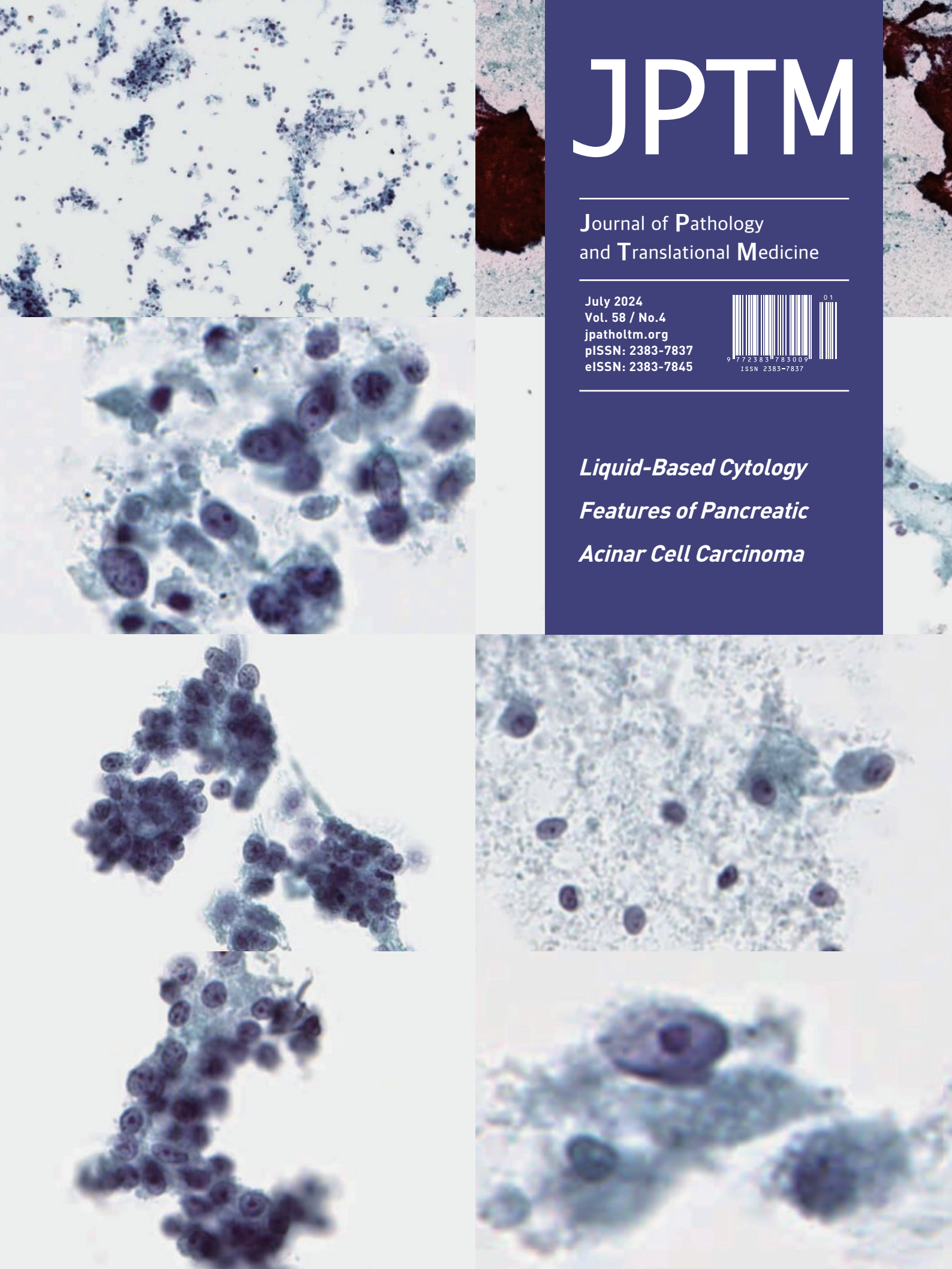




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*Liquid-Based Cytology
Features of Pancreatic
Acinar Cell Carcinoma*

Aims & Scope

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, brief case reports, and letters to the editor.

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Clinical practice recommendations for the use of next-generation sequencing in patients with solid cancer: a joint report from KSMO and KSP

Miso Kim¹, Hyo Sup Shim², Sheehyun Kim³, In Hee Lee⁴, Jihun Kim⁵, Shinkyo Yoon⁶, Hyung-Don Kim⁶, Inkeun Park⁶, Jae Ho Jeong⁶, Changhoon Yoo⁶, Jaekyung Cheon⁶, In-Ho Kim⁷, Jieun Lee⁷, Sook Hee Hong⁷, Sehhoon Park⁸, Hyun Ae Jung⁸, Jin Won Kim⁹, Han Jo Kim¹⁰, Yongjun Cha¹¹, Sun Min Lim¹², Han Sang Kim¹², Choong-Kun Lee¹², Jee Hung Kim¹³, Sang Hoon Chun¹⁴, Jina Yun¹⁵, So Yeon Park¹⁶, Hye Seung Lee¹⁷, Yong Mee Cho⁵, Soo Jeong Nam⁵, Kiyong Na¹⁸, Sun Och Yoon², Ahwon Lee¹⁹, Kee-Taek Jang²⁰, Hongseok Yun³, Sungyoung Lee³, Jee Hyun Kim⁹, Wan-Seop Kim²¹

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In recent years, next-generation sequencing (NGS)-based genetic testing has become crucial in cancer care. While its primary objective is to identify actionable genetic alterations to guide treatment decisions, its scope has broadened to encompass aiding in pathological diagnosis and exploring resistance mechanisms. With the ongoing expansion in NGS application and reliance, a compelling necessity arises for expert consensus on its application in solid cancers. To address this demand, the forthcoming recommendations not only provide pragmatic guidance for the clinical use of NGS but also systematically classify actionable genes based on specific cancer types. Additionally, these recommendations will incorporate expert perspectives on crucial biomarkers, ensuring informed decisions regarding circulating tumor DNA panel testing.

Key Words: Next-generation sequencing; Solid cancer; Precision medicine; Korea

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Over the past few years, next-generation sequencing (NGS)-based genetic testing has emerged as a crucial aspect of cancer patient care, with the number of tests performed rapidly increasing since its reimbursement by the national health insurance in Korea in 2017. However, as the use of NGS-based genetic testing continues to expand, there is an increasing need for maximizing benefits for patients while also considering cost-effectiveness.

The primary objective of NGS-based genetic testing is to identify targetable actionable genes that can guide treatment selection. However, its application has expanded to include diagnosis and exploration of resistance mechanisms, enabling more personalized treatment options. Moreover, biomarkers like homologous recombination deficiency (HRD), microsatellite instability-high (MSI-H)/mismatch repair deficiency (MMR-D), and high tumor mutational burden (TMB-H) have gained increasing significance. Consequently, NGS-based testing is now widely used to analyze these biomarkers and make well-informed treatment decisions.

With the expanding application of NGS-based genetic testing, there is a need for expert consensus on best practices and guidelines for its use. This recommendation aims to (1) provide guidance on the practical application of NGS in daily clinical practice and (2) classify actionable gene lists by cancer type, based on a comprehensive review of the literature and the consensus of experts. Furthermore, the recommendation will present expert opinions, based on existing evidence, regarding biomarkers including HRD, MSI-H/MMR-D, TMB, and circulating tumor DNA (ctDNA) panel testing.

MATERIALS AND METHODS

The Korean Society of Medical Oncology (KSMO) and the Korean Society of Pathologists (KSP) have collaborated to develop

subsequent clinical practice recommendations. These focus on key questions not addressed in the previous guidelines for NGS-based genetic testing and the molecular tumor board from the KSMO and Korean Cancer Study Group (KCSG) Precision Medicine Networking Group [1]. In March and April of 2022, the Steering Committee and Writing Committee were reestablished. They were comprised of medical oncologists, pathologists, and bioinformaticians convened by KSMO, KCSG, and KSP. Two main issues were addressed: the proper recommendations for NGS-based genetic testing in solid cancers, and the classification level determination of genes applicable in Korea. The committees initially conducted a survey to assess the appropriateness of key questions, achieving consensus through feedback from all committee members, to confirm the final selection of key questions. Subsequently, recommendations for these questions were drafted by the Steering Committee and further refined through extensive discussions with all committee members during a comprehensive workshop in September 2022. These modified recommendations were then finalized through a final survey in November 2022. Additionally, the Writing Committee classified actionable genes by cancer type using the Korean Precision Medicine Networking Group (KPMNG) scale for clinical actionability of molecular targets (Table 1). The references for determining the actionability of target genes include case series and clinical trials from all phases (phase I, II, III) published up to August 31, 2023. Studies that were part of basket trials were also considered for inclusion. Furthermore, significant abstracts from clinical trials presented at the American Society of Clinical Oncology Annual Meeting and the European Society for Medical Oncology (ESMO) Congress were incorporated. Subsequently, these gene lists, along with their corresponding references, were shared with disease-specific divisions within KCSG and KSP, where feedback and input from these committees were in-

Table 1. KPMNG scale of clinical actionability of molecular target (K-CAT) [1]

Level	Clinical implication	Required level of evidence
1	Treatment should be considered standard of care	MFDS, FDA, EMA or equivalent-approved drug OR Prospective, randomized, phase III trials showing the benefit of survival endpoints
2	Treatment would be considered	Prospective phase I/II trials show clinical benefit ^a
3	Clinical trials to be discussed with patients	A: Retrospective study or case series show potential clinical benefit in a specific tumor type B: Clinical studies show potential clinical benefit in other indications
4	Preclinical data only, lack of clinical data	Preclinical evidence suggests the potential benefit
G	Suspicious germline variant on tumor tissue NGS	Suggestive actionable germline variant on tumor tissue testing
R	Predictive biomarker of resistance	FDA-recognized predictive biomarker of resistance

KPMNG, Korean Precision Medicine Networking Group; K-CAT, KPMNG scale of Clinical Actionability of molecular Targets; MFDS, Ministry of Food and Drug Safety; FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency; NGS, next-generation sequencing.

^aProspective phase I/II trials supporting level 2 targets include clinical trials across tumor types such as basket trials. In this case, the clinical benefit needs to be judged by expert consensus.

incorporated to further refine the rankings. The lists underwent one final review and confirmation by the entire committee. The finalized recommendations were presented at the 2023 KSMO annual meeting and announced at the 2023 KSP annual meeting. These recommendations have received endorsements from both KSMO and KSP.

KEY QUESTIONS AND RECOMMENDATIONS

Question 1. What are the appropriate recommendations for NGS-based genetic testing in solid cancers?

Recommendation 1. NGS-based genetic testing is recommended for patients with advanced or metastatic solid cancers who are eligible for systemic treatments.

There is mounting evidence that NGS-based matched treatments enhance outcomes in patients with advanced or metastatic cancers [2-6]. Even in tumor types like breast cancer, where the role of NGS has traditionally been less defined, a recent study has shown improved treatment outcomes when patients were matched to appropriate therapies through comprehensive genomic analysis, including NGS [7].

Genomic testing should be conducted in patients with advanced or metastatic solid cancers if there are approved treatments matching genomic biomarkers by a regulatory authority. For instance, several genetic tests, including those for *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *KRAS*, *ERBB2*, and *RET*, should be conducted in patients with non-squamous non-small cell lung cancer (NSCLC). In cases where multiple gene tests are required, NGS can efficiently utilize tumor tissue compared to testing individual genes. The National Comprehensive Cancer Network guideline for NSCLC also recommends panel-based genomic testing by NGS [8]. The use of a multi-gene panel by NGS is also recommended for tumors like ovarian cancer, prostate cancer, and pancreatic cancer. Testing for homologous recombination repair (HRR) related genes is required for these types of cancers to inform the use of poly(ADP-ribose) polymerase (PARP) inhibitors. Even for patients with cancers in which actionable genetic alterations are rarely found, NGS is recommended, taking into account tumor-agnostic biomarkers. MSI-H/MMR-D, TMB-H, *BRAF* V600E, *RET* fusion, and *NTRK* fusions have been approved by the U.S. Food and Drug Administration (FDA) as tumor-agnostic biomarkers [9-20]. In Korea, matched treatments for tumors with MSI-H/MMR-D and *NTRK* fusions have been approved.

If a biomarker-matched treatment showing clinical benefit

has not yet received regulatory approval, we strongly encourage patients to participate in clinical trials based on molecular profiles from NGS. Our goal is to provide maximum treatment options for individual patients with advanced or metastatic cancer. The probability of detecting actionable genetic alterations using NGS varies based on the cancer type [2]. Given that the potential benefits of NGS may vary among individuals, it is essential to discuss its aims and limitations with the patient. Furthermore, NGS is not recommended when systemic treatment is unfeasible due to factors including the patient's performance status, comorbidities, and socioeconomic conditions.

Recommendation 2. NGS-based genetic testing can be recommended for the pathological diagnosis of solid cancers.

Precise pathological diagnosis is a fundamental component of precision oncology and in predicting prognosis for patients with solid cancer. Notably, in the recently published classification of tumors by the World Health Organization (WHO), the diagnosis of tumors defined by genetic alterations is gradually expanding. Consequently, there are increasing cases in which a final pathological diagnosis is made based on NGS results. In addition, OncoKB [21], which is widely referred to in the interpretation of genetic alterations, provides information about diagnosis of hematologic malignancy by classifying the genetic alterations into 'Diagnostic' Level Dx1 (required for diagnosis), Dx2 (supports diagnosis), and Dx3 (investigational diagnosis). It is anticipated that this trend will soon be reflected in the diagnosis of solid cancers. We will briefly discuss the application of NGS in the diagnosis of bone and soft tissue sarcoma, renal cell carcinoma, and central nervous system tumors, using these as representatives.

Bone and soft tissue sarcomas

As more than half of soft tissue tumors and approximately a quarter of bone tumors harbor recurrent genetic alterations [22], molecular analysis is a strong diagnostic tool for the evaluation of bone and soft tissue sarcomas. There are several advantages of using NGS: simultaneous examination of multiple genomic regions, low-level tumor sample requirement and intuitive visualization of results [23]. NGS panels designed for sarcoma diagnosis utilize primers for the detection of fusions, amplifications, deletions and point mutations, which broadly cover genetic alterations in various sarcoma types. In daily practice, pathologists often encounter cases in which NGS provides the precise diagnosis by confirming or excluding differential diagnoses. Some cases can be even diagnosed toward unsuspected entities on the microscopic examination after NGS analysis [24].

NGS analysis may be applied for differential diagnosis of bone and soft tissue sarcomas as follows: (1) low-grade central osteosarcoma (*MDM2*) vs. fibrous dysplasia (*GNAS*); (2) chondroblastic osteosarcoma (chromosomal instability) vs. chondrosarcoma (*IDH1/2*); (3) malignant peripheral nerve sheath tumor (*CDKN2A*) vs. atypical neurofibroma; (4) liposarcoma (*MDM2*) vs. atypical pleomorphic lipomatous tumor (RB1); (5) alveolar rhabdomyosarcoma (*PAX3/7::FOXO1*) vs. embryonal rhabdomyosarcoma (mutations in *RAS-MAPK* pathway); (6) tumors of uncertain differentiation (Ewing sarcoma, round cell sarcoma with *EWSR1*-non-ETS fusions, *CIC*-rearranged sarcoma, sarcoma with *BCOR* genetic alterations, synovial sarcoma, alveolar soft part sarcoma, extraskeletal myxoid chondrosarcoma, clear cell sarcoma of soft tissue, etc.)

Renal cell carcinoma

NGS-based genetic panel test can be recommended for the pathological diagnosis of molecularly defined renal cell carcinoma (RCC), which includes fumarate hydratase (*FH*)-deficient RCC, succinate dehydrogenase (*SDH*)-deficient RCC, *TFE3*-rearranged RCC, *TFEB*-rearranged or *TFEB*-amplified RCC, *ELOC* (formerly *TCEB1*)-mutated RCC, *SMARCB1* (INI1)-deficient RCC, and *ALK*-rearranged RCC according to the recent 2022 WHO classification [25]. The molecular alterations of these renal tumors are as follows: biallelic *FH* mutation/inactivation in *FH*-deficient RCC; inactivating mutations of one of *SDH* genes, most commonly *SDHB*, followed by *SDHA* and *SDHC*, and rarely *SDHD* in *SDH*-deficient RCC; translocations involving *TFE3* in *TFE3*-rearranged RCC; translocations involving *TFEB* in *TFEB*-rearranged RCC; *TFEB* amplification in *TFEB*-amplified RCC; inactivating mutations exclusively at *TCEB1* Y79 in *ELOC* (formerly *TCEB1*)-mutated RCC; translocations or deletions involving 22q11.23 in *SMARCB1* (INI1)-deficient RCC; translocations involving *ALK* in *ALK*-rearranged RCC. In addition, NGS-based genetic panel test may also be recommended for morphologically defined renal tumors with characteristic molecular alteration. Clear cell RCC is characterized by the loss of chromosome 3p accompanied by the inactivation mutation or methylation of the remaining *VHL* gene. Papillary RCC commonly shows gains of chromosomes 7 and 17, and loss of the Y chromosome with *MET* alterations in the low-grade tumor. Chromophobe RCC has losses of multiple chromosomes including 1, 2, 6, 10, 13, 17, 21, and Y. Eosinophilic solid and cystic RCC can show *TSC* gene mutations or biallelic losses.

Central nervous system tumor

With the development of research techniques such as NGS, our understanding of the molecular and clinicopathological characteristics of brain tumors has advanced greatly. Based on these changes, following the 2016 Central Nervous System (CNS) WHO classification revised 4th edition [26] and cIMPACT-NOW [27], the 2021 CNS WHO classification 5th edition [28] fully included the molecular genetic characteristics of tumors in the WHO classification of brain tumors. In the 2021 CNS WHO classification, several molecular genetic characteristics such as gliomas, glioneuronal tumors, ependymomas, embryonic tumors (medulloblastoma, etc.), and meningiomas were introduced into the diagnostic criteria. Molecular genetic characteristics included in the diagnostic criteria range from those that can be identified with a single test (sequencing, fluorescence in situ hybridization, etc.) to those that require integrated identification of various genes involved in a specific pathway, as well as those that identify chromosomal arm-level copy number alterations. To cover all of these, NGS testing is essential. In addition, these molecular classifications determine the diagnosis of the tumor and further determine the WHO grade, which is a basic brain tumor grading system that determines the treatment strategy. The use of traditional histopathological morphological classification alone without NGS testing can mislead patients' treatment strategies.

Recommendation 3. NGS-based genetic testing can be repeated in patients with solid cancer in case of disease recurrence or development of drug resistance.

Acquired resistance inevitably occurs with the growing use of targeted agents targeting various driver oncogenes. Representatively, we have seen the successful development of osimertinib, the third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) during the last decade [29]. At the time of drug development, osimertinib was developed for the patients who revealed the acquired *EGFR* threonine to methionine at codon 790 (T790M) mutation at the time of treatment failure with first- or second-generation EGFR TKI [30]. Therefore, the detection of *EGFR* T790M has been crucial for making treatment decisions in patients who experienced treatment failure with first- or second-generation EGFR TKIs [8]. Apart from *EGFR* T790M, other types of acquired resistance mechanisms were revealed by NGS, such as *ERBB2* amplification or *MET* amplification [31]. Given the recent memorial imprint of resistance mechanism discovery, we have started using repeated NGS to detect acquired resistance in on-treatment tumor tissue, as well as in liquid biopsy samples.

Generally, acquired resistance can be classified into two categories: (1) target-dependent, such as target gene mutations, and (2) target-independent, such as gene aberrations in bypass pathways [32]. Beyond the *EGFR* T790M mutation, the *EGFR* C797S mutation is one of the most common *EGFR*-dependent resistance mechanisms against osimertinib [33]. *MET* amplification is another type of bypass pathway resistance mechanism across oncogene-driven subsets of NSCLC [34]. The *EML4::ALK* fusion, occurring in 3%–7% of all NSCLC cases, is currently treated with alectinib or brigatinib, the second-generation ALK TKIs, which are the standard treatments for treatment-naïve ALK-positive NSCLC patients [35–37]. *ALK* G1202R, solvent front mutation affecting drug binding to active site, is the most common target-dependent mutation [38]. Detecting the *ALK* G1202R mutation through NGS enables the prediction of a notable response with subsequent lorlatinib. *NTRK* fusion is a tumor agonistic driver oncogene, detected in less than 1% of solid cancers. With introduction of larotrectinib and entrectinib in clinic, several target-dependent point mutations were noted, which can be found by NGS [19,20]. Repotrectinib (TPX-0005) has demonstrated anti-tumor efficacy in patients previously treated with NTRK-targeting TKIs and who harbor target-dependent *TRK* mutations [39].

