid and as a venous vessel when there is a thick muscle wall or many red blood cells in the lumen. When it is difficult to distinguish between lymphatic vessels and small venules, classify them as lymphatic vessels.

IHC staining may be performed to better observe lymphatic or venous vessels. However, because H&E and other immunostained slides are obtained from different levels, they should be...
interpreted separately. A specimen is deemed to be positive even if invasion is observed on only one slide.

**Histologic classification**

Histologic classification of GC is based on the 5th edition of the World Health Organization (WHO) blue book [4]. Representative histopathologic types described in the WHO classification are summarized in Table 3 and Fig. 7. The diagnosis of GC is usually determined according to the component that occupies the largest portion of the tumor, but the diagnosis of special histologic subtypes is based on the diagnostic criteria of each subtype. The most common subtype is tubular adenocarcinoma, characterized by prominent dilated or slit-like tubules. Carcinomas composed of solid tumor clusters with rare tubule formation are also classified as tubular adenocarcinoma. Tumor cells can be columnar, cuboidal, or flat, and luminal mucin/cell debris is common.

Papillary adenocarcinoma shows a papillary tumor structure with a central fibrovascular core and columnar or cuboidal tumor cells. For a diagnosis of papillary adenocarcinoma, more than 50% of the tumor area must contain the papillary tumor component [60-62]. High rates of liver metastasis, lymphovascular invasion, lymph node metastasis, and poor prognosis are reported in papillary adenocarcinoma [61-64].

Mucinous adenocarcinoma is defined when more than 50% of the tumor area shows extracellular mucin. Tumor cells in mucinous adenocarcinoma can show a glandular growth pattern, solid pattern, or scattered single cell pattern, including SRC carcinoma [4]. Mucinous adenocarcinoma is classified as the intestinal, diffuse, or indeterminate type according to the main component of tumor cell differentiation [4]. Mucinous adenocarcinoma tends to be diagnosed at an advanced stage [65,66].

Poorly cohesive carcinoma (PCC) is the second most common subtype of GC and is composed of isolated or small groups of tumor cells without gland formation [4]. Until the 3rd edition of

![Table 3. Histopathologic classification of gastric carcinoma](https://jpatholtm.org)
the WHO classification, SRC carcinoma was an independent subtype, but since the 4th edition of WHO classification, SRC has been included in the PCC category. Recently, several studies have suggested that non-SRC PCC (PCC-NOS) has a relatively poor prognosis compared with SRC and that SRC and PCC-NOS have different molecular profiles [67-70]. The WHO classification defines SRC as “composed predominantly or exclusively of signet ring cell components” [4]. A European group suggested a PCC classification definition according to the percentage of the SRC component (SRC, > 90%; PCC-NOS, < 10%; PCC with SRC component, 10%–90%), but no definite criteria for diagnosing PCC-NOS and SRC have been established, so more studies are required [71].

Mixed adenocarcinomas, according to the WHO definition, are carcinomas with both glandular (tubular adenocarcinoma/papillary adenocarcinoma) and poorly cohesive (PCC/SRC) components [4]. Some reports recently suggested that mixed adenocarcinomas have poorer prognosis, such as frequent local recurrence and lymph node metastasis, than a pure subtype of carcinoma, especially in EGC [72,73]. However, no clear criteria have established a minimum ratio of glandular/poorly cohesive components for a diagnosis of mixed adenocarcinoma. Contrary to the WHO definition, many studies define mixed adenocarcinoma as PD adenocarcinoma or a PCC/SRC component mixed with gland-forming components; those studies also report that the prognosis of mixed adenocarcinoma in EGC is worse than that of pure subtypes [39,74,75]. Although mixed adenocarcinoma does not have a clear definition, it seems that EGC has a poor prognosis when a glandular component coexists with other components in the same tumor; therefore, when both a glandular component and other components are observed in an EGC, it is recommended that they be mentioned separately.

Adenocarcinoma with lymphoid stroma (medullary carcinoma with lymphoid stroma) was previously called ‘lymphoepithelioma-like carcinoma’ or ‘medullary carcinoma.’ Tumor cells of this subtype show irregular sheets, poorly defined clusters or tubules, trabeculae, or syncytial cells with dense lymphocytic infiltration and intraepithelial lymphocytes [4,76]. Such a tumor usually shows a well-defined margin without infiltrative growth and minimal desmoplasia. This type of tumor is frequently associated with Epstein-Barr virus (EBV) infection and sometimes shows microsatellite instability/mismatch repair deficiency [4,76]. Patients with this subtype show a lower number of lymph node metastases and better prognosis after surgery than those with other subtypes [77,78].

Hepatoid adenocarcinoma is composed of hepatocyte-like tumor cells, which are large polygonal cells with eosinophilic-abundant cytoplasm arranged in a trabecular pattern [4,79]. This alpha-fetoprotein-positive tumor is often diagnosed preoperatively with multiple liver and lymph node metastases [4,79].

Micropapillary adenocarcinoma is characterized by an inside-out pattern of tumor clusters, which are small tumor clusters without a fibrovascular core, in clear spaces [4,80]. Micropapillary adenocarcinoma can be diagnosed when more than 10% of the tumor comprises micropapillary components [4,81]. This subtype is associated with poor prognosis and lymph node metastasis [4,80,81].

Adenocarcinoma of the fundic-gland type is composed of tumor cells showing chief cell differentiation, parietal cell differentiation, or both. Because this tumor does not show obvious nuclear dysplasia or structural abnormalities, it would be reasonable to regard it as adenocarcinoma only when it invades the submucosal layer. Lymph node metastasis is very rare in this subtype [4,82,83].

Undifferentiated carcinoma is composed of anaplastic cells without specific differentiation [4]. Grossly, a large ulcerating or fungating mass with necrosis is common. Tumor giant cells and rhabdoid tumor cells are common in this subtype, and spindle sarcomatoid cells can be seen [84,85]. Most patients show dismal prognosis with distant metastasis.

Squamous cell carcinoma is a very rare gastric tumor and shows morphology similar to that found in other organs. Adenosquamous carcinoma has both glandular and squamous tumor components, with ≥25% squamous component [4]. Gastroblastoma is a biphasic tumor composed of spindle and epithelial cells.

Crawling-type adenocarcinomas are characterized by complex branching or anastomotic structures and low-grade nuclei and have not yet been classified as a distinct subtype in the WHO classification [4]. Because of their low-grade nuclear atypia, reactive looking structural change, and mucosal location, crawling-type adenocarcinomas were once called a very WD form of gastric adenocarcinoma. Recent studies have shown that large crawling-type adenocarcinomas are often accompanied by PD components, and one report indicates that lymph node metastasis occurs frequently when the cancer invades beyond the submucosal layer [86,87]. Although it has not yet been classified as a formal subtype, some research results on crawling-type adenocarcinoma have recently been published, and attention needs to be paid in terms of prognosis.

Tubular adenocarcinoma and papillary adenocarcinoma can be graded. When two or more differentiations are mixed in an adenocarcinoma, the differentiation grade reflects the largest tumor
area. A distinct glandular structure composed of columnar cells is classified as WD, and a small glandular structure composed of cuboidal or flat cells is classified as MD. In a tumor with an indistinct glandular structure, carcinoma forming frequent luminal structures is classified as MD, and that with a rare luminal structure is classified as PD (Fig. 8) [3]. Although the WHO recommends a two-tier grading system of low- (WD and MD) and high-grade (PD), most pathologists and clinicians use a three-tier grading system. We have agreed to use a three-tier grading system that can be easily switched to a two-tier grading system.

**Histologic types in biopsy specimens**

In endoscopic gastric biopsy samples, it is often difficult to diagnose a specific subtype of gastric carcinoma. However, histologic subtypes and differentiation are important in the selection of a treatment modality. We recommend reporting a histologic component or subtype if there is a PD component or subtypes associated with poor prognosis (such as PCC, PD tubular adenocarcinoma, or micropapillary feature), irrespective of the proportion. Some peculiar subtypes of adenocarcinomas, such as adenocarcinoma of the fundic-gland type and EBV-associated gastric carcinoma, have a lower rate of lymph node metastasis than other subtypes with similar invasion depth, especially in EGC [82,88,89]. Reporting these subtypes and testing for EBV in situ in biopsy specimens could thus be helpful for patient management [89].

**Lauren classification**

The Lauren classification has been one of the most commonly used classification systems for GC worldwide since its publication in 1965 (Table 3) [90]. According to the WHO 5th edition, WD and MD papillary adenocarcinoma and tubular adenocarcinoma are classified as the intestinal type, and PCC and SRC are classified as the diffuse type (Fig. 9). In the Lauren classification, the mixed type (not the same as mixed adenocarcinoma in the histological classification) is used when intestinal and diffuse tumor components coexist in similar proportions. Although a table in the WHO blue book indicates that solid type, PD adenocarcinoma is classified as indeterminate type, this does not mean that all PD adenocarcinoma should be classified as such, and there is some disagreement among pathologists about the definition of the indeterminate type. Further discussion is needed to decide whether other special histological types of adenocarcinoma are excluded from the Lauren classification or whether they can be classified as intestinal, diffuse, or indeterminate according to their morphology.

To determine the feasibility of an endoscopic resection of tumors, most clinical guidelines and studies apply the differentiated type (papillary adenocarcinoma, tubular adenocarcinoma, WD and MD)/undifferentiated type (tubular adenocarcinoma, PD and poorly cohesive carcinoma, including SRC carcinoma) criteria of the Japanese guidelines [57]. In these criteria, PD adenocarcinoma is classified as the undifferentiated type. To prevent confusion with undifferentiated carcinoma, we do not recommend using the ‘differentiated type/undifferentiated type’ criteria in pathology reports. Instead, using the histologic classification and/or Lauren classification can provide sufficient information to clinicians and researchers.

**Adenoma**

Neoplastic epithelial proliferation without stromal invasion is called either adenoma or dysplasia. This intraepithelial neoplasia is usually called an adenoma by Western pathologists when the tumor shows a protruding, polypoid appearance with a distinct border and dysplasia when the tumor appears as a flat, depressed lesion or elevated indistinct lesion [4]. The Japanese classification tends to refer to elevated, flat, and depressed intraepithelial lesions as adenomas. Both adenoma and dysplasia can be used as terms for intraepithelial neoplasia in Korea.

Gastric adenomas can be subclassified into the intestinal type, foveolar type, pyloric gland type, and oxyntic gland type. Intestinal-type adenomas are the most common adenomas and usually
show tubule formation and columnar cells with elongated nuclei, with or without goblet cells and Paneth cells [4]. Foveolar-type adenomas are the second most common type of gastric adenoma, and an apical mucin cap is characteristic [91]. Pyloric gland type adenomas consist of columnar cells with ground-glass-like cytoplasm, basally located nuclei, and closely packed tubular glands with occasional dilatation [92]. Oxyntic gland type adenomas, also called oxyntic gland neoplasms because they can be diagnosed as adenocarcinoma only when submucosal invasion is confirmed, can progress into adenocarcinoma of the fundic gland type. This adenoma is composed of tumor cells with an oxyntic gland (chief cells, parietal cells, and mucous neck cells) and exhibits structural irregularity and minimal to mild nuclear atypia [82,88].

A two-tier system (low-grade/high-grade) is recommended for grading adenomas. Low-grade adenomas are characterized by a simple tubular or papillary architecture, hyperchromatic elongated or ovoid nuclei, preserved cellular polarity with basally located nuclei, and relatively regular intervening stroma without structural disruption. Goblet cells, apoptotic features, and mild to moderate mitotic features can be observed in low-grade adenomas (Fig. 10A). High-grade adenomas show more complex structures such as fusion, crowding, and budding of glands and the formation of glands with varying diameters. Cellular atypia is more pronounced in high-grade adenomas, such as loss of polarity, a high nuclear/cytoplasm ratio, pleomorphic nuclei, frequent mitosis, and atypical mitosis [93,94]. Intraglandular necrotic debris is also a diagnostic clue for high-grade dysplasia and, more commonly, adenocarcinoma (Fig. 10B) [95]. A diagnosis of adenocarcinoma should be considered when more than one of the following is present: evidence of stromal invasion (including single cell invasion into stroma and desmoplastic reaction), marked structural atypia, and marked glandular crowding (Fig. 10C) [94].

**Helicobacter pylori**

*H. pylori* infection is the most common cause of gastric adenocarcinoma, and eradication of *H. pylori* is associated with metachronous GC [96,97]. To detect *H. pylori* infection in a pathology specimen, additional staining (such as the Wright-Giemsa stain or Warthin-starry stain) is recommended. The proportion of drug-resistant *H. pylori* is increasing, and in patients with clarithromycin-resistant *H. pylori* infection, the failure rate of standard eradication treatment is also increasing. In patients with *H. pylori* infection, testing for clarithromycin-resistance is helpful for *H. pylori* eradication.

**Molecular markers**

All molecular tests are optional, conditional data elements. All report forms for the pathologic diagnosis of molecular markers are shown in Table 4.

**Human epidermal growth factor receptor 2 testing**

Determination of human epidermal growth factor receptor 2 (HER2) status is critical to identify patients with advanced-stage cancer for appropriate precision therapy. HER2-positive GC patients are currently treated with trastuzumab in combination with chemotherapy as first-line therapy, and fam-trastuzumab deruxtecan-nxki, a.k.a. trastuzumab deruxtecan, was recently approved by the Food and Drug Administration as a third- or later-line treatment [5,7,98,99]. HER2 status is principally determined by IHC or in situ hybridization (ISH) assays. HER2-positivity is defined as IHC 3+ or IHC 2+/ISH-positive [100,101]. HER2 testing requires formalin-fixed paraffin-embedded biopsy tissues with an adequate number of tumor fragments (ideally at least four) or representative surgical specimens with more differentiated components [102,103].

In currently recommended testing algorithms, HER2 status should be initially established using IHC [7,100] to estimate the
immunoreactive intensity and percentage of basolateral membranous expression on cancer cells [7,104]. The score ranges from 0 to 3 based on ≥10% cutoff level of HER2 expression in surgical specimens and ≥5 clustered cells in biopsy specimens as follows: 0 (negative), no reactivity or membranous reactivity in < 10% of cancer cells from surgical specimens or any cancer cells in biopsy specimens; 1+ (negative), faint or barely perceptible membrane reactivity; 2+ (equivocal), weak to moderate complete or basolateral membrane reactivity; and 3+ (positive), strong complete or basolateral membrane reactivity (Fig. 11).

Cases with a score of 2+ or indeterminate by IHC should be confirmed with ISH techniques to determine the final HER2 status [7,100]. Positive HER2 amplification is defined as a HER2:CEP17 (centromeric region of chromosome 17) ratio ≥ 2.0. To evaluate the ISH results, first check the HER2 IHC slide to select the most strongly stained region that might predict a higher level of HER2 amplification. Next, at least 20 evaluable, non-overlapping invasive tumor cells should be counted. If CEP17 signals are ≥3 and the ratio of HER2:CEP17 is < 2.0, an average HER2 copy number > 6 signals/cell is considered positive for HER2 amplification by ISH and < 4 signals/cell is considered negative. If an average HER2 copy number is between four and six signals/cell, another 20 cells should be counted in a different area. Sometimes, the determination of HER2 status is uncertain due to sample problems or technical issues [103,105]. In that case, the test should be reported as “cannot be determined.”

Some studies have revealed a significant correlation between HER2 expression and histologic subtype in GC. The Trastuzumab for Gastric Cancer (ToGA) trial and other published studies showed that the HER2 positivity rate was higher in differentiated subtypes (Lauren intestinal type and WD and MD type) than in the Lauren diffuse type or PD type [106-108]. Furthermore, intratumor heterogeneity of HER2 expression was reported in approximately 50% of GC cases [106,109]. Inter-lesional heterogeneity of HER2 expression for either positive or negative shifting has been reported between primary carcinomas and synchronous or metachronous locoregional/distant metastases at a rate of 2%–14% [110-115]. Therefore, HER2 status should be re-evaluated for all newly diagnosed secondary, recurrent, and metastatic lesions, regardless of the HER2 status of the primary cancer because it affects the therapeutic strategy and prognosis of patients [116,117].

**Microsatellite instability and mismatch repair deficiency**

Microsatellites, also called short tandem repeats, consist of repeats of a sequence that ranges from 1–6 nucleotides in length [103,118,119]. DNA mismatch repair (MMR) is a highly conserved mechanism to recognize and replace or repair mismatched nucleotides during DNA replication [119]. MMR deficiency (dMMR) is commonly caused by a germline mutation or sporadic epigenetic silencing and leads to insertions or deletions of nucleotides in microsatellite regions during DNA replication [119,120].
The four genes that play an important role in this process are mutL homolog 1 (MLH1), mutS homolog 2 (MSH2), mutS homolog 6 (MSH6), and PMS1 homolog 2 (PMS2) [103,119-121]. When MMR does not function normally, it is called microsatellite instability ( MSI) [119,122].

MSI is the hallmark of Lynch syndrome and is found in many sporadic cancers [103,123]. MSI-high (MSI-H) is observed in 6.9%–22.7% of sporadic cases [124-127]. As a distinct molecular subtype, MSI-GC is characterized by the gastric CpG island methylator phenotype with MLH1 silencing [124]. The clinical characteristics of MSI-GC are antrum (distal) locations, intestinal type of Lauren histology, early disease stage, and favorable prognosis [5,103,125,126]. Clinically, MSI is an actionable predictive biomarker for resistance to 5-fluorouracil-based adjuvant chemotherapy and indicates good suitability for immunotherapy [128-132]. For this reason, clinician requests for MSI and/or MMR test are increasing. In the National Comprehensive Cancer Network Guidelines for Gastric Cancer V.2.2022, universal MSI and MMR testing is recommended for all newly diagnosed GC patients, in accordance with the CAP DNA Mismatch Repair Biomarker Reporting Guidelines [100].

The three main methods used to detect MSI/dMMR are as follows: (1) polymerase chain reaction (PCR) amplification of microsatellite sequences; (2) IHC staining to determine the expression of the four MMR proteins MLH1, MSH2, MSH6, and PMS2; and (3) next-generation sequencing (NGS) [103,119,120,133]. Additionally, a new kit enables diagnosis of MSI according to the number of deleted base mutations by using a melting curve analysis with a peptide nucleic acid (PNA) probe [134].

PCR can compare the allelic position of the microsatellite locus in the tumor with that in normal tissue [103,120,135]. The National Cancer Institute recommends the so-called Bethesda Panel as reference [133,135]. This panel is composed of two mononucleotide repeats (BAT-25 and BAT-26) and three dinucleotide repeats (D2S123, D5S346, and D17S250) [22,103,133,135]. These regions are amplified in parallel using fluorescent PCR, and their sizes are assessed by capillary electrophoresis [133,136]. However, because the dinucleotide markers are less sensitive and specific than the mononucleotide markers [137], an alternative panel with five poly-A mononucleotide repeats (NR-21, NR-24, NR-27 [or Mono-27], BAT-25, and BAT-26) has also been suggested [22,103,119].

MSI-H is defined as instability of two or more of five microsatellite loci; MSI-low (MSI-L) is defined as instability of one site, and microsatellite stable (MSS) is defined as no instability at any site. Currently, clinical studies tend to categorize MSI-L and MSS

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**Table 4. Report form for pathologic diagnosis using molecular markers**

<table>
<thead>
<tr>
<th>Molecular markers</th>
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<tbody>
<tr>
<td><strong>PD-L1 immunohistochemistry</strong></td>
<td></td>
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<tr>
<td>Positive (3+/+)</td>
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<tr>
<td>Positive (2+/+)</td>
<td></td>
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<tr>
<td>Positive (1+/+)</td>
<td></td>
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<tr>
<td>Positive (0+/+)</td>
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<tr>
<td>Undetermined (explain):</td>
<td></td>
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<tr>
<td><strong>HER2 (ERBB2) in situ hybridization</strong></td>
<td></td>
</tr>
<tr>
<td>Number of invasive cancer cells counted:</td>
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</tr>
<tr>
<td>Using dual-probe assay</td>
<td></td>
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<tr>
<td>HER2 (ERBB2)/CEP17 ratio:</td>
<td></td>
</tr>
<tr>
<td>Average number of HER2 (ERBB2) signals per cancer cell:</td>
<td></td>
</tr>
<tr>
<td>Average number of CEP17 signals per cancer cell:</td>
<td></td>
</tr>
<tr>
<td>Using single-probe assay</td>
<td></td>
</tr>
<tr>
<td>Average number of HER2 (ERBB2) signals per cancer cell:</td>
<td></td>
</tr>
<tr>
<td>Summary: Negative/Positive for HER2 (ERBB2) gene amplification</td>
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<tr>
<td>Undetermined (explain):</td>
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<tr>
<td><strong>Microsatellite instability (MSI)</strong></td>
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<tr>
<td>Summary:</td>
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<tr>
<td>Negative (loss of expression)</td>
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<tr>
<td>Positive (retained expression)</td>
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<tr>
<td>Undetermined (explain):</td>
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<tr>
<td><strong>DNA mismatch repair immunohistochemistry</strong></td>
<td></td>
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<td>MLH1:</td>
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<td>Positive (retained expression)</td>
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<tr>
<td>Negative (loss of expression)</td>
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<td>Undetermined (explain):</td>
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<td>MSH2:</td>
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<td>Positive (retained expression)</td>
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<td>Negative (loss of expression)</td>
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<td>Undetermined (explain):</td>
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<td>PMS2:</td>
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<td>Positive (retained expression)</td>
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<td>Negative (loss of expression)</td>
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<td>Undetermined (explain):</td>
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<td>MSH6:</td>
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<td>Positive (retained expression)</td>
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<td>Negative (loss of expression)</td>
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<tr>
<td>Undetermined (explain):</td>
<td></td>
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<tr>
<td>Summary:</td>
<td></td>
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<tr>
<td>DNA mismatch repair deficiency (was/was not) observed</td>
<td></td>
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<tr>
<td>Because it is difficult to determine DNA mismatch repair deficiency, PCR-based testing and/or NGS for MSI is recommended.</td>
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<tr>
<td><strong>In situ hybridization for Epstein-Barr virus-encoded small RNAs</strong></td>
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<tr>
<td>Positive (diffuse/heterogenous [focal and/or mixed intensity])</td>
<td></td>
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<tr>
<td>Negative</td>
<td></td>
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<tr>
<td>Summary: Epstein-Barr virus-associated gastric carcinoma</td>
<td></td>
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<tr>
<td><strong>PD-L1 immunohistochemistry</strong></td>
<td></td>
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<tr>
<td>PD-L1 [Antibody (22C3 PharmDx/22C3 conc. Ventana/28-8 PharmDx/others: ___________________________):</td>
<td></td>
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<td>CPS =</td>
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as one type. This PCR method enables a functional measure of dMMR by directly measuring DNA changes. However, the method does not identify the MMR gene to be investigated. When the PCR test fails or the interpretation of the results is difficult, the test should be reported as “undetermined,” and IHC testing or NGS is recommended.

IHC for MMR proteins in GC samples is a simple and useful practice to determine dMMR. This method shows performance characteristics similar to MSI detection by PCR and a high concordance rate (>90%) [138]. The use of all four proteins, MLH1, MSH2, MSH6, and PMS2, is recommended for the IHC test. However, in more than 90% of cases, MSI-GC is associated with MLH1 and/or PMS2 losses by hypermethylation of the MLH1 gene. Because this IHC method is based on the ubiquitous expression of the MMR proteins in cell nuclei, nuclear staining should be checked when determining MMR positivity [22,119]. The presence of internal positive controls such as normal mucosa, lymphocytes, or stromal cells is essential for the interpretation of results [119]. dMMR is determined when the nuclear expression of at least one MMR protein is absent (Fig. 12) [22]. Heterogeneity of IHC or abnormal staining (cytoplasmic or membranous staining) is sometimes observed [138-143]. When it is difficult to interpret the IHC results, the test should be reported as “undetermined,” and PCR-based testing or NGS is recommended to confirm the MMR status. Using both IHC and PCR analyses for the detection of MSI-H/dMMR can reduce indeterminacy in the results.

EBV testing

EBV-associated gastric carcinoma belongs to one of four types of molecular classification suggested by the Cancer Genome Atlas (TCGA) [124]. Virus-host interactions play a pivotal role in EBV-induced carcinogenesis [144]. In EBV-associated gastric carcinoma, BamHI-A rightward frame 1 (BARF1) and latent membrane 2A (LMP2A) are putative viral oncogenes [145-147]. Once EBV enters the epithelium, EBV DNA methylation occurs globally. Hypermethylation of the CpG island promoter occurs throughout human cellular progress, which inactivates tumor suppressor genes [148]. Unique methylation leading to CDKN2A (p16) downregulation seems to be essential [124]. Eventually, EBV-infected gastric epithelial cells begin clonal growth, and gene mutations in EBV-infected cells lead to carcinogenesis [144]. EBV-associated gastric carcinoma is molecularly characterized by frequent mutations in phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA) [124] and AT-rich interaction domain 1A (ARID1A) [125], rare TP53 mutations [124], and the overexpression of interferon-γ [149] and programmed death ligand 1 (PD-L1) [124,150].

EBV-associated gastric carcinoma has distinct histologic, genetic, and immune microenvironmental features. Notably, EBV-associated gastric carcinomas exhibit a dramatic response to pembrolizumab immunotherapy (100% overall response rate) [130]. EBV positivity can be a good indication for immunotherapy in GC. Moreover, in submucosal invasive GC, EBV positivity has been associated with a low risk of lymph node metastasis [151,152].
ISH for EBV-encoded small RNAs (EBERs) is the most suitable and widely used method to detect EBV in formalin-fixed paraffin-embedded tissues and cytology specimens [153,154]. It is a highly sensitive detection method because of the large number of EBERs (10^6–10^7 copies/cell) [19], but it cannot be used for quantitative analysis of viral particles. Several commercial probes for EBERs are available, in which EBERs labeled with biotin, digoxigenin, or fluorescein can be visualized by microscopic examination. In most EBV-associated GCs, EBER signals are observed with strong intensity in almost all cancer cell nuclei. In certain cases, EBER signals are heterogeneous, i.e., positive only in a focal portion of the cancer or mixed—weak to strong—intensity (Fig. 13). Recently, focal positivity of EBER signals was reported in 18% of EBV-associated GC cases in Germany [155]. In daily practice in Korea, however, intratumoral heterogeneity of EBER signals is not as high as in those German cases. Whether focal negative/weak intensity represents an absence of EBV infection or a subcritical or insufficient copy number of EBERs remains unclear [156]. EBER signals are rarely detected in intratumoral or peri-tumoral lymphocytes, which originate from peripheral B lymphocytes infected with EBV in a latent state.

**PD-L1 immunohistochemistry**

The programmed death-1 receptor (PD-1)–PD-L1 interaction is one of the major mechanisms of immune modulation that allow T-cell inactivation and tumor immune evasion [157]. Blocking the PD-1/PD-L1 pathway is a standard therapeutic strategy for various solid tumors, including GCs [158].

Pembrolizumab was granted accelerated FDA-approval as a third-line treatment of GC based on the findings of the phase 2 KEYNOTE-059 trial, which demonstrated its treatment benefit in advanced GC patients with PD-L1 combined positive score (CPS) positivity (CPS ≥1). Accompanying approval was granted for the PD-L1 IHC 22C3 pharmDx assay on the Autostainer Link 48 platform as a companion diagnostic assay [159]. However, the subsequent phase 3 KEYNOTE-061 trial failed to demonstrate a significant survival improvement in PD-L1-positive GC patients [160].

Another phase 3 trial, CheckMate-649, demonstrated the efficacy of nivolumab in combination with fluoropyrimidine and platinum-based chemotherapy as a first-line treatment for HER2-negative advanced or metastatic GC, gastroesophageal junction cancer, and esophageal adenocarcinoma patients with PD-L1 CPS ≥ 5 [161]. In that trial, PD-L1 expression was determined using the PD-L1 IHC 28-8 pharmDx assay on the Autostainer Link 48 platform. Recently, that assay earned the CE-IVD mark in Europe as a companion diagnostic for identifying candidates for nivolumab treatment.

Both assays share the CPS scoring system to determine PD-L1 expression, which is the number of PD-L1–stained cells (tumor cells, lymphocytes, and macrophages) divided by the total number of viable tumor cells, multiplied by 100. For adequate evalu-
ation, a specimen containing a minimum of 100 viable tumor cells is required [162]. A PD-L1–stained tumor cell should present partial or complete membrane staining of viable cells with more than faint staining intensity (≥1+). PD-L1–stained immune cells include only mononuclear inflammatory cells (lymphocytes or macrophages) within tumor nests and adjacent stroma and show membrane and/or cytoplasmic staining. Other stromal cells such as fibroblasts, neutrophils, and plasma cells should be excluded from the CPS numerator. If the result of the calculation exceeds 100, it is presented as a maximum score of 100. If the PD-L1 staining shows heterogeneous results, the final CPS should be estimated by calculating each area’s CPS result (Fig. 14).

Because two different PD-L1 assays have been approved based on different CPS cutoff values, the interpretation of PD-L1 positivity should be based on the CPS cutoff value appropriate to the assay used for evaluation. The PD-L1 IHC 22C3 pharmDx assay uses CPS ≥1 for CPS positivity, and the 28-8 pharmDx assay uses CPS ≥5. The report should specify the assay type and appropriate cutoff value used for the PD-L1 positivity interpretation.

Previous studies have reported changes in PD-L1 expression during chemotherapy [163,164] and discrepancies between primary and metastatic lesions [164,165]. Therefore, re-evaluation of PD-L1 IHC in secondary, recurrent, and metastatic lesions is recommended for GC patients.

**Next generation sequencing**

Recently identified molecular profiles are not only important for improving our understanding of driver alterations involved in gastric carcinogenesis, but also for identifying clinically relevant biomarkers and new potential therapeutic targets [124,125]. Therefore, the clinical need for NGS in AGCs is increasing.