Since the 2000s, the clinical use of NGS has expanded beyond the detection of driver oncogenes. It has paved the way for the discovery of novel targets associated with acquired resistance and provided valuable insights into potential targets for the next generation of targeted therapeutics. However, it's important to acknowledge certain limitations associated with the repetition of NGS testing. Challenges include the increased cost, difficulties in obtaining repeated tumor biopsies, and associated risks. Additionally, the likelihood of identifying actionable targets at the point of resistance can vary depending on the specific cancer type and drugs, with potential restrictions in drug availability. Nonetheless, it remains evident that NGS can play a crucial role in helping inform subsequent treatment decisions for certain patients who have experienced treatment failure with targeted therapy.

Question 2. How can we determine the classification level of genes applicable in Korea?

Advancements in NGS technologies have facilitated the identification of driver mutations in cancer, prompting a shift from a histology-based to a molecular-based approach in cancer treatment. Simultaneously, the advent of targeted therapies has allowed for treatments based on genetic alterations irrespective of

the tumor's origin. This concept, known as tissue-agnostic indication, has demonstrated promising results in recent studies and has become a crucial element in the standard care for cancer. Currently, the tissue-agnostic indications approved by the FDA are listed in Table 2 [9–20,40].

Taking into account both the evidence level of clinical research and clinical benefit, the committee members classified actionable genes for each type of cancer based on their level using KPMNG scale of Clinical Actionability of molecular Targets (K-CAT). We also included certain genes, such as *POLE* in endometrial cancer, that are clinically significant and thus necessitate testing. The actionable gene lists for NSCLC, breast cancer, esophageal cancer, stomach cancer, colorectal cancer, head and neck cancer, pancreatic cancer, biliary tract cancer, endometrial cancer, urothelial cancer, and kidney cancer are provided in Tables 3–17 [11–15,29,36,37,41–190]. Each table included genes corresponding to levels 1 through 3A.

Additional topics

Homologous recombination deficiency

Genomic instability is one of the most frequent underlying features of carcinogenesis, and defective DNA repair has been described as a cancer hallmark [191]. HRR is a series of interrelated pathways that function in the repair of DNA double-strand breaks and interstrand crosslinks [192]. Important genes involved in the HRR process include *BRCA1*, *BRCA2*, *RAD51*, *RAD51C*, *RAD51D*, *ATM*, *ATR*, *PALB2*, *MRE11*, *NBS1*, *BARD1*, *CHEK1*, and *CHEK2* [193,194]. However, it is essential to note that the list of genes known to be related to the HRR process is continually evolving through ongoing research. A defect in the HRR pathway has been linked to several cancers, including breast, ovarian, prostate and pancreatic cancer [117,142,153,195], and

Table 2. List of genetic alterations with tumor agnostic indications by FDA

Gene/Alteration	Matched treatment	K-CAT	Reference
<i>NTRK</i> fusion	Entrectinib Larotrectinib	1	[19,20]
<i>BRAF</i> V600E	Dabrafenib+trametinib (except colorectal cancer)	1	[11–17]
<i>RET</i> fusion	Selpercatinib	1	[18]
Microsatellite instability– high/Mismatch repair deficiency	Pembrolizumab	1	[9,40]
High tumor mutation burden	Pembrolizumab	1	[10]

FDA, U.S. Food and Drug Administration; K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

Table 3. List of genomic alterations level 1/2/3A according to K-CAT in advanced NSCLC

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>EGFR</i>	Exon 19 in-frame deletions, L858R, G719X, L861Q, S761I	30–46	1	[41–45]
	T790M	50 of treated <i>EGFR</i> mutant NSCLC	1, R	[29,46,47]
	Exon 20 in-frame insertion	3	1	[48,49]
<i>BRAF</i>	V600E	2–4	1	[12,13,50]
<i>ALK</i>	Rearrangement/Fusions	3–5	1	[36,37,51,52]
<i>KRAS</i>	G12C	13	1	[53,54]
<i>MET</i>	Exon 14 in-frame deletions, Exon 14 splice mutations	3–4	1	[55,56]
	Amplification	3–5	2	[56]
<i>RET</i>	Rearrangement/Fusions	1.7	1	[57,58]
<i>ROS1</i>	Rearrangement/Fusions	2.6	1	[59,60]
<i>ERBB2</i>	Exon 20 in-frame insertion	2.3	1	[61–64]
	Amplification	2.4–38	2	[65,66]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets; NSCLC, non-small cell lung cancer.

HRD can make tumors more sensitive to platinum-based chemotherapy and PARP inhibitors [196,197]. Thus, it is critical to develop methods for determining the HRD status in order to maximize clinical benefit from these drugs.

There are three main categories of available tests for HRD analyzing (1) the etiology of HRD (mutation/methylation sequencing), (2) the current homologous recombination status (functional assays), and (3) prior HRD exposure (genomic scars). Each type of cancer (ovarian, breast, pancreatic and prostate) requires different tests. The germline *BRCA* 1/2 mutation test is useful for predicting response to PARP inhibitors in ovarian and breast cancer [76,143–146,198]. In ovarian cancer, tumor (incorporating germline and somatic) as well as somatic *BRCA* 1/2 mutation testing exhibit good clinical validity by reliably identifying the subset of patients who benefit from PARP inhibitor therapy [146–148]. Evidence regarding the benefit of mutation tests for each non-*BRCA* HRR gene for predicting responses to PARP inhibitors remains insufficient in ovarian cancer. HRD tests using genomic instability scores (GIS) or loss of heterozygosity (LOH) scores are useful for predicting the responses to PARP inhibitors in ovarian cancer patients without *BRCA* 1/2 mutation [142,144,146]. The GIS from myChoice CDx (Myriad Genetics) represents the sum of LOH, large-scale transitions, and telomeric allelic imbalance and a GIS of 42 has been established as the threshold to determine HRD positivity [199,200]. To date, GIS is the only genomic scar assay that has been evaluated in first-line randomized controlled trials for ovarian cancer

Table 4. List of genomic alterations level 1/2/3A according to K-CAT in advanced breast cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>ERBB2</i>	Amplifications	15–20	1	[67–71]
	Oncogenic mutations	4	2	[72,73]
<i>PIK3CA</i> ^a	Oncogenic mutations	30–40	1	[74,75]
<i>BRCA1/2</i>	Germline oncogenic mutations	4	1	[76,77]
<i>BRCA1/2</i> ^b	Somatic oncogenic mutations ^c	3	2	[78–80]
<i>PTEN</i>	Oncogenic mutations	7	2	[81,82]
<i>ESR1</i>	Oncogenic mutations	10	R	[83]
	(mechanism of resistance)			
<i>AKT1</i>	E17K	5	2	[82,84]
<i>PALB2</i> ^d	Germline oncogenic mutations	0.5–1	2	[79,85]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets; PARP, poly(adenosine diphosphate [ADP]-ribose) polymerase; HRD, homologous recombination deficiency.

^aThis applies only to breast cancer that is hormone receptor-positive/HER2-negative and has mutations including E542K, E545A, H1047R, H1047Y, Q546E, H1047L, Q546R, E545G, E545D, E545K, C420R. Other oncogenic mutations not included in this category, caution is needed, since it is unknown whether other mutations are associated with response to phosphoinositide 3-kinase inhibitor therapy; ^bPhase III trials of PARP inhibitors have been conducted in patients with germline *BRCA* mutations, and their therapeutic effects have been confirmed. In some studies, the effects of PARP inhibitors have also been reported in patients with somatic *BRCA* mutations, and somatic tumor sequencing can identify many germline *BRCA* mutations; ^cIn addition to *BRCA* 1/2, there are several other genes associated with homologous recombination deficiency, including *ATRX*, *BLM*, *BRIP1*, *CHEK2*, *FANCA/C/D2/E/F/G/L*, *MRE11A*, *NBN*, *PALB2*, and *RAD50*. Although the discovery frequency of each gene is very low, they are collectively found in approximately 8% of all breast cancers; ^dThere are multiple germline mutations associated with HRD in breast cancer patients, but this table only includes the two most frequent ones.

[142,143]. The LOH test (FoundationOne CDx, Foundation Medicine) uses NGS to determine the percentage of genomic LOH and LOH-high is defined with a cut-off of 16% or higher, referencing The Cancer Genome Atlas data [201]. In metastatic pancreatic cancer, a germline *BRCA* 1/2 mutation test is recommended to evaluate the potential benefits of PARP inhibitors as maintenance treatment for patients whose tumors have not progressed after first-line platinum-based chemotherapy [117]. In castration-resistant prostate cancer, it is recommended to assess by sequencing for *BRCA* 1/2 mutations, at a minimum, using germline and/or somatic tumor DNA [153,202]. To date, insufficient evidence is available regarding the benefit of performing a HRD functional assays to predict response to PARP inhibitor; however, the potential for using functional assays in conjunction with HRR gene tests and genomic tests should be evaluated. While there have been multiple NGS assays to evaluate HRD status, only a limited number of tests are clinically accepted, and

Table 5. List of genomic alterations level 1/2/3A according to K-CAT in advanced esophageal cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>ERBB2</i>	Amplification	3.9–10	2	[86]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

Table 6. List of genomic alterations level 1/2/3A according to K-CAT in advanced stomach cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>ERBB2</i>	Amplification	15	1	[87-89]
<i>FGFR2</i> ^a	Amplification	5	2	[90]
<i>MET</i>	Amplification	2–5	2	[91]
<i>EGFR</i>	Amplification	5–10	3A	[92]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets; ctDNA, circulating tumor DNA.

^aFGFR2b overexpression or *FGFR2* amplification by ctDNA analysis.

Table 7. List of genomic alterations level 1/2/3A according to K-CAT in advanced colorectal cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>KRAS</i>	Oncogenic mutations	40	R	[93,94]
<i>NRAS</i>	Oncogenic mutations	3–5	R	[95,96]
<i>BRAF</i>	V600E	5–10	1	[96-98]
Mismatch repair deficiency	MSI-H/MMR-D	4–5	1	[99,100]
<i>ERBB2</i>	Amplification	4–5	1	[101]
<i>KRAS</i>	G12C	3	2	[102,103]
<i>POLE</i>	Exonuclease domain mutations	1–3	2	[104-106]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets; MSI-H, microsatellite instability-high; MMR-D, mismatch repair deficiency.

their technical details including evaluation criteria are unclear. Many methodological approaches have been proposed to measure HRD status using NGS data of various types, including whole genome sequencing (WGS), whole exome sequencing (WES) and targeted sequencing [203,204]. However, the absence of congruent measure remains a challenge to validate their reliability and consistency. Although WGS has not yet been approved for the diagnosis of HRD, it might become a promising diagnostic tool for HRD in the near future.

Microsatellite instability-high/mismatch repair deficiency

MSI-H/MMR-D has become an important biomarker of eligibility for immune checkpoint inhibitor (ICI) therapy as the FDA has approved ICIs for patients with unresectable or metastatic MSI-H/MMR-D solid cancers regardless of tumor types [9,40,205]. The polymerase chain reaction (PCR)-based assessment of selected microsatellite loci in a patient's tumor and

Table 8. List of genomic alterations level 1/2/3A according to K-CAT in advanced head and neck cancer

Gene	Alteration	Prevalence (%) ^a	K-CAT	Reference
<i>NOTCH1</i> , 2, 3	Oncogenic mutations	10–12	2	[107,108]
<i>ERBB2</i>	Amplification	30–40	2	[109-111]
<i>FGFR1</i> , 3	Amplification/ Oncogenic mutations	1–7	2	[112-114]
<i>MET</i>	Amplification	1	3A	[115,116]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

^aThe above prevalence is about the representative subtype among various subtypes of head and neck cancer.

Table 9. List of genomic alterations level 1/2/3A according to K-CAT in advanced pancreatic cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>BRCA 1/2</i>	Germline oncogenic mutations	1–4	1	[117,118]
<i>PALB2</i>	Oncogenic mutations	0.6	2	[118]
<i>KRAS</i>	G12C	2–3	2	[119,120]
<i>PIK3CA</i>	Oncogenic mutations	3	3A	[121]
<i>ERBB2</i>	Amplifications/ Oncogenic mutations	1–2	3A	[72,122]
<i>ALK</i>	Rearrangement/ Fusions	<1	3A	[123]
<i>NRG1</i>	Rearrangement/ Fusions	1	3A	[124]
<i>ROS1</i>	Rearrangement/ Fusions	<1	3A	[125]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

Table 10. List of genomic alterations level 1/2/3A according to K-CAT in advanced biliary tract cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>IDH1</i>	Oncogenic mutations	10–23	1	[126,127]
<i>FGFR2</i>	Rearrangement/Fusions	8–14	1	[128-130]
<i>BRAF</i>	V600E	5	1	[14,15]
<i>ERBB2</i>	Amplification	10	2	[131-133]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

matched non-neoplastic tissue had been accepted as the gold standard method before the era of NGS. Nevertheless, the PCR-based MSI test can be misleading in certain cases because the selected microsatellite loci (typically, 5 to 8 loci) may not cover all affected microsatellite regions [206]. Alternatively, MMR-D can be inferred through immunohistochemistry (IHC) of MMR proteins, such as MLH1, MSH2, MSH6, and PMS2, since most MMR-deficient tumors exhibit a loss of MMR protein expression. However, there are limitations to detecting MMR-D by the IHC method. Certain tumors harboring pathogenic missense or

Table 11. List of genomic alterations level 1/2/3A according to K-CAT in advanced endometrial cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>ERBB2</i>	Amplification	30 of uterine serous carcinoma	2	[134]
<i>AKT1</i>	E17K	2	2	[84]
<i>POLE</i> ^a	Oncogenic mutations	5–15	NA	[135,136]
<i>TP53</i> ^{ab}	Oncogenic mutations	5–15	NA	[135]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets; NGS, next-generation sequencing; IHC, immunohistochemistry; TCGA, The Cancer Genome Atlas; MMR, mismatch repair.

^aAdjuvant treatment of endometrial cancer based on molecular classification; ^bConsidering the coverage limitations of NGS for detecting p53 loss, a combined IHC approach is recommended. The TCGA approach results in the molecular stratification of endometrial cancer (EC) into four distinct molecular groups [137]: (1) ultramutated (> 100 mut/Mb) with pathogenic variations in the exonuclease domain of DNA polymerase epsilon (*POLE*)-ultramutated (*POLEmut*), (2) hypermutated (10–100 mut/Mb), microsatellite-unstable, (3) somatic copy number-high with frequent pathogenic variants in *TP53*, and (4) an MMR-proficient, low somatic copy number aberration subgroup with a low mutational burden. Extensive research on these surrogate markers has revealed a strong correlation with clinical outcome, thus proving their prognostic value [138–140]. *POLEmut* EC had generally has an excellent clinical outcome, while p53-abn EC has the worst, regardless of risk category, type of adjuvant treatment, tumor type, or grade. Adjuvant chemotherapy is beneficial in for patients with p53mut EC, while treatment de-escalation is being explored in patients with *POLEmut* EC [139], which exhibits a favorable outcome [141]. Consequently, all EC pathology specimens should undergo molecular classification, independent of histological type, using well-established IHC staining for p53 and MMR proteins (MLH1, PMS2, MSH2, MSH6), in conjunction with targeted tumor sequencing (*POLE* hotspot analysis). While *POLE* hotspot analysis is currently unavailable in Korea, and most NGS panels include the *POLE* gene, it has been incorporated into the recommendations. Moreover, since IHC plays a well-established role in identifying p53 mutations and NGS target sequencing of *TP53* is insufficient to identify all loss of P53 function, IHC confirmation of p53 is recommended over NGS testing as a priority.

Table 12. List of genomic alterations level 1/2/3A according to K-CAT in advanced ovarian cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>BRCA</i> 1/2	Oncogenic mutations	5–15	1	[142–149]
HRD score	GIS, LOH	50	1	[142–144, 146,148]
<i>AKT1</i>	E17K	2	2	[84]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets; HRD, homologous recombination deficiency; GIS, genomic instability scores; LOH, loss of heterozygosity.

in-frame insertion/deletion mutations of MMR genes may still show intact MMR protein expressions, and interpretation errors may occur when the staining quality is poor.

Since NGS is now widely used in clinical practice, it has been investigated whether NGS can be used to detect MSI-H/MMR-D in clinical setting. Numerous validation studies have demonstrated that NGS can accurately detect pathogenic or likely

Table 13. List of genomic alterations level 1/2/3A according to K-CAT in advanced urothelial cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>FGFR3</i>	Oncogenic mutations	13–15	1	[150]
	Rearrangement/Fusions			
<i>FGFR2</i>	Rearrangement/Fusions	Unknown	1	[150]
<i>ERCC2</i>	Oncogenic mutations	9–12	3A	[151,152]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

Table 14. List of genomic alterations level 1/2/3A according to K-CAT in advanced prostate cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>BRCA2</i>	Germline and/or somatic oncogenic mutations	3–13	1	[153,154]
<i>BRCA1</i>	Germline and/or somatic oncogenic mutations	1	1	[153,154]
<i>ATM</i>	Oncogenic mutations	6–7	1	[153,154]
<i>BRIP1, BARD1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D, RAD54L</i>	Oncogenic mutations	<1–5	1	[153,154]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

Table 15. List of genomic alterations level 1/2/3A according to K-CAT in advanced kidney cancer

Gene	Alteration	Prevalence (%)	K-CAT	Reference
<i>VHL</i>	Germline oncogenic mutations	0.2	1	[155]
<i>FH</i>	Germline oncogenic mutations	0.5	3A	[156,157]
<i>ALK</i>	Rearrangement/Fusions	0.3–0.5	3A	[158]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

pathogenic mutations affecting MMR genes and can determine MMR-D reliably. Thus, there is a consensus that NGS can replace the standard PCR-based MSI test. NGS can detect MSI-H/MMR-D in various ways [207]. Several computational tools for detection of MSI-H/MMR-D using NGS data are available: mSINGS [208], MSIsensor [209], MANTIS [210], and MOSA-IC [211]. Furthermore, NGS can detect MSI-H/MMR-D even in the absence of the patient's matched normal tissue [212,213]. Furthermore, pathogenic or likely pathogenic MMR gene mutations detected by NGS testing may select candidates of germline

genetic testing for Lynch syndrome. Finally, NGS-based MSI-H/MMR-D testing may provide information about eligibility for immunotherapy in tumor types where MMR IHC and/or PCR-based MSI tests have not been done during routine clinical practice.

Analysis of TMB by NGS panel

ICIs can enhance a durable anti-tumor immune response and prolong overall survival [214]. However, only a subset of the patients showed a dramatic response to immunotherapy, and the identification of predictive biomarkers was essential to identify responders to immunotherapy, such as programmed death-ligand 1 expression, MSI-H/MMR-D and TMB-H [215-217]. TMB is defined as the number of somatic mutations (mut) per megabase (Mb) of genomic sequence [217]. TMB is a surrogate marker for making immunogenic neopeptides shown on the surface of tumor cells by major histocompatibility complexes, which affect the anti-tumor immune response to ICIs [218,219].

In June 2020, the FDA authorized pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic TMB-H (≥ 10 mut/Mb) solid tumors, as determined

by FoundationOneCDx assay, that have progressed following prior treatment and who have no satisfactory alternative treatment options [220]. Therefore, determining the TMB value and identifying TMB-H tumors are among the most critical aspects in the clinical NGS analysis.

Although the TMB calculation can vary according to the test assays, the gold standard method for TMB estimation is WES with tumor tissues and matched normal samples. However, since WES has limitations in terms of time and costs to apply in clinical use, analytic methods and algorithms have been developed for calculating TMB from clinical targeted NGS panel tests [221,222]. Targeted NGS panel tests usually cover only a small limited size (about 1 to 2 Mb) of exonic regions, so sophisticated bioinformatic algorithms and statistical methods must be applied to filter out noise variants and artifacts caused by formalin-fixed tissues. For tumor-only sequencing, which is currently conducted in most targeted gene panels in Korea, germline variants are filtered out using genomic information from public databases or data on allele frequency in normal populations to avoid TMB overestimation. In several studies, the evaluated TMB from targeted NGS panel testing showed a high correlation with the TMB calculated by WES using analytic techniques [221,222].