According to the recent National Comprehensive Cancer Network (NCCN) guideline, the biomarkers implicated in clinical management of AGC include HER2, MSI, PD-L1, tumor mutation burden (TMB) status, and neurotrophic tyrosine receptor kinase (NTRK) gene fusion [100]. Among these, TMB can only be assessed using NGS, and NTRK fusion is best evaluated using NGS (preferential RNA sequencing) [166]. Alternatively, it can be screened with TRK IHC, and then sequencing can be performed in positive cases [166]. Some other targets also showed promising clinical results in advanced GC, such as fibroblast growth factor receptor 2 (FGFR2) amplification [167], epidermal growth factor receptor (EGFR) amplification [168], MET amplification [169], and alterations of homologous recombination deficiency–related genes [170]. In addition, there are very rare (prevalence <1%) targetable tissue–agnostic variants [171] such as BRAF V600E [172], anaplastic lymphoma kinase (ALK) fusion [173], and reactive oxygen species 1 (ROS1) fusion [174].

TMB is defined as the total number of somatic coding muta-

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Fig. 13. A representative figure of Epstein-Barr virus (EBV) in situ hybridization. Diffuse positive EBV-encoded small RNA (EBER) signals (A). Heterogenous pattern of EBER signals in cancer cells. EBER signals appear within a few intratumoral lymphocytes (B).
tions in a tumor and represents an emerging biomarker for immunotherapy response in cancer patients [175]. The exploratory analysis for KEYNOTE-062 suggested an association between TMB and the clinical efficacy of first-line pembrolizumab-based therapy in patients with advanced GC [176]. Although whole exome sequencing is considered the gold standard for TMB, recent targeted gene panels have also provided accurate quantification [175]. The lack of harmonization in panel-based TMB quantification and lack of robust predictive cutoffs are currently some of the main limitations of TMB as a biomarker in clinical practice [175].

The gold standard for MSI detection is PCR or IHC. Recently, several MSI detection methods based on NGS have shown high concordance (> 95%) with the conventional PCR-based assay [171,177,178]. The recent NCCN guidelines indicate that sequencing via a validated NGS assay may be used to determine MSI status and other biomarkers when limited tissue is available for testing [100].

Tissue preparation is one of the most important factors for getting accurate and reliable results from NGS. In general, the total DNA and RNA requirements range from 10 to 300 ng for targeted gene panels [179]. Tissue specimen requirements are formalin-fixed, paraffin-embedded tissue or cytology specimens [179]. The minimum sample requirement for reliable sequencing results is a specimen with a tumor fraction and surface area >10%–20% and 5 mm², respectively [179].

Mucin phenotype

GC is classified as the gastric type, intestinal type, mixed type, or unclassified type based on the expression of MUC5AC, MUC6, MUC2, and CD10 [3]. The gastric type is positive for MUC5AC and/or MUC6, and the intestinal type is positive for MUC2 and/or CD10. The mixed type is positive for both gastric and intestinal mucins, and the unclassified type is negative for both.

Easy methods for molecular classification

Molecular profiles of GCs have been published in recent studies by TCGA and the Asian Cancer Research Group (ACRG). TCGA classified GCs into EBV, MSI, genomically stable, and chromosomally unstable [124]. In contrast, ACRG published a molecular classification of MSI, microsatellite stable/epithelial mesenchymal transition (MSS/EMT), MSS/TP53+, and MSS/TP53– [125]. The MSS/EMT subtype is closely associated with the SRC and PCC histology and Lauren’s diffuse type, and patient survival is poor. The EBV and MSI subtypes are related to the histologic type of adenocarcinoma with lymphoid stroma and have relatively better prognosis. High TMB and increased expression of PD-L1 are commonly reported in the EBV and MSI subtypes.

Several studies have reported that these molecular classifications could be reproduced in GCs using simple techniques, including EBV ISH, MSI testing, MMR IHC, E-cadherin IHC, and p53 IHC [127,180,181]. Using those tests, GC is classified as EBV, MSI, EMT, altered p53, and not altered p53. Those molecular subtypes showed distinct clinicopathologic characteristics.

Supplementary Information

The Data Supplement is available with this article at https://doi.org/10.4132/jptm.2022.12.23.

Ethics Statement

Not applicable.

Availability of Data and Material

Data sharing not applicable to this article as no datasets were generated or analyzed during the study.

Code Availability

Not applicable.

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Conflicts of Interest
S.H.L., a contributing editor of the Journal of Pathology and Translational Medicine, was not involved in the editorial evaluation or decision to publish this article. All remaining authors have declared no conflicts of interest.

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Standardized pathology of gastric cancer


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Standardized pathology of gastric cancer


위암 병리보고서 기재사항 표준화 2판

서론(Introduction)


한편, 암종의 조직병리학적 분류 및 예후 예측에 관계된 몇몇 병리학적 특징을 포함하여 위암 병리에 있어서 그동안 상당한 변화가 있었다[4,5]. 또한 표적 치료제나 면역항암요법의 도입 등 위암의 치료 전략이 빠르게 발전함에 따라 위암에 대한 분자병리학적인 검사는 필수적인 것으로 바뀌었다[6,7]. 따라서 병리의사들을 위해 위암 진단의 최근 변화를 반영하는 표준화된 보고서 제2판을 마련함의 필요성이 대두되었다.

2022년 3월 대한병리학회 소화기병리학연구회는 보고서 개정을 위한 위원회를 구성하였다. 위원회는 (1) 위절제 검체, (2) 내시경절제 검체, (3) 조직학적 분류 그리고 (4) 위암의 분자표지자 등 네 가지 주제를 논의하는 소위원회로 구성되었다. ‘위암 병리보고서 기재사항 표준화 2판’은 전체 위원회와 소위원회의 여러 회의를 거쳐 개발되었다.

표준화된 위암 병리보고서의 목표는 표준화된 병리학적 진단을 가능하게 하고 암종의 유병률을 통한 전문가의 커뮤니케이션을 통해 치료영역을 확장시키는 데 있다. 위암의 예후와 관련된 기본적인 병리학적 소견은 “표준 기재사항” 부분에 설명되어 있으며, 분자표지자 등을 포함한 진단 및 보조 치료와 관련된 다른 항목들은 “선택 기재사항” 부분에서 다루고 있다. 병리의사들이 적극적으로 활용할 수 있도록 본 보고서는 영문판과 함께 한글판으로도 작성하였다.

표준화 병리보고서의 적용

본 표준화 병리보고서는 원발성 위암에 적용된다. 신경내분비종양(neuroendocrine tumors), 림프종(lymphomas), 위장관절종양(gastrointestinal stromal tumors, GIST) 및 기타 육종(sarcomas)은 적용 대상에서 제외한다. American Joint Committee on Cancer (AJCC) 제8판에 정의된 바와 같이, 위식도접합부(esophagogastric junction, EGJ)를 침범하면 EGJ을 중심으로 위측 2 cm 이하에 병변의 중심이 있는 양은 원위식도암(distal esophageal carcinoma)으로 간주되므로 역시 적용 대상에서 제외한다[8]. 본 병리보고서는 잔여(항암화학요법 후 또는 내시경절제술 후) 암종에도 적용된다.
I. 위절제 검체

위절제 검체 종류(Gastrectomy type)

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrectomy (specimen) type</td>
</tr>
<tr>
<td>□ Total gastrectomy</td>
</tr>
<tr>
<td>□ Distal (subtotal) gastrectomy</td>
</tr>
<tr>
<td>□ Proximal gastrectomy</td>
</tr>
<tr>
<td>□ Wedge resection</td>
</tr>
<tr>
<td>□ Others ( ____________ )</td>
</tr>
</tbody>
</table>

해설: 수술기록을 참조하여 위절제술의 종류를 표시해야 한다.

육안 형태(Gross type)

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross type</td>
</tr>
<tr>
<td>□ EGC type</td>
</tr>
<tr>
<td>□ EGC type I/IIa/IIb/IIc/III</td>
</tr>
<tr>
<td>□ Mixed EGC type ( ____________ )</td>
</tr>
<tr>
<td>□ AGC type</td>
</tr>
<tr>
<td>□ Borrmann type 1/2/3/4/unclassifiable</td>
</tr>
<tr>
<td>□ Others ( ____________ )</td>
</tr>
</tbody>
</table>

해설: 병변의 육안적 형태를 기술해야 한다(여러 개일 경우 별개적으로 기술한다). 조기위암(early gastric cancer, EGC)은 일본의 분류법(type 0의 하위분류)을 따르며[9], 진행성위암(advanced gastric cancer, AGC)은 Borrmann 분류법을 따른다. 분류가 불가능한 유형(unclassifiable)은 일본의 분류법에 따라 Borrmann 5형에 해당한다[9]. 육안 형태는 육안 검사를 통해 결정된다. 따라서, 육안 소견과 현미경 소견 간에 차이가 있을 경우, 즉 육안 소견상 EGC이지만 현미경적으로는 중앙이 근육을 침범하는 경우(AGC), 육안적 형태는 육안 소견 항목에 남겨놓아야 하며 현미경 소견에 따라 수정하지 않는다. 이러한 경우, 다음과 같이 기재할 것을 권장한다: AGC, mimicking EGC type X 혹은 EGC, mimicking Borrmann type X. 육안적으로 AGC가 의심될 경우 가장 깊은 침윤을 포함하여 최소 4개의 대표적인 부위를 슬라이드 절편으로 제작해야 하며, 종양에 가장 가까운 장막 표면에 잉크를 칠해야 한다. 병변이 육안적으로 EGC일 경우 4–5 mm 폭으로 조직구축학적 검사(매핑, grid mapping)를 시행한다.
사전 치료(Previous treatment)

Standard data elements

Residual with previous treatment (when applicable)

<table>
<thead>
<tr>
<th></th>
<th>□ Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□ Previous treatment</td>
</tr>
<tr>
<td></td>
<td>□ Chemotherapy</td>
</tr>
<tr>
<td></td>
<td>□ Chemoradiotherapy</td>
</tr>
<tr>
<td></td>
<td>□ Endoscopic mucosal resection</td>
</tr>
<tr>
<td></td>
<td>□ Endoscopic submucosal dissection</td>
</tr>
<tr>
<td></td>
<td>□ Unknown</td>
</tr>
<tr>
<td></td>
<td>□ Others ( ____________ )</td>
</tr>
</tbody>
</table>

해설: 수술 전에 사전 치료를 받은 경우 이를 기록하고, 종양이 남아있을 경우에는 이것이 잔여 종양임을 기술해야 한다. 항암화학요법 후 위절제술을 시행 받은 경우에는 종양의 경우, 병변이 크고 명백히 관찰된다면 대표적인 슬라이드 절편을 제작하는 것으로 충분하다. 다만, 대표 절편에 잔여 암세포가 없거나 육안적으로 잔여 병변이 작거나 잘 보이지 않는 경우에는 종양 부위 전체를 슬라이드 절편으로 제작하여 현미경으로 검사해야 한다. 수술에서 내시경 절제술 후 추가 수술을 시행 받은 경우에도 종양 부위 전체를 슬라이드 절편으로 제작해야 한다.

종양 개수(Tumor focality)

Standard data elements

Tumor focality

<table>
<thead>
<tr>
<th></th>
<th>□ Single</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□ Multiple</td>
</tr>
</tbody>
</table>

해설: 단일 종양인지 다발성 병변인지 종양의 개수를 기록해야 한다. 두 개 이상의 종양이 발견될 경우 침음 깊이가 가장 깊은 종양부터 시작하여 각각의 종양에 대해 육안 및 현미경 소견 모든 항목을 적는다. 다만, 국소립프절전이(regional lymph node metastasis), 연관소견(associated findings) 및 독립병변(separate lesions)은 가장 깊은 종양에만 적는다.

종양 위치(Tumor location)

Standard data elements

Tumor location

<table>
<thead>
<tr>
<th></th>
<th>□ Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□ Esophagus/Upper/Middle/Lower third of the stomach/Duodenum</td>
</tr>
<tr>
<td></td>
<td>□ Center</td>
</tr>
<tr>
<td></td>
<td>□ Cardia/Fundus/Body/Antrum/Pylorus</td>
</tr>
<tr>
<td></td>
<td>□ Lesser curvature/Greater curvature/Anterior wall/Posterior wall</td>
</tr>
<tr>
<td></td>
<td>□ Others ( ____________ )</td>
</tr>
</tbody>
</table>

해설: 종양의 위치는 침범 부위(involvement)와 중심부(center) 두 부분으로 기술한다. 종양의 침범 부위는 가장 많이 침범한 순서대로 세 군데까지 기록한다. 위의 upper, middle 및 lower third의 구분은 일본 분류법을 따른다[9]. 종양의 중심부는 종양학 국제질병분류(International classification of
diseases for oncology, ICD-O 분류법에 따른 위치(cardia, fundus, body, antrum, pylorus, lesser curvature, greater curvature) [10] (cardia, fundus, body, antrum, pylorus, lesser curvature, greater curvature)와 전벽 및 후벽의 조합을 사용하여 표시한다[11]. 만일 종양의 위치를 적절하게 기술할 수 없을 때에는 ‘other’를 사용한다.

종양 크기(Tumor size)

**Standard data elements**

**Tumor size**

*One largest dimension*

□ _____ cm

**Conditional data elements**

*Secondary or tertiary tumor dimensions*

□ ___ x ___ cm  
□ ___ x ___ x ___ cm


종양치료반응등급(Tumor regression grade)

**Standard data elements**

**Tumor regression grade** *(when applicable)*

□ Grade 0: Complete response (no viable cancer cells)  
□ Grade 1: Near complete response (single cells or rare small groups of cancer cells)  
□ Grade 2: Partial response (residual cancer with evident tumor regression, but more than single cells or rare small groups of cancer cells)  
□ Grade 3: Poor or no response (extensive residual cancer with no evident tumor regression)

**Conditional data elements**

**Lymph node tumor regression** *(when applicable)*

□ Not identified  
□ Present


첨부 길이(Depth of invasion)

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of invasion (pT)</td>
</tr>
<tr>
<td>□ Invades lamina propria (pT1a)</td>
</tr>
<tr>
<td>□ Invades muscularis mucosae (pT1a)</td>
</tr>
<tr>
<td>□ Invades submucosa (sm1 / sm2 / sm3) (pT1b)</td>
</tr>
<tr>
<td>□ Invades proper muscle (pT2)</td>
</tr>
<tr>
<td>□ Invades subserosa (pT3)</td>
</tr>
<tr>
<td>□ Invades serosa (visceral peritoneum) (pT4a)</td>
</tr>
<tr>
<td>□ Directly invades adjacent structure (pT4b)</td>
</tr>
</tbody>
</table>

Specify (___________)

해설: 종양 침음의 길이는 AJCC 제8판과[8] 일본의 가이드라인을 따르고 있으나[9], 일본 가이드라인은 제자리암(carcinoma in situ, pTis)을 인정하지 않는다. AJCC 제8판에서는 pTis는 고유관(lamina propria)을 침범하지 않은 상피내 종양으로 정의되며, 이는 고등급 이형성에 해당한다. pT1b는 sm1, sm2, sm3로 세분된다. 암세포가 점막하조직과 고유근층(proper muscle layer)을 나누는 가상의 선 아래에 존재할 경우 암세포가 실제로 근육 섬유내에 있지 않더라도 pT2로 간주한다. 또한 궤양으로 인해 고유근층이 없는 경우 암세포가 고유근육 하단 경계를 잇는 가상의 선 아래에 존재할 경우 pT3로 간주한다. 그물막(omentum)과 위주변부 지방(perigastric fat) 침범은 pT3로 간주한다. 장막(내장폭막, visceral peritoneum) 침범을 제대로 평가하려면 육안 검사 시 종양과 가장 인접한 장막 표면에 잉크를 칠해야 한다. 암세포가 중피세포(mesothelial cells)에 부착되거나 중피세포를 넘어 노출된 경우는 pT4a로 간주한다. 결정관막(mesocolon)과 대망(greater omentum) 및 소망(lesser omentum)을 포함한 장막(gastric serosa)의 발생학적 기원이 다르기 때문에 결정관막의 침범은 pT4b로 간주한다. 그러나, 전정부(antrum)의 후벽, 위장막, 횡행결장 장막(transverse mesocolon)의 앞쪽과 같은 일부 부위는 단단히 유합되어 있으므로, 일본 가이드라인에서는 횡행결장 장막 침음의 경우 결정의 혈관(colic vessel)까지 침범하거나 결정관막 후면을 관통하지 않는 한 pT4b가 아니라고 명시하고 있다[9]. 따라서 결정관막의 침범은 종양의 위치에 따라 pT4a 또는 pT4b가 될 수 있다. 체장막(pancreas capsule) 침범은 pT4b로 간주한다.
십이지장이나 또는 식도로 직접 침범한 경우는 pT4b로 간주하지 않는다. 간, 체장, 대장, 비장, 횡격막 또는 신장에 비롯한 다른 장기가 침범된 경우는 이를 기록한다. 림프관 또는 혈관 내의 암세포는 침윤 깊이를 결정하는데 있어서 고려 대상은 아니지만[8], 이러한 혈관 침범의 존재는 괄호 안에 별도로 기록해야 한다(e.g., tumor invades proper muscle [involvement of subserosa by lymphatic emboli]).

### 절제면(Resection margin)

**Standard data elements**

<table>
<thead>
<tr>
<th>Proximal margin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>Involved by carcinoma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Distal margin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free from carcinoma (safety margin, ___ cm)</td>
</tr>
<tr>
<td>Involved by carcinoma</td>
</tr>
</tbody>
</table>

**Conditional data elements**

- Circumferential resection margin
  - Applied in EGJ or cardia cancer
    - Free from carcinoma (safety margin, ___ cm)
    - Involved by carcinoma

### 국소 림프절 전이(Regional lymph node metastasis)

**Standard data elements**

<table>
<thead>
<tr>
<th>Regional lymph node metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least 16 regional lymph nodes should be assessed</td>
</tr>
<tr>
<td>no metastasis in ____ regional lymph nodes</td>
</tr>
<tr>
<td>metastasis in ___ out of ___ regional lymph nodes</td>
</tr>
</tbody>
</table>

**Conditional data elements**

- Isolated tumor cell clusters
  - Applied in incidentally identified tumor cell cluster less than 0.2 mm in greatest dimension with no other regional lymph node metastasis (pN0)
    - Present [pN0 (i+)]

찾았다면 더 많은 림프절을 찾아 욕안 재검사를 시행해야 하며 추가 결과를 보고해야 한다. 그러나 이는 이전에 위 부분절제술, 수술 전 항암화학방사선치료를 한 경우에는 해당되지 않는다. 림프절 평가를 위해서는 전체적인 한 단면을 현미경적으로 관찰한다. 일반적으로 림프절에서 관찰되는 전이의 크기가 ≤0.2 mm이면 고립 종양 세포(isolated tumor cells, ITC), 0.2 mm 초과 2 mm 이하이면 미세전이(micrometastasis)라고 한다. 위암에는 미세전이 항목이 없으므로 이를 pN 병기에 반영한다[8]. AJCC 제8판에 따르면 다른 림프절 전이가 없는 경우 ITC는 pN 병기에 반영하지 않고 pN0 (i+)으로 보고해야 한다. 그러나 hematoxylin and eosin (H&E) 염색 슬라이드에서 충분히 관찰할 수 있는 ITC를 무시하기는 어렵다. 따라서 대부분의 경우 림프절 내의 모든 전이중양세포에 대해 크기에 관계없이 pN 병기에 반영하며 cytokeratin 면역조직화합염색(immunohistochemistry, IHC)을 통해 부차적으로 발견된 ITC만 pN 병기에서 제외한다. 림프절 구역(station)은 해당 라벨에 별도로 명명해오지 않는 한 보고하지 않는다. 종양침착(tumor deposit)은 식별 가능한 림프절 조직, 혈관 또는 신경 구조가 없이 원발종양과 떨어져서 림프절 구역에 존재하는 별개의 종양 결절로 정의된다(Fig. 1) [8]. 대장암과 달리 종양침착은 림프절 전이로 간주하여 pN 병기에 반영된다. 복막 전이(peritoneal seeding)는 pM1이므로 종양침착과 장막(복막) 전이 결절은 구분되어야 한다. 원위 림프절 전이(distant lymph node metastasis)는 pM1이며 이는 pN 병기에 반영하지 않는다. AJCC 제8판과 일본 가이드라인에서의 원위 림프절 전이의 정의는 다르며, 저자들은 AJCC 제8판의 정의를 따르 것으 권고한다. 따라서 상위 장간막 림프절 전이(superior mesenteric lymph node metastasis)는 pM1로 간주한다[8].

### Lymph node metastasis

<table>
<thead>
<tr>
<th>Conditional data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extranodal tumor extension</td>
</tr>
<tr>
<td>□ Not identified</td>
</tr>
<tr>
<td>□ Present</td>
</tr>
</tbody>
</table>

**해설:** 염색소가 림프절의 피막(capsule)을 넘어 주위 지방 조직까지 침범을 보이면 림프절 피막외 침범(extranodal tumor extension)으로 보고할 수 있다. 림프절 피막외 침범은 위암에서 좋지 않은 예후와 관련이 있는 것으로 알려져 있다[28-30].

### Lymphovascular invasion

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphovascular invasion</td>
</tr>
<tr>
<td>□ Not identified</td>
</tr>
<tr>
<td>□ Present</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conditional data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous invasion</td>
</tr>
<tr>
<td>Applied when identified in large vessels with an identifiable smooth muscle layer or elastic lamina</td>
</tr>
<tr>
<td>□ Not identified</td>
</tr>
<tr>
<td>□ Present</td>
</tr>
</tbody>
</table>

**해설:** 림프혈관 침범은 림프관과 혈관 침습 모두를 포함한다. 림프관과 혈관은 특히 작은 크기인
경우 H&E 슬라이드 상에서는 구분이 어렵다 (Fig. 2A, B). D2-40 또는 CD31에 대한 IHC를 시행할 수 있지만 위암에서 림프관과 혈관 침범 간의 예후적인 차이가 아직 충분히 평가되지 않았으므로 [12], 저자는 림프혈관 침범(lymphovascular invasion)을 사용할 것을 권장한다. 그러나 식별 가능한 평활근층이나 탄력막(elastic lamina)을 가지는 큰 혈관에서 종양 침범이나 색전(tumor invasion or emboli)이 관찰되는 경우 이를 '정맥 침범(venous invasion)'이라 하며 선택기재사항으로 보고할 수 있다 (Fig. 2C). 정맥 침범은 EGC와 [31,32] AGC에서 [33] 재발의 위험 인자로 보고된 바 있다.

신경침범(Perineural invasion)

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perineural invasion</td>
</tr>
<tr>
<td>□ Not identified</td>
</tr>
<tr>
<td>□ Present</td>
</tr>
</tbody>
</table>

해설: 신경침범은 암세포가 신경 내 또는 신경 주변에서 관찰될 때 보고한다 [34].

기존 선종(Pre-existing adenoma)

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-existing adenoma (when present)</td>
</tr>
<tr>
<td>Used if the carcinoma is within the adenoma</td>
</tr>
<tr>
<td>□ Tubular/Tubulovillous/Villous adenoma</td>
</tr>
<tr>
<td>□ Low grade dysplasia/High grade dysplasia</td>
</tr>
</tbody>
</table>

해설: 기존 선종은 선종 내에서 암종이 관찰되면 보고한다. 선종이 암종과 별개로 떨어져 있는 경우에는 독립병변(separate lesions)으로 보고한다.

연관소견(Associated findings)

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associated findings (when present)</td>
</tr>
<tr>
<td>□ Tumor perforation</td>
</tr>
<tr>
<td>□ Serosal (peritoneal, mesenteric) seeding</td>
</tr>
<tr>
<td>□ Distant metastasis</td>
</tr>
<tr>
<td>Other organ, specify: __________________</td>
</tr>
<tr>
<td>Distant lymph node</td>
</tr>
</tbody>
</table>

해설: 종양 천공, 장막(복막, 장간막) 전이 및 특정 부위를 포함한 원격전이가 존재할 경우 보고한다.
독립병변(Separate lesions)

Standard data elements

Separate lesions (when present)

☐ Peptic ulcer
☐ Adenoma
☐ GIST
☐ Others (__________ )

해설: 소화성 궤양, 선종, 위장관 기질 종양 및 별도의 다른 병변이 있을 경우 보고한다.

II. 내시경절제 검체

검체 기술(Description of the specimen)

Standard data elements

Specimen size

☐ ___ x ___ cm

Gross type of tumor

Same as method of surgical specimen

해설: 검체의 크기는 가장 긴 축의 길이와 이에 수직인 길이로 표현한다. 종양의 크기는 가장 긴 축의 길이로만 나타났다. 종양의 육안 형태(gross type)는 위절제 검체와 동일한 방식으로 기술한다.

육안 검사(Sectioning of the specimen)

해설: 검체의 모든 측부 절제면(lateral margin)과 심부 절제면(deep margin)에 잉크를 칠하여 현미경상으로 평가할 수 있도록 한다. 검체 전체를 2mm 간격으로 연속 절편하여 파라핀 블록을 제작한다. 네 방향의 측부 절제면 중 가장 가까운 절제면과 종양이 절편방향에 같이 포함되어야 한다.

위장관 검체의 경우, 일반적으로 원위부를 9시 방향에 두고 육안 사진을 촬영한다. 각각의 측부 절제면으로부터의 거리가 특별히 다르지 않을 경우, 일반적으로 검체를 같은 방향으로 연속 절편한다. 그러나 육안상 가장 가까운 측부 절제면이 절편 방향에 포함되어 있지 않다고 판단되는 경우에는 사진 방향이 달라지더라도 가장 가까운 절제면과 종양이 함께 보일 수 있도록 검체의 방향 또는 매핑틀(frame)을 돌려야 한다(Fig. 3).

조직학적 유형 및 구성요소(Histologic type and components)

Standard data elements

Histologic type

According to the principles described in “Histologic classification” section

☐ WHO
☐ Lauren

Conditional data elements

Histologic components
해설: 종양의 조직학적 유형은 수술 검체와 동일한 방식으로 기술한다. 각 유형에 대한 진단기준 및 설명은 “III. 조직학적 분류” 부분에 기술한 내용을 참고한다. 종양의 주된 조직학적 유형은 반드시 기술해야 하며, 이는 별도로 종양 세포의 조직학적 다양성이 “조직학적 구성성분(histologic component)”에 추가로 기술할 수 있다. 종양 내에서 다양한 조직학적 아형이 관찰될 경우 조직학적 분류에 따라 모든 종양 구성성분을 기술하도록 한다. 이 경우 종양 구성요소의 양적 우위성을 나타낼 수 있도록 기술해야 하며, 기관별로 나름의 기술 방법을 사용할 수 있다. 예) 순서대로 기록: well differentiated (WD)–moderately differentiated (MD) > poorly differentiated (PD) > signet ring cell carcinoma (SRC); 간격변수(interval variable): WD-MD>50%, PD<50%, SRC<10%; 연속변수(continuous variable): WD-MD 65%, PD 30%, SRC 5%. 많은 연구에서 분화형(differentiated-type)과 미분화형(undifferentiated-type)의 구성성분이 혼합된 종양이 하나의 구성성분만 가지는 종양보다 림프절 전이 위험이 더 높다고 보고되어 있다.”III. 조직학적 분류”에서 분화형과 미분화형은 기록할 수 있도록, 설명을 위해 ‘분화형(고분화/중등도분화 관세양암종과 유두모양암종)과 미분화형(저분화 관세양암종, 저응집암종(poorly cohesive carcinoma, PCC), 반지세포암종[signet-ring cell carcinoma, SRC])을 적어두었다[35-40]. 미분화형 내에서 반지세포(signet ring cell) 유형은 다른 미분화형보다 림프절 전이 빈도가 높으며 분화형과 유사한 수준으로 보고되어 있다[41-43]. 또한 반지세포와 다른 유형의 구성성분이 혼합된 경우보다 완전히 반지세포로만 구성된 증례들의 림프절 전이 빈도가 더 낮다는 보고도 있다[44-47]. 다만, 지금까지는 내시경 절제에 있어서 근치적 절제의 판정기준은 조직학적 유형만을 고려하며 조직학적 구성성분의 다양성에 따른 차이는 고려하지 않기 때문에 이는 표준기재사항이 아닌 선택적기재사항에 반영된다. 현재 대한병리학회 소화기병리학연구회는 한국병원의료연구원의 연구과제로 내시경 절제술의 근치적 절제 판정기준에 대한 병리학적 연구를 진행 중이며, 향후 이 연구에서 중요한 결과를 얻을 수 있다면, 본 가이드라인 항목에 반영하는 것을 고려해볼 수 있다.

종양 크기(Size of tumor)

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size</td>
</tr>
<tr>
<td>One largest dimension</td>
</tr>
<tr>
<td>□ _____ cm</td>
</tr>
</tbody>
</table>

해설: 조직학적으로 확인된 종양의 가장 큰 축의 길이만 기록한다.

침윤 깊이(Depth of invasion)

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of invasion (pT)</td>
</tr>
<tr>
<td>□ Invades lamina propria (pT1a)</td>
</tr>
<tr>
<td>□ Invades muscularis mucosae (pT1a)</td>
</tr>
<tr>
<td>□ Invades submucosa (submucosal depth: _____ mm or µm)</td>
</tr>
<tr>
<td>□ Invades proper muscle (pT2)</td>
</tr>
</tbody>
</table>
<table>
<table>
<thead>
<tr>
<th>Conditional data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth of invasion (pT)</td>
</tr>
<tr>
<td>In case of submucosa invasion, the invasion width can be additionally described</td>
</tr>
<tr>
<td>□ Invades submucosa (submucosal depth: _____ mm or µm) (submucosal width: _____ mm)</td>
</tr>
</tbody>
</table>

해설: 침윤 깊이를 기술하는 방법은 기본적으로 위결제 검체의 것과 동일하다. 차이점은 점막하 침윤 중래의 경우 점막하층의 침윤 깊이를 측정하고 기술한다는 것이다. 실제 측정된 값을 mm 또는 µm 단위로 기재한다. 측정 방법은 점막근층의 가장 아랫면부터 가장 깊은 침윤 지점까지의 깊이를 측정하는 것이다. 중앙의 침윤에 의해 점막근층이 변형되는 경우(hypertrophied, displaced, completely disappeared)가 있을 수 있다. 이러한 경우 중앙에 의해 변형되지 않은 정상부위의 인접점막근층을 연장하는 가상의 선으로부터 측정한다(Fig. 4A). 이 방법을 적용하였을 때, 모든 증례에서 변형되지 않은 점막근층의 가장 낮은 면을 기준으로 사용해야 한다는 점을 명심해야 한다. 인접 점막근층의 진행경로가 곡선을 이루는 경우 가상의 선도 곡선을 따라 그리도록 한다(Fig. 4B).