Since the targeted gene panels currently used in the clinic have different analysis pipelines for variant calling and apply various filtering criteria to select variants used in TMB calculation, TMB values vary among the tests, and the criteria for TMB-H are diverse [223]. Also, the distribution of TMB values and criteria for TMB-H are different by tumor type, even when calculating TMB with the same panel. In general, more than TMB of 10 mut/Mb has been used for the definition of TMB-H tumors, but the reliable value of TMB-H can be different among the test

Table 16. List of genomic alterations level 1/2/3A according to K-CAT in advanced melanoma

Gene	Alteration	Prevalence (%)	K-CAT	Reference
BRAF	V600E/K	35–50	1	[11,159-162]
	V600 (excluding V600E/K)	~5	1	[163]
KIT	D579del and 12 other oncogenic mutations	1–7	2	[164,165]
NRAS	Oncogenic mutations	~20	2	[166,167]
BRAF	Rearrangement/Fusions	3–7	3A	[168,169]
	K601, L597	< 1	3A	[170-173]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets.

Table 17. List of genomic alterations level 1/2/3A according to K-CAT in advanced sarcoma

Gene	Alteration	Prevalence (%)	K-CAT	Reference
KIT	Oncogenic mutations	~75–80 in GIST	1	[174,175]
PDGFRA	Oncogenic mutations	~8–10 in GIST	1	[175-177]
PDGFB	Rearrangement/Fusions mostly COL1A1::PDGFB	~90 in DFSP	1	[178,179]
ALK	Rearrangement/Fusions	~50 in IMT	1	[180-182]
SMARCB1	Deletion	~83 in ES	2	[183]
IDH1	Oncogenic mutations	~65 in chondrosarcoma	2	[184]
TSC2	Oncogenic mutations	~30 in PEComa	2	[185,186]
MDM2	Amplification	~90 in WDLPS/DDLPS; frequent in IS, low grade OSA	2	[187,188]
CDK4	Amplification	~90 in WDLPS/DDLPS; frequent in IS, low grade OSA	2	[187,189]
MET	Oncogenic mutations, Rearrangement/Fusions, Amplification	< 1%	2	[190]

K-CAT, Korean Precision Medicine Networking Group scale of Clinical Actionability of molecular Targets; GIST, gastrointestinal stromal tumor; DFSP, dermatofibrosarcoma protuberans; ES, epithelioid sarcoma; IMT, inflammatory myofibroblastic tumor; WDLPS/DDLPS, well-differentiated/de-differentiated liposarcoma; IS, intimal sarcoma; OSA, osteosarcoma.

panels and requires caution in interpreting the estimated TMB value. In some studies, the TMB of 17–20 mut/Mb is considered TMB-H and a candidate for immunotherapy conservatively [224]. Therefore, standardization of TMB analysis among test panels, validation of TMB-H tumors with different assays, and establishing reliable criteria for TMB-H will be needed for the further precise application of TMB analysis with the clinical tumor NGS panels.

Clinical utility and limitations of ctDNA-based genetic panel tests using blood sample

As the growing number of druggable oncogenic drivers has been identified in solid cancer [225], ctDNA-based approach can be used as an alternative approach for facilitating the identification of tumor tissue genotype. However, ctDNA can be influenced by multiple preanalytical factors and the methodology of analysis [226]. Since the ctDNA detection rate is highly related to tumor burden and is affected by various factors such as plasma levels of ctDNA, assay sensitivity, and tumor biology, a negative result from the ctDNA test may not necessarily indicate a true negative. In particular, low analytical sensitivity may occur because ctDNA assay are performed solely on DNA derived from tumor cells [227]. Recent studies have reported that gene fusions and splice variants have higher detection rates when sequencing is performed with RNA transcripts [228,229]. In addition, in the case of copy number variations (CNVs), determining the presence of CNVs remains challenging due to its dependence on ctDNA fractions [230,231]. Hence, ctDNA-based test reports should include essential elements, including pre-analytical elements, sequencing results, potential factors related to the germline variants, and limitations of assays to assist the interpretation of the report to the clinician [232].

ctDNA-based genotyping can be used as either complementary to tissue genotyping or as the first choice in certain circumstances. ctDNA-based genotyping has advantages over tissue-based genotyping in a short turnaround time, invasiveness, and feasibility in serial assessment [233–235]. Due to the limitation of tissue-based genotyping, which can be affected by tissue accessibility or tumor purity, ctDNA-based genotyping can be conducted as initial genotyping in the rapidly growing aggressive tumor when challenges or delays in sample acquisition are anticipated. In addition, the ctDNA-based genotyping first approach can be preferred for the evaluation of emerged resistance mechanism [236]. ctDNA-based genotyping can also be used as a complementary method, either concurrently or sequentially with tissue-based genotyping in case of incomplete tumor geno-

typing or foreseen inadequate results due to uncertain adequacy of tissue [237].

Before genotyping ctDNA sequences, the concentration of cell-free DNA in plasma can be used as a prognostic biomarker [238,239]. The sensitivity of ctDNA assay varies among the primary sites and tumor types and should be considered at applying ctDNA test in clinical use [240]. Similarly, the metastatic site of the tumor affects the ctDNA detection and should be taken into account for using ctDNA assay [241]. Additionally, MSI-H/MMR-D and TMB-H, as determined by ctDNA assay, have been widely studied [242–244]. Improving the accuracy of the MSI detection and TMB calculation from ctDNA and defining reliable criteria for MSI-H/MMR-D and TMB-H in the ctDNA assay is anticipated to broaden the use of ctDNA tests.

CONCLUSION

NGS-based genetic testing has become an essential tool in treating patients with advanced solid cancers. This report provides clinical recommendations for the application of NGS in such patients, offering expert opinions on its diagnostic uses, and gene classification in accordance with K-CAT, while taking the domestic Korean context into consideration.

As cancer genomics advances and new therapies emerge, the current gene classification is subject to dynamic changes, and the application of NGS is anticipated to continuously evolve. Consequently, healthcare providers and researchers are encouraged to stay abreast of the latest advancements in the field of precision oncology to ensure optimal patient care and further cancer research.

Ethics Statement

Not applicable.

Availability of Data and Material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability

Not applicable.

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Conceptualization: JHK, WSK, MK. Data curation: all authors. Writing—original draft: all authors. Writing—review & editing: all authors. Approval of final manuscript: all authors.

Conflicts of Interest

S.Y.P., the editor-in-chief and H.S.L. and S.O.Y., contributing editors of the *Journal of Pathology and Translational Medicine*, were 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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Welcoming the new, revisiting the old: a brief glance at cytopathology reporting systems for lung, pancreas, and thyroid

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This review addresses new reporting systems for lung and pancreatobiliary cytopathology as well as the most recent edition of The Bethesda Reporting System for Thyroid Cytopathology. The review spans past, present, and future aspects within the context of the intricate interplay between traditional morphological assessments and cutting-edge molecular diagnostics. For lung and pancreas, the authors discuss the evolution of reporting systems, emphasizing the bridge between past directives and more recent collaborative efforts of the International Academy of Cytology and the World Health Organization in shaping universal reporting systems. The review offers a brief overview of the structure of these novel systems, highlighting their strengths and pinpointing areas that require further refinement. For thyroid, the authors primarily focus on the third edition of The Bethesda System for Reporting Thyroid Cytopathology, also considering the two preceding editions. This review serves as an invaluable resource for cytopathologists, offering a panoramic view of the evolving landscape of cytopathology reporting and pointing out the integrative role of the cytopathologist in an era of rapid diagnostic and therapeutic advancements.

Key Words: Thyroid gland; Lung; Pancreas; Cytology; Review literature as topic

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Cytopathology has entered an exciting phase highly influenced by rapid advancements in molecular technologies. These developments have elevated the role of cytology in molecular diagnostics, enabling targeted therapies and personalized medicine. With these innovations comes the imperative for global standardization of organ-based reporting systems to ensure seamless integration of fast-paced developments into a cohesive framework that supports evolution of the field. These systems draw from insights and data documentation of previous reporting systems such as the Papanicolaou Society of Cytopathology (PSC) System [1] and the Japan Lung Cancer Society/Japanese Society of Clinical Cytology system [2], which are unified under the

foundational World Health Organization (WHO) pathology guidelines, tailored for worldwide application. This integration streamlines the practice of cytopathology, ensuring consistency and clarity across the discipline.

The International Academy of Cytology, in collaboration with the International Agency for Research on Cancer (IARC) and the WHO, has released in 2022 the inaugural editions of the WHO reporting systems for lung and pancreatobiliary cytopathology. These pioneering resources are accessible at the WHO website and in print [3,4].

The introductory sections of the WHO lung and pancreatobiliary reporting systems outline the essential elements of a cy-

topathology report and the role of cytopathology in a diagnostic workup. These sections also define the reporting categories, indicate the risk of malignancy (ROM) associated with each category based on available literature, and provide recommendations for additional diagnostic procedures.

Following the introduction, there is a detailed chapter in each guidance document on optimal sampling techniques for lung, pancreatic, and biliary tissue. Protocols to obtain lung samples include sputum collection, bronchial washings and brushes, bronchoalveolar lavage, and fine-needle aspiration (FNA) as guided by imaging, while those to obtain pancreatic and biliary samples include percutaneous or endoscopic ultrasound-guided FNA and biliary brushings. This section also discusses specimen triage and preparation, as well as the utility of ancillary tests.

Subsequent sections delve into individual diagnostic categories, providing definitions, context, suggested ROMs, and management recommendations, which are key elements of a solid reporting system. These sections also provide sample reports to promote standardized documentation, along with guidance to align with local practices and system constraints.

The WHO Cytopathology Blue Books also discuss differential diagnoses for a given pattern of cytopathological features and current best practices for ancillary testing to help ensure the systems are applicable globally, including in low- and middle-income countries where access to additional testing may be limited.

To increase consistency across the field of cytopathology, the third edition of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has been released in 2023. This recent update builds upon the 2007 and 2017 editions and upon a robust compendium of research and experience garnered through clinical application. While operating independently from the World Health Organization's reporting systems, the 2023 edition of the TBSRTC shared a similar objective to refine and harmonize cytopathological practices on an international scale.

In this review, our goal was to present a concise guide to the essential aspects of these reporting systems in practice. We highlight the most substantial modifications of categorization schemes that have been included in the recent updates to the lung, pancreaticobiliary, and thyroid cytopathology reporting systems. We also endeavored to identify and document the strengths and limitations of these reporting systems from the perspective of practicing cytopathologists to inform improvements for future editions.

LUNG

Pulmonary masses and nodules are increasingly recognized

by imaging techniques and subsequently targeted for cytologic and/or small-volume biopsy evaluation, responsible for the diagnosis of an estimated 70% of pulmonary malignancies and allowing disease staging [3]. In fact, lung cancer is mostly a time-sensitive condition, as a significant fraction of patients present with or rapidly progress to advanced stages, hampering a surgical approach and relying on cytological and histological samples to further determine potential therapies.

Rationale, historical background, and state of the art

In 1999, the PSC Task Force on Standards of Practice issued detailed guidelines for handling each type specimen obtained using lower-respiratory-exfoliative or FNA techniques and briefly outlined six recommended categories for reporting these samples (Fig. 1) [1,5,6]. The 1999 guidelines emphasized safety and efficiency and underlined that, despite the main use of diagnosis of malignancy, the guidelines could also be used to identify benign conditions [7].

The 2016 update of the guidelines proposed a new standardized terminology, recognizing that the lack of homogeneity in reporting could be hindering the clinical decision-making process [1]. The diagnostic criteria were refined, specific diagnoses/entities were listed, expected ROM rates were reported, and the categories were renamed and sequentially numbered (Fig. 1). The assessment of regional lymph nodes (mediastinal and hilar) was nested under the same classification system. Recommendations for ancillary studies were also included [1], primarily addressing the evaluation of predictive markers (such as programmed death-ligand 1 [PD-L1] and potential oncogenic genetic changes) using immunohistochemistry and molecular techniques. Institutional data [8] confirmed the overall ROM for each category and shed light on inconsistencies in overall diagnostic accuracy and ROM estimation across the various procedural approaches used to assess a given lesion.

The PSC System for Reporting Respiratory Cytology [9] was subsequently published in 2019. This atlas expanded the morphologic criteria and was supported by an extensive assortment of photographs and explanatory notes, sample reports, and updated ROM rates (stratified by primary lesion and nodal assessment). The directives for ancillary studies were included but indicated that PD-L1 testing lacked comprehensive validation and did not provide specific recommendations.

In 2022, the International Academy of Cytology and the IARC, which oversees the WHO Classification of Tumors, combined forces to develop an analogous series of WHO reporting systems for lung cytopathology. Indeed, the so-called WHO Blue Books

1999	2019	2022
Papanicolaou Society of Cytopathology Guidelines	Papanicolaou Society of Cytopathology System for Reporting Respiratory Cytology	WHO Reporting System for Lung Cytopathology
Categories	Categories	Categories
Nondiagnostic	I. Nondiagnostic	Insufficient/inadequate/Nondiagnostic
Benign (specific lesions)	II. Atypical	Benign/negative for malignancy
Atypical cells present, probably benign	IIIa. Neoplasm, benign	Atypical
Atypical, suspicious for malignancy	IIIb. Neoplasm, undetermined malignant potential/ low-grade malignancy	Suspicious for malignancy
Malignancy present	IV. Suspicious for malignancy	Malignant
	V. Malignant	
		ROM
		Management recommendations
		CLIN-IMG-MICRO correlation → MDT meeting → repeat FNA (± CNB)
		CLIN-IMG-MICRO correlation; if BENIGN → 3-6 months follow-up; if unclear → repeat FNA
		CLIN-IMG-MICRO correlation → MDT meeting; if BENIGN → 3-6 months follow-up; if unclear → repeat FNA (+ ROSE ± CNB)
		CLIN-IMG-MICRO correlation → MDT meeting; if MALIGNANT → treatment; if unclear → repeat FNA (+ ROSE ± CNB)
		CLIN-IMG-MICRO correlation → MDT meeting; if MALIGNANT → treatment; if unclear → repeat FNA (+ ROSE ± CNB)

Fig. 1. Lung cytopathology reporting: historical perspective and state of the art. CLIN-IMG-MICRO, clinical, imaging, and microbiological findings; CNB, core needle biopsy, including endobronchial biopsy; FNA, fine-needle aspiration biology; MDT, multidisciplinary team; ROSE, rapid onsite evaluation [1,5,6].

and the WHO cytopathology system are directly linked and are both offered in print and online forms. The main goal was to define a universal lexicon and specify criteria by which robust cytopathological diagnoses could be achieved worldwide, even by laboratories with limited resources. Five categories applicable to all types of specimens were established (Fig. 1; see also below). Under the banners of “Benign” and “Malignant,” the authors explained specific lesions or entities through subsections that echo the WHO Blue Books structure. Additional sections described best practices for specimen collection and handling, provided recommendations for ancillary testing (formally including PD-L1 determinations) and for management of each diagnostic category, emphasizing the role of multidisciplinary study and rapid onsite evaluation (ROSE) [3]. The five primary diagnostic categories are presented as follows.

Insufficient/inadequate/nondiagnostic

This category applies to specimens that cannot be reliably diagnosed due to inadequate cellularity, poor preparation, or obscuring factors. Each institution should resort to a single term to label this category and document the reasons for specimen insufficiency. The presence of any atypical cells upgrades the specimen to a higher category. The overall ROM for this category ranges from 40% to 60%; repeated sampling increases the sensitivity, especially of exfoliative specimens.

Benign

Specimens in this category show clear cytopathologic signs of

benign processes or neoplasms. It is essential to thoroughly compare these findings with imaging results; any disparities should be noted, with recommendations for further diagnostic steps, including a conservative approach, prompt reassessment of the morphological lesion, or (in such cases as an infection secondary to bronchial obstruction), surgical treatment. The ROM was projected to be 20% to 40%, and further assessment is necessary to refine this estimate.

Atypical

This category includes specimens displaying predominantly benign characteristics but featuring worrisome findings that raise the suspicion for malignancy, without sufficient evidence for conclusive diagnosis. These cases require correlation with clinical and imaging data and carry an ROM of 50% to 60%.

Suspicious for malignancy

Specimens that show features indicative of malignancy but lack conclusive evidence for a definitive diagnosis fall into this category. This category implies a degree of uncertainty while maintaining a high positive predictive value, with an ROM around 82%. Further investigation is typically warranted, and ancillary techniques can help refine the diagnosis.

Malignant

This definitive category is used when the specimen exhibits clear-cut features of malignancy without ambiguity; subclassification based on cytopathologic features and immunocytochem-

istry markers may also be undertaken. The ROM in this category exceeds 90%, and the diagnosis should be supported by clinical and imaging data to guide appropriate treatment.

Cytopathological practice

The *WHO Reporting System for Lung Cytopathology* reflects a convergence of principles from previous classifications and ongoing initiatives to address the challenges in lung cancer diagnosis. The framework is structured to document and interpret small-volume biopsies, standardize procedures, and stratify the ROM, the latter of which is particularly important given early detection can significantly impact treatment outcomes.

Faced with continuing instrument development, pathologists in the field must make deliberate efforts to adhere to the directives to reduce subjectivity and variability. The ROM should be considered with a critical outlook, and professionals should assume responsibility for institutional cytohistological correlation series, on whose account the ROM will be periodically revisited.

Future perspectives

Like so many other fields in pathology, the scientific knowledge of lung cancer is constantly evolving, not only with respect to basic science, but also with respect to clinical trial data that may quickly alter the standard of care; the WHO Reporting System must remain adaptable to this dynamic landscape. In particular, the emergence of liquid biopsies has the potential to provide deeper insights into the molecular profile of each tumor, while likely relying on morphological correlation for validation.

First, determination of PD-L1 in ethanol-fixed non-cellblock specimens should be validated through large-scale studies. Second, a more structured role could be carefully outlined for ROSE, potentially involving a dedicated and abbreviated classification, with consideration of the use of telecytopathology platforms, an invaluable resource for a growing number of institutions.

Digital pathology, artificial intelligence, and machine learning could streamline workflows by pre-selecting samples warranting examination and possibly identifying viral cytopathic effects or microorganisms. These measures may reduce the need for supplementary investigations that can consume both the often limited sample material and economic resources. In addition, subtle morphological changes that could otherwise be overlooked may be detected, enhancing accuracy and promoting consistency across categories.

PANCREAS

The *WHO Reporting System for Pancreaticobiliary Cytopathology* is part of a new series aligned with the fifth edition of the Classification of Digestive System Tumors [10,11]. This system standardizes reporting based on modifications of the 2015 PSC System [12]. The new system introduces seven categories, including “Pancreaticobiliary neoplasm: low-risk/grade” and “High-risk/grade,” based on a two-tiered stratification of cytological atypia. Notably, neuroendocrine tumors and solid pseudopapillary neoplasms are now in the “Malignant” category, while benign tumors like serous cystadenoma are classified as “Benign/Negative for malignancy.” The following sections offer a concise overview of these changes, providing insights into diagnostic categories, rationale for updates, and management implications.

Rationale, historical background, and state of the art

The *WHO Reporting System for Pancreaticobiliary Cytopathology* updates the 2015 PSC system for reporting Pancreaticobiliary Cytopathology [12]. Many entities have been reclassified in other categories in alignment with the *WHO Classification of Digestive System Tumors*. Ancillary studies like fluid biochemical assays, immunocytochemistry, fluorescent in situ hybridization (FISH), and next-generation sequencing (NGS) are essential in the diagnosis of pancreatic cysts and cases with suspicious morphology. Pancreatic FNA specimens and bile duct cytology have different ROMs owing to the inherent nature of the lesion and sampling techniques.

In the new system, there are seven categories compared to six in the PSC system. Tumors that were placed in the “Neoplastic: other” category like pancreatic mucinous neoplasm, ductal lesions, biliary and pancreatic intraepithelial neoplasia (PanIN) are placed in “Pancreaticobiliary neoplasm: low-risk/grade or high-risk/grade” based on the cytological atypia.

The seven diagnostic categories are as follows.