점막근층이 변형되었을 때 침윤 깊이를 측정하는 방법에 대한 명확한 설명이나 연구결과는 없다. 일본 가이드라인의 경우, 2010년 제14판에 처음 기재되었으며 “궤양으로 인해 점막근층이 잘 보이지 않을 경우 인접한 정상 근층을 기준으로 가상선으로부터 깊이를 측정하여야 한다”고 기술하였다[9]. 그 후 2017년 제15판에서는 중앙의 표면으로부터 깊이를 측정하도록 변경되었다[48]. 우리나라는 병리의사들 중에는 점막근층이 변형되었을 경우, 변형된 근층의 가장 낮은 근섬유부터 측정하는 병리의사도 있고 인접한 정상근층 부위의 가상선으로부터 측정하는 병리의사도 있다. 이와 관련한 두 개의 국내 연구 모두 점막근층이 변형된 경우에 인접한 정상근층 부위의 가상선으로부터 측정하는 것이 적절하다고 보고하였으며[49,50], 이에 따라 본 가이드라인에서 표준 측정방법으로 권장한다.

점막하 침윤의 경우 침윤 깊이뿐만 아니라 침윤 넓이 또한 림프절 전이의 유의미한 위험요인이라는 연구결과가 있다[50,51]. 그러나 이에 대해 아직 다기관 연구의 수가 적고 근치적 절제기준에는 포함되어 있지 않기 때문에 선택적 기재사항으로 반영하였다. 현재 소화기병리학연구회 주관으로 진행 중인 내시경 절제 중래에서의 근치적 절제 판정 기준에 대한 연구에서 이 주제도 연구되고 있다. 침윤 넓이를 측정하는 방법은 다음과 같다(Fig. 5). 점막하 침윤이 한 슬라이드에서만 관찰되는 경우 슬라이드 내에서 실제로 측정된 해당 부분의 크기를 기록한다. 점막하 침윤이 두 개 이상의 슬라이드에서 관찰되는 경우 다음 두 값을 더 큰 값을 기록한다. (1) 침윤 넓이가 가장 큰 슬라이드에서 측정한 실제 크기 또는 (2) 침윤 범위가 걸쳐 있는 슬라이드의 수x2 mm (절편 두께).

<table>
<thead>
<tr>
<th>Standard data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resection margin</td>
</tr>
<tr>
<td>□ Lateral margin</td>
</tr>
<tr>
<td>□ Free from carcinoma (safety margin, ____ cm)</td>
</tr>
<tr>
<td>□ Involved by carcinoma</td>
</tr>
<tr>
<td>□ Deep margin</td>
</tr>
</tbody>
</table>
Free from carcinoma (safety margin, ___ cm)
Involved by carcinoma

Examples
a) Lateral: 1.0 cm; b) deep: 0.02 cm
a) Lateral: 0.2 cm (anterior wall); b) deep: 0.02 cm
a) Lateral: positive (anterior wall), 0.1 cm (distal); b) deep: 0.02 cm

Conditional data elements
Resection margin
- Proximal margin
  - Free from carcinoma (safety margin, ___ cm)
  - Involved by carcinoma
- Distal margin
  - Free from carcinoma (safety margin, ___ cm)
  - Involved by carcinoma
- Anterior margin
  - Free from carcinoma (safety margin, ___ cm)
  - Involved by carcinoma
- Posterior margin
  - Free from carcinoma (safety margin, ___ cm)
  - Involved by carcinoma
- Deep margin
  - Free from carcinoma (safety margin, ___ cm)
  - Involved by carcinoma

Examples
a) Distal: 0.1 cm; b) proximal: 0.4 cm; c) anterior wall: positive; d) posterior wall: 0.8 cm; e) deep: 0.02 cm

Conditional data elements
Ulceration
- Absent
- Present

Examples
- Ulceration
 Ulceration

□ Absent
□ Non-significant (≤4 mm)
□ Significant (>4 mm)

해설: 궤양은 활동기와 반흔 모두를 포함하여 점막근층의 전층 탈락으로 정의되며, 내시경 소견이 아닌 조직학적 소견에 의해 진단된다[5,9,52]. 궤양의 유무는 점막암(mucosal cancer)에서 내시경 절제술의 근처적 절제 여부 판단을 위한 중요한 기준이 되므로, 점막암 병리보고서에 반드시 기술되어야 한다[5]. 궤양이 내시경 절제술의 적용중에 포함되기 때문에 내시경 소견으로 궤양의 유무를 판단하지만, 궤양적 절제의 병리 검사를 통해 확인해야 한다. 점막결손(mucosal break)이 없는 경우 내시경적 진단이 어려우며[53], 내시경 소견으로는 궤양 음성이지만 병리학적으로 양성인 경우가 4.6~5.5%의 증례에서 보고되었다[54,55].

임상에서 발생하는 또 다른 문제는 작은 크기의 궤양과 원래 궤양이 없었지만 내시경 성장 후에 발생한 생검에 의한 변형(biopsy-induced changes)을 구별하는 기준이 명확하지 않다는 것이다. 내시경 소견에서 궤양 판별의 정확도가 낮기 때문에, 내시경 성장 상 궤양이 없었다는 것이 생검에 의한 변화라는 것을 보장하지는 못한다. 이에 대한 진단 기준은 Shimoda 등이 제시한 바 있으며[56], 이를 인용하여 일본 위암 치료 가이드라인에서 다음과 같이 기술하고 있다: “생검유래 흉터(biopsy-derived scar)는 조직학적으로 대개 점막근층 바로 아래의 작은 부위에 국한된 섬유화로 관찰된다. 궤양 흉터와 구별이 가능하지 않은 경우 UL1으로 분류해야 한다”[57]. 이 기준은 또한 미분화형 EGC에 대한 임상 연구인 JCOG1009/1010에도 사용되었다: “고유근층이 완전히 파괴된 경우와 점막하층의 섬유화가 파괴된 고유근층의 범위보다 넓은 경우에 UL (궤양)이 존재하는 것으로 판단한다”[58]. 본 소위원회의 경우 현재 진행중인 근처적 절제 기준에 대한 소화기병리학연구회 주관 연구에서 궤양의 크기를 측정하였으며, 이 문제에 대하여 구분이 가능한 기준을 조사한 결과, 궤양 크기가 4 mm 이하인 경우와 궤양이 없는 경우 국소절제 전이 위험은 동일한 것으로 판명되었다. 이들 기준으로 삼는다면 국소절제 전이 위험인자에서 아주 작은 궤양을 제외할 수 있으므로 생검에 의한 변화와 구별할 필요가 없다. 따라서 이를 반영하여 궤양 크기의 등급을 선택기제사항에 포함하였다. 궤양의 크기를 측정하는 방법은 다음과 같으며 점막하 천침 측정 방법과 유사하다(Fig. 6). 궤양(점막근층의 완전한 파괴)이 한 슬라이드에서만 관찰되는 경우 해당 슬라이드 낭에서 실제 측정된 크기를 기록한다. 궤양이 두 개 이상의 슬라이드에서 관찰되는 경우 다음 두 개 간 더 큰 값을 기록한다: (1) 점막근층의 파괴 폭이 가장 넓은 슬라이드에서 측정한 실제 크기 또는 (2) 점막근층의 파괴 범위가 적절히 있는 슬라이드의 수×2 mm (슬라이드 두께), 궤양의 크기는 오직 종양내에서만 측정한다. 궤양이 종양과 주변 점막에 걸쳐 있는 경우에는 종양에 해당하는 부위에서만 크기를 측정한다.

선종 성분이 있는 증례(Cases with adenoma components)

<table>
<thead>
<tr>
<th>Standard data elements</th>
<th>Cases with adenoma components</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Absent</td>
<td>□ Present</td>
</tr>
</tbody>
</table>

Specify: ______________
Example

Adenocarcinoma, tubular, well differentiated (intestinal)
- Size: 1.0 cm (total tumor: 2.0 cm)
- Margin: a) lateral (distal): positive (carcinoma); b) deep: 0.02 cm

Pre-existing adenoma: tubular adenoma, low grade

<table>
<thead>
<tr>
<th>Standard data elements</th>
<th>En bloc resection</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Yes</td>
<td>No (piecemeal/tearing)</td>
</tr>
</tbody>
</table>

**해설:** 선종의 조직학적 소견이 명확하고 종양내 부위가 선암종의 성분과 뚜렷이 구별될 때만 선종 성분에 대해 기술해야 한다.

진단에는 선암종에 대한 것만 기술하고, 선종은 추가 항목으로써 별개로 기술해야 한다. 종양의 크기는 선암종의 크기를 먼저 기술하고 전체 종양의 크기를 추가로 기술한다. 절제면으로부터의 거리는 그 성분이 어떤 것이든 상관없이 모든 종양의 성분으로부터 가장 가까운 거리를 기술한다. 절제면에 종양이 포함되어 있거나 0.2 cm 이내로 근접해 있다면, 그 성분에 대해 기술해야 한다.

대장암과 달리 위암은 소수의 사례에서만 선종-선암종 경로(adenoma-adenocarcinoma pathway)를 거쳐 발생하고, 아주 작은 크기의 선암종 또한 흔하다. 또한 많은 고분화 선암종에서 구조적 이상이 심하지 않은 경우가 많아서 선종과 구분하기 어려운 부분이 종양에 섞여 있을 수 있다. 따라서 조직학적 소견이 명확하고 종양내의 구역이 선암종의 성분과 뚜렷이 구별되는 경우에만 주변부 선암(visit adenoma)을 언급한다. 혼합된 성분을 구별하기 어려운 경우, 전체를 선암종으로 취급한다(예를 들어, 일부에 선암종으로 판단되는 성분이 있으면서 다른 부위는 이형성은 심하지만 선암종으로 판정하기는 어려운 성분이 있을 때, 전체를 선암종 성분으로 취급한다). 선종의 경우 선종 성분이 존재하는지에 대해서만 별도의 섹션(separate section)에서 간략히 기술한다.

<table>
<thead>
<tr>
<th>Standard data elements</th>
<th>Lymphatic invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Not identified</td>
<td>□ Present</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard data elements</th>
<th>Venous invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Not identified</td>
<td>□ Present</td>
</tr>
</tbody>
</table>

**해설:** 종양이 분할절제(piecemeal resection) 되었는지 또는 전층 파열(full-thickness tearing)이 보이는지를 조직학적 검사로 확인하여 기록하여야 한다. 검체가 여러 조각으로 절제되었다 할지라도 종양 전체가 온전하게 한 조각 안에만 존재한다면 그 종양은 분할절제 된 것이 아니다.

<table>
<thead>
<tr>
<th>Standard data elements</th>
<th>Lymphatic invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Not identified</td>
<td>□ Present</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard data elements</th>
<th>Venous invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Not identified</td>
<td>□ Present</td>
</tr>
</tbody>
</table>

**해설:** 위절제 검체와 달리 림프관 및 정맥 침범은 별도로 기록한다. 이는 두 인자의 림프절 전이 위험도가 다르기 때문이다. 림프관 침범과 정맥 침범은 모두 근치적 절제 여부를 결정하는
기준이다. 그러나 림프관 침범의 림프절 전이 위험은 정맥 침범보다 2-3배 높고 따라서 림프절 전이 위험이 예측 모델에서도 림프관 침범의 점수가 더 높다[59]. 이 정보는 임상의가 환자의 상태를 고려해 치료계획 여부를 결정할 때 도움이 되므로 별도로 보고하는 것이 좋다. 이들을 구분하기 위한 표준은 H&E 염색 슬라이드이다. H&E 염색법을 기반으로 이 둘을 구분하는 기준은 다음과 같다: 얇은 벽이 있거나 림프액이 있는 경우 림프관으로 판단하고, 두꺼운 근육벽이나 내강에 적혈구가 많이 있다면 정맥 혈관으로 판단한다. 림프관과 작은 세정맥 (venule)을 구분하기 힘든 경우, 림프관으로 분류한다.

림프관 또는 정맥 혈관을 더 잘 관찰하기 위해 IHC를 시행할 수 있다. 그러나 H&E 슬라이드와 면역염색 슬라이드는 각각 다른 층의 절편에서 얻어지므로 각각을 구별해서 해석해야 하며, 침범이 한 슬라이드에서만 관찰되는 경우라도 양성으로 판단한다.

III. 조직학적 분류

위암의 조직학적 분류(Histologic classification)

<table>
<thead>
<tr>
<th>WHO classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Tubular adenocarcinoma</td>
</tr>
<tr>
<td>□ Tubular adenocarcinoma, well differentiated</td>
</tr>
<tr>
<td>□ Tubular adenocarcinoma, moderately differentiated</td>
</tr>
<tr>
<td>□ Tubular adenocarcinoma, poorly differentiated</td>
</tr>
<tr>
<td>□ Papillary adenocarcinoma</td>
</tr>
<tr>
<td>□ Mucinous adenocarcinoma</td>
</tr>
<tr>
<td>□ Poorly cohesive carcinoma</td>
</tr>
<tr>
<td>□ Poorly cohesive carcinoma, signet-ring cell type</td>
</tr>
<tr>
<td>□ Poorly cohesive carcinoma, not otherwise specified</td>
</tr>
<tr>
<td>□ Mixed adenocarcinoma</td>
</tr>
<tr>
<td>□ Adenocarcinoma with lymphoid stroma</td>
</tr>
<tr>
<td>□ Hepatoid adenocarcinoma</td>
</tr>
<tr>
<td>□ Micropapillary adenocarcinoma</td>
</tr>
<tr>
<td>□ Adenocarcinoma of fundic-gland type</td>
</tr>
<tr>
<td>□ Undifferentiated carcinoma</td>
</tr>
<tr>
<td>□ Squamous cell carcinoma</td>
</tr>
<tr>
<td>□ Adenosquamous carcinoma</td>
</tr>
<tr>
<td>□ Gastroblastoma</td>
</tr>
<tr>
<td>□ Others (specify: __________________ )</td>
</tr>
</tbody>
</table>

해설: 위암의 조직학적 분류는 기본적으로 World Health Organization (WHO) 분류 5판을 따른다[4]. WHO 분류에 따른 대표적인 조직병리 아형들은 Fig. 7에 소개하였다. 위암의 조직학적 진단은 종양의 대부분을 차지하는 아형에 따라 이루어지지만, 특수한 조직학적 아형의 진단은 각 아형의 진단기준에 따라 이루어진다. 위암에서 가장 흔한 조직학적 아형은 관세암종(tubular adenocarcinoma)으로, 확장된 내강 또는 틈새 형태의 내강을 가진 세관 구조를 특징으로 한다. 종양의 일부에서만 세관을 형성하고, 대부분의 종양이 세관을 형성하지 않는 고형의 종양
단어로 이루어져 있는 경우도 관세포종으로 분류할 수 있다. 종양을 이루는 세포들은 원주형, 입방형, 또는 납작한 형태를 보일 수 있으며, 내강의 점액이나 세포 부스러기가 흔히 관찰된다.

유두모양세포종 papillary adenocarcinoma은 원주형 혹은 입방형의 종양세포들이 중심부의 세포유합관색 주변에서 유두모양으로 증식하는 형태로 보인다. 유두모양의 종양 성분이 전체 종양의 50%를 넘을 경우 유두모양세포종을 진단할 수 있다[60-62]. 유두모양세포종 환자는 간세포를, 밸렬혈관 관찰, 밸렬혈관 전이가 높은 비율로 관찰되며 나쁜 예후를 보인다는 보고들이 있다[61-64].

점액세포종 mucinous adenocarcinoma는 종양 면적중 세포바갈 점액이 전체의 50%를 넘게 차지하는 종양으로 정의된다. 점액세포종의 종양 세포들은 생육 혹은 고형구조를 이루며, SRC와 같이 특별한 구조를 만들지 않는 흔적 세포의 형태를 보일 수 있다[4]. 종양 세포의 구성 성분에 따라 점액세포종은 Lauren 분류에서 정형, 미만형, 혹은 불확정형으로 분류될 수 있다[4]. 점액세포종은 진행위암의 단계에서 진단이 되는 경우가 많다[65,66].

PCC는 두 번째로 흔한 위암의 아형으로, 생육세포를 형성하지 않는 흔적 단독 세포나 작은 집락을 이루는 종양세포로 구성된다[4]. SRC는 WHO 분류 3판 까지는 독립된 아형으로 분류되었지만, WHO 분류 4판 이후로 SRC는 PCC에 포함되었다. 최근 비반지세포 저응집암종(adenocarcinoma with lymphoid stroma, PCC-NOS)이 SRC에 비해 예후가 더 나쁘고, 서로 다른 분자 프로필을 보인다는 연구들이 발표되었다[67-70]. SRC는 “주된 종양세포가, 혹은 거의 대부분의 종양세포가 반지세포인 경우”로 WHO에 정의되어 있다[4]. 아직까지 PCC-NOS와 SRC를 가릴 수 있는 확실한 진단 기준은 없으나, 유럽의 한 연구진들은 반지세포성분의 비율(SRC, >90%; PCC-NOS, <10%; 반지세포성분을 가진 저응집암종, 10-90%)에 따라 PCC의 분류 정리의 제시하였고, 앞으로 확실한 분류를 위해서는 추가적인 연구들이 더 필요할 것이다[71].

혼합세포종 mixed adenocarcinoma은 WHO 정의에 따르면 생육조를 만드는 종양(관세포종/유두모양세포종) 성분과 저응집세포(SRC 포함) 성분을 둘 다 가지고 있는 종양을 일컫는다[4]. 최근의 일부 보고에서는 혼합세포종이 순수아형의 임상에서 특이 EGC에 깊은 국소 재발이나 밸렬혈관 전이 등 나쁜 예후와 관련이 있다고 제시하였다[72,73]. 그렇지만, 아직까지 혼합세포종의 진단을 위한 생/저유동 성분의 최소 비율에 대한 명확한 기준은 없다. 다른 많은 연구에서는 WHO의 혼합세포종 진단 기준과 달리 저응집암종 뿐 아니라 분화가 나쁜 생육성 성분이 생육조를 만드는 종양 성분과 섞여 있는 경우도 혼합세포종으로 정의하고 있지만, 이 연구들에서도 역시 EGC에서 혼합세포종이 순수아형의 임상에 비해 예후가 나쁘다고 보고하고 있다[39,74,75]. 혼합세포종이 명확히 정의되지 않은 경우가 많았지만, 생육조의 종양성분과 생육조를 만들지 않는 종양성분이 같이 있는 EGC는 나쁜 예후를 가지는 것으로 보인다. EGC에서 생육조를 만드는 종양성분과 생육조를 만들지 않는 종양성분이 같이 관찰될 경우에는 이를 별도로 인급해 주는 것이 권장된다.

팀프구면중과반만생암 방(adenocarcinoma with lymphoid stroma) 또는 팀프구면중과반만생암 방(adenocarcinoma with lymphoid stroma)은 이전에 혼합성유증세포종 혹은 ‘수질암’으로 불렸다. 이 아형에서 종양세포는 불규칙한 패 구조, 경계가 나쁜 군집/세관, 혹은 용합세포로 구성되며, 이 종양세포들 사이로 팀프구의 첨유가 밀도 높게 관찰된다[4,76].
종양 경계부에서 있는 섬유조직형성이나 침윤성 성장이 거의 관찰되지 않아 종양의 경계가 분명하게 주변과 구분되어 보이는 것이 특징이다. 많은 경우, 림프구비행질동반생암종은 Epstein-Barr virus (EBV) 감염성 위암과 관련이 있고, 일부는 허미부수체불안정 위암과 관련이 있다[4,76]. 이 아형은 수술 후 낮은 림프절 전이율 및 좋은 예후와 관련이 있다고 보고되어 있다[77,78].

간세포모양샘암종(hepatoid adenocarcinoma)은 기등 모양으로 배열된 풍부한 호산성 세포질을 가진 다각형 세포로 구성되어 간세포암종과 유사한 형태를 보인다[4,79]. 간세포모양샘암종은 IHC에서 알파태아단백 양성이며, 수술 전 이미 간의 다발성 전이나 림프절 전이가 있는 경우가 많다[4,79].

미세유두모양샘암종(micropapillary adenocarcinoma)은 섬유혈관핵이 없는 작은 종양군집들이 투명한 공간에 떠 있는 형태로 관찰된다[4,80]. 미세유두성분이 전체 종양의 10% 이상으로 높은 경우에서 전단될 수 있다[4,81]. 이 아형은 불량한 예후 및 림프절 전이와 관련이 있다[4,80,81].

위바닥샘형샘암종(adenocarcinoma of fundic-gland type)은 위의 주세포나 벽세포, 혹은 양쪽 모두의 분화를 보이는 세포로 구성되어 있다. 이 종양은 명백한 핵의 이형성이나 구조적 이상을 보이지 않는 경우가 많기 때문에, 점막하층으로 종양 침윤이 있는 경우에만 생암종으로 진단하는 것이 적절하다. 이 아형에서 림프절 전이는 매우 드문 것으로 알려져 있다[4,82,83].

미분화암종(undifferentiated carcinoma)은 특정 분화를 보이지 않는 역행성 세포로 구성된 암종이다[4]. 육안적으로, 커다란 궤양성/융기형 종양이 많은 경우, 고장이 혼합 관찰된다. 거대 종양세포나 횡문근육모양 세포가 혼히 관찰되며, 점막하층에서의 전이가 드문 것으로 알려져 있다[84,85].

편평세포암종(squamous cell carcinoma)은 위의 원발암으로는 아주 드문 종양으로, 다른 장기에서 관찰되는 편평세포암종과 형태적으로 유사하다. 생편평세포암종(adenosquamous carcinoma)은 생암종과 편평세포암종 성분이 섞여 있는 종양으로, 편평세포암종 성분이 종양의 25% 이상일 경우 진단할 수 있다[4]. 편평세포암종(gastroblastoma)은 방추세포와 상피세포로 구성된 이상성 종양이다.

포복형샘암종(crawling-type adenocarcinomas)은 종양세포 핵의 이형성이 뚜렷하지 않고 복잡하게 분지 혹은 연결되는 구조가 조직학적 특징인 종양으로 WHO 분류에서 아형으로 분류되어 있는 것이 아니다[4]. 이 종양은 핵의 변화가 미미하고 변용성 변화처럼 보이는 구조적 특성으로 인해 한 때 고분화 형태의 위암종으로 생각되었다. 정식 아형으로 아직 분류되지 않았지만, 크기가 큰 포복형샘암종은 분화가 나쁜 종양 성분이 동반되는 경우가 많으며, 점막하층 이상으로 침윤을 보이는 경우 림프절 전이가 혼하다는 최근의 보고들이 있기 때문에 예후와 관련하여 눈여겨 봐야 할 종양이다[86,87].

생암종의 분화도는 관세포암과 유두모양샘암종에 적용될 수 있다. 한 종양 내에서 두가지 혹은 그 이상의 분화가 혼합되어 관찰될 경우, 가장 많은 부위에서 관찰되는 분화에 따라 등급을 결정할 수 있다. 원주세포로 구성되어 뚜렷한 납작한 생 구조를 만드는 종양은 고분화로 분류되며, 입방형 혹은 납작한 생 구조로 구성된 작은 생구조로 구성된 종양은 종등도 분화로 분류되며, 내강
구조를 거의 만들지 않는 종양은 저분화로 분류된다(Fig. 8) [3]. WHO 분류에서는 저등급(고분화 및 중등도 분화)과 고등급(저분화)의 두 단계의 등급 분류체계를 권장하지만, 대부분의 병리의사들이 더 익숙한 세 등급의 분화도 분류체계를 여전히 사용하고 있는 점과, 세 등급의 분류체계는 두 등급의 분류체계로 쉽게 전환이 가능한 점을 고려하여, 우리는 고분화, 중등도 분화, 저분화의 세 등급 분류체계를 사용하는 것을 권장한다.

생검 검체의 병리 진단(Histologic types in biopsy specimen)

내시경 생검 검체에서 위암의 특정 아형을 진단하는 것은 어려운 경우가 많다. 그렇지만, 위암의 조직학적 아형과 분화도는 치료방법을 선택하는 데 있어서 중요한 역할을 하기 때문에, 저분화 성분이 생검 조직 내에 포함되어 있거나 나쁜 예후와 관련이 있는 아형(PCC, 저분화 관샘암종, 미세유두모양 성분 등)이 포함되어 있는 경우에는 나쁜 예후와 관련 있는 부분을 병리 진단지에 기재해 주기를 권장한다. 위암의 특정 아형들(위바닥형성암종, EBV관련위암 등)의 경우 유사한 병기의 다른 아형의 위암들과 비교하여 림프절 전이 빈도가 낮은 것으로 알려져 있기 때문에, EBV관련위암이 의심될 경우 생검 검체에서 EBV제자리부합 검사를 시행하고, 이런 아형이 확인되는 경우 병리진단에 기술하는 것이 환자 치료방침 결정에 도움이 될 수 있다[82,88,89].

Lauren 분류(Lauren classification)

<table>
<thead>
<tr>
<th>Lauren classification</th>
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</thead>
<tbody>
<tr>
<td>□ Intestinal</td>
</tr>
<tr>
<td>□ Diffuse</td>
</tr>
<tr>
<td>□ Indeterminate</td>
</tr>
<tr>
<td>□ Mixed</td>
</tr>
</tbody>
</table>

해설: Lauren 분류는 1965년 발표된 이후 전세계적으로 가장 많이 사용되는 위암의 분류 방법들 중 하나이다[90]. WHO 제5판에 따르면, 고분화 및 중등도 분화의 관샘암종/유두모양암종은 장형으로 분류되며, PCC와 SRC는 미만형으로 분류된다(Fig. 9). Lauren 분류에서 혼합형은 조직학적 분류에서 언급한 혼합암종과는 정의가 동일하지 않으며, 장형과 미만형의 중앙 성분이 유사한 비율로 같은 중앙에서 관찰될 경우 사용할 수 있다. WHO 제5판의 Lauren 분류 관련 표에서는 고형 구조를 보이는 저분화 생암종을 불확정형으로 표시하고 있지만, 이것이 모든 저분화 생암종을 불확정형으로 분류하여야 한다는 의미는 아니며, 병리의사들 간에도 불확정형의 진단 기준에 대해서는 아직 협의가 완전히 이루어져 있지 않다. 일부 특정 아형의 위암들을 Lauren 분류에서 제외하는 불확정형으로 진단해야 할 지, 아니면 이들 중 일부는 형태에 따라 장형, 미만형, 또는 불확정으로 분류해야 할 지는 추가적인 논의와 연구가 필요하다.

위암에서 내시경적점막하박리술적 치료가 가능한지 결정하기 위해, 대부분의 임상 진료 지침과 연구에서는 일부 가이드라인의 분화형(고분화/중등도분화 관샘암종과 유두모양암종)과 미분화형(저분화 관샘암종, PCC, SRC) 분류 기준을 사용한다[57]. 이 기준에 저분화 생암종은 미분화형으로 분류된다. 용어 사용시 미분화암종과의 혼동을 방지하기 위해, 병리보고서에 이러한
분화형/미분화형 기준의 사용은 권고하지 않는다. 조직학적 분류와 Lauren 분류를 사용하면 내시경적점막하박리술 치료의 대상이 되는 지 여부의 정보를 임상의사와 연구자들에게 충분히 제공할 수 있을 것으로 본다.

선종(Adenoma)

기질의 침윤을 동반하지 않는 상피세포의 종양성 증식을 선종(adenoma) 또는 이형성(dysplasia)이라고 부른다. 서구에서는 상피내 종양이 뚜렷한 경계를 보이고 내강으로 돌출된 용종의 형태를 보일 때 선종으로 부르고, 편평하거나 혼합된 병변과 경계가 뚜렷하지 않으면서 상승된 병변은 이형성으로 칭한다[4]. 반면에 일본의 분류는 편평, 헤 NavParams 상피내 병변들을 모두 선종이라고 부르는 경향이 있다. 한국에서는 선종과 이형성이 모두 상피내 종양을 칭하는 용어로 사용할 수 있다.

위의 선종은 정형(intestinal-type), 위로행(foveolar-type), 유문샘형(pyloric gland-type)과 산분비형(oxyntic gland-type)으로 분류할 수 있다. 정형 선종은 가장 흔한 선종으로, 보통 세관을 형성하는 구조를 가지며, 간격에 가진 원주형 세포로 구성되고 솔잔세포나 파네트세포가 섞여 있을 수 있다[4]. 위로행 선종은 두번째로 흔한 선종으로 세포 정족의 점액소가 특징적이다[91]. 유문샘형 선종은 간유리 점액을 보이는 원주형 세포들의 구조로 구성되어 있는데 핵들은 세포바닥에 위치하고 있고 조밀하게 배열된 생구조를 보이며 간혹 생 내강이 늘어나 있는 형태를 보여준다[92]. 산분비형 선종은 같은 세포학적 소견을 보이더라도 점막하 침윤이 있는 경우 생암종으로 진단할 수 있고, 점막하 침윤이 없음을 확인한 경우 선종으로 진단할 수 있기 때문에, 생검 진행시에는 산분비형 신생물로도 불린다. 산분비형 선종이 진행하여 점막하 침윤을 보이는 경우 위바닥생활생암종으로 진단할 수 있다. 산분비생을 구성하는 세포들(주세포, 벽세포, 목유성세포)로 구성되며, 구조적인 이상을 보이지만 경도의 핵 이형성만을 보이는 경우가 많다[82,88].