Insufficient/inadequate/nondiagnostic

This category has three options depending on the context and institutional practice. The categorization of tissue requires clinical and radiological correlation. If native tissue is sampled, it is prudent to categorize it as “Inadequate” rather than “Benign.” In contrast, even when extracellular mucin is devoid of epithelial cells, if the cyst fluid shows increased carcinoembryonic antigen (CEA) and corroborating radiological findings, it can be diagnostic. The ROM range for this category is 5%–25% [13]. Bile-duct stricture brushings have a higher ROM of 28%–69% due

to sampling bias.

Benign/negative for malignancy

This category combines the nomenclature of the PSC system and the *WHO Classification of Digestive System Tumors*. Either terminology can be used. These terms include both non-neoplastic entities such as pancreatitis, pseudocyst, and lymphoepithelial cyst and benign neoplasms such as serous cystadenoma and, rarely, schwannoma, or lymphangioma. The ROM is 0%–15% [13,14]. For bile-duct brushings, the ROM is 55%. This increased risk is due to the high threshold for malignancy leading to false negative cases.

Atypical

This category applies to cases that have architectural and cytological features that suggest more than a reactive process but for which there is insufficient evidence for placement in definite categories such as “Pancreaticobiliary neoplasm, low-risk/grade (PaN-low),” “Pancreaticobiliary neoplasm, high-risk/grade (PaN-high),” or “Malignant” [15]. Such limitations could be due to low cellularity, artifacts, or the inherent nature of the lesion. In cases of mass lesion in pancreas, the atypia can be due to reactive atypia in pancreatitis or poor sampling of malignant lesions. In cystic lesions, only a minority of the cases may judiciously be placed in this category, after utilization of integrated approach to place them in the specific “PaN-low” category. The ROM is 30%–40% for pancreatic FNA samples and 25%–61% for bile-duct brushings [13,16].

Pancreaticobiliary neoplasm, low risk/grade

Pancreaticobiliary neoplasm (low risk/grade) is a new category incorporating the remaining entities of PSC “Neoplastic: other” after exclusion of solid pseudopapillary neoplasm and neuroendocrine tumors. This category includes cystic neoplasms and intraductal neoplasms with low-grade epithelial atypia. The two-tiered stratification (i.e., low-grade and high-grade atypia) is similar to the histological classifications provided in the fifth edition of the *WHO Classification of Digestive System Tumors* [17]. The included entities are intraductal papillary mucinous neoplasm (IPMN) low-grade, mucinous cystic neoplasm low-grade, biliary intraepithelial neoplasia low-grade, PanIN low-grade, intraductal papillary neoplasm of bile duct low-grade, and low-grade spindle cell neoplasm.

In cystic neoplasms with mucin, the cellularity may be sparse with low- to intermediate-grade atypia [18]. The cells can be arranged in sheets and papillae. The background may show thick

colloid-like mucin. Testing the cyst fluid for elevated CEA (above 192 ng/mL) is useful to identify neoplastic mucinous cysts [19]. In addition, testing for *KRAS*, *GNAS*, and *RNF43* mutations in suspected cases of IPMN is advisable [20,21]. The estimated ROM for this category is 5%–20% [13]. The ROM for bile-duct brushings is not available.

Pancreaticobiliary neoplasm, high risk/grade

This category includes cystic neoplasms and intraductal neoplasms, as described above, that also display high-grade epithelial atypia (HGEA), as well as intraductal oncocytic papillary neoplasm and intraductal tubulopapillary neoplasm. High-grade dysplasia and invasive carcinoma can be difficult to distinguish based on cytology alone. An HGEA is defined as a cell smaller than a duodenal enterocyte (12 μ m) with high nucleus/cytoplasm ratio and chromatin abnormalities with or without necrosis [22]. Intermediate-grade dysplasia is placed in the histological “low-grade” group, creating a diagnostic dilemma. Mutation testing for *TP53*, *CDKN2A* (p16), and *SMAD4* deletion may indicate progression to malignancy [23,24]; p53 immunostaining (overexpression or null type) and loss of *SMAD4* may also aid in diagnosis. The estimated ROM for this category is 60%–95% [13].

Suspicious for malignancy

This category is used when the features are suspicious but not diagnostic of malignancy. This uncertainty could be due to low cellularity, difficulty in interpretation due to inflammation/stenting, or inadequate tissue for ancillary testing. This category may be used to reduce false-positive cases and when diagnostic features are seen in only a small number of fragments [25]. Consensus review and ancillary testing can help guide further management [26]. The ROM is 80%–100% for pancreatic FNAs and 74%–100% for bile-duct brushings [13].

Malignant

Pancreatic ductal adenocarcinoma is the most common pancreatic malignancy. Other tumors that may share overlapping morphology are acinar cell carcinoma, neuroendocrine carcinoma, and metastatic carcinoma, which must be distinguished by ancillary studies [27]. Neuroendocrine tumors and solid pseudopapillary neoplasms are included in this category in accordance with the fifth edition of the *WHO Classification of Digestive System Tumors*. False-positive results may occur due to florid reactive atypia in autoimmune pancreatitis and primary sclerosing cholangitis. Integrating ancillary studies such as FISH and NGS

will help guide further management [28]. The ROM is 99%–100% for positive pancreatic FNA and is 96%–100% for biliary tract cytology [13,16].

THYROID

FNA cytology plays a pivotal role in the management of thyroid nodules, reducing the need for unnecessary surgery. In 2010, TBSRTC introduced a six-tiered system to categorize thyroid FNA findings and their associated ROM [29], based on the proceedings of the October 2007 National Cancer Institute Thyroid FNA State of the Science Conference in Bethesda, Maryland [30].

The third edition of the TBSRTC, released in 2023, further refines these categories, emphasizing clarity in reporting using explicit category names. The 2023 TBSRTC updates the ROM based on recent large-scale studies and responds to the ambiguous ROM associated with indeterminate diagnoses, not distinguished despite molecular testing.

Rationale, historical background, and state of the art

In 1996, the PSC Task Force on Standards of Practice first released guidelines [30] pertaining to the evaluation of thyroid nodules by FNA. The document summarized technical matters, addressed interdisciplinary approaches, and proposed four ten-

tative diagnostic groups (Fig. 2) [29,31-33].

In 2010, the proposed TBSRTC (six-tiered system) (Fig. 2) was comprehensively explained and, regardless of being a first edition of the classification, attempted to establish the ROM of each category. The reporting system was globally acclaimed across medical specialties and readily adapted to the various national settings [34].

In 2018, a second edition of TBSRTC was published [32], in which the nomenclature (Fig. 2) and general criteria remained largely unchanged while embracing a role for molecular pathology. The tiered ROMs were recalculated based on pooled data from multiple cyto-histological correlation series published after 2010, with an effort to forecast the ROM for the newly distinguished noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Additionally, characterization of the type of atypia observed in Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS) was encouraged, loosely into classifiers of “cytologic,” “architectural,” “cytologic and architectural,” “Hürthle cell aspirates,” “not other specified,” and “atypical lymphoid cells.”

In 2023, the third edition of the TBSRTC was released [33,35], reflecting the continuous effort to integrate clinical perspectives and data from imaging and genetic studies. The 2023 TBSRTC simplified the diagnostic criteria and terminology (Fig. 2) and

1996	2010	2017	2023
Papanicolaou Society of Cytopathology Guidelines	The Bethesda System for Reporting Thyroid Cytopathology		
Diagnostic groups	1 st edition	2 nd edition	3 rd edition
Benign non-neoplastic lesions	Categories ROM	Categories ROM	Categories ROM (adults) ROM (pediatric) Management recommendations (underlined for pediatric population)
Cellular follicular lesions	ND / U -	ND / U 5-10%	ND 5-20% 0-33% <u>Repeat FNA (+ US)</u>
Hürthle cell neoplasm	B 0-3%	B 0-3%	B 2-7% 0-27% <u>Follow-up (CLIN + US)</u>
Malignant	AUS / FLUS ~5-15%	AUS / FLUS ~10-30%	AUS 13-30% 11-54% <u>Repeat FNA or molecular testing or surgery (lobectomy) or surveillance</u>
	FN / SFN 15-30%	FN / SFN 25-40%	FN 23-34% 28-100% <u>Molecular testing or surgery (lobectomy)</u>
	SM 60-75%	SM 50-75%	SM 67-83% 40-100% <u>Molecular testing or surgery (lobectomy vs. thyroidectomy)</u>
	M 97-99%	M 97-99%	M 97-100% 86-100% <u>Surgery (lobectomy vs. thyroidectomy)</u>

Fig. 2. Thyroid cytopathology reporting: historical perspective and state of the art [29,31-33]. ND, nondiagnostic; U, unsatisfactory; B, benign; AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; FN, follicular neoplasm; SFN, suspicious for follicular neoplasm; SM, suspicious for malignancy; M, malignant; FNA, fine-needle aspiration; US, ultrasound; CLIN, clinical.

aligned them with the 2022 WHO Thyroid Tumor Classification [36] by adopting updated histopathological nomenclature and discarding outdated terms like “Hürthle cell.” This 2023 edition continues to differentiate atypia on the basis of nuclear or architectural patterns, reflecting their heterogeneous implications for the ROM, and distinguishing the qualifiers of “atypia of undetermined significance” into “nuclear atypia” and “other.” The ROMs were revised and stratified by adult and pediatric age, and the bias introduced by NIFTP was acknowledged, with an estimated projected percent reduction of the ROM by category. New sections offer insights into radiologic correlations, molecular diagnostics, and pediatric-specific management.

Cytopathology practice

Given TBSRTC is among the most established cytopathology reporting systems, it is commonly referenced by the many disciplines encompassed by thyroidology. It provides a standardized language for communication clarity and consistency as pathologists can quickly craft a detailed report that conveys their reasoning without concern for potential interpretation bias, and intra- and interdepartmental datasets can be evaluated across institutions or even countries and continents. Use of the TBSRTC also contributes to a solid stratification and management, by serving as a blueprint to guide multidisciplinary decisions. The six tiers can serve as a helpful starting point for pathologists faced with challenging cases, allowing them to approach report drafting considering the clinical outcome first, rather than focusing solely on labeling a diagnosis.

The system is not free from criticism. As expected for any non-dichotomic classification (benign versus malignant), categories that are less determinate (grey zones) can become mired in uncertainty; some of the uncertainty can be resolved by molecular pathology, but this technique is not available at many institutions. In addition, the overlap between some categories complicates a precise ROM calculation and selection of the most appropriate management. The ROM data stem from retrospective studies with a selection bias (i.e., lesions undergoing surgery), and tissue samples are obtained from very diverse populations and frequently from tertiary institutions, with significant variability in the criteria applied by cytopathologists and surgical pathologists, and questionable histological correlation with aspirated nodules [34].

In this context, the contrast between Asian (namely Korean) and Western settings must be addressed. As reported in the literature, the former population is enriched for the conventional form of papillary thyroid carcinoma (PTC), allowing strict nu-

clear criteria assessment and targeted molecular *BRAF* V600E testing in cytology samples without loss of sensitivity. On the other hand, Western practices tend to be less conservative to not underdiagnose so-called RAS-like neoplasms and, in some instances, rely on broader molecular panels or even diagnostic lobectomy/thyroidectomy; this approach leads to surgical series filled with low-grade neoplasms that could be successfully managed through watchful monitoring, as established in Asian settings. These studies also underline the burden on healthcare systems stemming from the evaluation of minute nodules (< 1 cm in diameter) without overt clinical or radiological malignant features, which cytopathology teams should strongly advise against in multidisciplinary settings [37–41].

Future perspectives

A consistent classification scheme should be revised periodically to address new information. In particular, the ROMs merit an update, especially with regard to stratification for adult and pediatric ages and the advent of entities like NIFTP. Moreover, several authors have noted that the “Follicular Neoplasm” category could be improved by further subdivision to account for the presence of nuclear features of PTC [42].

Novel molecular data are frequently published and should be incorporated into cytology classification schemes, paving the way for tailored approaches. Special efforts should be devoted to developing inexpensive surrogate markers for actionable oncogenic variants and morphologic techniques with a high positive predictive value to identify high-risk lesions.

Finally, digital pathology, artificial intelligence, and machine learning are poised to assume a more substantial—synergistic—role in the future. Potential applications may include automating triage tasks in centers with high workloads or limited staff potential, as well as identifying subtle nuclear features and performing overall pattern analysis to aid pathologists in faster and more accurate diagnoses, which may be particularly valuable in cases with artifacts or processing issues.

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

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Immunohistochemical expression in idiopathic inflammatory myopathies at a single center in Vietnam

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Background: The identification of idiopathic inflammatory myopathies (IIMs) requires a comprehensive analysis involving clinical manifestations and histological findings. This study aims to provide insights into the histopathological and immunohistochemical aspects of IIMs. **Methods:** This retrospective case series involved 56 patients diagnosed with IIMs at the Department of Pathology, University of Medicine and Pharmacy at Ho Chi Minh City, from 2019 to 2023. The histology and immunohistochemical expression of HLA-ABC, HLA-DR, C5b-9, Mx1/2/3, and p62 were detected. **Results:** We examined six categories of inflammatory myopathy, including immune-mediated necrotizing myopathy (58.9%), dermatomyositis (DM; 23.2%), overlap myositis (8.9%), antisynthetase syndrome (5.4%), inclusion body myositis (IBM; 1.8%), and polymyositis (1.8%). The average age of the patients was 49.7 ± 16.1 years, with a female-to-male ratio of 3:1. Inflammatory cell infiltration in the endomysium was present in 62.5% of cases, perifascicular atrophy was found in 17.8%, and fiber necrosis was observed in 42 cases (75.0%). Rimmed vacuoles were present in 100% of cases in the IBM group. Immunohistochemistry showed the following positivity rates: HLA-ABC (89.2%), HLA-DR (19.6%), C5b-9 (57.1%), and Mx1/2/3 (10.7%). Mx1/2/3 expression was high in DM cases. p62 vacuole deposits were noted in the IBM case. The combination of membrane attack complex and major histocompatibility complex I helped detect IIMs in 96% of cases. **Conclusions:** The diagnosis of IIMs and their subtypes should be based on clinical features and histopathological characteristics. Immunohistochemistry plays a crucial role in the diagnosis and differentiation of these subgroups.

Key Words: Idiopathic inflammatory myopathies, immunohistochemistry, HLA-ABC, HLA-DR, C5b-9, Mx1/2/3, p62

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Inflammatory idiopathic myopathies (IIMs) are uncommon but manageable conditions defined by muscle weakness and the presence of inflammatory cells, mainly T lymphocytes, in the muscle tissue. IIMs are categorized into five subgroups: dermatomyositis (DM), polymyositis (PM), inclusion body myositis (IBM), immune-mediated necrotizing myopathy (IMNM), and nonspecific myositis. These are all autoimmune disorders that are linked to distinct autoantibodies in different subcategories such as antisynthetase syndrome (ASS) and overlap myositis (OM). To conduct a comprehensive evaluation of myopathy patients, it is necessary to perform clinical assessment, electromyography, measurement of muscle enzymes, serological testing, imaging techniques, and histological muscle biopsies. Accurate subgroup clas-

sification is essential because of the diverse disease processes and therapeutic responses.

Certain subgroups exhibit positive responses to immunosuppressive medications. Immunohistochemistry is essential for diagnosing these disorders; however, there is ongoing discussion regarding the choice of a suitable panel for diagnosis. The objective of this study was to investigate the histological and immunohistochemical features of IIMs.

MATERIALS AND METHODS

The study involved a cohort of 56 Vietnamese individuals diagnosed with myositis at the Department of Pathology, Univer-

sity of Medicine and Pharmacy at Ho Chi Minh City. The research period spanned from January 1, 2019, to June 30, 2023. Sample selection criteria were cases diagnosed with myositis based on clinical assessments that adhered to the categorization criteria specified by the European League Against Rheumatism (EULAR) in 2017, with a probability surpassing 55% (particularly, those classified as definite or probable).

We categorized patients into one of six subgroups based on their ultimate clinical presentation, antibody panel results obtained through immunoblot assay, and findings from muscle biopsy. Furthermore, we adhered to the diagnostic criteria outlined for DM by the European Neuromuscular Centre (ENMC) in 2018 [1], for IMNM by the ENMC in 2016 [2], and for IBM by the ENMC in 2011 [3].

Exclusion criteria encompassed evidence indicating other causes of myopathy such as drug-induced myopathy, exposure to toxic substances, infectious myopathy, endocrine disorders, or severe neurological disorders in internal medicine conditions. Additionally, evidence from family history, clinical characteristics, genetic testing, or histopathology suggestive of genetic etiology was considered.

Muscle biopsy samples were preserved using liquid nitrogen and isopentane, followed by staining with hematoxylin and eosin, modified Gomori Trichrome, periodic-acid Shiff, and NADH. Immunohistochemistry was conducted on frozen sections using the following panel of antibodies: HLA-ABC (W6/32, Invitrogen, Carlsbad, CA, USA), HLA-DR (LN3, Invitrogen), C5b-9 (aE11, Invitrogen), Anti-SQSTM1/p62 (GT1478, Invitrogen), and Mx1/2/3 (sc166412, Santa Cruz, Dallas, TX, USA).

The study collected clinical data of age, sex, sites of muscle weakness, muscle strength, creatine phosphokinase (CK) level in the blood, electromyography findings, and specific autoantibodies recorded in the pathology requisition form. Histological and immunohistochemical analysis was used to evaluate the presence and distribution of inflammatory cells in muscle tissue, as well as the occurrence of perifascicular atrophy (PFA), necrosis, phagocytosis, and rimmed vacuoles.

The Pearson's χ^2 test (or Fisher's exact test) was used to evaluate the relationship between pairs of categorical variables. All statistical analyses were performed using SPSS ver. 20.0 (IBM Corp., Armonk, NY, USA). A p -value $< .05$ was considered statistically significant.

RESULTS

Clinical characteristics of inflammatory myopathies

According to our records, there are six categories of inflammatory myopathy with the following numbers of cases and percentages as determined in this study: IMNM 33 (58.9%), DM 13 (23.2%), OM 5 (8.9%), ASS 3 (5.4%), IBM 1 (1.8%), and PM 1 (1.8%). The average age was 49.7–16.1 years, with the youngest patient being 17 years old and the oldest being 79. The age with the highest disease prevalence was 43 years. The majority of patients was female, accounting for 73.2% of the cases. The female-to-male ratio was 3:1.

The average duration from symptom onset to diagnosis was six months, with no notable distinction between groups, except for cases of IBM, which exhibited an extended diagnostic period of 36 months. While IBM is characterized by a prominent manifestation of distal weakness in the upper limbs, the other groups predominantly displayed proximal weakness.

Skin lesions, a hallmark of DM and also present in ASS, were documented in 67% of cases. In the DM subgroup, 100% of cases presented with skin lesions, among which specific lesions (heliotrope sign, Gottron's sign, and Gottron's papules) were observed in seven of 13 cases (53.8%). The remaining skin lesions included poikiloderma (38.4%), V-sign (46.1%), mechanics' hands (46.1%), and nonspecific lesions (46.1%). In the ASS subgroup, all three patients (100%) had mechanic's hands, and one case also exhibited Gottron's sign, while two cases had nonspecific skin lesions (Table 1).

Difficulty in swallowing was noted in 16% of cases. Pulmonary involvement, as diagnosed by computed tomography scan, was observed in 21% of cases, with respiratory failure more commonly occurring in the DM, ASS, and OM groups compared to the IMNM group. The mean blood CK level at the time of diagnosis was 3,997 U/L. The IMNM group exhibited a higher CK level in the blood compared to the DM group.

In serological assessments, the diagnostic positivity rate was 32 of 43 cases (74.4%). Among them, nine cases were positive for more than two antibodies (excluding Ro-52), exhibiting distinct clinical features associated with MDA5-PL7, Mi2-SRP, NXP2-SRP, PM-Scl-EJ, and PM-Scl-Jo1. Additionally, 23 cases (53.5%) positive for a single antibody contributed to the classification of myositis subgroups.

Pathological findings of inflammatory myopathies

Among the total 56 cases, the described pathological features on biopsy had diagnostic and classificatory significance for in-

inflammatory myositis. PFA was found in 10 cases (17.8%), in 100% of the ASS group and 50% of the DM group. Infiltration of lymphocytes was observed in 62.5% of the cases, with the highest frequency in the OM group with five cases (100%) and the lowest in the IMNM group. Fiber necrosis was observed in 75.0%, with the highest percentage in the ASS group (100%), 93.9% in the IMNM group, 23.1% in the DM group, 80.0% in the OM group, and zero in the IBM group. Endomysial fibrosis was noted in 23% of the cases. Vasculitis was detected in 5.3% of the cases, with the highest incidence in the OM group (40.0%). Rimmed vacuoles, a distinctive feature of IBM, were identified in only one case of IBM in our study (Table 2, Fig. 1).

Regarding immunohistochemical staining, major histocompatibility complex I (MHC-I, HLA-ABC) expression was noted in 89% of the cases. Abnormal expression of MHC-II (HLA-DR) was observed in 19.6% of the cases. Mx1/2/3 (MxA), a distinctive marker for DM, showed abnormalities in 10.7% of the total cases, all of which belonged to the DM and ASS groups. In 57% of the cases, we observed abnormal membrane attack complex (MAC) expression in the sarcolemma and endomysial capillaries, with C5b-9 deposited on capillaries and perivascular inflammation noted in 25% of cases. Additionally, MAC expression was observed in muscle fiber necrosis, serving as a nonspecific

marker. However, there was no significant difference in MAC deposits between PM and DM, while p62 expression was noted in the one IBM case (Table 3, Fig. 2).

The combined use of MHC-I and MAC can identify 96% of inflammatory myositis cases. Among the cases examined, only two DM cases (3.5%) exhibited no expression of MHC-I, MHC-II, MAC, Mx1/2/3, or p62.

DISCUSSION

Regarding the disease subgrouping in our study, the highest proportion of patients were in the IMNM group (58.9%), which is consistent with the study conducted by Watanabe et al. [4]. In the Watanabe study, the DM subgroup had a higher prevalence compared to our study. Conversely, the OM subgroup had a higher prevalence in our study compared to the Gupta et al. [5] and Ohnmar et al. [6]'s studies. Only one case was categorized into the IBM subgroup in our study, and none were reported in the studies by Gupta et al. [5] and Ohnmar et al. [6]. In contrast, IBM cases accounted for 16% of the Watanabe et al.'s study [4]. This difference could be due to the smaller sample size in our study as well as variations in ethnic characteristics. IBM is a more common subgroup in the white population over 50 years of age.

Table 1. Clinical findings of IIMs

	IMNM (n=33)	DM (n=13)	ASS (n=3)	OM (n=5)	PM (n=1)	IBM (n=1)
Specific skin lesions	0	7 (53.8)	1 (33.3)	2 (40.0)	0	0
Non-specific skin lesion						
Shawl sign	-	3 (23.0)	-	-	-	-
Mechanic hand	-	6 (46.1)	3 (100)	-	-	-
V sign	-	6 (46.1)	-	-	-	-
Poikiloderma	-	5 (38.4)	2 (66.6)	1 (20.0)	-	-
Others skin rash	5 (15.1)	6 (46.1)	-	-	-	-
Dysphagia	5 (15.1)	1 (7.6)	1 (33.3)	2 (40.0)	0	0
ILD/CT scan	7 (21.2)	0	3 (100)	2 (40.0)	0	0

Values are presented as number (%)

IIMs, idiopathic inflammatory myopathies; IMNM, immune-mediated necrotizing myopathy; DM, dermatomyositis; PM, polymyositis; IBM, inclusion body myositis; ASS, anti-synthetase syndrome; OM, overlap myositis; ILD, interstitial lung disease.

Table 2. Histological findings of IIMs

Pathological findings	PFA	Lymphocytic infiltration	Fiber necrosis	Vasculitis	Endomysial fibrosis	Rimmed vacuole
ASS (n=3)	3 (100)	2 (66.6)	3 (100)	1 (33.3)	1 (33.3)	0
DM (n=13)	6 (46.1)	8 (61.5)	3 (23.1)	0	3 (23.1)	0
IBM (n=1)	0	0	0	0	1 (100)	1 (100)
IMNM (n=33)	0	19 (57.6)	31 (93.9)	0	5 (15.1)	0
OM (n=5)	1 (20.0)	5 (100)	4 (80.0)	2 (40.0)	3 (60.0)	0
PM (n=1)	0	1 (100)	1 (100)	0	0	0

Values are presented as number (%)

IIMs, idiopathic inflammatory myopathies; IMNM, immune-mediated necrotizing myopathy; DM, dermatomyositis; PM, polymyositis; IBM, inclusion body myositis; ASS, anti-synthetase syndrome; OM, overlap myositis.

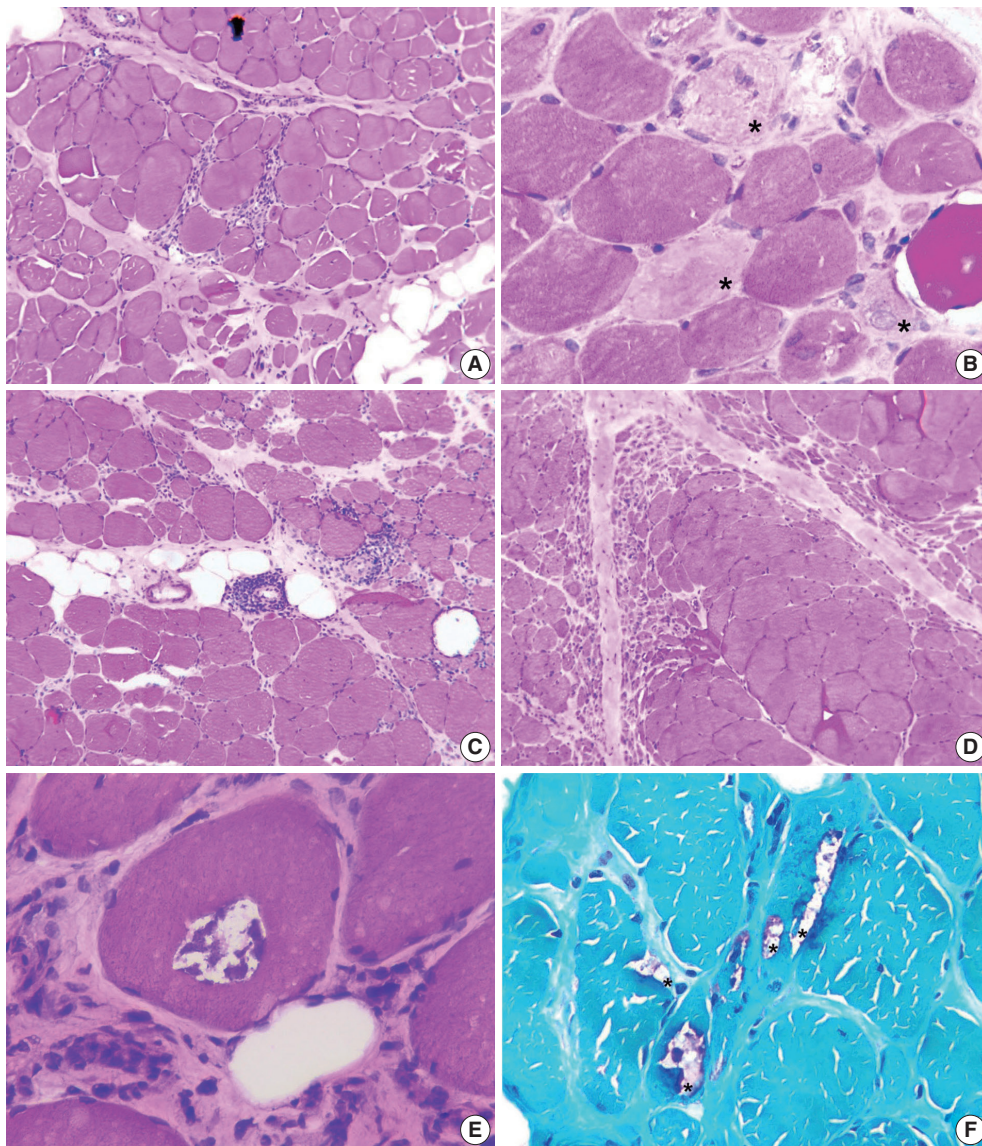


Fig. 1. (A) Infiltration of mononuclear inflammatory cells in the endomysial region. (B) Myofiber necrosis and myophagocytosis indicated by asterisks. (C) Infiltration of mononuclear inflammatory cells in the interfascicular region and perivascular area. (D) Perifascicular atrophy. (E) Rimmed vacuole. (F) Rimmed vacuole indicated by asterisks (modified Gomori trichrome).

Clinical features of the IBM subgroup include distinct weakness patterns involving upper and lower limb muscles and a slowly progressive course over many years, making it challenging to diagnose, with common misdiagnosis.

The age of onset in our study exhibited a standard distribution, with a mean age of 50 and a peak disease frequency at 43 years of age, which is consistent with the study by Chen et al. [7], who conducted a retrospective population-based study on the Chinese population with a mean age of 51.2 years. The mean age in our study was slightly higher compared to the study by van der Meulen et al. [8]. Among the inflammatory myopathy

subgroups, age of onset did not show significant differences. This finding is in line with other epidemiological studies [5,8-10].

The infiltration of lymphocytes into the biopsy tissue is a characteristic feature initially described in the histopathological criteria for DM diagnosis by Bohan and Peter [9] in the 1970s. However, this phenomenon can also be encountered in other conditions characterized by muscle fiber breakdown, such as Duchenne or Becker muscular dystrophy. Lymphocytic infiltration may not be detected in cases of amyopathic DM, and it can be absent in patients who have received prior immunosuppressive therapy. In cases with nonspecific findings on muscle biopsy, the diagno-

Table 3. Immunohistochemical findings of IIMs

Marker expression	Membranous and cytoplasmic HLA-ABC expression	Membranous and cytoplasmic HLA-DR expression	Membranous and cytoplasmic MAC expression	MAC deposit on endomysial capillaries	Mx1/2/3 in PFA	p62 deposit in vacuole	Cytoplasmic p62 expression
ASS (n=3)	3 (100)	1 (33.3)	2 (66.7)	1 (33.3)	0	0	2 (66.7)
DM (n=13)	11 (84.6)	0	4 (30.7)	2 (15.4)	5 (38.5)	0	2 (15.4)
IBM (n=1)	1 (100)	0	1 (100)	1 (100)	0	1 (100)	0
IMNM (n=33)	31 (93.9)	9 (27.3)	15 (45.5)	7 (21.2)	0	0	8 (24.2)
OM (n=5)	4 (80.0)	1 (20.0)	1 (20.0)	2 (40.0)	0	0	0
PM (n=1)	0	0 (100)	1 (100)	1 (100)	0	0	0

Values are presented as number (%)

IIMs, idiopathic inflammatory myopathies; IMNM, immune-mediated necrotizing myopathy; DM, dermatomyositis; PM, polymyositis; IBM, inclusion body myositis; ASS, anti-synthetase syndrome; OM, overlap myositis; PFA, perifascicular atrophy.

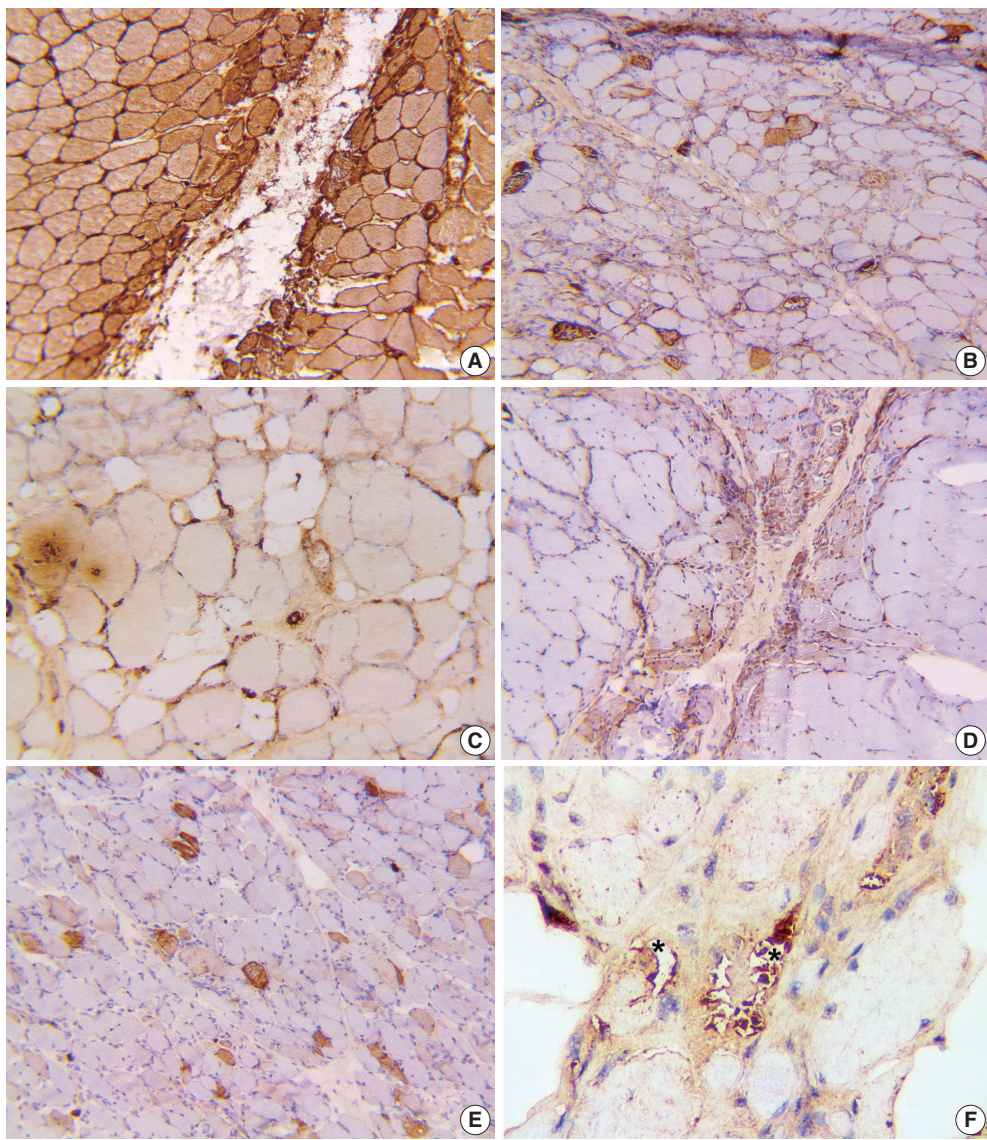


Fig. 2. (A) Membranous and cytoplasmic MHC-1 expression. (B) Membranous and cytoplasmic membrane attack complex (MAC) expression. (C) Deposits of MAC on endomysial capillaries. (D) Mx1/2/3 expression on perifascicular atrophy. (E) Cytoplasmic p62 expression. (F) Deposits of p62 in rimmed vacuoles indicated by asterisks.

sis of OM in our study primarily relied on autoantibody testing (positive for PM-Scl and anti-Ku) and clinical features indicative of multisystem involvement, muscle stiffness on examination, and evidence of systemic vasculitis.

PFA, which is specific for DM, was observed in 46% of the DM cases in our study, which is lower than the study conducted by Uruha et al. [10]. Consensus on the diagnostic criteria for DM from the ENMC indicates that approximately 50% of cases exhibit this feature [1]. The lower PFA rate observed in our study could be linked to expertise and biopsy site selection. Utilizing Mx1/2/3 immunohistochemistry is instrumental in improving the identification of PFA fibers, which highlights the crucial role of Mx1/2/3 immunohistochemistry in the diagnosis of DM.

PFA is specific for DM; however, it also can be observed in ASS. According to the findings of Uruha et al. [10], PFA was observed in 13% of ASS cases. Mx1/2/3 or MxA expression helps differentiate between DM and ASS when both are present with PFA. In our study, no cases of ASS tested positive for Mx1/2/3. These results align with the study by Inoue et al. [11], who observed similar clinical manifestations between DM and some ASS cases but found no positivity for Mx1/2/3.

In our study, fiber necrosis was observed in 42 of 56 cases (75%). The prevalence of fiber necrosis varied among subgroups as follows: IMNM (93.9%), DM (23.1%), OM (80.0%), ASS (100%), and PM (100%) and was not observed in the IBM subgroup. The degree of fiber necrosis was also notably higher in the IMNM group compared to the DM group (94% vs. 23%). In the ASS subgroup, fiber necrosis was consistently observed at a very high rate (100%), surpassing the 48% reported in the study by Noguchi et al. [12].

Based on immunohistochemistry, our study recorded the following positivity rates: HLA-ABC (MHC-I, 89.2%), HLA-DR (MHC-II, 19.6%), C5b-9 (MAC, 57.1%), and Mx1/2/3 (10.7%), notably all of positive Mx1/2/3 staining cases were DM. MAC deposits were identified in endomysial capillaries in 25% of cases. Our study showed that MHC-I expression had the highest sensitivity for detecting abnormalities, while MHC-II, Mx1/2/3, and MAC showed lower positivity rates but higher specificity in myositis sub-classification. Compared with previous studies by Das et al. [13], Rider et al. [14], and Uruha et al. [15], our study showed similarity in the positivity rate of MHC-I, while the other markers exhibited differences mainly due to variations in the initial classification criteria. The Das et al.'s [13] and Rider et al.'s [14] studies only classified PM and DM in their diagnosis, and Uruha et al.'s study [15] primarily relied on antibody screening for the initial sample selection.

While MHC-I has been demonstrated to have high sensitivity, this immunohistochemical staining method can yield unusual results in various myopathies due to different underlying causes. According to van der Pas et al. [16], 11% of cases with dysferlinopathy tested positive for MHC-I, as did 4% of other non-inflammatory myopathies. Another study by Confalonieri et al. [17] found relatively high rates (70%) of dysferlinopathy with MHC-I positivity and 20% with MHC-II positivity, but no cases of Duchenne muscular dystrophy tested positive. Many other studies have reported varying positivity rates, demonstrating that, while MHC-I has high sensitivity, it may not be highly specific. In a retrospective study by Rodriguez Cruz et al. [18] analyzing biopsy samples from groups with and without inflammation (inflammatory myopathies, non-inflammatory myopathies, genetic myopathies, drug-induced myopathies, severe medical conditions), they observed 98% positivity for MHC-I in inflammatory myopathies and 92% positivity in non-inflammatory myopathies. For MHC-II, they found 60% positivity in inflammatory myopathies and 10.1% in non-inflammatory myopathies. These rates, in comparison to our study, show similarity in MHC-I positivity and lower MHC-II positivity. Notably, no cases in that previous study were MHC-II positive without MHC-I positivity, which aligns with our findings.

The analysis of inflammatory cells, MHC-I expression, and MAC deposits plays a role in distinguishing dysferlinopathy from IIMs [19]. In our study, the combined use of MAC and MHC-I was effective in identifying 96% of inflammatory myositis cases in muscle biopsies. Another study indicated that MAC deposits on capillaries were observed in childhood DM, suggesting that MAC deposits on endomysial capillaries could serve as a valuable indicator of early-stage DM [20].

Of the cases studied, only two DM cases (3.5%) did not exhibit positivity with any immunohistochemical staining method. However, the presence of other clinical features and biochemical abnormalities also contributed to the final diagnosis in these cases. This underscores the importance of a combination of clinical parameters and biopsy for an accurate diagnosis. It is essential to consider the location of muscle biopsy as cases with extensive fibrosis may not be helpful for diagnosis.

The sample size for the current study is limited, and several subgroups have a small number of cases, possibly not fully representing the groups in terms of clinical and laboratory characteristics, especially for the Vietnamese population. Nevertheless, considering the rarity of these diseases, the initial assessments based on pathological and immunohistochemical findings offer neurologists valuable insights for diagnosis and treatment. Still,

immunohistochemical staining supports the diagnosis, and muscle biopsy aids in diagnosing specific muscle diseases, accurately classifying the group of inflammatory myopathies in close alignment with clinical findings.

The integration of muscle biopsy and antibody testing is essential for accurately categorizing and diagnosing myositis, as it is a highly sensitive clinical procedure. To diagnose inflammatory myopathies through muscle biopsy, it is essential to perform immunohistochemistry with a variety of markers, including MHC-I, MHC-II, C5b9, and Mx1/2/3. p62 staining is essential in cases of suspicion of IBM. The diagnosis of myositis is, however, a multi-modal process, where pathology and immunohistochemical staining play a supporting role. Successful pathological diagnosis of myositis may vary depending on the biopsy location, the condition of the biopsy sample, and the expertise of the pathologist.