선종의 등급 분류는 저등급과 고등급의 두 등급 분류 체계를 권장한다. 저등급 선종은 단순한 세관 혹은 유두모양 구조가 특징적으로, 심한 이형성이 없고 간격화한 과압색질 핵을 가진 세포들로 구성되어 있다. 저등급 선종에서는 핵이 부정형성의 소실이 없고 세포바닥쪽에 위치하고, 구조가 부풀림이 없이 비교적 일정한 간격으로 배열되어 있다. 또한, 솔잔세포나 세포자멸사, 경도나 중등도의 유사분열이 관찰될 수 있다(Fig. 10A). 고등급 선종은 생구조의 용함이나 방아, 밀집된 모습을 보일 수 있고, 저등급 선종에 비해 다양한 직경의 생구조로 구성될 수 있다. 세포극성의 소실이나 높은 핵/세포질 비율, 다형성 핵, 빈번한 유사분열, 비정형 유사분열이 더 흔히 관찰된다[93,94]. 또한 중앙 생 내부에서 관찰되는 괴사 세포 파편은 생암종에서 더 흔히 관찰되나, 고등급 선종의 진단에도 도움이 될 수 있다(Fig. 10B) [95]. 생암종의 진단은 중앙세포의 기질 침윤(기질내 단일세포 침윤이나 기질내 유사조직형성반응), 심한 구조적 이상, 심하게 밀집된 중앙 생구조가 있을 때 고려해야 한다(Fig. 10C) [94].

헬리코박터 파일로리(Helicobacter pylori)
헬리코박터 파일로리 감염은 위샘종 발생의 가장 흔한 원인이며, 내시경적 절제술로 위암 치료 후 헬리코박터 파일로리 제균 치료를 하면 추가적인 염발생이 줄어드는 것으로 보고되어 있다[96,97]. 병리 검체에서 헬리코박터 파일로리 감염을 진단하기 위해서는 추가적인 염색(Wright-Giemsa 염색, Warthin-starry 염색)이 추천된다. 약물 저항성 헬리코박터 파일로리 비율이 증가하고 있으며, 클라리스로마이신 내성 헬리코박터 파일로리 감염 환자에게서 표준 제균 치료가 실패하는 비율 또한 증가하는 추세이다. 클라리스로마이신 내성 검사가 헬리코박터 파일로리 환자의 제균치료 방법 선택에 도움을 주는 것으로 알려져 있다.

Ⅳ. 분자표지자

모든 분자 검사는 선택 사항으로, 이는 “선택기재사항” 항목에 반영되어 있다.

**HER2 검사(HER2 testing)**

*Conditional data elements*

<table>
<thead>
<tr>
<th>HER2 immunohistochemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Negative (0/1+)</td>
</tr>
<tr>
<td>□ Equivocal (2+)</td>
</tr>
<tr>
<td>□ Positive (3+)</td>
</tr>
<tr>
<td>□ Undetermined (explain):</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HER2 (ERBB2) in situ hybridization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of invasive cancer cells counted: _____ cells</td>
</tr>
<tr>
<td>□ Using dual-probe assay</td>
</tr>
<tr>
<td>□ <em>HER2 (ERBB2)/CEP17</em> ratio: _____</td>
</tr>
<tr>
<td>□ Average number of <em>HER2 (ERBB2)</em> signals per cancer cell: _____</td>
</tr>
<tr>
<td>□ Average number of CEP17 signals per cancer cell: _____</td>
</tr>
<tr>
<td>□ Using single-probe assay</td>
</tr>
<tr>
<td>□ Average number of <em>HER2 (ERBB2)</em> signals per cancer cell: _____</td>
</tr>
</tbody>
</table>

**Summary:** Negative/Positive for HER2 (ERBB2) gene amplification

| Undetermined (explain): |

해설: Human epidermal growth factor receptor 2 (HER2) 검사 및 진단은 진행성 위암 환자에서 적절한 표적항암치료의 선택을 위해 매우 중요하다. 현재, HER2 양성 환자는 위암 환자에서는 1차 항암화학요법으로 trastuzumab과 화학요법을 병용하고 있으며, 3차 이상 항암화학요법으로 fam-trastuzumab deruxtecan-nxki (또는 trastuzumab deruxtecan)이 미국 Food and Drug Administration에서 승인되었다[5,7,98,99]. HER2 검사는 IHC와 제자리 부합법(in situ hybridization, ISH)으로 시행된다. HER2 양성은 IHC 3+인 경우 또는 IHC 2+이며 ISH 양성인 경우로 정의한다[100,101]. HER2 검사는 포르말린 고정 파라핀 포매(formalin-fixed paraffin-embedded) 조직에서 시행하는데, HER2의 중앙내 이질성(intratumoral heterogeneity)을 고려하여 내시경 생검인 경우 4조각 이상의 생검 조직이 권장되고, 외과적 절제술 검체인 경우 장상 분화를 보이는 부분이 포함된 검체가 권장된다[102,103].

현재 권장되는 HER2 검사의 순서는 먼저 IHC를 시행해야 한다[7,100]. IHC는 면역염색의 강도 및
암세포 중 기저외측막 발현 비율을 추정한다[7,104]. 생검 검체에서는 HER2 발현이 5개 이상의 암세포 군집에서 관찰되는 경우를 기준으로 하고 수술 검체에서는 10% 이상의 암세포에서 관찰되는 경우를 기준으로 발현 강도에 따라 0에서 3점으로 판독한다. 발현 강도는 0 (negative)는 음성 또는 암세포의 10% 미만에서 세포막에 양성인 경우, 1+ (negative)은 희미하거나 거의 감지되지 않게 세포막에 약양성인 경우, 2+ (equivocal)은 약하거나 중등도로 세포막에 완전하게 또는 기저외측으로 양성인 경우, 3+ (positive)는 강하고 완전하거나 기저외측 세포막에 양성인 경우로 판독한다(Fig. 11).

HER2 면역염색 결과 2+ (equivocal)인 경우에는 ISH로 확인해야 한다[7,100]. HER2 ISH 진단 기준은 CEP17 (17번 염색체의 동원체 부위, centromeric region of chromosome 17)에 대한 HER2 copy의 비율이 2 이상인 경우로 정의된다. ISH 결과를 평가하기 위해서는 먼저 HER2 IHC 슬라이드에서 HER2 유전자의 증폭이 예상되는 가장 강하게 염색된 부위를 확인한 후 ISH 슬라이드의 같은 부위에서 최소 20개의 평가 가능하며 증폭되지 않는 침윤성 암세포에서 copy 수를 세어야 한다. 추가적으로 평균 CEP17 copy 수가 3개 이상이고 HER2:CEP17 비율이 2 미만인 경우에는 세포당 평균 HER2 copy 수가 6개를 초과하면 HER2 증폭 양성으로 진단하고, 4 미만이면 음성으로 진단한다. HER2 copy 수가 평균 4개에서 6개인 경우에는, 종양의 다른 부분에서 추가로 20개의 암세포를 평가해야 한다. 때때로, HER2 검사의 진단은 검체 문제 또는 기술적 문제로 인해 병리의사가 해결할 수 있는 수준을 벗어나는데[103,105], 그런 경우에 검사 결과는 ‘undetermined’로 보고해야 한다.

HER2 양성 위암은 임상적 병리학적으로 HER2 음성 위암과 구별되는 특성을 가진다. ToGA 임상시험 및 이전 연구들에서 HER2 양성 위암은 장형 또는 고분화 아형에서 미만형 또는 저분화 아형보다 높은 비도로 관찰되었고[106-108], HER2 발현의 중앙내 이질성도 50%에서 보고되었다[106,109]. 또한 HER2 발현의 중앙간 이질성(원발성 암종과 동시성 또는 이시성 국소 재발/원격 전이 사이의 이질성)이 2–14%로 보고되었다[110-115].

따라서, HER2 발현의 이질성은 표현형양치료제의 선택 및 환자의 예후에 영향을 미치기 때문에 원발암의 HER2 결과와 상관없이 새롭게 진단되는 모든 이차 원발성, 재발성, 전이성 병변에 대해 HER2 검사의 추가 시행이 권장된다[116,117].

현미부수체 불안정성 및 불일치복구 결핍(Microsatellite instability and mismatch repair deficiency)

<table>
<thead>
<tr>
<th>Conditional data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microsatellite instability (MSI)</strong></td>
</tr>
<tr>
<td>Summary:</td>
</tr>
<tr>
<td>□ Microsatellite stable (MSS)</td>
</tr>
<tr>
<td>□ Microsatellite instability–low (MSI-L)</td>
</tr>
<tr>
<td>□ Microsatellite instability–high (MSI-H)</td>
</tr>
<tr>
<td>□ Undetermined (explain)*</td>
</tr>
<tr>
<td><strong>DNA mismatch repair immunohistochemistry</strong></td>
</tr>
<tr>
<td>MLH1:</td>
</tr>
<tr>
<td>□ Positive (retained expression)</td>
</tr>
<tr>
<td>□ Negative (loss of expression)</td>
</tr>
<tr>
<td>□ Undetermined (explain):</td>
</tr>
</tbody>
</table>
MSH2:
- Positive (retained expression)
- Negative (loss of expression)
- Undetermined (explain):

PMS2:
- Positive (retained expression)
- Negative (loss of expression)
- Undetermined (explain):

MSH6:
- Positive (retained expression)
- Negative (loss of expression)
- Undetermined (explain):

Summary:
- DNA mismatch repair deficiency (was/was not) observed
- Because it is difficult to determine DNA mismatch repair deficiency, PCR-based testing and/or NGS for MSI is recommended.

Because it is difficult to determine MSI status, mismatch repair immunohistochemistry and/or NGS is recommended.

**해설**: 단연쇄 반복(short tandem repeats)으로도 불리는 현미부수체(microsatellites)는 뉴클레오타이드 1-6개 길이의 염기서열의 반복으로 구성된다[103,118,119]. DNA 불일치복구(mismatch repair, MMR)는 고도로 보존된 기전으로 DNA 복제 시 불일치 뉴클레오타이드를 인식하고 교체 및 복구하기 위해 설계된다[119]. MMR 결핍(deficient MMR, dMMR)은 주로 DNA 복제 동안 현미부수체 부위에서 뉴클레오타이드의 삽입 또는 결실로 이어지는 생성세포 돌연변이(germline mutation) 또는 산발적 후생 유전적 사일런싱(sporadic epigenetic gene silencing)에 의해 발생한다[119,120]. 이 과정에서 중요한 역할을 하는 네 개의 유전자는 MLH1, MSH2, MSH6, PMS2이다[103,119-121]. MMR이 정상적인 기능을 하지 않는 현상을 현미부수체 불안정성(MSI)이라 한다[119,122].


MSI-H 또는 dMMR을 발견하기 위해 사용되는 3가지의 대표적 검사 방법은 (1) 현미부수체 염기서열의 중합효소 연쇄반응(polymerase chain reaction, PCR)을 이용한 증폭, (2) MLH1, MSH2, MSH6, PMS2의 4개 MMR 단백질의 발현을 보기 위한 IHC, (3) 차세대 염기서열분석(next generation sequencing, NGS)이다[103,119,120,133]. 또한 PNA probe를 이용한 melting curve 분석을 통해
핵산증폭산물의 길이변화를 검출하는 새로운 MSI 진단 키트가 개발되었다[134].

PCR 방법은 정상조직 대비 종양조직에서의 핵산증폭산물의 길이 변화를 비교하는 방법이다[103,120,133]. 미국 National Cancer Institute (NCI)는 MSI 검사에 베데스다 패널(Bethesda panel)을 권고하였다[133,135]. 이 패널은 두 개의 모노뉴클레오타이드 마커(monomonucleotide repeats, BAT-25, BAT-26) 및 세 개의 다이뉴클레오타이드 마커(dinucleotide repeats, D2S123, D5S346, D17S250)로 구성된다[22,103,133,135]. 이러한 부위는 형광 PCR를 사용하여 증폭되며, 그 크기들은 모세관전기이동(capillary electrophoresis)으로 평가한다[133,136]. 그러나 모노뉴클레오타이드 마커가 다이뉴클레오타이드 마커보다 민감도와 특이도가 높다고 알려지면서[137], 5개의 플리-A 모노뉴클레오타이드 마커(NR-21, NR-24, NR-27 [또는 모노-27], BAT-25, BAT-26)로 구성된 대체 패널이 제시되었다[22,103,119].

MSI-H의 진단 기준은 5개의 현미부수체 마커 중 두 개 이상에서 불안정성(unstable)을 보이는 것이고, MSI-low (MSI-L)는 하나의 마커에서 불안정성을 보이는 반면, microsatellite stable (MSS)은 5개 모든 마커에서 불안정성이 나타나지 않을 때이다. MSI-L과 MSS는 임상적으로 차이가 뚜렷하지 않아 하나로 분류하는 경향이 있다. 이러한 PCR 방법은 DNA의 변화를 직접 측정하여 MMR의 기능적 측정을 가능하게 하지만, 이 방법으로는 MMR 유전자 중 어느 유전자의 변이인지 알 수 없다. PCR 검사의 오류나 실패, 또는 PCR 결과의 해석이 어려운 경우, 검사를 "undetermined"으로 보고해야 하며, 이 경우에는 IHC 검사 또는 NGS 검사가 추천된다.

위암 검체에서 MMR 단백질에 대한 IHC는 dMMR을 판단하기에 간단하고 유용한 검사법이다. 이 방법은 PCR에 의한 MSI 검사와 유사한 진단율을 보이며 두 검사 방법은 높은 일치율(>90%)을 보인다[138]. MMR 검사는 MLH1, MSH2, MSH6, PMS2의 4개 단백질에 대한 면역염색 검사가 권장된다. 그러나 MSI-H 위암의 90% 이상에서 MLH1 유전자의 과메틸화에 의한 MLH1 및 PMS2 발현 소실과 관련이 있다. 이러한 IHC 방법은 정상 세포의 세포핵에 MMR 단백질이 항상 발현하는 현상을 기반으로 하기 때문에, MMR 양성을 결정할 때 핵에 양성인지 확인해야 하며[22,119], 정상 절막, 리프가 모모 두께 세포와 같은 내부 양성 대조물질에서 세포핵의 양성 소견이 MMR 진단에 필수적이다[119]. dMMR은 MMR 단백질 중 한 개 이상에서 세포핵 발현이 소실될 때 진단할 수 있다(Fig. 12) [22]. 때때로 IHC의 이질성 또는 비정형적 염색(세포질 또는 막염색)이 관찰되기도 한다[138-143]. IHC 결과를 해석하기 어려운 경우 "undetermined"로 진단되어야 하며, MMR 상태를 확인하기 위해 PCR 기반의 MSI 검사 또는 NGS 검사가 추천된다. PCR 기반 MSI 검사와 MMR IHC를 함께 시행하는 경우 결과 오류를 줄일 수 있다.

**엠스타인-바 바이러스 검사(Epstein-Barr virus testing)**

<table>
<thead>
<tr>
<th>Conditional data elements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In situ hybridization for Epstein-Barr virus-encoded small RNAs</strong></td>
</tr>
<tr>
<td>□ Positive [diffuse/heterogenous (focal &amp;/or mixed intensity)]^a,b</td>
</tr>
<tr>
<td>□ Negative</td>
</tr>
<tr>
<td><strong>Summary:</strong> Epstein-Barr virus-associated gastric carcinoma</td>
</tr>
</tbody>
</table>

^Checking the signal pattern is optional; aThe term “Epstein-Barr virus-associated gastric carcinoma” applies to positive cases.

해설: 엠스타인-바 바이러스(EBV) 연관 위암(EBV-associated gastric carcinoma)은 The Cancer Genome
E-payment is currently not supported. Please consider alternative means of payment.
위암에서는 pembrolizumab이 PD-L1 combined positive score (CPS)가 양성(CPS ≥1)인 진행성 위암 환자에서 생존율 향상을 증명한 제2상 KEYNOTE-059 임상시험의 결과를 근거로 3차 항암화학요법을 위한 FDA 승인을 받았으며, Autostainer Link 48 플랫폼 상의 PD-L1 IHC 22C3 pharmDX 분석법이 동반 진단 분석법으로 함께 승인되었다[159]. 이후 제3상 KEYNOTE-061 임상시험에서는 PD-L1 양성 위암 환자에서 통계적으로 유의한 생존율 향상을 증명하는데 실패하였다[160].

또 다른 제3상 임상시험에서는 CheckMate-649가 PD-L1 CPS ≥5인 HER2-음성 진행성 또는 전이성 위암, 위장관 질환, 식도 선암종 환자의 일차 항암화학요법 치료를 위해 fluoropyrimidine 및 platinum 기반 항암화학요법을 병용한 nivolumab의 치료 효능을 입증하였다[161]. 이 임상시험에서 PD-L1 발현은 Autostainer Link 48 플랫폼을 이용한 PD-L1 IHC 28-8 pharmDX 검사를 사용하여 확인하였고, 이는 최근에 nivolumab 치료 대상군을 파악하기 위한 동반 진단으로 CE-IVD 마크를 유럽에서 획득하였다[159].

이 두 가지의 검사법은 임상 시스템을 공유하여 PD-L1 발현을 CPS로 평가하는데, 이는 PD-L1 양성인 종양세포, 림프구, 대식세포의 수를 종양세포의 전체 수로 나누고 100을 곱한 수이다. 충분한 평가를 위해 100개 이상의 생존한 종양세포가 포함된 검체가 필요하다[162]. 종양세포는 희미한 염색 강도(≥1+) 이상으로 부분적이거나 완전하게 세포막에 염색된 경우 양성으로 평가한다. 면역세포는 종양 내부 또는 종양 주변 기질 안에 있는 단백 염증 세포(림프구 또는 대식세포)의 세포막 또는 세포질에 양성인 경우를 포함한다. 섬유아세포, 종양세포, 형질세포 등은 CPS 평가 시 분차에서 제외되어야 한다. 만약 점수가 100을 초과한다면 CPS 결과는 100으로 평가한다. PD-L1 염색이 종양내 이질성을 보이는 경우, 최종 CPS는 각 부위의 CPS 결과를 계산하여 통합하여야 한다(Fig. 14).

두 개의 다른 PD-L1 검사법이 각기 다른 CPS 점수를 기준으로 승인되었기 때문에, PD-L1 양성의 진단은 사용된 검사법에 따라 각기 다른 CPS 기준을 기반으로 해야 한다. PD-L1 IHC 22C3 pharmDX 검사법은 CPS 양성에 대해 CPS ≥1 기준을 사용하며, 28-8 pharmDX 검사법은 CPS≥5를 사용한다. 따라서 보고서에는 사용한 검사법이 명시되어야 하고 적절한 기준을 PD-L1 양성 해석에 적용하여야 한다.

이전 연구에서 항암화학요법 후에 PD-L1 발현이 변할 수 있고[163,164], PD-L1 발현은 원발성 병변과 전이성 병변 간의 불일치가 있다고 보고한 바 있다[164,165]. 따라서 위암 환자의 진단, 재발성 및 전이성 병변에서 PD-L1 IHC의 재평가가 권장된다.

차세대 염기서열분석(Next generation sequencing)
최근에 확인된 분자유전학적 특성은 위암 발생에 수반된 driver mutation에 대한 이해도를 향상시키는 데 중요할 뿐만 아니라 임상적으로 적절한 바이오마커 및 새로운 잠재 치료표적을 파악하는 데에도 도움이 된다[124,125]. 따라서, 진행성 위암에서 NGS에 대한 임상적 요구가 증가하고 있다.

NCCN의 최근 가이드라인에 따르면 진행성 위암에서 임상적으로 필요한 바이오마커로는 HER2, MSI,
PD-L1, TMB, 그리고 NTRK fusion이 포함된다[100]. 이들 가운데 TMB는 NGS를 통해서만 평가될 수 있으며, NTRK fusion은 NGS (우선적으로 RNA 염기서열분석)으로 가장 잘 평가할 수 있다[166]. 또는 이것은 TRK IHC로 스크리닝할 수 있으며, 이후 양성 사례에서 염기서열분석을 시행할 수 있다[166]. 또한 fibroblast growth factor receptor 2 (FGFR2) 증폭[167], epidermal growth factor receptor (EGFR) 증폭[168], MET 증폭[169], homologous recombination deficiency-related genes의 변이[170]와 같은 바이오마커는 진행성 위암에서 임상적으로 높은 가능성을 보였다. 한편, 모든 고형성 종양에서 표적치료의 바이오마커로 보고된 BRAF V600E 돌연변이[171], ALK fusion[172], ROS1 fusion [173] 등은 위암에서 1% 미만으로 매우 드물게 관찰된다[174].

TMB는 종양에서 somatic coding mutation의 전체 수로 정의되며 암 환자에서 면역항암제 반응을 예측할 수 있는 바이오마커이다[175]. KEYNOTE-062 임상시험의 분석 결과에서 진행성 위암 환자에서 pembrolizumab 기반 1차 항암치료요법으로 TMB와 임상 효능 사이의 연관성을 제시하였다[176]. 전장 웰ɔ 염기서열분석(whole exome sequencing)이 TMB를 위한 최적의 표준 검사로 고려되는 반면에 최근 표적 유전자 패널[targeted gene sequencing] 및 정확한 TMB값을 제공한다[175]. 그러나 사용되고 있는 다양한 패널들은 대한 표준화가 되어 있지 않고, 면역치료반응 예측을 위한 TMB값의 기준이 명확하지 않아 실제 임상에서 바이오마커로 TMB를 적용하는데 한계가 있다[175].

MSI 검사의 표준 검사법은 PCR 기반 분석법이나 면역조직화학법이다. 최근 NGS를 기반으로 한 여러 MSI 진단방법이 기존의 PCR 기반 분석법과 95% 이상의 높은 일치율을 보여주었다[174,177,178]. 최신 NCCN 가이드라인에 의하면 검사를 위해 제한된 조직만을 사용할 수 있을 때, MSI 검사를 위해 탐성을 입증된 NGS로 다른 바이오마커의 검사와 함께 한 변에 시행할 수 있다고 언급하였다[100].

마지막으로 무엇보다도 적절한 검체의 준비는 NGS로 정확하고 신뢰할 만한 결과를 얻기 위해 가장 중요한 요소 중 하나이다. 일반적으로 중 DNA 및 RNA는 표적 유전자 패널 시행을 위해 10~300 ng가 필요하다[179]. 사용할 수 있는 조직 검체는 포르말린 고정 파라핀 포매 조직 또는 세포검사 검체이다[179]. 신뢰할 수 있는 NGS 결과를 위한 최소 검체 요건은 종양 분율은 10-20% 이상이어야 하고 종양의 표면적은 5 mm² 이상이어야 한다[179].

유신 표현형(Mucin phenotype)

위암은 MUC5AC, MUC6, MUC2, CD10의 발현 여부를 기준으로 위형(gastric type), 장형(intestinal type), 혼합형(mixed type), 미분류형(unclassified type)으로 분류된다[3]. 위형은 MUC5AC 또는 MUC6에 대해 양성이며, 장형은 MUC2 또는 CD10에 대해 양성이다. 혼합형은 위형과 장형 유신에 대해 모두 양성이며, 미분류형은 이 두 가지에 모두 음성인 경우이다.

분자 분류(Molecular classification by easy methods)

위암의 분자유전학적 분류는 TCGA group과 the Asian Cancer Research Group (ACRG) 등의 연구에서 발표되었다. TCGA 연구에서는 위암이 EBV, MSI, genomically stable, chromosomal instability로 분류될 수 있다고 하였다[124]. ACRG 연구에서는 MSI, microsatellite stable/epithelial mesenchymal transition (MSS/EMT), MSS/TP53+, MSS/TP53-를 포함한 네 가지 아형의 분자 분류를 발표하였다[125]. MSS/EMT
아형은 signet-ring cell (PCC)의 조직학적 소견 및 Lauren의 미만형과 밀접하게 연관되어 있으며, 불량한 환자 생존률을 보인다. EBV 및 MSI 아형은 림프 기질을 가진 선암종의 조직학적 형태와 관련이 있으며 비교적 더 좋은 예후를 갖는다. 높은 TMB와 PD-L1의 발현 증가가 EBV 및 MSI 아형에서 흔히 관찰된다.


대한병리학회 소화기병리학연구회 위암 기재사항 표준화 개정 소위원회

위원: 박영수, 국명철, 김백희, 이해승, 강동욱, 구미진, 신옥란, 최영희, 이원애, 김현기, 송인혜, 김경미, 김희성, 강구현, 박인정, 김준미, 최윤정, 장희경, 조미연, 안수민, 장미수, 한송희, 곽윤진, 서안나

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Infections and immunity: associations with obesity and related metabolic disorders

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About one-fourth of the global population is either overweight or obese, both of which increase the risk of insulin resistance, cardiovascular diseases, and infections. In obesity, both immune cells and adipocytes produce an excess of pro-inflammatory cytokines that may play a significant role in disease progression. In the recent coronavirus disease 2019 (COVID-19) pandemic, important pathological characteristics such as involvement of the renin-angiotensin-aldosterone system, endothelial injury, and pro-inflammatory cytokine release have been shown to be connected with obesity and associated sequelae such as insulin resistance/type 2 diabetes and hypertension. This pathological connection may explain the severity of COVID-19 in patients with metabolic disorders. Many studies have also reported an association between type 2 diabetes and persistent viral infections. Similarly, diabetes favors the growth of various microorganisms including protozoal pathogens as well as opportunistic bacteria and fungi. Furthermore, diabetes is a risk factor for a number of prion-like diseases. There is also an interesting relationship between helminths and type 2 diabetes; helminthiasis may reduce the pro-inflammatory state, but is also associated with type 2 diabetes or even neoplastic processes. Several studies have also documented altered circulating levels of neutrophils, lymphocytes, and monocytes in obesity, which likely modifies vaccine effectiveness. Timely monitoring of inflammatory markers (e.g., C-reactive protein) and energy homeostasis markers (e.g., leptin) could be helpful in preventing many obesity-related diseases.

Key Words: Metabolic disorders; Infections; COVID-19; Parasites; Immune cells

Several investigators have reported that obesity or obesity-related complications appeared to be associated with increased risk of hospitalization of coronavirus disease 2019 (COVID-19) patients and death [1-3]. In general, overweight or obese persons are at higher risk for infections and respond poorly to therapies [4,5]. Being obese or overweight corresponds to a state of energy imbalance and is caused by inappropriate intake of energy-dense foods and physical inactivity. According to the World Health Organization (WHO), more than 1.9 billion adults were overweight (body mass index [BMI], 25.0 to < 30) in 2016; of these over 650 million were obese (BMI, 30 or higher). In addition, over 340 million children and adolescents aged 5–19 were overweight or obese in 2016. Elevated BMI is an important risk factor for various non-communicable diseases such as type 2 diabetes and insulin resistance, hypertension, coronary artery disease, stroke, osteoarthritis, and certain cancers such as postmenopausal breast cancer, endometrial cancer, and renal cell carcinoma. With increasing BMI, the risk for these diseases also increases.

Obesity and its intimately associated disorder—type 2 diabetes or insulin resistance— are associated with gradual alterations in cellular physiology. Many investigators believe that the poor outcomes observed in individuals with obesity and type 2 diabetes/insulin resistance are due to immune system dysfunction that is triggered by chronic low-grade inflammation present in both health problems [6]. Interestingly, these patients also are at increased risk of infections and mortality from sepsis. Recent data suggest that infections may precipitate insulin resistance via multiple mechanisms such as the pro-inflammatory cytokine response,
the acute-phase response, and alteration of nutrient status [7]. In general, excessive adipose tissue in the body is known to hinder immune function, altering leucocyte counts as well as cell-mediated immune responses [8]. These immune cells are an intimate part of adipose tissue and an important source of pro-inflammatory cytokines/products, which ultimately contribute to the development of local adipose tissue inflammation and several metabolic complications [9].

In fact, adipose tissue acts as an endocrine organ; it secretes a number of hormone-like cytokines or adipokines, e.g., leptin, tumor necrosis factor α (TNF-α), monocyte chemoattractant protein-1 (MCP-1/CCL2), plasminogen activator inhibitor-1, resistin, adipin, and adiponectin among others. The majority of these adipokines participate in pro-inflammatory processes in obesity and perpetuate the state of insulin resistance [10]. Among these adipokines, several studies have suggested that leptin has an important role in major obesity-related health problems such as type 2 diabetes, hypertension, and different cancers [11]. Under normal conditions, leptin primarily maintains energy homeostasis through the central/hypothalamic anorexigenic pathway. However, in obesity, leptin possibly acts differently and supports a pro-inflammatory milieu. The long isoform of the leptin receptor (Ob-Rb) may play a key role in both physiologic and pathologic conditions. Interestingly, both long and short forms of the leptin receptor are expressed by different immune cells, e.g., B-cells, T-cells, neutrophils, eosinophils, monocytes, and macrophages [12-14]. Therefore, leptin can modulate both innate and adaptive immune response. An appropriate understanding of the complicated network of infectious pathologies and immune responses in obesity would help to formulate novel preventive strategies.

**CORONAVIRUS DISEASE, OTHER VIRAL INFECTIONS, AND OBESITY-RELATED PROBLEMS**

During the last two decades, there have been three coronavirus disease outbreaks. The first was the 2002–2004 outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV or SARS-CoV-1) that emerged in China. A similar disease, caused by Middle East respiratory syndrome coronavirus (MERS-CoV), was initially detected in Saudi Arabia in 2012. The latest coronavirus pandemic (COVID-19) caused by SARS-CoV-2 began in December 2019 in China. The rapid spread of this infectious disease has been documented in different parts of the world, and about 6.7 million deaths have been recorded so far. SARS-CoV-2 is an enveloped positive-sense single-stranded linear RNA virus; the envelope is coated with envelope (E) and membrane (M) proteins as well as a spike (S) glycoprotein that is responsible for binding to the host target cell receptor angiotensin-converting enzyme 2 (ACE-2). In addition, other cellular components such as extracellular matrix metalloproteinase inducer/CD147, transmembrane serine protease 2, and ADAM metallopeptidase domain 17 are implicated in viral endocytosis [15].

ACE catalyzes the conversion of angiotensin I to angiotensin II, which is an important step in the regulation of blood pressure via the renin-angiotensin-aldosterone system (RAAS). Moreover, ACE acts on several biomolecules including bradykinin, encephalin, substance P, and amyloid β-peptide (Ab), as well as in various physiological processes such as renal development, male fertility, hematopoiesis, and immune responses [16,17]. Conversely, ACE-2 is an important homolog of ACE and responsible for the conversion of angiotensin II to angiotensin 1-7, thereby counterbalancing ACE activity [18]. Both ACE and ACE-2 are cell membrane-anchored proteins, expressed in several organs, and functionally antagonistic to each other.