Ethics Statement

The study was approved by the Institutional Review Board of Biomedical Research at the University of Medicine and Pharmacy at Ho Chi Minh City (IRB number 476/HĐĐĐ-DHYD on September 29, 2021) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

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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Liquid-based cytology features of pancreatic acinar cell carcinoma: comparison with other non-ductal neoplasms of the pancreas

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Background: Acinar cell carcinoma (ACC) is a rare malignant epithelial neoplasm, which shares many cytomorphological features with other non-ductal pancreatic neoplasms such as pancreatic neuroendocrine neoplasm (PanNEN) and solid-pseudopapillary neoplasm (SPN). Due to the relative rarity of these tumors, pathologists are less familiar with the cytological features, especially on liquid-based cytology (LBC) which has been relatively recently introduced for endoscopic ultrasound-guided fine needle aspiration specimens. **Methods:** We evaluated the detailed cytological features of 15 histologically confirmed ACC (7 conventional smears [CS], 8 LBC), and compared them with the LBC features of SPN (n=9) and PanNEN (n=9). **Results:** Compared with CS, LBCs of ACC demonstrated significantly less bloody background. All ACCs demonstrated prominent nucleoli and macronucleoli on LBC. On comparison with the LBC features of SPN and PanNEN, most ACCs demonstrated a necrotic background with apoptotic debris while PanNEN and SPN did not show these features. Acinar structures were predominantly observed in ACC, while frequent pseudopapillary structures were seen only in SPN. Prominent nucleoli and macronucleoli were only seen in ACC. **Conclusions:** ACC had characteristic cytological features that could be observed on LBC preparations, such as high cellularity, necrotic/apoptotic background, nuclear tangles, acinar arrangement of cells, and macronucleoli. These findings also help distinguish ACC from PanNEN and SPN on LBC. It is important to be familiar with these features, as an accurate diagnosis on endoscopic ultrasound-guided fine needle aspiration cytology would have impact on the management of the patient.

Key Words: Acinar cell carcinoma; Cytology; Pancreatic neoplasms

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Acinar cell carcinoma (ACC) of the pancreas is a rare malignant epithelial neoplasm accounting for up to 1% of carcinomas of the exocrine pancreas [1]. Diagnostic accuracy is critical as symptoms are nonspecific and the prognosis is poor, with a median survival of 47 months and about one-half of patients presenting with metastasis at the time of diagnosis [1-3].

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is a well-established diagnostic method for solid pancreatic tumors, and thus an accurate diagnosis on aspiration cytology material is essential to guide the next steps for patient management [4,5]. While conventional smear (CS) cytology was the standard method for processing EUS-FNA cytology specimens, CS preparations often result in bloody smears, dry artifacts, and crushing artifacts, which could obscure the cytologic features and result in a suboptimal diagnosis [6]. As such, there is currently

increasing interest in implementing liquid-based cytology (LBC) preparations for EUS-FNA material to circumvent these innate limitation of CS specimens [6].

The cytological diagnosis of ACC is often challenging, most importantly due to the rarity of the tumor; ACC has a high rate of misdiagnosis by cytology and is often misinterpreted as other solid pancreatic neoplasms, such as pancreatic neuroendocrine neoplasms (PanNEN), solid-pseudopapillary neoplasms (SPN), and pancreatoblastoma, which demonstrate overlapping cytological features [3,4,7-10]. In addition, ACC may demonstrate scattered neuroendocrine cells in up to 40% of cases, which may falsely lead to a diagnosis of PanNEN [4]. Moreover, while immunohistochemistry may be performed on cell block specimens when available, there is also some immunophenotypical overlap between ACC with PanNEN, which adds to the diagnostic dif-

ficulty [3,11,12]. Finally, inadequate smears due to low cellularity or bloody smears may also lead to underdiagnosis of such cases [13].

While the cytological characteristics of ACC on CS have been described in the previous literature, the cytomorphology on LBC has been less well characterized [1]. The aim of this study was to evaluate the cytological features of ACC on LBC, by comparing CS and LBC features, and also by comparing the LBC cytology of ACC, SPN, and PanNEN.

MATERIALS AND METHODS

Case selection

In this retrospective study, histologically confirmed cases with a final histological diagnosis of ACC over a 9-year period from 23 December 2013 to 14 July 2022 were retrieved from the pathology database of Seoul National University Hospital (SNUH) and Ulsan University Asan Medical Center. All cytology specimens were obtained by EUS-FNA using 19- or 22-gauge needles. For CS, aspirated specimens were immediately smeared onto glass slides and fixed in 95% ethanol in the endoscopy room; the slides were sent to the cytopathology laboratory for further Papanicolaou stains. For all LBC cases, the specimens were immediately suspended in preservative fluid (CytoRich Red, Thermo Scientific, Waltham, MA, USA), sent to the cytopathology laboratory and further processed on the BD PrepStain Slide Processor (Becton Dickinson, Franklin Lakes, NJ, USA) with the NON-GYN protocol.

Among these cases, a total of 15 cases (7 CS and 8 LBC) had matching preoperative EUS-FNA cytology specimens. Of these 15 cases with a final histological diagnosis of ACC, six cases were initially interpreted as “ACC”, seven cases as “carcinoma”, and two cases as “adenocarcinoma” on cytology. Immunohistochemical stains were performed for all 15 cases.

In addition, EUS-FNA LBC specimens from nine PanNENs and nine SPNs (all of which were histologically confirmed) were retrieved from SNUH, for comparison with the LBC features of ACC.

The clinicopathological features and radiological features (magnetic resonance imaging and/or abdominal computed tomography) were reviewed for all ACC, SPN, and PanNEN cases.

Cytomorphological evaluation

The cytological features of LBC and CS specimens were reviewed by two pathologists (MK, HK), and the following parameters were evaluated: (1) cellularity (high, moderate, low);

(2) background (bloody or necrotic background, presence of apoptotic debris and/or nuclear tangles); (3) cytoarchitecture (2-dimensional sheets, acinar or (pseudo)papillary structures, single cells, naked nuclei, nuclear overlapping); (4) cytoplasmic features (granularity, cell membranes, cell shape); and (5) nuclear features (size, degree of pleomorphism, nuclear shape, nuclear: cytoplasmic ratio, nucleoli, macronucleoli, and chromatin pattern). The cytological features were initially evaluated independently, followed by discussion and review using a multiheaded microscope to render a final assessment.

Statistical analysis

Statistical analysis was performed by Chi-square tests and Fisher's exact test using the R software (v3.6.1, R Foundation for Statistical Computing, Vienna, Austria). Values of $p < .05$ were considered statistically significant and all p -values were 2-sided.

RESULTS

Baseline characteristics

The clinicopathological details of the ACC, SPN, and PanNEN cases are summarized in Table 1. Most of the patients with SPN (8/9, 88.9%) and PanNEN (6/9, 66.7%) were younger than 60 years of age, whereas 73.3% (11/15) of patients with ACC were aged 60 or older. There was no significant predilection for location within the pancreas for all tumors, although SPNs were more commonly located in the body/tail region than in the head/uncinate process. The gross appearance was predominantly solid in all three tumors.

The initial radiological impression for the ACC cases were as follows: (1) pancreatic cancer (9/15, 60.0%), (2) “atypical pancreatic cancer” with differential diagnoses including malignant lymphoma, metastases, PanNEN and sarcoma (5/15, 33.3%), (3) and SPN (1/15, 6.7%). The most common imaging diagnosis for SPN was “SPN versus PanNEN” (6/9, 66.7%), while PanNENs were mostly classified as either “PanNEN” (4/9, 44.4%) or “PanNEN or SPN” (3/9, 33.3%) on imaging.

Comparison between LBC and CS cytology features of ACC

The detailed cytomorphological features of ACC on CS and LBC are summarized in Table 2 and Fig. 1. When cellularity was evaluated, all eight LBC cases (100%) showed moderate to high cellularity, compared to that of CS (4/7 cases, 57.1%). A bloody background was more frequently seen in CS preparations (CS, 85.7%; LBC, 12.5%; $p = .010$). Necrotic backgrounds were seen in both CS (57.1%) and LBC (75.0%), and apoptotic

Table 1. Baseline characteristics

	ACC (n=15)	SPN (n=9)	PanNEN (n=9)
Age (yr)			
≤60	4 (26.7)	8 (88.9)	6 (66.7)
>60	11 (73.3)	1 (11.1)	3 (33.3)
Sex			
Male	8 (53.3)	4 (44.4)	3 (33.3)
Female	7 (46.7)	5 (55.6)	6 (66.7)
Tumor location			
Head	1 (6.7)	1 (11.1)	3 (33.3)
Uncinate process	7 (46.7)	0	1 (11.1)
Neck	2 (13.3)	2 (22.2)	0
Head and neck	0	1 (11.1)	0
Body	6 (40.0)	3 (33.3)	2 (22.2)
Tail	4 (26.7)	2 (22.2)	3 (33.3)
Gross feature			
Solid	15 (100)	8 (88.9)	7 (77.8)
Cystic	0	0	0
Solid and cystic	0	1 (11.1)	2 (22.2)
Tumor size (cm) ^a	3.5 (1.6–6.7)	1.8 (1.0–2.5)	2.5 (1.0–6.0)
WHO grade ^b			
Grade 1	NA	NA	4 (44.4)
Grade 2	NA	NA	4 (44.4)
Grade 3	NA	NA	1 (11.1)

Values are presented as number (%).

ACC, acinar cell carcinoma; SPN, solid-pseudopapillary neoplasm; PanNEN, pancreatic neuroendocrine neoplasm; WHO, World Health Organization; NA, not applicable.

^aTumor size was measured based on computed tomography images. Tumor sizes are presented as median (range); ^bApplicable to PanNEN cases only.

debris were frequently seen in both CS (85.7%) and LBC (87.5%) preparations. Next, we assessed the cytoarchitecture. Acinar structures was more frequently seen in LBC preparations compared with CS (CS, 28.6%; LBC, 75.0%). Notably, single cells and naked nuclei were frequently seen in ACC in both LBC (75.0%) and CS (71.4%), and nuclear overlapping was observed in all cases regardless of the type of preparation (CS, 100%; LBC, 100%).

Cytoplasmic granularity was a common finding of ACC in both LBC (100%) and CS (85.7%). The cell borders were often blurred in ACCs. Among the cases where the cell membranes could be discerned, the ACC tumor cells often assumed a plasmacytoid cell shape on LBC. As for the nuclear features, the ACC tumor nuclei were predominantly medium-to-large in both preparations (CS, 100%; LBC, 62.5%), and high nuclear:cytoplasmic ratio and irregular chromatin were observed in all ACC cases (CS, 100%; LBC, 100%). Interestingly, prominent nucleoli and macronucleoli could be appreciated in all LBC cases of ACC, while only 28.6% ($p = .010$) and 14.3% ($p = .001$) of CS cases

Table 2. Cytomorphological comparison of LBC and CS of acinar cell carcinoma

Cytomorphological feature	LBC (n=8)	CS (n=7)	p-value ^a (LBC vs. CS)
Cellularity			.070
Moderate/high	8 (100)	4 (57.1)	
Low	0	3 (42.9)	
Background			
Bloody	1 (12.5)	6 (85.7)	.010
Necrotic	6 (75.0)	4 (57.1)	.600
Apoptotic debris	7 (87.5)	6 (85.7)	> .999
Nuclear tangles	2 (25.0)	3 (42.9)	.610
Cytoarchitecture			
2-D sheets	0	0	
3-D structures	8 (100)	6 (85.7)	
Papillary structures	0	0	> .999
Acinar structures			.610
Frequent	6 (75.0)	2 (28.6)	
Some/rare/absent	2 (25.0)	5 (71.4)	
Single cells and naked nuclei			> .999
Frequent	6 (75.0)	5 (71.4)	
Some/rare	2 (25.0)	2 (28.6)	
Nuclear overlapping	8 (100)	7 (100)	> .999
Cytoplasm			
Granularity	8 (100)	6 (85.7)	.470
Poorly defined cytoplasm membrane			.200
Present	8 (100)	5 (71.4)	
Focal/absent	0	2 (28.6)	
Cell shape			
Plasmacytoid	4 (50.0)	0	.080
Round-oval	1 (12.5)	1 (14.3)	> .999
Not evaluable	3 (37.5)	6 (85.7)	
Nucleus			
Size			.200
Medium/large	5 (62.5)	7 (100)	
Small	3 (37.5)	0	
Pleomorphism			.570
Marked/moderate	1 (12.5)	2 (28.6)	
Mild	7 (87.5)	5 (71.4)	
Membrane			.610
Smooth	6 (75.0)	4 (57.1)	
Occasional convoluted	2 (25.0)	3 (42.9)	
Shape			> .999
Ovoid	8 (100)	7 (100)	
N/C ratio			> .999
> 1:2	8 (100)	7 (100)	
< 1:2	0	0	
Nucleoli			.010
Prominent	8 (100)	2 (28.6)	
Inconspicuous	0	5 (71.4)	
Macronucleoli	8 (100)	1 (14.3)	.001
Chromatin			> .999
Irregular	8 (100)	7 (100)	
Fine	0	0	

Values are presented as number (%).

LBC, liquid-based cytology; CS, conventional smear; N/C, nuclear-to-cytoplasmic.

^aFisher exact test.

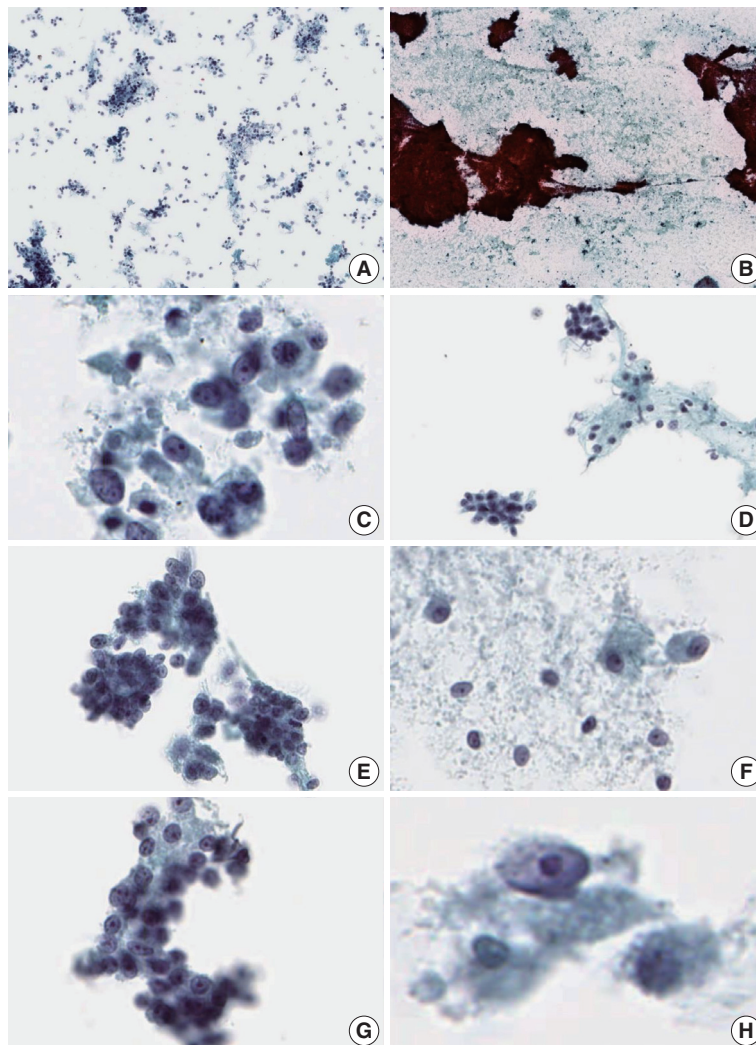


Fig. 1. Cytologic features of acinar cell carcinoma on liquid-based cytology (LBC) and conventional smear (CS). (A) Hypercellular background (LBC). (B) Bloody background (CS). (C) Necrotic background (LBC). (D) Acinar structure (LBC). (E) Nuclear overlapping (LBC). (F) Plasmacytoid cell shape (LBC). (G) Prominent nucleoli (LBC). (H) Macronucleoli (LBC).

demonstrated these features, respectively.

In summary, compared to CS, LBC cytology of ACC was characterized by less bloody backgrounds, relatively higher cellularity, more appreciable acinar structures, and prominent (or macro) nucleoli.

Comparison of LBC features between ACC, PanNEN, and SPN

Next, we sought to compare the LBC features of ACC with those of SPN and PanNEN. The results are summarized in Table 3 and Fig. 2. ACC and SPN cases more often showed moderate to high cellularity compared to PanNENs, although not statistically significant (ACC, 100%; SPN, 88.9%; PanNEN, 44.4%). Findings that were unique to ACC were (1) a necrotic

background (ACC, 75.0%; SPN, 0%; PanNEN, 0%; $p = .003$), (2) apoptotic debris in the background (ACC, 87.5%; SPN, 0%; PanNEN, 0%; $p < .001$), and (3) nuclear tangles (ACC, 25.0%; SPN, 0%; PanNEN, 0%). A bloody background was seen in a minority of ACC, SPN, and PanNEN LBCs (ACC, 12.5%; SPN, 11.1%; PanNEN, 11.1%).

As for cytoarchitecture, the presence of frequent pseudopapillary structures was a unique feature of SPN (ACC, 0%; SPN, 77.8%; PanNEN, 0%; $p = .003$); in contrast, rare papillary structures (with fibrovascular cores) were observed in PanNENs (22.2%). Acinar structures, on the other hand, were more frequently observed in ACC (ACC, 75.0%; SPN, 11.1%; PanNEN, 0%; $p = .007$ [ACC vs. PanNEN], $p = .020$ [ACC vs. SPN]). Singly scattered tumor cells and naked nuclei were seen

Table 3. Cytomorphological comparison of ACC, SPN, and PanNEN

Cytomorphological feature	ACC (n=8)	SPN (n=9)	PanNEN (n=9)	Total (n=26)	p-value ^a (ACC vs. PanNEN)	p-value ^a (ACC vs. SPN)
Cellularity					.090	>.999
Moderate/high	8 (100)	8 (88.9)	4 (44.4)	20 (76.9)		
Low	0	1 (11.1)	5 (55.6)	6 (23.1)		
Background						
Bloody	1 (12.5)	1 (11.1)	1 (11.1)	3 (11.5)	>.999	>.999
Necrotic	6 (75.0)	0	0	6 (23.1)	.003	.003
Apoptotic debris	7 (87.5)	0	0	7 (26.9)	<.001	<.001
Nuclear tangles	2 (25.0)	0	0	2 (7.7)	.300	.300
Cytoarchitecture						
2-D sheets	0	0	0	0		
3-D structures	8 (100)	8 (88.9)	9 (100)	25 (96.2)		
(Pseudo)papillary structures					.003	.003
Frequent	0	7 (77.8)	0	7 (26.9)		
Rare	0	2 (22.2)	2 (22.2)	4 (15.4)		
Absent	8 (100)	0	7 (77.8)	15 (57.7)		
Acinar structures					.007	.020
Frequent/some	6 (75.0)	1 (11.1)	0	7 (26.9)		
Rare/absent	2 (25.0)	8 (88.9)	9 (100)	19 (73.1)		
Single cells and naked nuclei					.930	.930
Frequent	6 (75.0)	5 (55.6)	4 (44.4)	15 (57.7)		
Some/rare	2 (25.0)	4 (44.4)	5 (55.6)	11 (42.3)		
Nuclear overlapping	8 (100)	9 (100)	9 (100)	26 (100)	>.999	>.999
Cytoplasm						
Granularity	8 (100)	8 (88.9)	9 (100)	25 (96.2)	>.999	>.999
Poorly defined cytoplasm membrane					.710	.710
Present	8 (100)	7 (77.8)	7 (77.8)	22 (84.6)		
Focal/absent	0	2 (22.2)	2 (22.2)	4 (15.4)		
Cell shape						
Plasmacytoid	4 (50.0)	7 (77.8)	6 (66.7)	17 (65.4)	.960	.960
Round-oval	1 (12.5)	0	0	1 (3.8)	.710	.710
Not evaluable	3 (37.5)	2 (22.2)	3 (33.3)	8 (30.8)		
Nucleus						
Size					>.999	.960
Medium/large	5 (62.5)	4 (44.4)	6 (66.7)	15 (57.7)		
Small	3 (37.5)	5 (55.6)	3 (33.3)	11 (42.3)		
Pleomorphism					.860	>.999
Marked/moderate	1 (12.5)	1 (11.1)	3 (33.3)	5 (19.2)		
Mild	7 (87.5)	8 (88.9)	6 (66.7)	21 (80.8)		
Membrane					.860	.860
Smooth	6 (75.0)	8 (88.9)	8 (88.9)	22 (84.6)		
Occasional convoluted	2 (25.0)	1 (11.1)	1 (11.1)	4 (15.4)		
Shape					>.999	>.999
Ovoid	8 (100)	9 (100)	9 (100)	26 (100)		
N/C ratio						
> 1:2	8 (100)	6 (66.7)	8 (88.9)	22 (84.6)		
< 1:2	0	3 (33.3)	0	3 (11.5)		
Not evaluable	0	0	1 (11.1)	1 (3.8)		
Nucleoli					>.999	>.999
Prominent	8 (100)	8 (88.9)	7 (77.8)	23 (88.5)		
Inconspicuous	0	1 (11.1)	2 (22.2)	3 (11.5)		
Macronucleoli	8 (100)	0	0	8 (30.8)	<.001	<.001

(Continued to the next page)

Table 3. Continued

Cytomorphological feature	ACC (n=8)	SPN (n=9)	PanNEN (n=9)	Total (n=26)	p-value ^a (ACC vs. PanNEN)	p-value ^a (ACC vs. SPN)
Chromatin					> .999	< .001
Irregular	8 (100)	0	9 (100)	17 (65.4)		
Fine	0	9 (100)	0	9 (34.6)		

Values are presented as number (%).