As mentioned earlier, to enter host cells, SARS-CoV-2 utilizes ACE-2 expressed in various organs, e.g., lung cells (pneumocytes and bronchial epithelium), gastrointestinal epithelium, and endothelial cells. Besides the lung parenchymal injury, there may be generalized endotheliitis (accumulation of lymphocytes, plasma cells and macrophages below the endothelium and in the perivascular spaces) [19]. In COVID-19, endothelial dysfunction is associated with the recruitment of immune cells and can result in many complications such as vasoconstriction, ischemia, inflammation, a pro-coagulant state, edema, and finally organ damage [20]. Moreover, abnormally increased levels of immune reaction and cytokines in different organs may cause a cytokine storm that can lead to additional organ dysfunction.

Obesity and insulin resistance are strongly connected to the activity of RAAS. Interestingly, expression of ACE-2, the functional receptor for viral entry, has been found to be higher in adipose tissue (and therefore higher in obesity) [21,22]. Furthermore, obesity and its complications such as hypertension, insulin resistance, and type 2 diabetes are associated with a higher risk of COVID-19 disease severity and mortality [22-24]. The impact of obesity and/or diabetes on SARS-CoV-1 infection has not been properly evaluated, although a few studies have investigated MERS-CoV–linked pathologies. One study of 32 MERS-CoV infected patients observed that mortality was significantly correlated with both obesity and diabetes [25]. A meta-analysis of 657 MERS-CoV cases revealed that diabetes and hypertension were present in roughly 50% of the patients [26]. Addition-
ally, in the 2009 H1N1 influenza pandemic, obesity was also identified as an important risk factor for a poor prognosis [27,28]. In general, a number of reports have documented an association between type 2 diabetes and chronic viral infections (Table 1) [29-48].

In addition to the disrupted immune response in obesity, other plausible mechanisms responsible for the poor prognosis of SARS-CoV-2 infection include obesity-associated pre-existing endothelial dysfunction, a reduction in respiratory compliance, dysregulated lipid metabolism, and an overabundance of pro-inflammatory cytokines [20,22,49]. It is worth mentioning that obese individuals have defective/decreased responses to vaccination [49]. As a result, the combined effects of obesity and viral infection may worsen the existing status of cytokines, which normally coordinate the immune system and physiological homeostasis.

**Table 1. Association between type 2 diabetes and common chronic viral infections**

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients' details</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ndiako et al. (2021); Nigeria</td>
<td>180 Diabetic patients and 100 non-diabetes controls</td>
<td>Higher risk of HBV infection among type 2 diabetic patients than non-diabetics</td>
</tr>
<tr>
<td>Iovanescu et al. (2015); Romania</td>
<td>246 Patients with chronic liver disease (136 chronic viral hepatitis, 110 viral liver cirrhosis)</td>
<td>A significant association between diabetes mellitus and HCV-induced chronic liver disease</td>
</tr>
<tr>
<td>Cheng et al. (2006); Hong Kong</td>
<td>2,838 Type 2 diabetes patients</td>
<td>HBV-infected patients had earlier onset of diabetes, higher frequency of retinopathy, and increased risk of end-stage renal disease than non-HBV-infected patients</td>
</tr>
<tr>
<td>Vinseda Chamorro et al. (2006); Spain</td>
<td>305 Patients who came for HCV assessment</td>
<td>A relationship between HCV infection and type 2 diabetes</td>
</tr>
<tr>
<td>Arao et al. (2003); Japan</td>
<td>866 Patients with chronic viral disease (707 HCV-infected and 159 HBV-infected)</td>
<td>HCV infection was closely associated with diabetes, and cirrhosis was an independent risk factor for diabetes</td>
</tr>
<tr>
<td>Dworzanski et al. (2019); Poland</td>
<td>173 Diabetic patients and 50 persons without diabetes</td>
<td>Prevalence of EBV, HPV, and EBV+HPV co-infection was significantly higher in diabetic patients than those without diabetes</td>
</tr>
<tr>
<td>Karjalta et al. (2011); USA</td>
<td>Data from the National Health and Examination and Nutritional Examination Survey (NHANES) 2007-2008</td>
<td>Obesity was significantly associated with HSV-1 infection</td>
</tr>
<tr>
<td>Fernandez-Real et al. (2007); Spain</td>
<td>74 Healthy middle-aged men from the general population</td>
<td>Significant positive relation between HSV-1 titer and fat mass</td>
</tr>
<tr>
<td>Sun et al. (2003); China</td>
<td>1,244 Inpatients (408 with dyslipidemia and 836 controls)</td>
<td>Prevalence of HSV-2 seropositivity was significantly higher in patients with dyslipidemia. BMI, diabetes, and hypertension were more common in patients with dyslipidemia than those without</td>
</tr>
<tr>
<td>Woelfle et al. (2022); Germany</td>
<td>From the German population-based KORA cohort (pre-diabetes, n = 1,257)</td>
<td>HSV-2 and CMV were associated with pre-diabetes incidence</td>
</tr>
<tr>
<td>Yoo et al. (2019); Korea</td>
<td>576 Adults with CMV diseases</td>
<td>Type 2 diabetes cases had a higher incidence of CMV diseases</td>
</tr>
<tr>
<td>Chen et al. (2012); Netherlands</td>
<td>549 Participants</td>
<td>CMV seropositive subjects were more likely to have type 2 diabetes</td>
</tr>
<tr>
<td>Roberts and Cech (2005); USA</td>
<td>113 Hemodialysis patients</td>
<td>A higher seroprevalence of anti-CMV IgG among diabetes patients</td>
</tr>
<tr>
<td>Chiu et al. (1997); Canada</td>
<td>Endarterectomy specimens from the 76 patients with carotid artery stenosis and 20 normal carotid artery and aortic tissue autopsy specimens</td>
<td>CMV was detected in carotid atherosclerotic plaques from 27 cases (35.5%)</td>
</tr>
<tr>
<td>Reinholdt et al. (2021); Denmark</td>
<td>Male population (n = 2,528,756), nationwide registry-based cohort study</td>
<td>Increased incidence rate of HPV-related anogenital intraepithelial neoplasia and cancer among men with diabetes than non-diabetic men</td>
</tr>
<tr>
<td>Sobti et al. (2019); UK</td>
<td>210 Patients with HNSCC</td>
<td>Prevalence of developing HPV-16–positive HNSCC was 3.79 times higher in diabetic patients than in those without diabetes. Moreover, diabetes was a risk factor for a poorer prognosis</td>
</tr>
<tr>
<td>Siima et al. (2021); USA</td>
<td>1,584 Men with pre-diabetes (793 with HIV, 791 without HIV, over a median 12-year follow-up)</td>
<td>40% higher risk for the development of diabetes among men with HIV</td>
</tr>
<tr>
<td>Kubiski et al. (2021); South Africa</td>
<td>1,369 Persons with HIV</td>
<td>Among adults with HIV, diabetes and pre-diabetes were common</td>
</tr>
<tr>
<td>Hema et al. (2021); Burkina Faso</td>
<td>4,259 Patients in a cross-sectional study</td>
<td>Prevalence of diabetes and hypertension was higher among persons with HIV on ART than the general population</td>
</tr>
<tr>
<td>Jeremiah et al. (2020); Tanzania</td>
<td>1,947 Adults (336 with HIV on ART, 956 with HIV ART-naive, 655 without HIV)</td>
<td>Prevalence of diabetes was high, particularly among HIV-infected ART-naive persons</td>
</tr>
</tbody>
</table>

HBV, hepatitis B virus; HCV, hepatitis C virus; EBV, Epstein-Barr virus; HPV, human papillomavirus; HSV, herpes simplex virus; BMI, body mass index; CMV, cytomegalovirus; HNSCC, head and neck squamous cell carcinoma; HIV, human immunodeficiency virus; ART, antiretroviral therapy.
COVID-19–ASSOCIATED OPPORTUNISTIC Fungal Diseases and Diabetes

Generally, the majority of COVID-19 patients are asymptomatic or have minor symptoms. Less than 20% of cases require medical attention. According to the National Institutes of Health (NIH, USA), approximately 65% of individuals with serious illness from SARS-CoV-2 infection also had metabolic disorders such as obesity, type 2 diabetes, hypertension, and heart issues. Disease severity is associated with an uncontrolled immune response involving macrophages, neutrophils, different complement components, and a number of cytokines including TNF-α and interleukin-6 (IL-6). All these factors ultimately lead to a ‘cytokine storm’ and accompanying problems such as acute respiratory distress syndrome (ARDS), widespread vascular inflammation, and disseminated intravascular coagulation.

Therefore, to prevent the aforementioned abnormal immune reactions, intervention with corticosteroids (like dexamethasone) has been considered [50]. Of note, apart from immune suppression, corticosteroid therapy can aggravate insulin resistance/type 2 diabetes, which is a risk factor for COVID-19 disease severity. Moreover, immune suppression can also allow the growth of opportunistic bacterial and fungal pathogens. Among hospitalized COVID-19 patients, investigators have isolated various bacterial strains such as coagulase-negative staphylococci, Klebsiella pneumoniae, and Pseudomonas aeruginosa, as well as a number of fungal agents including Candida albicans, Aspergillus fumigatus, and Cryptococcus neoformans [51-53]. Although Aspergillus and Candida species are commonly found in severely ill COVID-19 patients, studies have also documented other fungal pathogens. For example, researchers detected Histoplasma capsulatum complement fixation titers in patients with serious SARS-CoV-2 infection [53,54]. Other factors also thought to play a key role in fungal co-infection include hypoxemia/lack of tissue oxygenation, mechanical ventilation, and type 2 diabetes and associated hyperglycemia [55].

Inappropriately managed diabetes increases the risk of infection of various body organs. Furthermore, diabetes can hinder both innate and adaptive immune mechanisms [56]. In the second wave of the COVID-19 pandemic in India (roughly from March to July 2021), there was a mysterious outbreak of mucormycosis or black fungus infection among patients with SARS-CoV-2 infection, and diabetic patients were more susceptible to mucormycosis [57]. In the normal immune system, all three complement activation pathways (classical, lectin, and alternative) play an important role protecting against fungal pathogens through mechanisms such as opsonization, humoral immune response stimulation, and chemotaxis of immune cells [58,59]. In diabetes, glycation of complement components (functional low levels) may lead to an impaired immune response [55,60]. Consequently, the impaired immune response in severe COVID-19 illness allows fungal infection.

OTHER NON-VIRAL PATHOLOGIES: PRION AND PRION-LIKE DISEASES

Prions or protease-resistant misfolded proteins are unusual protein aggregates (also termed amyloids/amyloid fibrils) that have a high proportion of β-sheets. The first prion identified was the PrP protein [61]. The gene encoding the normal cellular prion protein (PrPC, misfolded–PrPȘ) is located on chromosome 20, and this glycoprotein is commonly present on the cell surface and can serve as a receptor for the Aβ peptide [62]. In addition, PrPȘ is expressed in different organs, particularly in the nervous and immune systems, and is thought to have numerous physiological functions such as cell surface scaffolding. Nevertheless, the mechanism of misfolding and aggregation into an abnormal prion-like conformation that again influences the misfolding of other associated protein copies in a self-propagating manner is indeed a unique biological process, and we can see this phenomenon even in yeast and fungi.

Apart from typical prion diseases, which include Creutzfeldt-Jakob disease (CJD), kuru, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia [63], there are other prion-like diseases such as Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, type 2 diabetes, and amyloidosis. Certain proteins such as Aβ, tau, α-synuclein, and serum amyloid-A, which share some pathological characteristics with prions, have been implicated in these prion-like diseases [64,65]. For example, in Alzheimer’s and Parkinson’s diseases, aggregates of Aβ, tau, and α-synuclein can transmit the disease-pathology to experimental animals [65]. Of note, the fundamental features of Alzheimer’s disease lesions are the formation of Aβ plaques and tau in tangles—both are β-sheet-rich misfolded variants of normal proteins.

Currently, about 50 different proteins are thought to form various human disease-related amyloid fibrils [66]. Furthermore, some of these abnormal proteins can cross species barriers and affect humans such as bovine spongiform encephalopathy or mad cow disease, which is linked to variant CJD (vCJD) [67]. Interestingly, aggregation of amylin or islet amyloid polypeptide has been found in the pancreatic islets of Langerhans in individuals with type 2 diabetes [66]. In addition, patients with type 2 dia-
betes have an increased risk of developing Alzheimer’s disease. Of note, a number of pathophysiological associations have been documented between Alzheimer’s disease and metabolic disorders such as type 2 diabetes, obesity, and metabolic syndrome [68]. On the other hand, both Alzheimer’s and prion diseases are neurodegenerative disorders, and there are several neuropathological commonalities and genetic connections between these diseases [69].

In humans, the most common prion disease is CJD with an incidence of about 1 case per 1 million population per year worldwide [70]. The majority (~85%) of cases of this rare disease occur sporadically, while ~10%–15% cases are due to familial or genetic mutations. The remaining cases (less than 1%) are linked to obvious environmental causes such as contaminated tissue transplant or surgical instruments (i.e., iatrogenic CJD) and contaminated meat consumption (i.e., vCJD). Another prion disease, kuru, once endemic in the Eastern Highlands of Papua New Guinea, disappeared rapidly after the cessation of ritual cannibalism. Clinically, kuru has a prodromal phase and three stages. Interestingly, in the second stage, obesity is a common feature, which could also exist in early disease in association with bulimia

[71]. However, unlike dissemination of microbial infections, prion disease is spread through ingestion or inoculation of contaminated materials (aside from sporadic and genetic inheritance). Therefore, different mechanisms by which conventional infectious diseases are spread, e.g., skin/mucosal contact, droplet/aerosol (airborne, coughs or sneezes), body fluids (like urine), fecal-oral route, vector-borne transmission, and fomites, have no role in the spread of prion diseases.

A number of investigators recorded an alteration of gut microbiota (dysbiosis) in prion disease and other neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease [72-76]. Gut microbiota dysbiosis has also been reported in obesity and metabolic disorders including type 2 diabetes [77-80]. Nonetheless, there is a close similarity between protein misfolding disorders and pathogenesis of prions (infectious/transmissible proteins). For example, misfolded Aβ and tau (of Alzheimer’s disease) spread in a way very similar to misfolded PrP [81-83]. As mentioned above, PrP acts as a cell surface receptor for Aβ. Fascinatingly, PrP acts in the propagation of prions as well as to transduce the neurotoxic signals from Aβ oligomers [82]. Experimentally, the transmission of kuru and CJD to various in

Table 2. Selected observations that displayed prion-like transmission characteristics of the most common neurodegenerative disease-related proteins

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayers et al. (2017) [87]</td>
<td>Injection of β-synuclein fibrils in M83 transgenic mice through different peripheral routes, i.e., intramuscular (hind limb muscle), intravenous (tail veins), and intraperitoneal.</td>
<td>Injection of α-synuclein fibrils via these peripheral routes in M83 mice induced a robust α-synuclein pathology in the central nervous system.</td>
</tr>
<tr>
<td>Betemps et al. (2014) [88]</td>
<td>Transgenic M83 mice were inoculated intracerebrally in the striato-cortical area with brain homogenates from sick M83 mice.</td>
<td>Disease acceleration following intracerebral inoculation suggests that disease propagation involves a prion-like mechanism.</td>
</tr>
<tr>
<td>Boluda et al. (2015) [89]</td>
<td>Intracerebral injection of Alzheimer’s disease brain extracts enriched in pathological tau in young mutant P301S tau transgenic mice (PS19) approximately 6–9 months before they show the onset of mutant tau transgene-induced tau pathology.</td>
<td>At 1-month post-injection, inoculated Alzheimer’s disease-tau in young PS19 mice induced tau pathology predominantly in neuronal perikarya (neuron cell body). With longer post-injection survival periods of up to 6 months, tau pathology spread to different brain regions distant from the inoculated sites.</td>
</tr>
<tr>
<td>Guo et al. (2016) [90]</td>
<td>2–3-Month-old C57BL/6 and C57BL/6/C3H F1 mice were intracerebrally inoculated with different tau fibrils; 15–19-month-old C57BL/6 mice were injected with Alzheimer’s disease-tau.</td>
<td>Intracerebral inoculation of tau fibrils purified from Alzheimer’s disease brains, but not synthetic tau fibrils, resulted in the formation of abundant tau inclusions in the brain of non-transgenic mice.</td>
</tr>
<tr>
<td>Lam et al. (2021) [91]</td>
<td>The posterior cingulate cortex areas of 1.5-year-old male mouse lemurs (Microcebus murinus) were inoculated with either Alzheimer’s disease or control brain extracts.</td>
<td>After 21 months, amyloid beta (Aβ) and tau pathologies developed in all Alzheimer-inoculated animals (n = 12) while no control brain extract-inoculated animals (n = 6) developed such lesions.</td>
</tr>
<tr>
<td>Morales et al. (2015) [92]</td>
<td>Brain extracts from 18-20 months old tg2576 mice (having significant amyloid deposits) were serially diluted (10⁻⁴ dilution) and intracerebrally injected into 50-55-day-old tg2576 mice.</td>
<td>Administration of misfolded Aβ significantly accelerated amyloid deposition in young mice.</td>
</tr>
</tbody>
</table>

*The M83 transgenic mouse model overexpresses A53T mutated human α-synuclein protein, which is connected with buildup of pathogonomonic Ser129-phosphorylated α-synuclein in the central nervous system. Abnormal accumulation of misfolded α-synuclein is linked to synucleinopathies including Parkinson’s disease; striato-cortical area: The corpus striatum (subcortical basal ganglia) and the adjacent cerebral cortex in the forebrain region; PS19 transgenic mouse expresses the P301S mutant form of human microtubule-associated protein tau. This hyper-phosphorylated and insoluble protein accumulates in the brain; posterior cingulate cortex: Situated at the posterior part of the cingulate gyrus in the medial part of the inferior parietal lobe, above the posterior end of the corpus callosum; The Tg2576 mouse model overexpresses a mutant form of amyloid precursor protein (APPswSWE, found in early-onset familial Alzheimer’s disease), which has the double mutation-APPK670M/E671L. The most common neurodegenerative diseases: Alzheimer’s disease and Parkinson’s disease.
vivo models has been performed by different laboratories [84-86]. Similarly, the pathologies of Aβ, tau, and α-synuclein could be transmitted in a prion-like manner to in vivo models by injecting the misfolded proteins. In this connection, the results of selected studies have been mentioned briefly in Table 2 [87-92].

Both in vitro and in vivo studies have identified a number of compounds that have anti-prion activity. Congo red, polyanionic glycans, quinacrine, and compB either inhibit the formation of PrPSc or enhance the degradation of PrPSc. Interestingly, in experimental studies, anle138b (a recently developed drug) has been documented to inhibit the formation of pathological aggregates of α-synuclein (Parkinson’s disease) and tau (Alzheimer’s disease) proteins in addition to inhibiting aggregation of prion protein (PrPSc) [93,94]. A clear understanding of protein misfolding and its association with metabolic disorders at the molecular level may provide insights into their precise pathogenesis and result in the development of prevention strategies. Furthermore, early diagnosis (preferably in the preclinical stage) and prompt intervention are critical when there are few pathological protein aggregates in the brain. Accordingly, identification of appropriate early diagnostic markers is essential.

PARASITES IN OBESITY-RELATED PATHOLOGIES

Parasitic infestations can have a diverse range of consequences/sequelae. Ryan et al. [95] posited that helminth-related anti-inflammatory mechanisms may be beneficial. Lifestyle-linked chronic diseases usually have a strong connection with inflammation. In this context, hookworm species, particularly Necator americanus, may have a protective effect in inflammatory bowel diseases such as Crohn’s disease and ulcerative colitis as well as in celiac disease. In addition, these authors reported an inverse association between human helminth infection and insulin resistance/type 2 diabetes. In an experimental study on male C57BL/6 wild-type mice, infection with Nippostrongylus brasiliensis (rodent hookworm) significantly decreased various diabetes-associated parameters such as fasting blood glucose and weight gain [96]. Similarly, studies in human subjects have demonstrated that infection with Strongyloides stercoralis (threadworm) can reduce the risk of type 2 diabetes by modulating the expression of different pro-inflammatory cytokines [97-99]. A study from Thailand showed that Opisthorchis viverrini (liver fluke) infection had a protective effect against hyperglycemia and metabolic disease risk [100]. In contrast, many studies have reported that individuals with parasitic diseases are more susceptible to diabetes or that diabetic persons are at higher risk of infection with various parasites, e.g., Ascaris lumbricoides, Echinococcus granulosus, Enterobius vermicularis, Schistosoma mansoni, S. haematobium, Hymenolepis nana, hookworm, and Taenia species, as well as protozoan parasites such as Giardia lamblia, Entamoeba histolytica, and Cryptosporidium species (Table 3) [101-116]. Some of these helminths are also considered to be responsible for cancer development. For example, S. haematobium can induce squamous cell carcinoma of the urinary bladder, and O. viverrini may cause cholangiocarcinoma/bile duct cancer [117]. Unprecedentedly, a report revealed the dissemination of cancer cells from H. nana to different organs of a human host [118].

Two major types of primary liver cancer, i.e., hepatocellular carcinoma (~75% of all liver cancers) and cholangiocarcinoma (10%–20% of cases), are uniquely linked to a diverse group of risk factors namely viral hepatitis (hepatitis B virus and hepatitis C virus), obesity, type 2 diabetes, alcohol consumption, smoking, and toxic substances including aflatoxins produced by Aspergillus species. Moreover, risk factors for cholangiocarcinoma are inflammatory bowel disease, parasitic infections, and hepatolithiasis. Along with O. viverrini, infection with Clonorchis sinensis (another liver fluke) can cause the development of cholangiocarcinoma, particularly in Southeast Asia [119]. In addition, C. sinensis and A. lumbricoides may promote hepatolithiasis [119,120]. Interestingly, the co-occurrence of O. viverrini infection and diabetes has been shown to be associated with hepatobiliary tract damage and malignant transformation [121,122].

For helminth infections that are predominantly connected with tissue migration, numerous studies have documented the presence of peripheral eosinophilia (or increased number of eosinophils in the peripheral blood) or Loeffler’s syndrome (i.e., accumulation of eosinophils in the lung) [123,124]. Although eosinophils play a significant role in various physiologic processes including innate and adaptive immunity, data on the precise role of human eosinophils in defense against helminths are limited. Data on the specific anti-parasitic role of mast cells and basophils, which behave functionally similar to eosinophils in hypersensitivity/allergic inflammation, are also inadequate [125-127]. By contrast, there is a growing body of evidence that neutrophils play a protective role against several parasitic infections such as E. histolytica, Leishmania, and Plasmodium infections [128-130]. Neutrophils may clear the parasites by a number of mechanisms including phagocytosis, generation of reactive oxygen species (ROS), and formation of neutrophil extracellular traps.
Table 3. Selected studies that recorded a positive association between metabolic disorders and protozoan parasites

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject</th>
<th>Important finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Udoh et al. (2020); Nigeria [105]</td>
<td>Cross-sectional study of 208 diabetic patients</td>
<td>Diabetic patients were reservoirs of asymptomatic Plasmodium falciparum.</td>
</tr>
<tr>
<td>Wyss et al. (2017); Sweden [106]</td>
<td>Retrospective observational study on 937 adults with malaria</td>
<td>Comorbidities, specifically obesity and diabetes, were risk factors for severe malaria in adults diagnosed with Plasmodium falciparum.</td>
</tr>
<tr>
<td>Dansu et al. (2010); Ghana [107]</td>
<td>Case-control study of 946 diabetic patients and 520 controls</td>
<td>Patients with type 2 diabetes had a 46% increased risk for infection with Plasmodium falciparum.</td>
</tr>
<tr>
<td>Vizzoni et al. (2018); Brazil [108]</td>
<td>Cross-sectional study of 619 patients with Chagas disease</td>
<td>Elderly patients had a high frequency of hypertension and other comorbidities such as diabetes and dyslipidemia.</td>
</tr>
<tr>
<td>dos Santos et al. (1999); Brazil [109]</td>
<td>Cross-sectional study of female patients with Chagas disease (n = 362) and controls (n = 285)</td>
<td>Diabetes/hyperglycemia was more prevalent in patients with the cardiac form of Chagas disease than in controls, or patients with gastrointestinal problems or the asymptomatic form of the disease.</td>
</tr>
<tr>
<td>Soltani et al. (2021); Iran [110]</td>
<td>Case-control study of 105 diabetic patients and 150 controls</td>
<td>Chronic Toxoplasma gondii infection was significantly associated with diabetes.</td>
</tr>
<tr>
<td>Li et al. (2018); China [111]</td>
<td>Case-control study of 1,200 diabetic patients (type 1, 2, and gestational) and 1,200 matched controls</td>
<td>Diabetic patients had a significantly higher Toxoplasma gondii seroprevalence than controls.</td>
</tr>
<tr>
<td>Reeves et al. (2013); Germany [112]</td>
<td>999 Randomly selected adults</td>
<td>Obese persons had significantly higher Toxoplasma gondii seropositivity than non-obese individuals.</td>
</tr>
<tr>
<td>Machado et al. (2018); Brazil [113]</td>
<td>Descriptive study of 156 diabetic individuals</td>
<td>Frequencies of Giardia lamblia were higher in individuals with type 2 diabetes than those without.</td>
</tr>
<tr>
<td>Sisu et al. (2021); Ghana [114]</td>
<td>Cross-sectional study of 152 diabetes patients</td>
<td>Diabetes patients appeared susceptible to infections with Giardia lamblia, Entamoeba histolytica, and Cryptosporidium parvum.</td>
</tr>
<tr>
<td>Akinko et al. (2013); Nigeria [115]</td>
<td>150 Diabetic patients and 30 controls</td>
<td>Diabetes was significantly associated with intestinal parasitic infections (like Entamoeba histolytica).</td>
</tr>
<tr>
<td>Aliyu et al. (2018); Ethiopia [116]</td>
<td>Cross-sectional study of 215 diabetic patients</td>
<td>Intestinal parasites were found more frequently in diabetic patients compared to data from other similar studies. Cryptosporidium parvum was the parasite found with the highest frequency.</td>
</tr>
</tbody>
</table>

**IMMUNE CELLS IN OBESITY**

White blood cells (WBCs or leukocytes) are fundamental components of the immune system. Several studies have reported a quantitative increase in WBCs among obese people [131-133]. A major component of the observed increase in WBC counts is neutrophils. In peripheral blood, more than half of the WBCs (up to about 70%) are neutrophils. It is notable that these cells are present in marginated (recoverable part) and circulating pools in almost equal proportions, while circulating cells have a remarkably short lifespan. Nonetheless, along with increased WBC counts, many investigators have noticed significantly higher levels of neutrophils in obese people [134-136]. Neutrophils from obese subjects have also been found to be functionally more active than neutrophils from lean controls. In obesity, the levels of neutrophil-released superoxides are significantly greater than in normal controls [137]. Apart from an elevated leukocyte count and release of ROS like superoxide (i.e., oxidative burst), the lymphocyte count is also elevated in obesity. In a community-based study of 116 obese women, investigators found that obesity was connected with an increase in certain lymphocyte subset counts, excepting natural killer (NK) and cytotoxic/suppressor T cells [138]. Multivariate analyses of 322 women who were longitudinally followed from 1999 through 2003 revealed that increasing body weight was independently related to higher WBC, total lymphocyte, CD4, and CD8 counts [139]. Similarly, a study of 119 Saudi female university students showed that both BMI and waist-to-hip ratio (WHR) were significantly correlated with WBC, neutrophil, and CD4 lymphocyte counts [140]. Furthermore, in a cross-sectional, retrospective study of 223 participants (female- 104), BMI was found to have a significant positive relationship with WBC, neutrophil, and lymphocyte counts [141]. These findings indicate that being overweight or obese may impact both innate and adaptive immune responses to numerous pathophysiological phenomena including infections by various pathogens.

Obesity is associated with chronic low-grade inflammation. A relatively inexpensive method to assess the systemic pro-inflammatory state is to determine the blood neutrophil-to-lymphocyte ratio (NLR) [142]. It is believed that this parameter also indicates the stress situation of our body. A healthy range is between 1 and 2; values more than 3 or less than 0.7 in adults are pathognomonic [142]. In a study that compared NLR between obese individuals with insulin resistance (n = 46) and those without (n = 51), both the neutrophil count and NLR were found to be significantly higher in the insulin resistance group [143]. It is worth
mentioning that insulin resistance or type 2 diabetes, which is a common sequela of obesity, is also associated with elevated total and differential WBC counts [131,132,144]. In a study of 600 subjects (BMI: 27.9 ± 4.7) selected from 474,616 patients who visited Severance Hospital, Seoul, South Korea between January 2008 and March 2017, NLR was significantly associated with intra-abdominal visceral adipose tissue volume [145]. In addition, WBCs and levels of the serum inflammatory marker high-sensitivity C-reactive protein (hs-CRP) were strongly correlated with visceral adipose tissue. In a cross-sectional study in Taiwan, a total of 26,016 subjects with metabolic syndrome were recruited between 2004 and 2013 [146]. Of note, the American Heart Association criteria for metabolic syndrome are central obesity, hypertension, high blood glucose and triglycerides, and lower high-density lipoprotein cholesterol levels in the blood. In this study, obesity and related anthropometric parameters such as WHR were positively associated with NLR and C-reactive protein (CRP) in both sexes. Another study of 1,267 subjects (1,068 female and 199 male) collected from the out-patient clinic of Düzce University Hospital, Turkey during 2012–2013 reported that while WBC, neutrophil, and lymphocyte counts as well as level of hs-CRP exhibited a significant interrelationship with BMI, BMI was not correlated with NLR [147]. NLR may not be a better pro-inflammatory indicator than CRP or hs-CRP. Nonetheless, a different study from Turkey of 306 morbidly obese subjects (BMI ≥40) demonstrated significantly higher NLR levels in these subjects than normal controls [148]. Moreover, the authors concluded that elevated NLR was an independent and strong predictor of type 2 diabetes in morbidly obese individuals. A cross-sectional study from the 2011–2016 National Health and Nutrition Examination Survey (NHANES 2011–2016, a US population database) recorded a positive association between BMI and NLR in healthy adult female participants (n = 3,201) [149]. The above studies indicate that being overweight or obese is linked with certain circulating markers that can be used affordably to evaluate systemic inflammatory status.