ACC, acinar cell carcinoma; SPN, solid-pseudopapillary neoplasm; PanNEN, pancreatic neuroendocrine neoplasm; N/C, nuclear-to-cytoplasmic.

^aFisher exact test.

most commonly in ACC although not statistically significant (ACC, 75.0%; SPN, 55.6%; PanNEN, 44.4%).

Cytoplasmic granularity and poorly defined cell membranes were seen in all three tumor types. Plasmacytoid cell morphology was observed in 50.0%, 77.8%, and 66.7% of ACC, SPN, and PanNEN, respectively. The nuclei were ovoid in shape for all tumor types. Nuclear pleomorphism was mild in most cases (ACC, 87.5%; SPN, 88.9%; PanNEN, 66.7%). Irregular nuclear membranes were more frequently observed in ACC (25.0%) compared to SPN (11.1%) and PanNEN (11.1%), although not statistically significant. High nuclear:cytoplasmic ratio and prominent nucleoli were observed in all ACC cases, and also in the majority of SPNs (66.7% and 88.9%, respectively) and PanNENs (88.9% and 77.8%, respectively). Macronucleoli were only observed in ACCs (ACC, 100%; SPN, 0%; PanNEN, 0%; $p < .001$). Irregular chromatin pattern was observed in all cases of ACC and PanNEN (ACC, 100%; SPN, 0%; PanNEN, 100%) while only SPN showed fine chromatin in all cases (ACC, 0%; SPN, 100%; PanNEN, 0%; $p < .001$).

DISCUSSION

Although EUS-FNA cytology is currently established as the procedure of choice for diagnosing pancreatic malignancies, detecting ACC on EUS-FNA cytology is a challenge due to its rarity. In this study, we aimed to evaluate the cytomorphologic features of ACC on LBC, and also to identify cytological features that help distinguish ACC from PanNENs and SPNs, which are more commonly encountered in everyday practice.

Although CS was the main preparation method for EUS-FNA specimens from the pancreas, many institutions are currently transitioning to LBC for reasons including less background blood or crushing/drying artifacts, resulting in superior sensitivity, accuracy, and negative predictive value [14,15].

When we compared LBC and CS preparations of ACC cases, ACCs commonly presented with hypercellular smears (100%) with frequent single cells and naked nuclei in the background

(75.0%) on LBC slides. Prominent nucleoli and macronucleoli could be observed in all LBC cases, while they were less discernible on CS preparations. Similarly to a previous study by Chun et al. [6], we also observed more frequent bloody backgrounds in CS (86.0%) than in LBC (13.0%). The bloody background on CS is more likely to obscure the characteristic cytological features of ACC, such as the acinar architecture and prominent nucleoli, leading to a different diagnostic interpretation. Indeed, in our cohort, the two ACC cases that were originally interpreted as adenocarcinoma were CS cases. Other background features that were often observed in ACCs, including necrotic, apoptotic debris, and nuclear tangles, were similarly present on both CS and LBC slides.

Next, we compared the LBC features of ACC, SPN, and PanNEN. Presence of necrosis and apoptotic debris in the background were significant cytological features suggestive of ACC, and nuclear tangles were only observed in ACC. As for the tumor cell architecture, frequent 3-dimensional pseudopapillary structures were only seen in SPNs. In contrast, the presence of frequent acinar structures was significantly more common in ACCs. As observed in one case in our cohort, acinar-like arrangements may be seen in SPNs; however, the characteristic pseudopapillary structures point to a diagnosis of SPN rather than ACC [16,17]. Acinar structures were not observed in PanNENs.

Cytoplasmic granularity was seen in ACC, SPN, and PanNEN; however, the coarse granularity due to zymogen granules could be better appreciated in ACCs due to the more abundant cytoplasm [16,18,19]. Although nucleoli were observed in all three tumor types, the presence of macronucleoli was unique to ACC, as previously reported [3,20]. The chromatin was irregular for all cases of ACC and PanNEN, while fine chromatin was a characteristic of SPN. In sum, we found that a necrotic background with apoptotic debris, nuclear tangles, acinar structure, presence of macronucleoli and irregular chromatin on LBC were significantly associated with ACC.

Other differential diagnoses to consider include benign acinar cells and other neoplastic lesions including intraductal oncocyt-

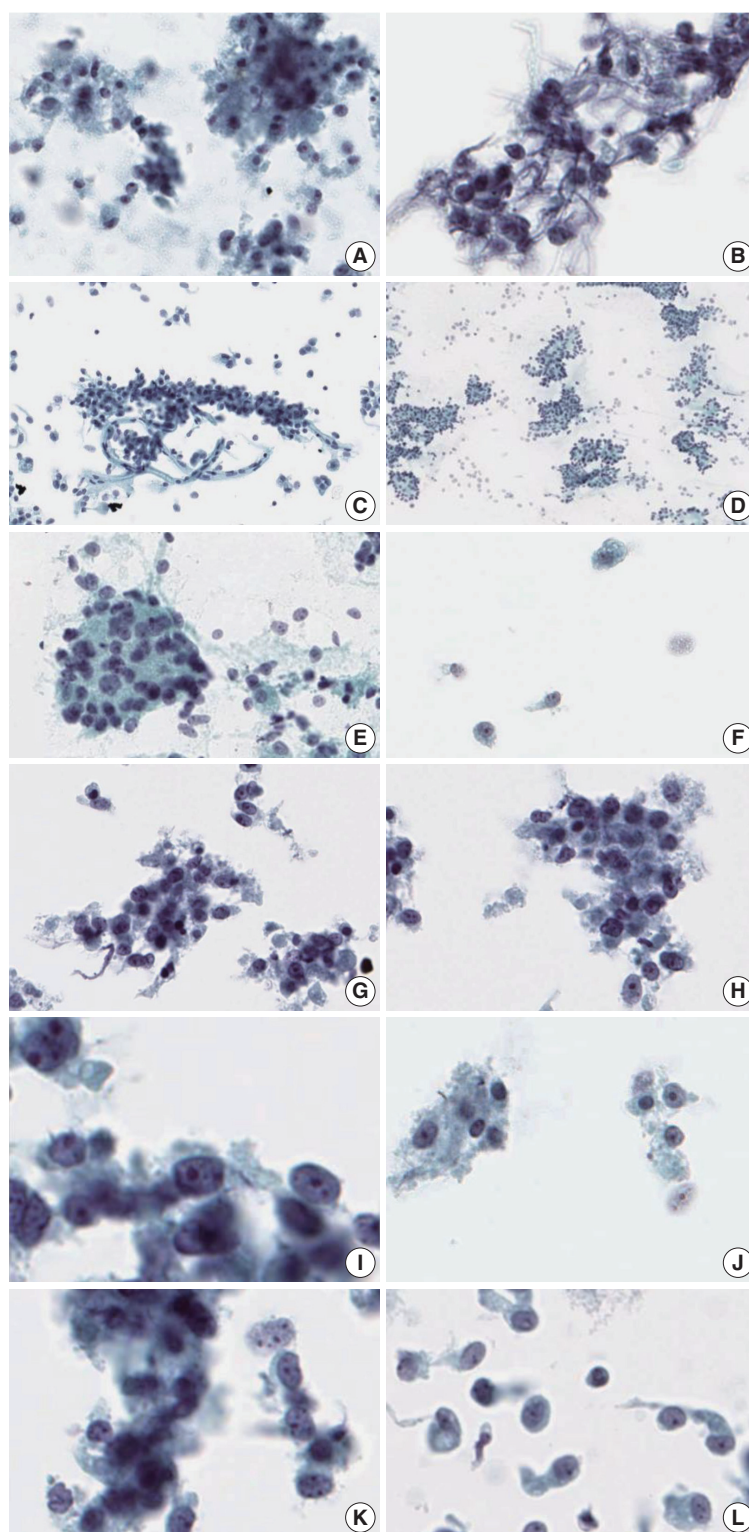


Fig. 2. Comparison of liquid-based cytology features between acinar cell carcinoma (ACC) and solid-pseudopapillary neoplasm (SPN). Liquid-based cytology of ACC demonstrates necrotic debris in the background (A) and nuclear tangles (B). Cell clusters showing three-dimensional pseudopapillary architecture in SPN (C) and the typical acinar architecture of PanNEN (D). Some scattered cells showing singly dispersed naked nuclei (E) with occasional plasmacytoid cells (F) in ACC. Tumor cells of ACC demonstrating ovoid nuclei with mild pleomorphism (G), and irregular nuclear membranes (H). Nucleoli are prominent in ACC, with some macronucleoli (I, J). Chromatin is irregular in ACC (K), compared with the fine chromatin observed in SPN (L).

ic papillary neoplasms or mixed acinar-ductal/neuroendocrine neoplasms. There are situations where reactive acini in pancreatitis could be misinterpreted as acinar cell neoplasia on cytology specimens [12,21]. HooKim et al. [5] demonstrated that the presence of syncytial clusters, prominent cherry red nucleoli and necrosis could be useful clues for the distinction between reactive pancreatic acini and ACC. ACC may show overlapping cytologic features with intraductal oncocytic papillary neoplasm; however, ACC is more mitotically active, and demonstrates a necrotic background [22]. Another differential diagnosis to consider is pancreatic neuroendocrine carcinoma (PanNEC), especially large-cell PanNEC, which is characterized by large cells with relatively abundant eosinophilic cytoplasm, prominent nucleoli, and often a necrotic background. These features overlap with those of ACC, and a definitive diagnosis may not be possible based on cytology without the help of immunocytochemical stains. Lastly, some mixed acinar-ductal or mixed acinar-neuroendocrine neoplasms are often difficult to diagnose accurately on cytology due to limitations such as sampling errors [11,13].

In conclusion, we found that ACC had characteristic cytological features that could be observed on LBC preparations, such as high cellularity, necrotic/apoptotic background, nuclear tangles, acinar arrangement of cells, and macronucleoli. These findings also help distinguish ACC from PanNEN and SPN on LBC. It is important to be familiar with these features, as an accurate diagnosis on EUS-FNA cytology would have impact on the management of the patient.

Ethics Statement

This study was approved by the Institutional Review Board of Seoul National University Hospital (IRB No. H-2402-011-1506), and informed consent was waived due to the retrospective nature of the study.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

Code Availability

Not applicable.

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Conflicts of Interest

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Concurrent intestinal plasmablastic lymphoma and diffuse large B-cell lymphoma with a clonal relationship: a case report and literature review

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Herein, we report a case of plasmablastic lymphoma (PBL) and diffuse large B-cell lymphoma (DLBCL) that occurred concurrently in the large intestine. An 84-year-old female presented with a palpable rectal tumor and ileocecal tumor observed on imaging analyses. Endoscopic biopsy of both lesions revealed lymphomatous round cells. Hartmann's operation and ileocecal resection were performed for regional control. The ileocecal lesion consisted of a proliferation of CD20/CD79a-positive lymphoid cells, indicative of DLBCL. In contrast, the rectal tumor showed proliferation of atypical cells with pleomorphic nuclei and abundant amphophilic cytoplasm, with immunohistochemical findings of CD38/CD79a/MUM1/MYC (+) and CD20/CD3/CD138/PAX5 (–). Tumor cells were positive for Epstein-Barr virus-encoded RNA based on in situ hybridization and MYC rearrangement in fluorescence in situ hybridization analysis. These findings indicated the rectal tumor was most likely a PBL. Sequencing analysis for immunoglobulin heavy variable genes indicated a common B-cell origin of the two sets of lymphoma cells. This case report and literature review provide new insights into PBL tumorigenesis.

Key Words: Plasmablastic lymphoma; Diffuse large B-cell lymphoma; Concurrent lymphoma; Sequencing analysis; Clonal relationship

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Plasmablastic lymphoma (PBL) is a rare histological type of aggressive lymphoma with morphologic and immunophenotypic features of plasmablasts that lacks expression of pan B-cell markers while harboring plasma cell markers such as CD38 and CD138 [1,2]. PBL mostly affects human immunodeficiency virus (HIV)-positive patients but also can occur in patients with other immunodeficient/immunocompetent conditions, including transplant recipients [2]. Epstein-Barr virus (EBV) infection, MYC rearrangement, and MYC protein overexpression are also reportedly associated with PBL [3-5]. However, the mechanisms of tumor evolution remain unclear.

PBL is sometimes detected as a metachronous tumor after treatment for other B-cell lymphomas, including chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) [6], follicular lymphoma [7], and mucosa-associated lymphoid tissue lymphoma [8]. Conversely, PBL concurrent with other lymphomas is extremely rare. Herein, we describe the clinicopathological features of an immunocompetent patient with concurrent intestinal PBL and diffuse large B-cell lymphoma (DLBCL) in whom a clonal relationship was proven. These findings provide insights into the pathogenesis and progression of PBL.

CASE REPORT

An 84-year-old female with no notable past or familial medical history was admitted to our hospital with a palpable tumor in the lower rectum. The patient experienced chronic diarrhea lasting 2 months after aluminum potassium sulfate and tannic acid sclerotherapy for internal hemorrhoids. Colonoscopy revealed a 50-mm-sized ulcerating localized tumor with 75% luminal circumference in the lower rectum. A complete blood count before surgery showed normocytic anemia with a red blood cell

count of $3.54 \times 10^6/\mu\text{L}$, hemoglobin of 10.8 g/dL, and hematocrit of 30.9%. The serum soluble interleukin-2 receptor (sIL-2R) level was 715.7 U/mL. Serum carbohydrate antigen 19-9 and carcinoembryonic antigen levels were within normal limits. Serological test results for HIV were negative. Fluorodeoxyglucose (FDG) positron emission tomography/computed tomography revealed increased FDG uptake from the cecum to the appendix vermiformis as well as the rectal tumor. Secondary endoscopic examination revealed a 20-mm-sized, slightly elevated polypoid lesion in the cecum (appendiceal orifice), and endoscopic biopsy of the ileocecal lesion revealed CD20/CD79a-positive large lymphoid cell proliferation, indicating DLBCL. In contrast, biopsy

specimen of the rectal tumor showed diffuse proliferation of discohesive, pleomorphic cells with immunoreaction of CD20 (–), CD79a (+), and CD3 (–); the diagnosis was “malignant lymphoma, suspected.” For regional tumor control, proctosigmoidectomy (Hartmann’s operation) and ileocecal resection were performed simultaneously.

Grossly, the resected ileocecal specimens showed a 40-mm tumor directly invading and thickening the appendiceal wall (Fig. 1A, B), and the resected rectum exhibited a 55-mm ulcerating localized tumor involving the entire thickness of the rectum (Fig. 2A, B). Both tumors had solid, whitish cut surfaces. Histologically, the ileocecal tumor was comprised of diffusely

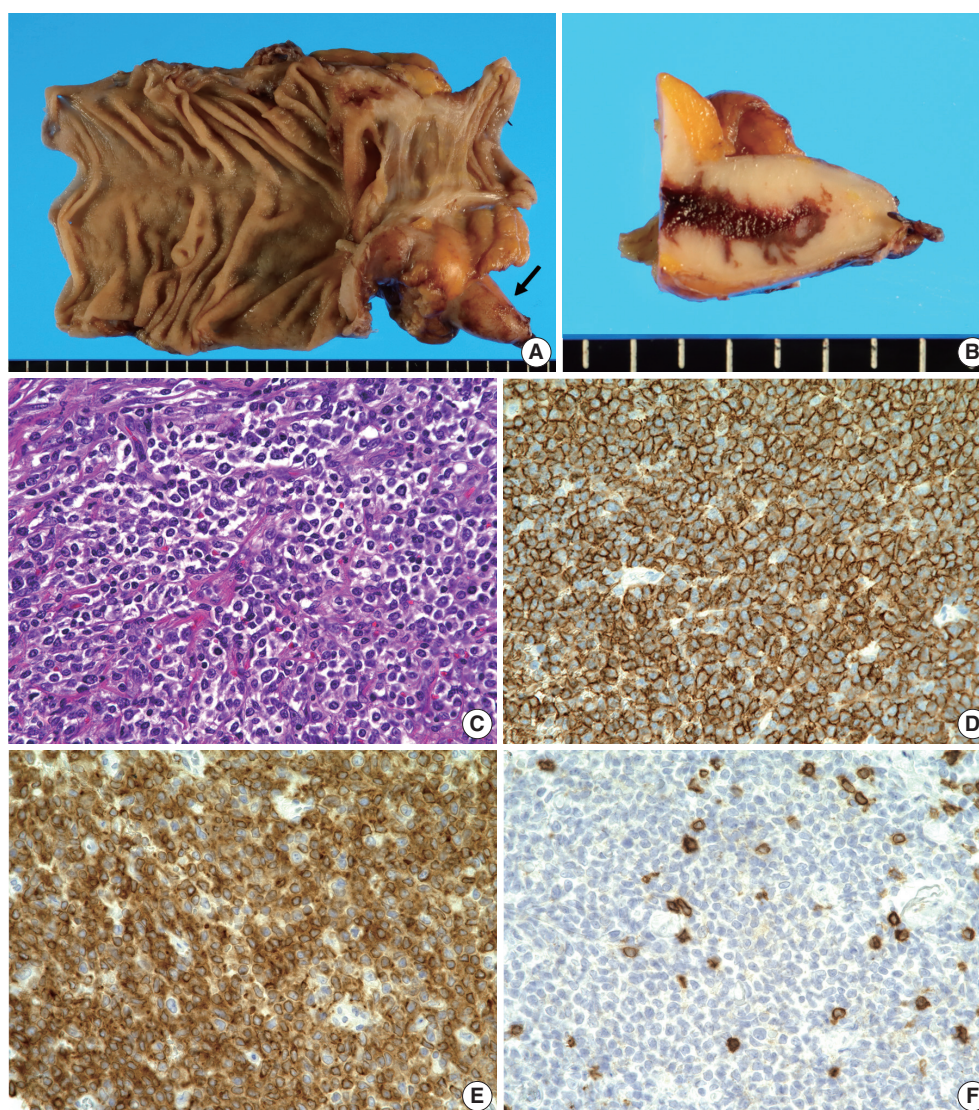


Fig. 1. Gross, microscopic, and immunohistochemical findings of the ileocecal tumor. (A) An ileocecal tumor, 40-mm in size, involving the cecum and appendix vermiformis (arrow). (B) The cut surface of the resected specimen showing a white solid tumor with luminal bleeding. (C) The tumor composed of a diffuse proliferation of medium- to large-sized lymphoid cells with prominent nucleoli. (D–F) The lymphoid cells were diffusely immunoreactive for CD20 (D) and CD79a (E) and negative for CD3 (F).