Lymphocytes (T-cells, B-cells, NK cells) play a significant role in obesity-linked inflammation [150]. A study from Germany revealed an impaired NK cells phenotype and subset alterations in obesity [151]. Another study of 169 subjects demonstrated an increase in total lymphocytes along with granulocytes and a decrease in the NK cell population among persons with metabolic syndrome and increased visceral adipose tissue [152]. Furthermore, an increase in memory cells was also documented in those subjects with an increased BMI and visceral adipose tissue. Impaired B-cell and T-cell function has been observed in high-fat (HF) diet-induced obese mice [153].

In HF diet-induced obese C57BL/6] female mice, investigators noted higher circulating monocytes in the HF group than the standard chow diet-fed mice [154]. Likewise, a number of studies involving human subjects have recorded an increased monocyte count in obese individuals [132,138,155,156]. However, other studies found no correlation between BMI and blood monocyte count [140,151]. Monocytes are the largest cells in our blood and normally up to 10% of WBCs are monocytes. Monocytes can be classified into three categories depending on their surface receptors: classical (CD14+), intermediate (CD14+ and low levels of CD16+), and non-classical (CD16+ along with lower levels of CD14+). In a study of 58 obese subjects and 25 metabolically healthy lean controls, numbers of both intermediate and non-classical monocytes were higher in obese subjects than lean controls [157]. Interestingly, these investigators also found that the levels of intermediate monocytes were positively and significantly related with the obese group’s serum triglyceride levels and mean blood pressure. Monocytes can differentiate into macrophages after migration to different tissues of our body—therefore, macrophages are present in the extracellular space. A number of reports have confirmed the accumulation of macrophages in the excess adipose tissue of obese individuals [157-159]. These infiltrated macrophages in adipose tissue create an inflammatory environment due to their production of several pro-inflammatory molecules. Consequently, macrophage infiltration and adipose tissue inflammation are important pathological processes that contribute to systemic inflammation and various complications such as insulin resistance and metabolic syndrome.

**INFLUENCE OF OBESITY ON VACCINE EFFECTIVENESS**

The efficacy and effectiveness of any vaccine varies considerably and no vaccines can provide 100% protection. According to the WHO, vaccine effectiveness is associated with a number of factors including age, gender, ethnicity, and other accompanying health conditions. The efficacy of a vaccine is evaluated by estimating the development of disease among vaccinated people in comparison with a placebo/control group in controlled clinical trials (i.e., ideal conditions). Conversely, vaccine effectiveness refers to how a vaccine actually performs in different populations.

The basic biological mechanisms underlying vaccine non-responsiveness are not well known. However, there is evidence that both carbohydrate and fat metabolic pathways are involved in responsiveness to vaccines [160]. Furthermore, obesity has been

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proposed to be associated with inadequate vaccine responsiveness [161]. Apart from well-established health-related problems such as insulin resistance and hypertension, the obesity-related chronic low-grade inflammatory state has adverse effects on the immune system [162]. Obviously, more research is needed to understand the effects of obesity on vaccine effectiveness.

**POTENTIAL INDICATORS OF OBESITY AND INFLAMMATION**

Obesity and CRP have been demonstrated to be positively correlated [163]. Furthermore, CRP is widely used as a marker of inflammation. Along with its role in inflammation, CRP also functions significantly in host defense against different pathogenic organisms [164]. CRP is present in at least two distinct forms: pentameric and monomeric (mCRP) isoforms, which have diverse activities and functional characteristics. Dissociation of the pentameric group into monomeric forms occurs at sites of inflammation and the monomeric form then may participate in local inflammation. CRP is primarily produced by the liver and its blood levels may increase from 0.8 mg/L (approximate normal value) to more than 500 mg/L in inflammatory conditions [165]. However, CRP is involved in several pathophysiological processes such as activation of the complement system, phagocytosis, promotion of apoptosis, release of nitric oxide (NO), and biosynthesis of various cytokines particularly pro-inflammatory cytokines such as TNF-α, MCP-1, and IL-6 [166]. In addition, it is believed that mCRP can stimulate the process of chemotaxis and recruitment of circulating WBCs to sites of inflammation. Studies have documented associations (both positive and negative) between CRP and various hormone-like cytokines (adipokines) that are released from adipose tissue [167-169]; in particular, with the pro-inflammatory adipokine leptin (Fig. 1).

Adipose tissue behaves like an endocrine organ. As mentioned earlier, several hormone-like cytokines or adipokines are secreted from adipose tissue or fat cells. In general, the majority of these adipokines are pro-inflammatory, for example, leptin, visfatin, and resistin. However, a few anti-inflammatory adipokines such as omentin, apelin, and adiponectin are also secreted. Nevertheless, the majority of published studies have focused mainly on two adipokines—pro-inflammatory leptin and anti-inflammatory adiponectin. These two adipokines are involved in a number of

![Fig. 1. Relationship among pro-inflammatory adipokines, interleukin-6 (IL-6), and C-reactive protein (CRP) in obesity. Infiltration of macrophages in excess adipose tissue is a common phenomenon. The pleiotropic pro-inflammatory cytokine IL-6, secreted by monocytes/macrophages, induces the biosynthesis of CRP from the liver.](https://patholtm.org/)

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biological mechanisms both under normal health conditions as well as under pathological circumstances. Interestingly, both adipokines are closely linked with our immune system. Leptin is a 16-kD protein produced primarily by adipocytes. Its main function is maintenance of energy homeostasis through regulation of the arcuate nucleus of the hypothalamus. Leptin is associated with both innate and adaptive immune responses [170]. This adipokine has a close connection with inflammatory molecules including IL-6, TNF-α, NO, eicosanoid, and cyclooxygenase (particularly cyclooxygenase 2) [171], as well as intracellular signaling pathways connected with inflammation such as mitogen-activated protein kinase, Janus kinase/signal transducer and activator of transcription, and phosphatidylinositol-3-kinase (Fig. 2). In addition, leptin promotes chemotaxis, phagocytosis, and release of ROS [170,172].

Higher circulating levels of both leptin and CRP have been demonstrated to be correlated with disease severity and poor prognosis in patients with COVID-19 [173-175]. In a recently published report from Italy, COVID-19 patients with pneumonia had increased circulating levels of leptin and IL-6 and lower adiponectin levels than age- and sex-matched healthy controls [176]. Similar findings were documented in another study from the Netherlands [177]. In contrast, a group of investigators hypothesized that increased blood levels of leptin could be due to patients’ obesity and unrelated to disease pathology [178,179]. A number of mechanisms have been proposed to explain the poor prognostic role of leptin in COVID-19. van der Voort et al. reasoned that SARS-CoV-2 infection, by inducing higher leptin production, might overactivate leptin receptors in pulmonary tissue, ultimately enhancing local inflammation in the lungs [177]. Higher leptin levels could also activate monocytes and thus upregulate the expression of pro-inflammatory cytokines in monocytes, resulting in dysregulation of immune responses, finally leading to ARDS and multiple organ failure [173,180]. As mentioned earlier, leptin receptors are present in all immune cells. Therefore, leptin may affect the functions of these cells. Understanding the precise role of leptin and its interactions with different adipokines (both pro-inflammatory and anti-inflammatory) and other classical hormones such as insulin, insulin-like growth factors, and estrogen, will help elucidate the relationships between obesity-related problems and immune mechanisms.

CONCLUSION

The recent COVID-19 pandemic has renewed interest in infectious diseases, and the disease pathology itself is a meeting place of both communicable and non-communicable diseases. For this reason, many authors have described the grave situation of 2020 and 2021 as ‘double pandemics’—the pandemic of COVID-19 and the long-continued global problem of obesity [1,181,182]. Apart from bacterial and fungal infections that are a common occurrence in obesity-related health conditions like type 2 diabetes, a number of prion-like diseases have a close link with these metabolic disorders. Regular assessment of a few markers such as CRP and leptin and adjustment of lifestyle factors could help protect against pathogens as well as metabolic diseases.

Ethics Statement

Not applicable.

Availability of Data and Material

The datasets generated or analyzed during the current study are available in the [MEDLINE] repository.

Code Availability

Not applicable.

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Single-cell and spatial sequencing application in pathology

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Traditionally, diagnostic pathology uses histology representing structural alterations in a disease's cells and tissues. In many cases, however, it is supplemented by other morphology-based methods such as immunohistochemistry and fluorescent in situ hybridization. Single-cell RNA sequencing (scRNA-seq) is one of the strategies that may help tackle the heterogeneous cells in a disease, but it does not usually provide histologic information. Spatial sequencing is designed to assign cell types, subtypes, or states according to the mRNA expression on a histological section by RNA sequencing. It can provide mRNA expressions not only of diseased cells, such as cancer cells but also of stromal cells, such as immune cells, fibroblasts, and vascular cells. In this review, we studied current methods of spatial transcriptome sequencing based on their technical backgrounds, tissue preparation, and analytic procedures. With the pathology examples, useful recommendations for pathologists who are just getting started to use spatial sequencing analysis in research are provided here. In addition, leveraging spatial sequencing by integration with scRNA-seq is reviewed. With the advantages of simultaneous histologic and single-cell information, spatial sequencing may give a molecular basis for pathological diagnosis, improve our understanding of diseases, and have potential clinical applications in prognostics and diagnostic pathology.

Key Words: Single-cell sequencing; Spatial sequencing; Pathology; Histology; Transcriptome; Diseases

Because tissue cells represent the fundamental unit of biology, deciphering the phenotypic heterogeneity between cells, the intercellular interactions, and the spatial organization of cells in tissues are crucial for understanding the pathophysiology of disease [1]. Recently developed high-throughput technologies, such as single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST), have brought revolutionary insights into diverse research areas, including developmental biology, cancer, immunology, and neuroscience [2,3]. However, the necessary tissue dissociation step of scRNA-seq destroys information on their spatial context, which is crucial to understand the intercellular interactions underlying normal and disease tissues [4]. Moreover, integrating scRNA-seq and ST data can address this limitation and thus provide novel insights for homeostasis, development, and disease microenvironment, which cannot be informed by scRNA-seq alone [4]. In this review, from a pathologist's view, we aim to suggest the overview of the integrative analysis of scRNA-seq and ST and describe representative research studies.

THE INTRODUCTION OF SINGLE-CELL RNA SEQUENCING

Conventional transcriptome technologies, including microarrays and bulk RNA sequencing (RNA-seq), have shown a way to assay only the average expression RNA expression signal of all cell types within the tissue. However, gene expression is heterogeneous between many tissue cells and even between the same cell types, and thus these technologies are likely to miss important cell-to-cell variability [2]. After the introduction in 2009 [5], the droplet-based scRNA-seq has been the most popular scRNA-seq technology that can capture the transcriptomes in tens of thousands of single cells per sample to dissect transcriptomic heterogeneity masked in bulk RNA-seq [6]. Since there have been numerous reviews that comprehensively introduce the technological aspects and various analysis methodologies of scRNA-seq [2,7-12] and ST [3,13-16], we briefly introduce the overview of principles and analytical methods of scRNA-seq and ST in this

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review. We summarize the strengths and limitations of clinical and experimental gene expression methods: recent scRNA-seq and ST, compared to conventional histopathology and bulk RNA-seq (Fig. 1).

The ordinary procedures for the generating scRNA-seq data include single-cell isolation and capture, cell lysis, reverse transcription, cDNA amplification, and library preparation [17]. Single-cell isolation and capture is the first process of acquiring high-quality single cells from a tissue in which all transcripts from single-cell will be uniquely barcoded. Polyadenylated mRNA molecules are captured by poly[T]-primers, reverse transcribed, polymerase chain reaction amplified, and resulting cDNA from every cell is pooled and sequenced by next-generation sequencing (NGS) [18]. Many methodologies are different in terms of the number of cell per sample (the breadth of cellular profiling) and the number of genes per cell (the depth of cellular profil-

![Fig. 1. Overview of strengths and limitations of clinical and experimental methods for gene expression. Conventional methods of histopathology (A) and bulk RNA sequencing (RNA-seq) (B). FISH, fluorescence in situ hybridization. Recent methods of single-cell RNA sequencing (scRNA-seq) (C) and next-generation sequencing (NGS)-based spatial transcriptomics (ST) (D).](https://jpatholtm.org/)
ing) [11]. Some provide full-length transcript coverage, while others only partial sequences from either 3’ or 5’ end of the transcript [7]. The preparation of high-quality single-cell suspension is key to successful scRNA-seq studies, and researchers should acknowledge the possible protocol-specific biases that can be developed during single-cell isolation [19,20]. While scRNA-seq needs prior tissue dissociation of fresh tissue for the preparation of single-cell suspension, single-nucleus RNA-seq capturing only transcripts present in the nucleus can be prepared from frozen or fresh tissue without the need for tissue dissociation [1,21].

Workflows for scRNA-seq analysis include pre-processing (quality control, normalization, data correction, feature selection, and dimensionality reduction) and cell/gene-level downstream analysis (clustering, cluster annotation, trajectory inference, and differential expression analysis). The best-practice recommendations and details for each step of the analysis pipeline are comprehensively described elsewhere [8]. There have been various tools for analyzing scRNA-seq data, among which R-package Seurat is the most commonly used tool for analysis and visualization of scRNA-seq data [22]. Researchers easily find the sample analysis pipeline using Seurat R-package with the representative dataset at https://satijalab.org/seurat/articles/pbmc3k_tutorial.html. Major applications of scRNA-seq include the clustering and identification of known or novel cell types, inferring cellular trajectory, and inferring gene regulatory networks [2]. Unsupervised clustering is preferred in most cases for clustering and identification of cell types, although supervised clustering using prior assumptions and canonical marker genes is also available [18]. Dimensional reduction and visualization are performed using algorithms such as principal component analysis, t-distributed stochastic neighbor embedding, and uniform manifold approximation and projection, followed by cell clustering into subpopulations with biological significance using algorithms such as a graph-based clustering [18,20]. In addition to the unsupervised clustering, cell types can be determined by reference-based annotation using reference expression profiles from bulk RNA-seq [23]. Trajectory inference reconstructs dynamic cellular trajectories during the cellular transition between cell identities underlying biological process of interest [2]. Tools for trajectory inference have been developed for ordering single cells in pseudotime, an abstract unit of progress through the single-cell trajectory, by taking advantage of individual cells’ asynchronous progression of transcriptional dynamics along the biological process [24]. For trajectory inference, RNA velocity that is the time derivative of the gene expression state, can be analyzed to predict the future state of individual cells by distinguishing between unspliced and spliced mRNAs [25]. From scRNA-seq data, gene regulatory networks underlying gene expression by transcription factors, co-factors, and signaling molecules can be inferred, which may help to pinpoint key factors that determine phenotype in healthy systems as well as in diseases [26,27].

THE INTRODUCTION OF SPATIAL TRANSCRIPTOMICS

Overview of ST was summarized in Fig. 2. One of the major limitations of scRNA-seq is the loss of spatial context since cells should be liberated from whole tissue before scRNA-seq. The spatial location of a cell can reveal helpful information for defining cellular phenotypes, cell states, intercellular interactions, and cell functions [15]. Although histopathology is the gold standard for diagnosis in most cases, it is limited by the type and number of cellular features delineated by stained agents [3]. Traditional methodologies for applying technologies for analyzing expression within tissues (in situ) include in situ hybridization (ISH) and immunohistochemistry; however, these methods limit analysis to, at most, a handful of genes or proteins at a time [13]. The recent development of ST technologies enables profile the whole transcriptome in spatially resolved way [13]. ST technologies can describe an unbiased picture of spatial composition that may provide valuable biological insights into development, physiology, and diseases microenvironment.

As technologies and computational approaches for generating and analyzing ST data are rapidly evolving, there are various options for ST technologies that differ in terms of the number of genes and the size of tissues that can be assayed [13]. ST technologies are primarily categorized as (1) NGS-based, encoding positional information onto transcripts; and (2) imaging-based approaches, comprising in situ sequencing-based or ISH-based methods [28,29]. Recently introduced and widely used NGS-based ST have shown increased resolution (55 μm spot diameter with 100 μm center-to-center distance) and sensitivity (more than 10,000 transcripts per spot) compared to the previous ones [13]. Currently, commercial kits utilize fresh-frozen tissues for ST; however cutting-edge technologies have shown successful ST application to formalin-fixed, paraffin-embedded (FFPE) tissues that will expand the usages of ST for numerous FFPE samples in biobanks [30]. ISH-based high-plex RNA imaging (HPRI) is a targeted ST method that localizes and quantifies RNA transcripts of hundreds of genes in an intact tissue through multiplexed fluorescent microscopy [4]. Depth is a limiting factor for
NGS-based techniques, while lack of transcriptome-wide coverage is for HPRI. Therefore, current ST technologies themselves still cannot reveal the deep transcriptomic information in tissue at single-cell level with accuracy, although they can shed light on the architecture of the cell-type distribution or the niches enriched for a specific gene set [4]. Until HPRI improves the transcriptome coverage and applicability of untargeted methods, the ST may stay advantageous, especially for obtaining an unbiased characterization of the spatial transcriptomics [4].

Compared to scRNA-seq, the workflows for ST analysis and its integration with scRNA-seq have emerged recently and rapidly evolved. As previously described, the workflows for ST analysis are akin to those in scRNA-seq. Several additional points are needed for analyzing ST: (1) to identify genes with coherent spatial patterns, (2) spot deconvolution or mapping single cells, and (3) analysis and visualization in the intercellular interactions [3,15]. Researchers can utilize various ST methodologies for clustering analysis of spatially coherent domains and identification of spatially domain-enriched genes [3]. Unsupervised clustering and subsequent characterization aim to identify clusters of spots and sets of genes with biological significances [13]. A cluster of spots may be characterized by pathological findings or by molecular marker genes [13], indicating pathologists’ roles in the biological interpretation of ST data. Alternative to the unsupervised clustering, researchers may focus on a specific region of interest, for example a specific layer in the brain or the interface between cancer and microenvironment, or on context-specific genes, for example known gene sets or highly variable genes [13]. Widely used techniques for ST utilize 50–100-μm spot diameters with mixture of 10–20 cells, indicating that the spatial spots in the ST dataset may correspond to mixture expressions of several cells [31]. The proportion of cell type (deconvolution) or the designation of cell type (mapping) can be analyzed using both scRNA-seq and ST data, which will be explained in detail in the later chapter of the manuscript [4]. Surely, while scRNA-seq analysis cannot distinguish short-distance (juxtacrine and paracrine) and long-distance (endocrine) intercellular signaling due to lack of spatial information, ST dataset can seek the spatial coordinate of cell signaling [3].

While currently often underused, the tissue image from ST analysis can be improved to show high-resolution information when combined with the knowledges in the field of histopathology [13]. For example, a previous study revealed that integrating ST data with high-resolution histology image data could improve the resolution of ST data [32]. Pathologists may play the main role in the integrative analysis and biological interpretation of ST for histopathology.

Fig. 2. Overview of spatial transcriptomics (ST) analysis. (A) Example of tissue slide (Visium technology) for ST. Original tissue image, detected area, spot clustering of ST data by unsupervised clustering (spatial spots colored by spot clusters), and magnified view of spatial spots are shown. Distance between spatial spots were 100-μm, and each spot has a diameter of 55-μm. (B) ST can measure genome-wide expression profiles in each 55-μm spatial spots. (C) Analysis strategies for ST data.
INTEGRATIVE ANALYSIS OF SINGLE-CELL AND SPATIAL RNA SEQUENCING

Integrated analysis of scRNA-seq and ST is summarized in Fig. 3. The major limitations for scRNA-seq and ST are loss of spatial information and low resolution, respectively. Furthermore, the lack of reliable ST methods to implement deep sequencing necessitates the need to integrate scRNA-seq and ST data. Thus, simultaneous measurements followed by the integrated analysis of scRNA-seq and ST from the same tissue may improve the data quality. Herein, we summarize strategies for integrating scRNA-seq and ST data: deconvolution, mapping, and spatially informed ligand-receptor analysis.

Because of the high read-depth and single-cell resolution of scRNA-seq compared with ST, cell subpopulations need to be defined firstly by scRNA-seq in a given tissue. There are two primary approaches for integration of scRNA-seq and ST data: first, deconvolution for ST without single-cell resolution such as spatial barcoding and second, mapping for ST with single-cell resolution such as HPRI [4]. Deconvolution refers to the process of quantifying the relative proportion of each cell type in spatial spots [15]. There are two main ways of the deconvolution: (1) inferring the proportion of cellular subtypes for a given spot, and (2) scoring a given spatial transcriptomic spot for how strongly it corresponds to a single cellular subtype. SPOTlight tool is good at validating the deconvolution analysis in terms of the accuracy, sensitivity, and specificity of cell-type detection [31]. The mapping has two facets: mapping assigned scRNA-seq-based cell subtypes to each cell and mapping each scRNA-seq cell to a specific niche or region of a tissue [13]. For mapping, pciSeq is one of the popular tools that have shown effectiveness in classifying cell type [33]. Researchers can adopt following statistical models for deconvolution and mapping: regression-based deconvolution and probabilistic modeling for deconvolution, and cell-type scoring and cluster-based mapping for mapping [4]. The possible mismatch between cell subtypes present in scRNA-seq data and those in spatial sequencing data that may complicate deconvolution and mapping should be acknowledged [4].

Spatial data from ST, for example, the spot clusters from unsupervised clustering and the areas of pathological interests, can be analyzed for deconvolution or mapping of cell types identi-
Unsupervised clustering of ST data can be performed using either gene expression of spatial spots alone or in combination with gene expression and histopathology [15], where pathologists may play an important role in the interpretation of pathological findings between identified cell subpopulations. Instead, the areas of pathological interests can be determined from the matched histological images by the pathologists, which are further investigated for distinct molecules of the areas: proportion of cell subtype, distinct expression, and intercellular interactions. For example, in cancer tissues, interesting areas of the tumor core and leading edges can be annotated by the pathologists for further investigations [34].

Since intercellular interactions, especially juxtracrine and paracrine communications, are spatially restricted, ST data is well suited to validate the ligand-receptor interactions computed from scRNA-seq [4,35]. Standard algorithms for predicting ligand-receptor interaction pairs adopt both scRNA-seq data and a known database for ligand-receptor interactions, such as CellphoneDB [36]. In this, researchers can use ligand-receptor and ligand-receptor-target co-expression restriction to establish intercellular communications from scRNA-seq data. From ST data, a further restriction can be applied by ligand-receptor proximity where the spatial context can enhance the intercellular interaction analysis. Integrated analysis of scRNA-seq and ST can be used to nominate the receptors and ligands that mediate communication between the proximal cell subpopulations. The Giotto workflow is one of widely used tools for anticipating the likelihood that a given ligand-receptor interaction is used more or less based on the proximity of all of the co-expressing cells [37].

**EXAMPLES FOR THE INTEGRATION OF SINGLE-CELL RNA SEQUENCING AND SPATIAL TRANSCRIPTOMICS TECHNOLOGIES**

Our group previously published the integrative analysis of scRNA-seq and ST of a foreign body reaction, which character-

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**Fig. 4.** Example study of integrated analysis [38]. Findings from single-cell RNA sequencing (scRNA-seq) (A), spatial transcriptomics (ST) (B), and integrated analysis of scRNA-seq and ST (C) are summarized. FBR, foreign body reaction.
ized the molecular signatures and cellular interactions of epithelioid cells and multinucleated giant cells in the foreign body re-
action (Fig. 4) [38]. From the scRNA-seq data, we designated three putative clusters of macrophages, such as M2-like macro-
phage, epithelioid cells, and giant cells; however the designation of cell types cannot be completely from scRNA-seq data alone
[38]. Unsupervised clustering of the ST data using the same for-
eign body reaction tissue found a cluster with the smallest num-
ber of spatial spots with scattered distribution, in which patho-
logical examination found a relatively higher proportion of giant
cells compared with other spot clusters [38]. Consistently, de-
convolution analysis also supported a relatively high proportion
of giant and epithelioid cells in this cluster [38]. scRNA-seq dis-
covered the marker genes for giant cells, which were further vali-
dated by both ST and immunohistochemistry [38].

We also present another two integration studies for human cancer transcriptomics. Profiling of human cutaneous squamous
cell carcinoma (cSCCs) and matched normal tissues was performed
via scRNA-seq, ST, and multiplexed ion beam imaging in ten
cSCC patients [39]. In the scRNA-seq, tumor cells in cSCC showed four subpopulations, three recapitulating normal skin epidermis and a tumor-specific keratinocyte population unique
to the cancer [39]. Integration analysis of the scRNA-seq and ST found that tumor-specific keratinocytes expressing epithelial-to-
mesenchymal signature were mainly located to the tumor leading
edges with enrichment of adjacent stroma of fibrovascular niche,
thus being a hub for intercellular communication [39]. Multi-
explexed ion beam imaging, ST technology at a single-cell level,
was further performed for validation [39]. Next, an integrative
study of scRNA-seq and ST were performed in two tumors from
patients with pancreas ductal adenocarcinomas [40], where high
concordance was found between pathological annotation by his-
tological features and unsupervised clustering of ST data [40].

A statistical approach to overlap cell type-specific and tissue re-
gion-specific gene sets, called the multimodal intersection analy-
sis, was used for identification and mapping of cell-type subpop-
ulations across tissue regions [40]. A multimodal intersection
analysis found that the subpopulations of ductal cells, macro-
phages, dendritic cells and cancer cells were spatially restricted
[40]. Co-enrichment analysis of the multiple cell types found that in-
flammatory fibroblasts and cancer cells shared a stress-response
gene module, which was further supported by a cancer genome
database and immunofluorescence experiments [40].

These example studies in area of tissue pathology highlight
the importance of integrated analysis of scRNA-seq and ST
along with histopathological features, in which the integrated
approach helps to overcome the limitations of any individual
method [38-40]. These studies confirmed that, the cellular sub-
types in spatial spots by deconvolution or mapping from inte-
grated scRNA-seq and ST were concordant with the histological
findings, supporting the robustness of the integrative analysis
[38-40]. Also, spatially informed ligand-receptor analysis sug-
gested candidates of pivotal intercellular interactions in the path-
ological area of interest, which would lead to further mechanistic
studies [38-40].

CONCLUSION

Integrated analysis of scRNA-seq and ST spatially maps cell
subtypes identified from scRNA-seq to decipher how cell pop-
ulations are spatiotemporally participated in shaping tissue
phenotypes. Such integration can also see the high-resolution
maps of cellular subpopulations and intercellular interactions
within the tissues, bridging the gap between the molecular char-
acterization of a disease by the transcriptomics and the classical
histological approaches. Among various available options, the
study methodology of scRNA-seq and ST should be carefully de-
dsigned and selected according to the biological questions. Espe-
cially for pathologists, coupling scRNA-seq and ST information
with traditional morphological details will suggest novel insights
for the molecular characterization as well as the cellular and spa-
tial context of a disease, which can help diagnose and manage the
diseases. The analytic tools in ST are rapidly evolving, especially
in the area of scRNA-seq and ST integration. Given the rapid
development, we expect new methodologies of a genome-wide
ST at high sensitivity with a real single-cell resolution. In addi-
tion, the future direction of this area would be multiple integra-
tive analyses of scRNA-seq and ST with other single-cell multi-
omics technologies such as the genome, chromatin accessibility,
and DNA methylation sequencing [41-43].

Ethics Statement
Not applicable.

Availability of Data and Material
Not applicable.

Code Availability
Not applicable.

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Conflicts of Interest
The authors declare that they have no potential conflicts of interest.

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References
cell RNA sequencing, J Invest Dermatol 2022; 142: 3232–42.
The introduction of single-cell RNA sequencing (scRNA-seq) opened a new era in cell biology, where cellular identity and heterogeneity can be defined by transcriptome profiling due to their fragility, large size, tight interconnections, and other factors. Single-nucleus RNA sequencing (snRNA-seq) is an alternative or complementary approach for cells that are difficult to isolate. In this review, we will provide an overview of the experimental and analysis steps of snRNA-seq to understand the methods and characteristics of general and tissue-specific snRNA-seq data. Knowing the advantages and limitations of snRNA-seq will increase its use and improve the biological interpretation of the data generated using this technique.

**Key Words:** Single-cell analysis; RNA sequencing; Transcriptome

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The introduction of single-cell RNA sequencing (scRNA-seq) opened a new era in cell biology, where cellular identity and heterogeneity can be defined by transcriptome data [1]. One cell type with tremendous phenotypic and functional heterogeneity is neurons in the brain. Indeed, scRNA-seq has revealed diverse neuronal cell types in the mouse brain [2]. However, cell isolation by enzymatic tissue dissociation may damage neurons, and most human brain samples are not available as fresh tissues. Among the alternative approaches attempted, the isolation of single nuclei and subsequent RNA sequencing have enabled high-throughput transcriptome profiling at a single-cell resolution [3,4]. In addition to neurons, single-nucleus RNA sequencing (snRNA-seq) has been applied to diverse hard-to-dissociate tissues and cell types, including the kidney, heart, liver, adipocytes, and myofibers [5-9]. For most tissues, snRNA-seq is more powerful at recovering attached cell types, whereas scRNA-seq is biased towards immune cell types [5,10-12]. Moreover, the enzymatic dissociation required for scRNA-seq induces a stress response that alters the cellular transcriptome [9,10,13]. Using snRNA-seq can reduce cellular and stress response biases. The different gene expression fractions in the nucleus and cytoplasm make it necessary to generate snRNA-based data references, and these have recently been provided [14]. Combining scRNA-seq and snRNA-seq data will enable more comprehensive transcriptome profiling and cell-type annotation in tissues.

**EXPERIMENTAL PROCEDURES FOR SINGLE-NUCLEUS RNA SEQUENCING**

snRNA-seq was developed as a method to obtain transcriptome data from cells that cannot be successfully dissociated due to their size and/or fragility, such as neurons, adipocytes, and epi-

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thelial cell types from the kidney (Fig. 1A). Multi-nucleated cells, such as trophoblasts, osteoclasts, and skeletal myocytes, can be inimitably interrogated using snRNA-seq. Archived frozen tissues with broken cell membranes are also the primary targets of this method. The isolation of single nuclei, instead of whole cells, is achieved by cell membrane lysis, and nuclear transcriptome data are generated using scRNA-seq workflows (Fig. 1B). Both chemical and mechanical forces are used for cell membrane lysis. Competent buffers with nonionic detergents that disrupt cell membranes, but preserve nuclear membranes, have been tested for nuclear isolation [12]. Mechanical force is exerted using a Dounce homogenizer or other types of tissue lysers. To obtain high-quality nuclei containing transcripts, the buffers and wash conditions are important and need to be optimized for different tissue types.

The inclusion of bovine serum albumin and a high concentration of RNase inhibitors, during and after the isolation process, is critical. After isolation, nuclear morphology indicative of intact nuclei are confirmed by microscopy at 40–60× magnification (Fig. 1C). Overlysis results in clumping and poor transcript recovery, whereas under-lysis causes contamination by cytoplasmic RNAs.

In addition to the isolation of intact nuclei, brain tissues require an additional clean-up process to remove excessive myelin debris.

---

**Fig. 1.** Summary of the single-nucleus RNA sequencing (snRNA-seq) experimental process. (A) Representative cell types and tissues fit for snRNA-seq–based transcriptome profiling. (B) Experimental workflow to isolate intact nuclei for snRNA-seq. Frozen tissue is dissected, chemically and mechanically lysed, and then filtered to obtain a single-nucleus suspension. Sucrose gradient centrifugation or flow cytometry analysis is used for nuclei enrichment (Optional). After reverse transcription and amplification, a cDNA library is constructed for sequencing. (C) Representative image of extracted nuclei stained with Trypan blue. High-quality (blue arrowhead) and poor-quality (red arrowhead) nuclei are marked. Scale bar = 20 μm. FACS, fluorescence-activated cell sorting; FSC, forward scatter; SSC, side scatter.
Iodixanol (OptoPrep, San Diego, CA, USA) or a sucrose gradient [15], a myelin removal column (Miltenyi, Bergisch Gladbach, Germany), and sorting by flow cytometry have been used for this extra clean-up process (Fig. 1B). The most frequently used buffer recipe for neurons in the brain is a combination of 250–320 mM sucrose and a low-concentration of non-ionic detergent, whereas the commercial EZ Prep Kit (Sigma, St. Louis, MO, USA) is the method of choice for kidney preparations. Sorting by flow cytometry is not recommended for kidney tissue. The use of commercial buffers other than EZ Prep, such as those from 10× Genomics (Pleasanton, CA, USA) is also increasing, because of the minimal optimization requirement. Studies providing nuclear isolation protocols for snRNA-seq are listed in Table 1. The details of the buffer recipes and complete protocols can be found in these publications.

### SINGLE-NUCLEUS RNA SEQUENCING DATA PROCESSING AND ANALYSIS

The data analysis pipeline for snRNA-seq is similar to the pipeline used for scRNA-seq (Fig. 2A). The most frequently used sequencing procedure for snRNA-seq is Chromium 3’ scRNA-seq (10× Genomics), and the sequencing read mapping process (Cell Ranger 7.0, 10× Genomics) currently used is identical for scRNA-seq and snRNA-seq. During this process, both exonic and intronic reads that map the sense orientation to a single gene are used for gene counting using the default option. In previous Cell Ranger versions, intronic mapped reads were not used for the default read count option in the scRNA-seq pipeline, and the option parameter, "--include-introns = true" needed to be added for snRNA-seq read counting. The inclusion of intronic reads in snRNA-seq is critical, as more than 50% of nuclear RNAs are typically intronic compared to 15%–25% of total RNAs [13,16]. Immune cell populations such as neutrophils and other granulocytes are more likely to be identified when intronic reads are included. Detection of neutrophils is difficult because of their low RNA content and low gene count [17]. Since neutrophils have a higher amount of introns compared to other cell types [18,19], the inclusion of intronic reads may enhance the recovery of neutrophils. According to the guideline by 10× genomics, experimental steps are also important to enhance the neutrophil recovery such as immediate processing, sample preparation at room temperature, and using a non-ionic detergent.

---

### Table 1. Representative studies reporting nuclei isolation protocols for the single-nucleus RNA sequencing

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>PMID</th>
<th>Nuclei extraction buffer components and additional nuclei clean up steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen human brain</td>
<td>26890679</td>
<td>250 mM Sucrose/0.1% Tritonx-100 optional iodixanol gradient/FACS</td>
</tr>
<tr>
<td>Frozen human brain</td>
<td>27339828</td>
<td>1% NP40 or nuclear extraction buffer (320 mM sucrose, 0.1% Triton X-100/iodixanol gradient)</td>
</tr>
<tr>
<td>Frozen human/mouse brain</td>
<td>28846088</td>
<td>EZ lysis buffer</td>
</tr>
<tr>
<td>Mouse brain</td>
<td>29220646</td>
<td>250 mM Sucrose/0.1% Tritonx-100/sucrose gradient</td>
</tr>
<tr>
<td>Frozen human/mouse brain</td>
<td>31932797</td>
<td>0.025% NP-40/sucrose gradient</td>
</tr>
<tr>
<td>Mouse</td>
<td>32507042</td>
<td>10× Genomics reagent and protocol</td>
</tr>
<tr>
<td>Human brain</td>
<td>32997934</td>
<td>320 mM Sucrose/0.1% Igepal (0.1%)/iodixanol gradient</td>
</tr>
<tr>
<td>Frozen human/mouse brain</td>
<td>33456272</td>
<td>1% Formaldehyde fixation/100 mM sucrose/0.5% Triton-X-100/sucrose gradient</td>
</tr>
<tr>
<td>Frozen human brain</td>
<td>33972803</td>
<td>0.05% NP-40/iodixanol gradient</td>
</tr>
<tr>
<td>Human kidney</td>
<td>31249312</td>
<td>320 mM Sucrose/0.1% Triton X-100</td>
</tr>
<tr>
<td>Human kidney</td>
<td>31506348</td>
<td>EZ lysis buffer</td>
</tr>
<tr>
<td>Mouse kidney</td>
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</tr>
<tr>
<td>Mouse kidney</td>
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<td>EZ lysis buffer</td>
</tr>
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<td>32673289</td>
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</tr>
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<td>Mouse kidney</td>
<td>34155061</td>
<td>EZ lysis buffer</td>
</tr>
<tr>
<td>Mouse heart</td>
<td>30939177</td>
<td>320 mM Sucrose/0.2% Triton-X-100</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>33116305</td>
<td>0.1% CHAPS (human) or EZ lysis buffer (mouse)</td>
</tr>
<tr>
<td>Human pancreas</td>
<td>33212097</td>
<td>250 mM Sucrose</td>
</tr>
<tr>
<td>Mouse skeletal muscle</td>
<td>33311454</td>
<td>250 mM Sucrose/0.4% Triton-X-100/FACS</td>
</tr>
<tr>
<td>Mouse skeletal muscle</td>
<td>34382019</td>
<td>0.1% NP-40/FACS</td>
</tr>
<tr>
<td>Human liver</td>
<td>34792289</td>
<td>CST/NST/TST</td>
</tr>
<tr>
<td>Human liver</td>
<td>35581624</td>
<td>0.1% IGEPAL</td>
</tr>
<tr>
<td>Human tumor</td>
<td>32405060</td>
<td>EZ lysis buffer or ST (salts and tris) with 0.49% CHAPS (CST), with 0.03% Tween20 (TST) or with 0.2% NP-40 (NST)</td>
</tr>
</tbody>
</table>

FACS, fluorescence-activated cell sorting.  
*Cell membrane disruption was achieved using isotonic sucrose and/or a nonionic detergent. The other buffer components were omitted from the analysis.*
Fig. 2. Summary of single-nucleus RNA sequencing (snRNA-seq) and single-cell RNA sequencing (scRNA-seq) analyses. (A) Schematic workflow of snRNA-seq and scRNA-seq analysis processes. (B) Distribution of confidently mapped snRNA-seq and scRNA-seq reads. Transcriptome, the fraction of reads mapped to the exons of an annotated transcript. Genome, fraction of reads mapped to exonic and non-exonic loci. PC, principal cells; PCA, principal component analysis; UMAP, uniform manifold approximation and projection.

From the filtered cell by gene matrices of snRNA-seq data, further quality control (QC) filtering, normalization, feature selection, scaling, dimensional reduction, and clustering can be performed for cell-type annotation, as in scRNA-seq data analyses. Mitochondrial or ribosomal gene contents, which are often used as QC parameters for scRNA-seq, are not robustly used in snRNA-seq, as mitochondria and ribosomes are excluded during the extension, increasing polymerase chain reaction cycles during cDNA amplification, adding RNase inhibitors in the wash and suspension buffers, and enrichment by fluorescence-activated cell sorting into 0.04% bovine serum albumin solution in scRNA-seq [20].
experimental procedure. The differences in sequencing reads between scRNA-seq and snRNA-seq are shown in Fig. 2B.

Differential expression analysis using bulk RNA sequencing data has demonstrated a high correlation between nuclei and whole-cell samples [21,22]. However, at the single-cell or single-nucleus levels, cell-to-cell or nucleus-to-nucleus correlations decrease and replicate variations become larger than the bulk samples [22]. Direct comparisons of matched scRNA-seq and snRNA-seq data from S1 cortex neurons have demonstrated differences in genomic read mapping to coding sequences, introns, or untranslated regions [23]. Significant gene length bias exists, such that nuclear-biased genes show a length of 17 kb compared with 188 kb for genes detected in both whole cells and nuclei. The total gene expression correlation between single-cell and single-nucleus data ranges from 0.21 to 0.74. In a study of adipocytes, the average gene expression correlation between whole-cell and nuclei data for white cells was found to be 0.5 or 0.6 (after normalization)

[24]. Despite the relatively low correlations, diverse batch correction algorithms allow the co-clustering of identical cell types at a global scale in scRNA-seq and snRNA-seq data [24].

While data integration allows the combined clustering analysis of scRNA-seq and snRNA-seq data, direct comparisons of the two are difficult because of the differences in cellular and nuclear gene expression patterns. In addition, over-representation of immune cells by scRNA-seq and the superior representation of epithelial cell types by snRNA-seq suggest that complementary analysis is more appropriate than integrated analysis (Fig. 3).

SPECIFIC TISSUE OR CELL TYPE APPLICATIONS

Neurons in the brain

A high-throughput snRNA-seq protocol has been described for transcriptomic analysis of individual neurons from archived

**Fig. 3.** Comparison of cell types detected by single-nucleus RNA sequencing (snRNA-seq) and single-cell RNA sequencing (scRNA-seq). (A) Uniform manifold approximation and projection (UMAP) plots of snRNA-seq and scRNA-seq data for the human kidney. A bar plot representing the percentages of annotated nuclei and cell identities. AMB, ambiguous; CD, collecting duct; DT, distal tubule; IC, intercalated cells; LH, loop of Henle; LOH (AL), loop of Henle, ascending limb; LOH (DL), loop of Henle, distal limb; NK, natural killer; PC, principal cells; PT, proximal tubule. (B) UMAP plots of snRNA-seq and scRNA-seq data for lung tumors from a lung cancer patient.
postmortem human brain tissues [25]. Before the introduction of high-throughput applications, low-throughput methods, such as intracellular tagging by transcription in vivo analysis [26] and extraction of the cytoplasmic contents using a glass microcapillary [27,28] or laser-capture microdissection [29] were explored, along with low-throughput snRNA-seq [22]. Lake and colleagues [4] applied snRNA-seq and identified 16 neuronal subtypes of the cerebral cortex from a postmortem brain.

Currently, snRNA-seq is extensively used to determine brain cell type complexity. The U.S. government’s Brain Research Through Advancing Innovative Neurotechnologies (BRAIN) Initiative [30] launched a project known as the BRAIN Initiative Cell Census Consortium to pursue a comprehensive human brain cell atlas [31]. These resources will serve as a reference for delineating brain functions and alterations in neurodegenerative and neurological diseases. To construct the brain cell atlas, electrophysiological, morphological, and transcriptional features were used for neuronal cell type specifications, signifying the importance of transcriptome-based cell type annotation in functional and anatomical contexts. Transcriptome-based neuronal identification was accomplished using both scRNA-seq and snRNA-seq [31] after regional dissection. Due to the under-representation of neuronal cell types in scRNA-seq data and the availability of frozen postmortem brains, more recent cell applications have concentrated on snRNA-seq.

Nonetheless, differences in the nuclear and cytoplasmic gene expression patterns, and limitations of snRNA-seq in the capture and characterization of non-neuronal cell types [32] necessitate the complementary use of scRNA-seq and snRNA-seq for cell type identification in the brain.

**Epithelial cells in the kidney**

Whereas the studies agree that average nephron number is approximately 900,000 to 1 million per kidney, numbers for individual kidneys range from approximately 200,000 to >2.5 million [33]. Each nephron contains a glomerulus, which is a bundle of vessels through which waste materials are filtered from the blood. The glomerulus is enclosed in Bowman’s capsule, and filtered water, ions, and small molecules are collected in Bowman’s space. Podocytes in the epithelial lining of the Bowman’s capsule wrap around the capillaries of the glomerulus and leave filtration slits between them. Filtered materials leave Bowman’s space through a proximal tubule where reabsorption occurs. Epithelial cells lining the proximal tubule are covered with dense microvilli to facilitate transport. The modular characteristics of the kidney make biopsy an accessible and efficient sampling method for the characterization of the glomerulus.

Glomerular cell types in the kidney have been characterized using both scRNA-seq and snRNA-seq protocols. For the mouse kidney, an snRNA-seq experimental protocol yielded 20-fold more podocytes than an scRNA-seq protocol [9,34]. The Kidney Precision Medicine Project developed a reference tissue atlas for the human kidney with single-cell resolution and spatial context [35]. Rare epithelial cell types and states can be captured by snRNA-seq, however, immune components in the kidney are not well captured by snRNA-seq (Fig. 3A) [36]. Thus, the kidney atlas data incorporate snRNA-seq and scRNA-seq data for tissue atlas generation [37].

**Tumors from frozen tissues**

A diverse range of solid tumor tissues have been subjected to scRNA-seq, and the biological features of tumor cells and their surrounding microenvironments have been extensively studied. However, scRNA-seq data shows a heavy bias towards immune cell types when compared with bulk tissue data after cell type deconvolution (Fig. 3B). The use of snRNA-seq data may resolve this problem [12]. Side-by-side comparisons of scRNA-seq and snRNA-seq analyses of hepatocellular carcinoma [38] demonstrated the predominant capture of hepatocytes and carcinoma cells in snRNA-seq data compared with the immune cell-dominant landscape in scRNA-seq data. In a pancreatic cancer study, a combination of snRNA-seq and digital spatial profiling revealed that gene expression programs in malignant tumor cells and fibroblasts were enriched after chemotherapy and radiotherapy [39]. In addition to tumor-centric data analysis, snRNA-seq can be performed on longitudinal samples stored as frozen tissues. Similar to brain and kidney examples, immune cells in the tumor microenvironment can be efficiently captured by scRNA-seq.

**CONCLUSION**

Transcriptome-based cell type profiling by scRNA-seq has remarkably enhanced our understanding of cellular diversity. While scRNA-seq shows good performance at capturing immune cell diversity, the cellular landscape depicted is biased against attached cell types and is missing fragile cells. In most tissues, snRNA-seq can be used to obtain more information about these cell types, including epithelial cells, fibroblasts, neurons, and adipocytes. In addition, snRNA-seq can be used for frozen tissues, such as postmortem brain and archived tumor samples. After the successful isolation of nuclei, experimental and analysis pipelines used for scRNA-seq can be adopted for snRNA-seq. In the anal-

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https://doi.org/10.4132/jptm.2022.12.19
ysis, data from the two methods should be combined with caution, considering the differences in cellular and nuclear RNA gene expression patterns.

**Ethics Statement**
Not applicable.

**Availability of Data and Material**

**Code Availability**
Not applicable.

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**Author Contributions**
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**Conflicts of Interest**
The authors declare that they have no potential conflicts of interest.

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**References**
Single-nucleus RNA sequencing

Inflammatory bowel disease–associated intestinal fibrosis

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Fibrosis is characterized by a proliferation of fibroblasts and excessive extracellular matrix following chronic inflammation, and this replacement of organ tissue with fibrotic tissue causes a loss of function. Inflammatory bowel disease (IBD) is a chronic inflammation of the gastrointestinal tract, and intestinal fibrosis is common in IBD patients, resulting in several complications that require surgery, such as a stricture or penetration. This review describes the pathogenesis and various factors involved in intestinal fibrosis in IBD, including cytokines, growth factors, epithelial-mesenchymal and endothelial-mesenchymal transitions, and gut microbiota. Furthermore, histopathologic findings and scoring systems used for stenosis in IBD are discussed, and differences in the fibrosis patterns of ulcerative colitis and Crohn’s disease are compared. Biomarkers and therapeutic agents targeting intestinal fibrosis are briefly mentioned at the end.

Key Words: Fibrosis; Inflammatory bowel disease; Crohn disease; Colitis, ulcerative

Fibrosis is an irreversible process that occurs as a consequence of chronic inflammation. It results in persistent luminal narrowing and strictures. Anti-inflammatory agents do not prevent or treat fibrosis in IBD, even if they improve the inflammation [13]. Because the fibrotic process is not affected by various IBD treatments, researchers have focused on inflammation-independent mechanisms, such as genetic factors, environmental risks, and the gut microbiota, which are known to affect the prognosis of fibrosis [14]. Moreover, intestinal fibrosis can be observed alongside epithelial-mesenchymal and endothelial-mesenchymal transitions, and gut microbiota, which are known to affect the prognosis of fibrosis [14].
cessive deposition of ECM and activated mesenchymal cells in the intestinal wall [15]. The main known drivers of this fibrosis mechanism are soluble molecules (cytokines and growth factors), the epithelial-mesenchymal transition (EMT), the endothelial-mesenchymal transition (EnMT), and the gut microbiota.

**Genetic factors**

Genetic research on IBD has proposed several genetic pathways for its pathogenesis [16,17]. However, relations between those gene mutations and intestinal fibrosis have not yet been well studied. In one bioinformatics study, researchers hypothesized that similar molecular pathways would be involved in fibrosis in various organs and thus measured gene expressions found in kidney fibrosis and liver cirrhosis in Crohn’s disease (CD) and ulcerative colitis (UC). They found that fibrosis in different organs had different gene signatures. C-X-C motif chemokine ligand 9 (CXCL9) and CD52 were upregulated in both CD and UC, whereas thrombospondin 2 (THBS2), matrix gla protein (MGP), protein tyrosine phosphatase receptor type C (PTPRC), and decorin (DCN) were upregulated only in CD. In UC, CXCL9, CD52, and granzyme A (GZMA) were upregulated, and DCN, which was elevated in CD, was downregulated [18].

Nucleotide binding oligomerization domain containing 2 (NOD2) has been most studied in association with IBD. Various polymorphisms related to the NOD2 gene have been reported and are known to be related to fibrogenesis in UC and CD [19–23]. The NOD2 gene was also suggested as a predictive marker for the progression of CD fibrosis [24]. Within the innate immune system, Toll-like receptors 4 (TLR) and signal transducers and activators of the transcription 3 (STAT3) might be a mechanism for intestinal fibrosis [19,25,26], and interleukin-23 receptor (IL23R), interleukin-12 subunit beta (IL12B), and Janus kinases 2 (JAK2), which are related to the Th17 pathway, could also be involved [25,27–29]. CX3CR1-mediating chemokines [30] and autophagy genes (autophagy related 16 like 1 [ATG16L1] and immunity-related GTPase family M protein [IRGM]) were reported to have an association with stricture disease [25,31]. However, the exact mechanism remains obscure because fucosyltransferase 2 (FUT2) appears to change the composition of the gut microbiota, which is presumed to be able to induce fibrosis [32]. Transforming growth factor β (TGF-β) plays a broad role in initiating inflammation and fibrosis [33,34]. Matrix metalloproteinase 3 (MMP3) encodes a proteinase that degrades most components of the ECM. Membrane-associated guanylate kinase inverted 1 (MAGI1), which is associated with a mechanism that disrupts the tight junction of intestinal epithelial cells, is a gene factor potentially associated with fibrosis (Table 1) [35,36]. Because the number of studies is too small for generalization, more genome-wide association studies and next generation sequencing studies are needed to reveal genetic factors involved with fibrosis in IBD [16].

**Cytokines and growth factors**

Local fibroblasts in fibrotic foci proliferate in response to various growth factors and cytokines. platelet derived growth factor (PDGF), basic-fibroblast growth factor, insulin like growth factor 1, epidermal growth factor, CTFG, tumor necrosis factor α (TNF-α), IL-1β, and IL-6 can act as major proliferating factors.

**Table 1. Potential genetic factors of IBD-associated fibrosis**

<table>
<thead>
<tr>
<th>Related genes</th>
<th>Disease entity</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD2</td>
<td>CD and UC</td>
<td>Apoptosis and activates NF-κB, induce interleukin 1-beta</td>
<td>[19-23]</td>
</tr>
<tr>
<td>TLR4</td>
<td>CD</td>
<td>Initiating innate immune responses</td>
<td>[19]</td>
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<td>IL23R</td>
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<td>Activation of Th17 lymphocytes</td>
<td>[27,28]</td>
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<tr>
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<td>CD</td>
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<td>[25]</td>
</tr>
<tr>
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<td>[29]</td>
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<td>CD</td>
<td>Leukocyte chemotaxis and adhesion</td>
<td>[30]</td>
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<td>Innate immune mechanisms</td>
<td>[25,26]</td>
</tr>
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<td>CD</td>
<td>Affects the composition of the gut microbiota</td>
<td>[32]</td>
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<tr>
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<td>CD</td>
<td>Initiation of inflammation</td>
<td>[33,34]</td>
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<td>MMP3</td>
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<td>Mediate degradation of components of the extracellular matrix</td>
<td>[35]</td>
</tr>
<tr>
<td>MAGI1</td>
<td>CD</td>
<td>Disruption of epithelial barrier via abnormality of tight junction of intestinal epithelial cells</td>
<td>[36]</td>
</tr>
</tbody>
</table>

IBD, inflammatory bowel disease; NOD2, nucleotide-binding oligomerization domain-containing protein 2; CD, Crohn disease; UC, ulcerative colitis; NF-κB, nuclear factor κB; TLR4, Toll-like receptors 4; IL23R, interleukin-23 receptor; IL12B, interleukin-12 subunit beta; JAK2, Janus kinases 2; CX3CR1, C-X3-C motif chemokine receptor 1; STAT3, signal transducers and activators of the transcription 3; ATG16L1, autophagy-related 16-like 1; IRGM, immunity-related GTPase family M protein; FUT2, fucosyltransferase 2; TGF-β, transforming growth factor beta; MMP3, matrix metalloproteinase-3; MAGI1, membrane-associated guanylate kinase inverted 1.
The proliferating fibroblasts recruit various inflammatory cells, T cells, eosinophils, and mast cells. Inflammatory mediators, such as PDGF-A, PDGF-B, IGF-1, and fibronectin, are also involved in local fibroblastic proliferation and the migration of fibroblasts to the ECM of an inflamed area. In addition, intestinal stellate cells are differentiated into fibroblasts at inflammatory sites using TGF-β [38]. Furthermore, though the molecular pathway is not well established, the capacity of adult bone marrow to derive fibroblast precursors recently became clear, and several cytokines, such as IL-10 or other growth factors, are considered to be part of that pathway [14,39,40].

Critical role of adipose tissue

The role of adipose tissue is essential in inducing hyperplasia of the muscularis propria and subsequent stricture formation in CD [41]. In IBD research, interest is increasing in the role of various bioactive substances secreted by mesenteric fat [42]. Creeping fat is a unique and pathognomonic phenomenon in CD that was first reported by Crohn in 1932 [43]. Creeping fat is defined as > 50% coverage of the exterior intestinal surface with proliferation and ectopic extension of mesenteric adipose tissue (Fig. 1). In the proliferated adipose tissue surrounding the intestine, numerous mediators play crucial roles in inflammation and immunity that lead to the development and progression of IBD [41,44]. Mediators secreted by fat tissue include adipokines (adiponectin, leptin, resistin, C1q/TNF-related protein 3 [CTRP-3], and fatty acids), cytokines (TNF-α, peroxisome proliferator-activated receptor-γ [PPAR-γ], macrophage colony-stimulating factor, monocyte chemoattracted protein-1, IL-1, IL-6, IL-8, IL-10, and chemokine (C-C motif) ligand 5), and growth factors (ghrelin and vascular endothelial growth factor). These secretions attract and activate various immune cells [45,46]. Therefore, cases of cobblestone mucosa and proper muscle hyperplasia are common in specimens surgically resected to treat CD complications and result in a thick intestinal wall and stricture formation (Fig. 1) [47].

EMT and EnMT

The EMT is a well-known phenomenon in malignant neoplasms and literally describes a phenomenon in which tumor cells with epithelial features acquire a mesenchymal tendency to break the resistance of the surrounding ECM, facilitate local migration and invasion, and exhibit aggressive behavior [48]. It is also a mechanism of distant metastasis, in which epithelial cells are attacked by immune cells, or an apoptotic program is initiated when epithelial cells float away from their location, especially when they enter the blood flow, which is the starting point of distant metastases [49].

In IBD, damaged intestinal epithelial cells are activated, and the EMT pathway is initiated. This change can be shown by a loss of epithelial marker expression (such as cytokeratins and E-cadherin) in the enterocytes of inflamed foci and increased expression of mesenchymal markers (especially fibroblast markers, MMP-2, MMP-9, ferroptosis suppressor protein 1, α-smooth muscle actin [α-SMA], and vimentin) [50].

In addition, IBD can damage vascular endothelial cells [14]. In the presence of an excessive inflammatory response, such as hypoxia and secondary mechanical stress, endothelial cells are activated and converted into cells with fibroblast properties. This is called the EnMT, in which endothelial markers (VE-cadherin, Von Willebrand factor, and CD31) expressed in cells are lost, and the expression of fibroblast markers increases. In the EnMT, TGF-β, insulin-like growth factor 2, and IL-1b or TNF-α, which are pro-inflammatory components, are inducers [48,51,52]. Occasionally, this process is reversible. Bone morphogenetic protein-7 or hepatocyte growth factor can convert fibroblasts back into endothelial cells. However, the conditions under which this reversal pathway works remain unknown [53,54]. Cellular transitions, i.e., the EMT and EnMT, are sources of new fibroblasts and result in excessive ECM deposition. Fibroblasts also exhibit enhanced migratory ability. Therefore, the submucosal layer, which is composed of loose connective tissue, is replaced with various ECM components. As a result, the condition of the intestinal wall impedes its flexible movement [13].

Gut microbiota

The gut microbiota is one key factor in the development of fibrosis in IBD [16]. It consists of bacteria, viruses, archaea, pro-

Fig. 1. Gross finding of resected large intestine specimen of 30-year-old patient with Crohn’s disease. Creeping fat covers more than 50% of the intestinal circumference surface with mesenteric proliferation. Cobblestone mucosa and proper muscle hyperplasia that result in intestinal stricture are also noted.
tists, and yeast. The composition of the intestinal microbiota affects the host metabolism and immune systems in various ways, and chronic inflammatory status caused by the gut microbiota can ultimately produce the complications of intestinal fibrosis or strictures [55]. The stability of the intestinal microbiota supports the barrier function of intestinal epithelial cells [56]. If the balance between beneficial and harmful bacteria is destroyed, the intestinal microbial barrier and anti-inflammatory regulatory pathway can be damaged, which can eventually cause severe colitis [57,58]. In one study, the intestinal bacterial diversity of mice was reduced using intestinal radiation and an antibiotic cocktail treatment, and that produced decreased levels of TGF, phosphorylated SMAD³, and SMA proteins, which in turn reduced the chronic inflammation that plays a crucial role in intestinal fibrosis [59]. Preliminary studies of fibrosis and the microbiome have been done [24]. Several studies have suggested that fibronectin and collagen deposition in the intestinal wall is a response to bacterial stimulation of the intestine [60,61]. In addition, several studies have reported that strictures are more frequent in patients with CD who have higher levels of antibacterial antibodies [56,58].

**HISTOPATHOLOGY**

In IBD-associated fibrosis, the change in the muscular layer, which contributes to the presence of a thickened bowel wall, is notable (Fig. 2A). This results in both hypertrophy and hyperplasia [47]. Although chronic inflammation, represented by basal plasmacytosis, is predominant, IBD can also show a mixed inflammation pattern that is accompanied by active inflammation (Fig. 2B, C). As the disease progresses, increased activation of intestinal myofibroblasts results in the gradual synthesis of ECM and contractile proteins (α-SMA and MYLK) [60]. Young fibroblasts begin to be deposited and gradually progress to fibrosis with increased deposition of ECM (collagen, fibronectin, etc.) [55]. In addition, slowed blood flow is caused by damage to highly branched vessels (Fig. 2D). This condition often becomes refractory to medication. Neuronal cell changes are usually observed in surgical specimens from patients with chronic constipation without a certain organic cause, and similarly in IBD patients, neuronal hypertrophy can be a secondary reactive change [62]; however, it can also act as a mechanism for intestinal stiffening (Fig. 2E). In trichrome stain results, dissection between hyperplastic smooth muscle bundles is observed to be interspersed with fibrosis (Fig. 2F).

**Histopathology scoring systems for stenosis**

In IBD, many scoring systems have been developed to express disease activity, including the Geboes score and Nancy Index of UC and the Crohn’s Disease Activity Index [63,64]. These evaluation methods consider not only the presence of ulcers but also the degree of infiltration of inflammation, submucosal fibrosis, and thickened muscularis propria. Following a recent discussion at the Stenosis Therapy and Antifibrotic Research Consortium, a

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**Fig. 2.** Histologic findings of Crohn’s disease. (A) Hypertrophy and hyperplasia of the submucosa and muscularis propria are present. (B) Chronic inflammation with lymphoid aggregates and lymphoid follicles is dominant. (C) Although chronic inflammation is predominant, active inflammation that consists of extensive neutrophilic infiltrates is also noted. (D) Fibromuscular hyperplasia of damaged submucosal vessels causes slow blood flow. (E) Neural hypertrophy is also noted. (F) Trichrome stain reveals that dissection between the hyperplastic smooth muscle bundles is interspersed with fibrosis.
Components of each mural layer were constructed to include an evaluation of the inflammatory and fibrotic components of each mural layer [55]. In this artificial intelligence era, researchers have endeavored to develop a deep learning model to evaluate intestinal fibrosis in surgical specimens for postoperative recurrence prediction [65,66].

Differences of intestinal fibrosis in UC and CD

UC and CD, which belong to the same IBD category but differ in their mechanisms of development and clinical features, also show differences in IBD-related fibrosis [67]. In UC, fibrotic changes are limited to the mucosal and submucosal layers [55]. This can shorten or stiffen the intestine, leading to motility disorders. Because strictures in UC are rare and can be either benign or malignant, a persistent stricture should raise suspicions of cancer [68]. Complications that are mainly due to bowel wall thickening, such as stricture and stenosis, are problematic in CD. Furthermore, diffuse transmural collagen layers down to the muscularis propria and proliferative fibroblastic infiltration are observed. In UC, on the other hand, the progression of intestinal fibrosis does not correlate with disease duration or location; however, inflammatory activity does correlate with medical treatment. In contrast, in CD, the duration and location of the disease and type of treatment are related to the risk of intestinal fibrosis [55,69,70].

Biomarkers and potential antifibrotic agents

Gene variants, epigenetic modifiers, antimicrobial antibodies, ECM components, and clinical, endoscopic, or environmental factors can be used to evaluate and predict fibrosis in IBD patients [71]. Fibrosis in CD, which causes several serious sequelae, is reversible, and thus it is important to develop therapeutic agents targeting it [72]. However, no effective therapeutic agents are available to prevent or repair the progression of fibrosis except by suppressing inflammation, though diverse potential antifibrotic therapies have been proposed. Although their mechanism is still unknown, statins (simvastatin) have been reported to effectively inhibit the progression of CD fibrosis [73,74]. In CD, pirfenidone, Rho kinase inhibitors, TGF-β signaling inhibitors, IL-13 inhibitors, and G31P (an antagonist of CXCL8) are also known to be effective [75,76]. GED-0507-34, an agent with a strong affinity for PPAR-γ, has been suggested as an anti-fibrotic agent in UC patients [77].

CONCLUSION

IBD-related intestinal fibrosis is the starting point for serious complications in patients with refractory and poorly controlled chronic IBD. If the uniquely activated profibrotic pathway observed in IBD can be identified, it could be used as a biomarker for targeted therapy. In addition, the gene expression signature of fibrogenesis at diagnosis could predict the risk of surgery. Intestinal fibrosis is an unfavorable result of the harmonic action of intestinal epithelial cells, the microbiota, and various mesenchymal components, such as the adipose tissue, fibroblasts, smooth muscle, neural tissue, and vascular endothelial cells, at the lesion site. It is thus necessary to pay attention to these mechanisms, from analyzing the ECM to developing therapeutic agents that target the main factors affecting pathogenesis, as well as elucidating the mechanisms involved by using various advanced research methods.

Ethics Statement

Not applicable.

Availability of Data and Material

All data generated or analyzed during the study are included in this published article (and its supplementary information files).

Code Availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the study.

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Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

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Cystitis glandularis (CG), a common glandular lesion of the urinary bladder, is a cystic structure with a glandular lumen surrounded by outer urothelial cells that usually evolve from von Brunn’s nests after long-standing inflammation or irritation [1]. CG with intestinal metaplasia (IM) or the presence of mucus-containing goblet cells can produce abundant extracellular mucin that requires differentiation from primary adenocarcinoma, urachal adenocarcinoma, or metastatic adenocarcinoma from other organs [1,2]. CG can also develop a mass-like florid lesion that mimics bladder cancer on radiologic and cystoscopic examinations [3].

In addition to the clinical and histopathological disguise characteristics of CG, its relationship with malignancy has been discussed in studies with contrasting results [2]. For example, the co-occurrence of CG and adenocarcinoma has been frequently reported in the urinary bladder, which may indicate a connection between these two entities [1]. Furthermore, prior studies, using single gene or chromosome assays, underlaid the molecular basis supporting the premalignant nature of CG with or without IM by elucidating telomere shortening and chromosomal instability [4], p53 loss of heterozygosity and overexpression [5], and nuclear β-catenin expression [6]. In contrast, other studies failed to find a clear indication that CG, with or without IM, increased the future risk of developing bladder cancer during years of retrospective observation [7,8]. Comprehensive characterization would greatly help identify the pathobiology and clinical implications of CG.
In this study, we compared the proteomic landscape of CG with that of urothelial carcinoma (UC) and normal urothelium (NU). Differentially expressed proteins (DEPs) with functional enrichment were analyzed in CG, UC, and NU to identify the oncologic significance of CG at the molecular level.

**MATERIALS AND METHODS**

**Patient selection**

From the Seoul National University Hospital, 10 CG, 12 UC, and 9 NU specimens were collected after excluding patients who previously had any bladder tumor or intravesical treatment. Experienced pathologists (M.J. and H.S.R.) reviewed the diagnoses using hematoxylin-eosin slides based on the 2022 World Health Organization (WHO) classification [9]. All samples were obtained by transurethral resection of the urinary bladder, except for one NU, which was obtained from the ureter.

**Liquid chromatography-tandem mass spectrometry**

Formalin-fixed paraffin-embedded slides were scrapped and lysed in a sodium dodecyl sulfate-extraction buffer. After protein isolation, samples were sonicated and precipitated using acetone. Proteins were digested according to the filter-aided sample preparation procedure [10]. Tandem mass tag 6-plex labeling was employed for the peptide samples according to the manufacturer’s instructions, with modifications. After pooling the labeled peptides, the sample was separated into 12 fractions using Agilent 1290 bioinert high-pH reverse-phase liquid chromatography (Agilent, Santa Clara, CA, USA). For each peptide fraction, liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was conducted using a Q-ExactivePlus mass spectrometer (Thermo Fisher, Waltham, MA) with an Ultimate 3000 RSLC system (Dionex, Sunnyvale, CA, USA), as previously described, with modifications [11]. Peptides were identified using a false discovery rate (FDR) of 1% as cutoff. Raw data are available in the ProteomeXchange Consortium (PXD027602) [12,13].

**Proteomic data analysis**

The MS raw data were processed using MaxQuant ver. 1.5.3.1 (Max Planck Institute of Biochemistry, Munich, Germany) [14] with the Andromeda engine [15]. For label-free quantification, the iBAQ algorithm was used on MaxQuant platform [16]. Using normalized abundance, we identified DEPs among CG, UC, and NU based on an analysis of variance (ANOVA) [14] test as a cutoff for permutation-based FDR < 0.05. Subsequently, the DEPs were hierarchically clustered based on Euclidean distance. Following a review of their abundance, we enlisted “UC-like” DEP clusters as “UC-like signature”, for which CG and UC overlapped but NU did not. All analyses were conducted using Perseus ver. 1.6.14.0 (Max Planck Institute of Biochemistry).

**Functional enrichment analysis**

To determine the biological processes represented by the lists of DEPs, we investigated protein-protein interactions (PPIs) using String database [17], Gene Ontology-biologic process (GOBP) annotation using Topgene Suite [18], and the Molecular Signatures Database (MSigDB) Hallmark gene sets using pre-ranked Gene Set Enrichment Analysis (GSEA) [19]. To examine the associations among GOBPs, we reconstructed a network model using REVIGO [20]. Network models of PPI and GOBP were visualized using Cytoscape ver. 3.7.2 [21]. The z-scores of the selected proteins were compared between the group with and without IM using a two-tailed t-test.

**RESULTS**

We investigated 31 urothelial specimens, including 10 CGs, 12 UCs, and nine NUs, using a proteomic approach. A schematic outline of this study is shown in Fig. 1. The median ages were 40, 70, and 63.5 years, respectively, and the male-to-female ratios were 1.0, 0.4, and 0.9, respectively. Half of the specimens that contained CG were accompanied by IM. All UCs were confined to the mucosa (stage T1), and eight (67%) were of WHO high grade.

From LC-MS/MS analysis, we identified 9,890 proteins across all samples and 1,139 DEPs among the three entities (ANOVA FDR < 0.05). With unsupervised hierarchical clustering, a substantial number of DEPs were discovered to overlap with CG/NU in a way distinct from UC (Fig. 2). CG was not differentiated by the presence of IM (Fig. 2, asterisks). Interestingly, we found a subset of DEP clusters (n = 53, 5%) that were differentially expressed in NU but similarly expressed between CG and UC (Fig. 2, arrows); thus, these proteins were named as “UC-like signature” (Fig. 3A). Since the signature were similar within each group, the patterns of these signatures were thought to represent the general profile of CG.

We hypothesized that this signature might represent the UC-like characteristics of the CG. Using PPI analysis, we explored cellular processes represented by the “UC-like signature”. This resulted in sets of DEP networks within the “UC-like signature”, which were enriched for reactive oxygen species (ROS) and energy metabolism [22-24], growth and DNA repair [25,26], transport [27,28], motility and epithelial-mesenchymal transition (EMT)
Proteomics in cystitis glandularis

[29,30], and cell survival [31,32] (Fig. 3B). The unit associated with ROS and energy metabolism, which was connected to the one with growth and DNA repair functions, included the top-ranked DEPs (e.g., SOD2, PRKCD, CYCS, and MRPL23) (Fig. 3B). Using the official pre-ranked GSEA of the “UC-like signature”, we also identified significant enrichment of ultraviolet (UV) response (normalized enrichment score = 1.772, FDR = 0.03) for SOD2, PRKCD, and MRPL23 (Fig. 3C). UV radiation stimulates carcinogenic sequences by promoting ROS generation and DNA damage [33]. These data suggest that CG is predominantly a benign lesion, but oxidative stress might be a gateway theme representing oncogenic potential in CG.

To further characterize the “UC-like signature”, we shortlisted the top 10 most significant DEPs (Fig. 3A, asterisk), followed by GOBP analysis (Fig. 4A). Subsequently, we constructed a network of connections between the GOBP terms. Consistent with the aforementioned single-protein networks, functional elements related to ROS metabolism, development, and transport were highlighted by GOBPs (Fig. 4B), which suggests that these functions could collectively substantiate the cancerous risks in CG.

Of note, GOBPs were represented almost exclusively by four molecules, i.e., SOD2, PRKCD, CYCS, and HCLS1 (Fig. 4A), and their abundance in UC/CG compared to NU accordantly pointed toward oncologic functions. For example, previous studies have shown an association of decreased level of SOD2 and increased level of PRKCD, as observed in CG/UC versus NU, with ROS production [22,24]. Overt oxidative stress by activating oncogenic signaling pathways, DNA mutations, EMT, and stromal remodeling promotes bladder cancer development and progression [22]. In line with this, altered expression of CYCS and HCLS1 might deregulate oxidative respiration and the RAS signaling pathway, thereby enhancing the malignant behavior of bladder cancer [23,34]. We further examined whether these four statistically and functionally significant molecules were differentially expressed with IM and found no significant differences related to IM (Fig. 4C).

**DISCUSSION**

The malignant risk of CG and its association with bladder can-
cer have been disputed [1,2]. Considering the high prevalence of CG, there is a dire need to clarify its oncologic implications. Using LC-MS/MS, we revealed the proteomic landscape of CG in relation to malignant (UC) and normal (NU) urothelial tissues. CG generally presented an NU-like profile, in contrast to UC, indicating the overall benign nature of CG. By retrospectively observing a handful of patients with CG, consistent with this result, previous studies failed to confirm clear association between UC and CG [7,8]. Conversely, we confirmed that some of the clustered DEPs showed an opposite pattern across the diagnosis; these proteins were shared by CG and UC but not by NU. Using functional and network analyses, we found that these DEPs coded for ROS and energy metabolism, growth and DNA repair, transport, motility and EMT, and cell survival, further supporting the relation of this “UC-like signature” with the malignant risk in CG. To our knowledge, this is the first study to determine the oncogenic characteristics of CG using a comprehensive proteomic analysis. Further large-scale studies are required to determine the risks of UC development in patients with CG.

Using an interconnected functional network analysis of the

![Fig. 2. Unsupervised hierarchical clustering of 1,139 differentially expressed proteins identified by an analysis of variance test of urothelial carcinoma (UC), cystitis glandularis (CG), and normal urothelium (NU). Arrows indicate a “UC-like signature” that showed disparity between UC/CG and NU. Asterisks indicate CG samples showing intestinal metaplasia.](https://jpatholtm.org/)
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shortlisted proteins, including SOD2, PRKCD, CYCS, and HCLS1. ROS metabolism, structural development, and transport functions were concordantly found to be significantly enriched in the “UC-like signature.” ROS are tightly regulated in normal cellular environments, and increased ROS act as versatile transducers for the generation and progression of bladder cancer [22]. For example, both UC and CG showed downregulation of SOD2, a scavenging molecule, and upregulation of PRKCD, an oxidative enzyme associated with ROS accumulation. ROS imbalance induces oxidative DNA damage, mutations, propagation of oncogenic signals including RAS, mitogen-activated protein kinases, phosphoinositide 3-kinase, and nuclear factor κB pathways, EMT, and stromal modification in bladder cancer [22,24]. Consistent with this, a previous study suggested that alterations in ROS metabolism participate in inflammation-associated cancer sequences [35,36]. In addition, upregulation of PRKCD can further support a malignant phenotype in bladder cancer by promoting migration and invasion [37]. CYCS is instrumental.

Fig. 3. Pathobiologic characteristics of the “urothelial carcinoma (UC)-like signature”. (A) The UC-like signature proteins. Asterisks denote the 10 top-listed proteins by false discovery rate (FDR). (B) Protein-protein interaction networks of the “UC-like signature” proteins with their common functions. (C) Gene Set Enrichment Analysis of the “UC-like signature” shows enrichment of response to ultraviolet in molecular hallmark function. CG, cystitis glandularis; EMT, epithelial-mesenchymal transition; FWER, family wise error rate; NES, normalized enrichment score; NU, normal urothelium; ROS, reactive oxygen species.
in regulating oxidative phosphorylation and energy metabolism, and high maintenance of CYCS, as identified in UC/CG, could induce metabolic modification in bladder cancer, or Warburg effect [23]. In addition, UC and CG showed lower HCLS1 levels than NU. HCLS1, an actin-binding molecule supporting cellular transport and trafficking, has been shown to result in

Fig. 4. Imperative functions enriched in the “urothelial carcinoma-like signature”. (A) Gene Ontology-biologic processes (GOBPs) represented by the top 10 shortlisted proteins of “urothelial carcinoma-like signature”. (B) Network analysis of the GOBPs identifies reactive oxygen species (ROS) metabolism, structure development, and transport as common functional themes. (C) SOD2, PRKCD, CYCS, and HCLS1 were comparable between cystitis glandularis with and without intestinal metaplasia (IM). The value is expressed with z-scores of the proteome abundance. FDR, false discovery rate.
adverse outcomes in bladder cancer [34,38]. We believe that the “UC-like signature” might reflect the oncogenic pathway related to altered ROS and energy metabolism and structural modification in CG, and the expression of these signature proteins deserves further study to determine the risk of aggression in CG.

This study lacked specimens with adenocarcinoma in the analysis. The malignant association of CG with IM has been conjectured, typically in terms of bladder adenocarcinoma, but definite evidence has not been confirmed [1]. Instead, altered ROS and energy metabolism have been implicated in the transformation of dysplasia to adenocarcinoma in the esophagus, consistent with the functions enriched in the “UC-like signature” [35]. In addition, the overall proteome profile and biomarkers relevant to such functions showed no difference regardless of the presence of IM in CG. However, previous studies have suggested that CG with IM may be more advanced than CG without IM regarding tumorigenic potential, as exemplified by more robust telomere shortening or β-catenin activation [4,6]. Therefore, we reasonably speculate that the “UC-like signature” might reflect the malignancy risk encoded in CG irrespective of IM at a global level.

In conclusion, using comprehensive proteomic profiling, we identified a predominantly non-neoplastic landscape of CG that is closer to NU than to UC. Furthermore, we confirmed a small subset of common DEPs in UC and CG, suggesting altered functions of ROS metabolism that might imply potential cancerous risks in CG.

**Ethics Statement**

All procedures performed in the current study were approved by the Institutional Review Board (IRB) (I-2009-163-1168; 05 October 2020) per the 1964 Helsinki Declaration and its later amendments. Formal written informed consent was not required, with a waiver by the appropriate IRB.

**Availability of Data and Material**

The datasets generated or analyzed during the current study are available in the ProteomeXchange Consortium (http://www.proteomexchange.org/) via the PRIDE database (accession: PXD027602).

**Code Availability**

Not applicable.

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**Conflicts of Interest**

The authors declare that they have no potential conflicts of interest.

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11: 2301-19.
Primary lymphoma of the bone and joint is rare. Malignant lymphomas related to metallic implants from an orthopedic procedure, called metallic implant-associated lymphomas, are extremely rare [1]. Only seven cases of malignant lymphomas complicating total knee replacement arthroplasty have been reported and occur several years after operation; all cases were large B-cell lymphoma [1-7].

Herein, we describe the first reported case of anaplastic large cell lymphoma (ALCL) presenting as periprosthetic joint infection occurring 10 years after total knee replacement arthroplasty, suggesting that patients with an inflammatory response to orthopedic prostheses should be monitored carefully for an extended time.

**CASE REPORT**

An 80-year-old female patient was admitted because of right knee pain for 2 years. The pain had recently increased, although there was no history of trauma. She had undergone total knee replacement arthroplasty 10 years prior. Computed tomography demonstrated an irregular osteolytic mass-like lesion in the right lateral femoral condyle, adjacent to the metallic prosthesis. Histologic findings revealed sheets of anaplastic tumor cells that were positive for CD2, CD4, CD5, CD43, and CD30 but negative for CD3, CD20, CD15, and anaplastic lymphoma kinase. Epstein-Barr encoding region in situ hybridization was negative. Analysis of T-cell receptor \(\gamma\) gene rearrangement studies using BIOMED-2-based multiplex polymerase chain reaction confirmed monoclonal T cell proliferation. The woman was finally diagnosed with ALK-negative anaplastic large cell lymphoma.
rangement studies using BIOMED-2–based multiplex polymerase chain reaction confirmed monoclonal T-cell proliferation (Fig. 3). The woman was finally diagnosed with ALK-negative ALCL. Whole body bone scan showed high $^{18}$F-fluorodeoxyglucose uptake in bone and synovium at the right knee arthroplasty removal site, with overlying soft tissue inflammation and joint effusion. There was no evidence of other site involvement in systemic workup, consistent with stage 1 disease. Postoperative definite radiotherapy was administered, and the patient was in good health at the latest follow-up (1 year). Follow-up position emission tomography–computed tomography (PET-CT) revealed no residual mass.

DISCUSSION

Medical devices such as breast, hip, knee, and vascular prostheses can be associated with malignant lymphomas. A prototype is an entity related to breast implants, called breast implant-associated anaplastic large cell lymphoma (BIA-ALCL), and more than 300 cases have been described in the literature [8]. Genomic characterization of BIA-ALCL shows abnormalities similar to those of systemic ALCL [8]. The characteristic clinical features are late-onset implant-associated seroma occurring greater than 1-year post-operative and an indolent course. Most patients are cured by implant removal [9]. In addition to ALCL, other types

Fig. 1. Computed tomography shows an irregular osteolytic mass-like lesion (arrow) in the right lateral femoral condyle, adjacent to the metallic prosthesis.

Fig. 2. Anaplastic tumor cells have irregularly folded nuclei, prominent nucleoli, and a moderate amount of amphophilic cytoplasm (A). Tumor cells are positive for CD30 (B), CD4 (C), and CD43 (D).
other chronic inflammatory conditions such as chronic osteomyelitis and pyothorax, and the two share several clinicopathologic features including development in the setting of prolonged chronic inflammation, localization to a confined body space, a long latency period, and the presence of large cell phenotype [11]. Most metallic implant-associated lymphomas were of B-cell origin, in contrast to breast implants. To the best of my knowledge, only one case of ALCL for a tibial metal implant has been published in the English literature [11], and another case of metallic dental implant-associated mucosal CD30-positive T-cell lymphoproliferative disorder was reported in Korea [12].

Only seven cases of malignant lymphomas complicating total knee replacement arthroplasty have been reported in the medical literature [1-7]. The mean time between implantation of the prostheses and lymphoma diagnosis was 7 years (range, 6 months to 16 years). All tumors were classified as large B-cell lymphomas. All cases arose in the bone and five of them were found in wear debris adjacent to the prosthesis or periprosthetic membrane. Three cases were presented as osteolytic bone lesions with soft tissue extension as is the case described above. Four patients were treated with radiotherapy and chemotherapy, one was treated with radiotherapy, one was treated with chemotherapy, and one patient was not treated. Follow-up was reported in six patients, all of them being free of disease for 8 months to 3 years. In particular, one patient was alive for 2 years without evidence of disease, even though no additional treatment was received other than revision arthroplasty [6].

In conclusion, this is the first reported case of ALCL complicating total knee replacement arthroplasty, which can cause prosthesis failure long after the placement operation. Periprosthetic primary lymphoma of the bone should be included in the differential diagnosis of a patient presenting with knee pain, knee mass, or lytic destruction after knee arthroplasty [5].

**Ethics Statement**
The Institutional Review Board of Dankook University Hospital (2022-03-028) approved this case report and informed consent was waived.

**Availability of Data and Material**
All data generated or analyzed during this study are included in this published article and available from the corresponding author.

**Code Availability**
Not applicable.

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**Conflicts of Interest**
The author declares that I have no potential conflicts of interest.
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References
What’s new in neuromuscular pathology 2022: myopathy updates and gene therapies

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Table 1. IIM subtypes and their associated autoantibodies

<table>
<thead>
<tr>
<th>IIM Subtype</th>
<th>Associated Autoantibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatomyositis (DM)</td>
<td>Mi2, NXP2, TIF1γ, MDA5, SAE</td>
</tr>
<tr>
<td>Inclusion body myositis (IBM)</td>
<td>cN1A*</td>
</tr>
<tr>
<td>Immune mediated necrotizing myopathy (IMNM)</td>
<td>SRP, HMGCR</td>
</tr>
<tr>
<td>Anti-synthetase syndrome (ASyS)</td>
<td>Jo-1, PL7, PL12, EJ, OJ, KS, Zo, Ha</td>
</tr>
</tbody>
</table>

*MSAs are usually mutually exclusive and specific for IIM subclasses, with the exception of cN1A

Antisynthetase syndrome (ASyS)
- Defined by the presence of an antibody to aminocyl tRNA synthetase (ARS), together with a single or combination of the following clinical manifestations:
  - Myositis
  - Polymyositis
  - Interstitial lung disease
  - Mechanic hands
  - Raynaud phenomenon
- ASyS patients with a DM-like rash are not classified as DM, but as “ASyS with a DM-like rash.”
- Pathology is characterized by:
  - Perifascicular pathology with necrotic myofibers and nonnecrotic fibers with sarcolemmal C5b-9 (MAC) expression
  - Perimysial connective tissue with substantial edema, fragmentation, and mixed mononuclear infiltration
  - MHC1 sarcoplasmic expression and nuclear actin inclusions in myofibers

Immune mediated necrotizing myopathies (IMNM)
- Anti-HMGCR and anti-SRP autoantibodies are considered specific for IMNM.
- Patients with anti-HMGCR antibody and a DM-like rash will be classified as having “anti-HMGCR myopathy with a DM-like rash,” while patients with anti-SRP antibody and a DM-like rash will be classified as having “anti-SRP myopathy with a DM-like rash.”
- Pathology requires diffusely scattered necrotic fibers at different stages of resolution and macrophage dominant, pauci-lymphocytic inflammation.
Sporadic inclusion body myositis (sIBM)
- The pathologic criteria for sIBM include:
  - Endomyosial T cell inflammation with invasion of non-necrotic fibers
  - Rimmed vacuoles
  - P62 or TDP-43 positive protein aggregates or 15-18 nm filamentous (ubulofilamentous inclusions) on electron microscopy
- Anti-cN1A autoantibody is present in 30%–70% of sIBM patients but has also been found in DM and other systemic autoimmune diseases such as Sjögren’s and lupus.
- Clinically, elderly patients with asymmetric muscle weakness and atrophy of proximal and distal muscle groups, with predilection for wrist and finger flexors and knee extensors.
- Usually refractory to immunosuppressive therapies.

Limb girdle muscular dystrophies (LGMD)
- The definition and nomenclature of LGMD have been re-defined in the 2017 ENMC international workshop as a genetically inherited condition that primarily affects skeletal muscle leading to progressive, predominantly proximal muscle weakness at presentation caused by a loss of muscle fibers.
- All LGMD subclasses must fulfill all of the following:
  - Described in at least two unrelated families
  - Patients have achieved independent walking (to proximal muscle weakness at presentation)
  - Elevated serum creatine kinase
  - Degenerative changes on muscle imaging over the course of disease
  - Dystrophic changes on muscle histology, ultimately leading to end-stage pathology
- New nomenclature: change from the alphanumerical system to include the name of the affected protein and mode of inheritance (D for dystrophic changes on muscle histology, R for Rippling muscle disease).
- Some previous LGMD subclasses no longer fulfill the new LGMD definition (Table 3).

Table 2. Old vs new LGMD nomenclature

<table>
<thead>
<tr>
<th>Previous name</th>
<th>Gene</th>
<th>New name</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD 1D</td>
<td>DNAJB6</td>
<td>LGMD D1 DNAJB6-related</td>
</tr>
<tr>
<td>LGMD 1I</td>
<td>CAPN</td>
<td>LGMD D4 Calpain3-related</td>
</tr>
<tr>
<td>LGMD 2A</td>
<td>CAPN</td>
<td>LGMD R1 Calpain3-related</td>
</tr>
<tr>
<td>LGMD 2B</td>
<td>DYSF</td>
<td>LGMD R2 Dysferin-related</td>
</tr>
</tbody>
</table>

Table 3. Previous LGMD subtypes that are no longer considered LGMD

<table>
<thead>
<tr>
<th>Previous name</th>
<th>Gene</th>
<th>New name</th>
</tr>
</thead>
<tbody>
<tr>
<td>LGMD 1A</td>
<td>MYOT</td>
<td>Myofibrillar myopathy</td>
</tr>
<tr>
<td>LGMD 1B</td>
<td>LMNA</td>
<td>Emery–Dreifuss muscular dystrophy</td>
</tr>
<tr>
<td>LGMD 1C</td>
<td>CAV3</td>
<td>Rippling muscle disease</td>
</tr>
<tr>
<td>LGMD 1E and 2R</td>
<td>DÆS</td>
<td>Myofibrillar myopathy</td>
</tr>
<tr>
<td>LGMD 1H</td>
<td>unknown</td>
<td>n/a</td>
</tr>
<tr>
<td>LGMD 2V</td>
<td>GAA</td>
<td>Pompe disease/acid maltase deficiency</td>
</tr>
</tbody>
</table>

Myofibrillar myopathies (MFM)
- MFM is a group of disorders associated with myofibrillar degradation that begins in the Z disk.
- Histologically characterized by sarcoplasmic pleomorphic amorphous, granular or hyaline protein aggregates on Gomori trichrome stain.
- Most contain mutations in Z disk associated protein coding genes: DES, CRYAB, MYOT, ZASP, FLNC, BAG3.
- Patients with mutations in FH1L1, DNAJB6, HSBP8, TTN, ACTA1, PLEC, and LMNA have also been associated with MFM phenotype.

Myotonic dystrophy (DM1/DM2)
- Autosomal dominant multi-system diseases with the common features of myotonia and progressive muscle weakness. There are two main forms: DM1 and DM2.
- DM1 is caused by CTG trinucleotide repeats in the 3‘ untranslated region of DMPK. DM1 shows striking anticipation, with age at onset decreasing by 20–30 years per generation.
- DM1 muscle pathology is characterized by markedly increased internalized nuclei, often in chains and ring fibers, in a background of chronic myopathy.
- DM2 is caused by CCTG repeat expansion in intron 1 of CNBP (ZNF9). Additionally, CLCN1 and SCN4A are disease modifying genes whose mutations may exaggerate DM2 phenotype; they therefore should be included in DM2 genetic screening.
- DM2 muscle pathology is characterized by type 2 atrophy and frequent internalized nuclei predominantly in type 2 fibers.

GENE THERAPIES
Gene replacement therapies for hereditary neuromuscular diseases
- Adeno-associated virus (AAV) based gene delivery vectors can produce replacement proteins in patients with loss of function mutations, such as spinal muscular atrophy (SMA) or Duchenne’s muscular dystrophy. The vector does not integrate into the patient’s genome and has a low immunogenicity.
- CRISPR-Cas9-mediated gene editing does incorporate into the patient’s genome and can permanently replace a deleterious mutation in patient with conditions such as hereditary transthyretin-mediated (hATTR) amyloidosis.
- Both methods entail only a single intravenous injection and thus have a clear advantage over siRNA based and antisense oligonucleotide (ASO) based therapies, which require serial infusions.

FDA approved gene therapy for neuromuscular diseases
- Zolgensma (Novartis) is the first ever FDA approved (2019), intravenously delivered, AAV9 vector mediated SMN gene therapy for spinal muscular atrophy.

New gene therapies currently in clinical trials
- NTLA-2001 is a CRISPR-Cas9-mediated gene editing construct that targets hATTR amyloidosis in a phase II-III trial for adults with polyneuropathy or cardiomyopathy (NCT04601051).
- SRP-9001 (Sarepta), SGT-001 (Solid Biosciences) and PF-06939926 (Pfizer) are AAV based micro-dystrophin constructs in phase III trials for DMD (NCT03375164, NCT03769116, NCT04281485).
- SPK-3006 (Spark Therapeutics) is an AAV based human GAA gene construct in a phase I/II trial for adult onset Pompe disease (NCT04933549).

Meet the Author
Dr. Chunyu Cai has been part of the PathologyOutlines.com editorial board and the Deputy Editor in Chief for Neuropathology since 2020. He is a pathologist and an Associate Professor at University of Texas Southwestern Medical Center. His research focuses on neuromuscular diseases and brain tumors.
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