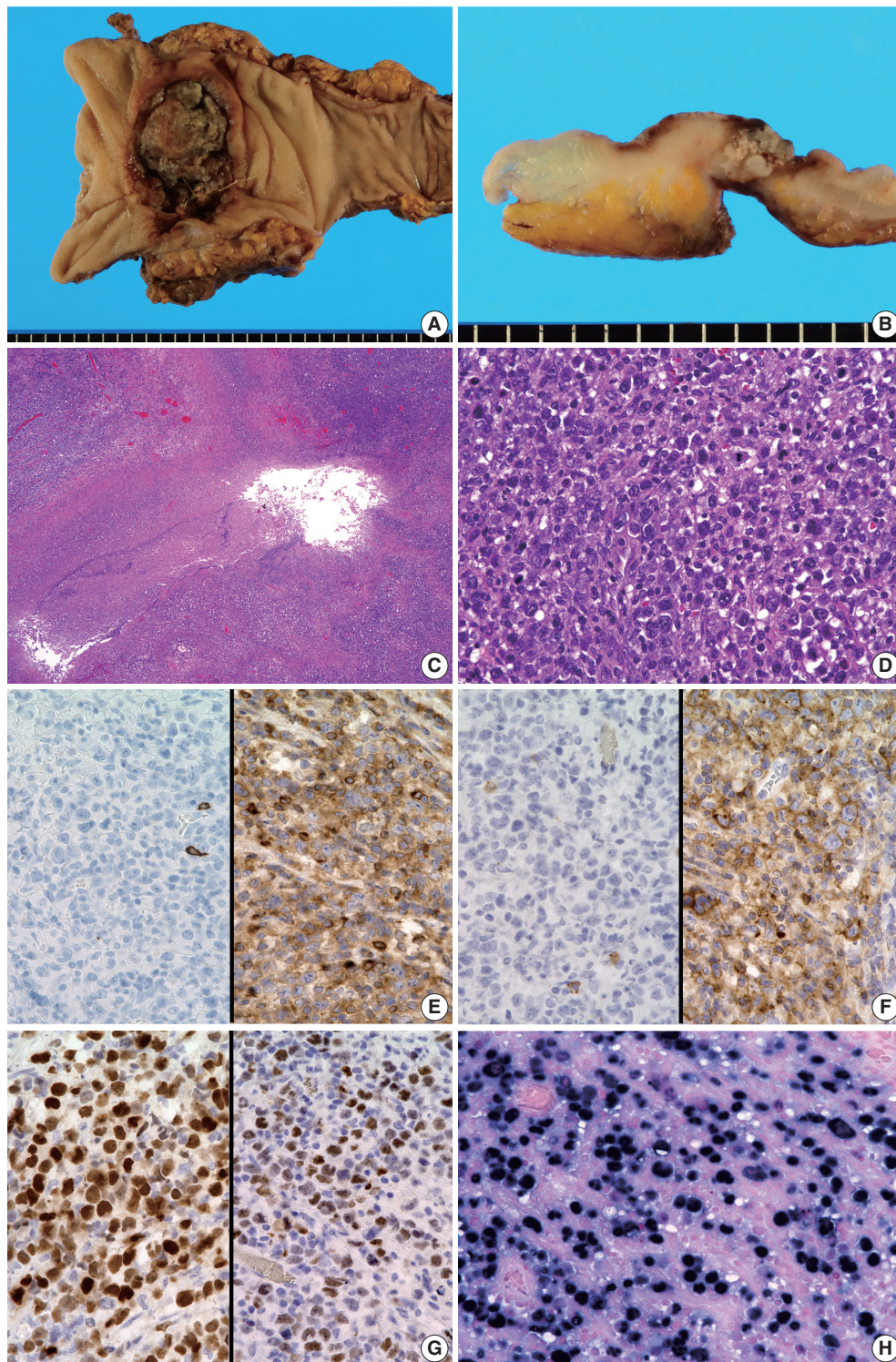


Fig. 2. Gross, microscopic, and immunobiological findings of the rectal tumor. (A) A 55-mm-sized ulcerating localized rectal tumor in the surgically resected specimen (B) The cut surface of the rectal tumor involving the whole rectal wall. (C) The tumor showing a dense cellular infiltration with geographic coagulative necrosis. (D) The tumor consisting of highly atypical lymphoid cells with pleomorphic nuclei and abundant amphophilic cytoplasm. Mitotic figures were frequently observed. (E–G) Immunohistochemically, tumor cells were (E, left) CD20 (–), (E, right) CD79a (+), (F, left) CD138 (–), (F, right) CD38 (+), (G, left) MUM1 (+), and (G, right) MYC (+, >40%). (H) Tumor cells were positive for in situ hybridization of Epstein-Barr virus-encoded small RNA.

proliferating medium- to large-sized lymphoid cells with swollen nuclei containing prominent nucleoli (Fig. 1C). These lymphoid cells were diffusely immunoreactive for CD20, CD79a, BCL2, and BCL6 and negative for CD3, CD10, CD30, CD38, CD138, cyclinD1, MYC, and MUM1 (Fig. 1D–F). A few tumor cells tested positive for EBV-encoded RNA (EBER) on in situ hybridization. These findings indicated a possible diagnosis of ileocecal DLBCL, germinal center B-cell type. In contrast, the rectal tumor showed proliferation of atypical lymphoid cells with pleomorphic nuclei and abundant amphophilic cytoplasm (Fig. 2C, D). Multinucleated, bizarre tumor cells were scattered, and geographic coagulative necrosis was observed. Immunohistochemical staining showed these atypical cells to be positive for CD38, CD30, CD79a (focally), MUM1, and MYC (>40%) and negative for CD45, CD20, CD3, CD4, CD8, CD138, BCL2, BCL6, PAX5, and CD56 (Fig. 2E–G). Most tumor cells were positive for EBER on in situ hybridization (Fig. 2H). Fluorescence in situ hybridization (FISH) for detecting MYC rearrangement was performed using ZytoLight SPEC MYC Dual Color Break Apart Probe (ZytoVision GmbH, Bremerhaven, Germany); the split signal was detected in the rectal tumor cells (Fig. 3A) but not in the cecal tumor cells (Fig. 3B), indicating MYC rearrangement in the rectal tumor but not in the cecal tumor. Based on these data, the rectal tumor was most likely PBL. Regional lymph node biopsy at the time of surgery, bone marrow trephine biopsy, and cerebrospinal fluid cytology revealed no

lymphoma cell involvement.

To confirm pathological diagnosis of PBL and identify the cell of origin of these two lymphomas that concurrently occurred in this patient, DNA was extracted from formalin-fixed paraffin-embedded sections for semi-nested polymerase chain reaction (PCR) and the complementarity-determining region (CDR) 3 in the immunoglobulin heavy variable (IGHV) genes sequenced as previously described [9]. Primer sequences were as follows: upstream consensus V region primer (FR2A), 5'-CCGGAAR RGTCTGGAGTGG-3'; consensus J region primer (LJH, for 1st PCR), 5'-CTTACCTGAGGAGACGGTGACC-3'; and consensus J region primer (VLJH, for 2nd PCR), 5'-GTGAC CAGGGTNCCTTGCCCC-3'. The CDR3 sequences of the ileocecal and rectal tumors matched, indicating that the two emerged from a common clonal B-cell (Fig. 3C). Consequently, a diagnosis of concurrent ileocecal DLBCL and rectal PBL was confirmed.

The patient was treated with six courses of rituximab, cyclophosphamide, doxorubicin hydrochloride, oncovin, and prednisolone (R-CHOP). The sIL-2R level was within the normal range, and recurrence was not observed on clinical and imaging examinations 1 year after surgery.

DISCUSSION

The usual immunophenotype of PBL is the expression of plasmacytic markers including CD138, CD38, and MUM1 and

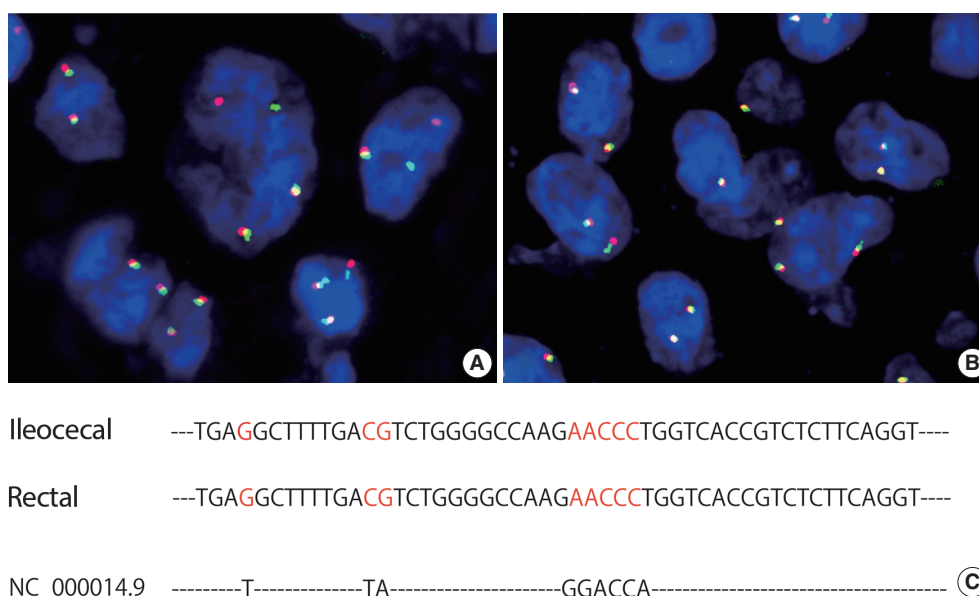


Fig. 3. Fluorescence in situ hybridization and sequencing analyses. (A, B) Split signals indicating MYC rearrangement were detected in tumor cells of the rectal tumor (A) but not in those of the cecal tumor (B). (C) Sequences of the complementarity-determining region 3 (CDR3) region in the immunoglobulin heavy variable genes of the ileocecal tumor and rectal tumor. The sequence of the CDR3 region in the two tumors matched. Genomic alterations from reference (NC_000014.9) are indicated as red letters.

Table 1. Clinicopathological features of previously reported cases with concurrent double lymphoma consisting of PBL and other lymphoma

Case No.	Age (yr)/ Sex	Immunological status	Location of PBL	Type and location of other tumors	Same clonality of lymphomas	Chemoradiotherapy	Immunohistochemical analysis of PBL						Outcome, post diagnosis of PBL	Study
							CD20	CD79a	CD138	CD38	MUM1	MYC	EBER	
1	67/M	Immunocompetent, HIV (-)	Ileocecal valve, left humerus	CLL, bone marrow	N/A	Bortezomib and dexamethasone	-	+	+	+	+	N/A	+	DOD, 2.5 mo Hatzimichael et al. [10]
2	61/M	Immunocompetent, HIV (-), EBV (-), HHV-8 (-)	Lymph node	SLL, the same lymph node as PBL	Detected	Hyper-C-VAD	-	N/A	+	N/A	+	N/A	-	DOD, a couple of months Ronchi et al. [11]
3	48/M	Immunocompetent, HIV (-)	Left supraclavicular area, duodenum	CLL, bone marrow	N/A	R-CHOP, bortezomib, ifosfamide, etoposide, carboplatin, mesna, brentuximab vedotin, and radiation to the neck lesion	N/A	+	+	+	+	N/A	N/A	DOD, N/A Holderness et al. [12]
4	57/F	Postchemotherapy for CLL, HIV (-), CMV IgM (-), CMV IgG (+)	Mandible, bone marrow	CLL, bone marrow	Not detected (different clones)	VAD and CHOP	-	N/A	N/A	N/A	N/A	N/A	N/A	DOD 6 mo Robak et al. [13]
5	69/M	Postchemotherapy for CLL, EBV (+)	Nasopharynx	CLL, bone marrow; cHL, left cervical lymph node	Not detected (CLL and PBL) N/A (cHL and PBL)	R-CHOP and radiation to the nasopharynx	-	-	+	N/A	+	N/A	+	DOD 9 mo Foo et al. [14]
6	37/M	EBV (+)	Urinary bladder	DLBCL, right nasal cavity	Detected	R-CHOP, intrathecal infusions of methotrexate, cytarabine, and hydrocortisone	-	-	+	N/A	N/A	+	N/A	Recurrence of DLBCL, 4 yr Hashimoto et al. [9]
7	84/F	Immunocompetent	Rectum	DLBCL, cecum and appendix vermiformis	Detected	R-CHOP	-	+	-	+	+	+	+	No recurrence, 11 mo Present case

PBL, plasmablastic lymphoma; EBER, Epstein-Barr virus-encoded RNA; M, male; HIV, human immunodeficiency virus; CLL, chronic lymphocytic leukemia; N/A, not available; DOD, death of disease; EBV, Epstein-Barr virus; HHV-8, human herpes virus-8; SLL, small lymphocytic lymphoma; Hyper-C-VAD, cyclophosphamide, vincristine, adriamycin, and dexamethasone; R-CHOP, rituximab, cyclophosphamide, doxorubicin, oncovin, and prednisolone; CMV, cytomegalovirus; VAD, vincristine, adriamycin, and dexamethasone; cHL, classical Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma.

the absence of pan B-cell markers including CD20 and PAX5 [15]. Despite the absence of CD138 expression, morphologically and immunohistochemically, the rectal tumor in the present case was most likely PBL. In addition, *MYC* rearrangement based on FISH analysis was detected only in the rectal PBL. The present case also showed a concurrent ileocecal tumor with features of DLBCL, namely discordant double lymphoma. Sequencing analysis of the CDR3 region in the IGHV genes revealed a common B-cell origin of the tumor cells of the two lymphomas. Based on these findings, the rectal tumor was confidently diagnosed as PBL and showed a clonal relationship with the concurrent ileocecal DLBCL.

The concomitant occurrence of PBL with other types of lymphoma is extremely rare. Review of the English literature revealed only six case reports in which the histopathological findings of two lymphomas were described [9-14]. The clinicopathological features of these previous cases and the present case are summarized in Table 1. Two patients were in the post-chemotherapeutic state (cases 4 and 5), and no patient was infected with HIV. Histological types concurrent with PBL were CLL/SLL in four cases, DLBCL in two cases, and classical Hodgkin lymphoma and CLL/SLL (concurrent triple lymphoma) in one case. Based on sequencing analysis of the CDR3 region in IGHV genes, the same and different clonality between PBL and other-type lymphomas were detected in three and two cases, respectively. Clonal analysis was not performed in two cases. All patients, except the present case, died of the disease or experienced tumor recurrence, indicating a generally poor patient prognosis.

PBLs are assumed to originate from plasmablasts that are precursor plasma cells derived from activated B lymphocytes. Although HIV/EBV infection and *MYC* rearrangement have been indicated in the disease development, the pathogenesis of PBL remains unclear [3-5]. Using whole-exome sequencing and RNA-sequencing analysis for 33 PBLs, Witte et al. [16] described a significant accumulation of the JAK signal transducer mutations and evidence of frequent perturbances of nuclear factor κ B signaling (NFKB2 and BTK), which is distinct from mutational and transcriptomic status of DLBCL and plasmacytic myeloma. Conversely, in the present case, the clonal status of the rectal PBL was the same as that of concurrent ileocecal DLBCL, indicating a developmental relationship of these tumors. Hashimoto et al. [9] reported a case of discordant lymphoma consisting of PBL in the urinary bladder and DLBCL in the nasal cavity and proved the clonality of the two lymphomas using sequencing analysis of the CDR3 region in the IGHV genes. In these two cases, *in situ* hybridization of EBER was diffusely positive in tumor cells of

PBL, and only scattered positive tumor cells were observed in DLBCL. In contrast, in three cases that were analyzed for clonality between concurrent PBL and CLL/SLL, one (case 2) showed the same clonal relationship, while two (cases 4 and 5) harbored different clonal relationships, indicating a case-specific pathogenesis of these tumors [11,13,14]. Gene expression analysis and comprehensive genomic and transcriptomic analyses for each set of tumor cells in such concurrent lymphoma cases would help to elucidate the pathogenesis of PBL.

The treatment of patients with two concurrent lymphomas is generally challenging. R-CHOP was selected for the present case, similar to a previous case of concurrent DLBCL and PBL that showed recurrence of DLBCL after 4 years [9]. Other cases of PBL co-existing with CLL/SLL were also refractory to R-CHOP and/or vincristine, adriamycin, and dexamethasone (VAD) treatment (Table 1, cases 1–5) [10-14]. Basically, standard treatment for PBL has not been established due to the rarity of the disease and its aggressive clinical course. The National Comprehensive Cancer Network guidelines recommend strong regimens such as dose-adjusted etoposide, vincristine, and doxorubicin with bolus doses of cyclophosphamide and prednisone (DA-EPOCH) based on evidence from other aggressive lymphomas [17,18]. In addition, chemotherapy based on bortezomib, a proteasome inhibitor, was effective as frontline treatment for patients with PBL in several case series [19,20]. However, review of the literature indicates that even bortezomib-based regimens are not effective for PBL concurrent with other lymphomas (Table 1, cases 1 and 3) [10,12]. In the present case, intensified chemotherapy, such as bortezomib plus DA-EPOCH, was necessary due to tumor recurrence.

In summary, we described a case of concurrent intestinal DLBCL and PBL in which the same clonality was detected on sequencing analysis. Data accumulation and molecular genetic analysis of such concurrent lymphoma cases are essential to elucidate their pathogenesis and could provide primary evidence for a treatment strategy in this challenging clinical situation.

Ethics Statement

The Institutional Review Board at National Defense Medical College approved publication of this article (Registration number, 4802). Informed consent from the participant has been waived by Institutional Review Board.

Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

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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Tubular adenoma arising in tubular colonic duplication: a case report

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Colonic duplication constitutes a rare congenital anomaly, characterized by the presence of hollow cystic or tubular structures exhibiting an epithelial-lined intestinal wall. Diagnostic challenges persist due to its low incidence and manifestation of nonspecific symptoms such as abdominal pain or constipation, resulting in a reluctance to pursue surgical resection. As associated malignancies in colonic duplication are rare, the inherent malignant potential of these anomalies remains undetermined. Additionally, despite reported instances of associated malignancies in colonic duplication, there is an absence of reports in the literature detailing tubular adenoma within these cases. The histologic features of the presented case are particularly noteworthy, situated at the precancerous stage, intimating potential progression towards adenocarcinoma within colonic duplication.

Key Words: Colon; Congenital abnormalities; Cysts; Adenoma

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Gastrointestinal duplication manifests as a cystic or tubular structure connected to the broader gastrointestinal tract, featuring a standard epithelial lining and smooth muscle layer. This congenital anomaly is rarely observed, with an incidence rate of one in every 4,500 individuals [1]. Although it can arise anywhere along the gastrointestinal tract, the ileum predominates as the most common site [2]. Colonic duplication, constituting 4%–18% of all gastrointestinal duplication cases [3], typically becomes evident in early childhood, with approximately 80% of cases identified before the age of 2 [4]. Clinical presentations of colonic duplication exhibit variability contingent upon the type and location of the duplication. Patients may manifest chronic or acute abdominal pain, constipation, abdominal distension, or detect a palpable mass. The diagnostic challenge inherent in colonic duplication is compounded by its low incidence and presence of nonspecific symptoms, often resulting in diagnostic confusion with other intestinal disorders.

The risk of malignancy arising in colonic duplication remains unclear [5]. However, cases of adenocarcinoma arising in colonic duplication have been reported [6]. Although tubular adenoma is a precancerous lesion leading to adenocarcinoma in the colon, there is a paucity of reports documenting the occurrence of tu-

bular adenoma within colonic duplication in the literature. This case report highlights a rare occurrence of tubular adenoma developing within a colonic duplication in a 40-year-old male.

CASE REPORT

A 40-year-old male patient visited the outpatient clinic for chronic constipation and abdominal pain. He reported a history of difficulty in defecation since childhood and had been hospitalized at the age of 10 for acute abdominal pain. Physical examination and laboratory assessments yielded no abnormalities.

The primary physician considered the possibility of a congenital anomaly or aganglionic megacolon. The patient was subsequently transferred to our hospital for further evaluation. An abdominal computed tomography scan revealed a large, stool-filled tubular structure on the right side (Fig. 1A), suggesting the diagnosis of a colonic duplication cyst communicating with the sigmoid colon. Colonoscopy further identified a bifurcation or outlet connecting to the duplication cyst within the sigmoid colon (Fig. 1B). The colonic duplication had expanded, and the examination revealed a mixture of liquid stool containing a large number of seeds and nuts.

The subsequent laparoscopic exploration identified a tubular colonic duplication measuring approximately 30 cm in length, connected to the sigmoid colon (Fig. 1C). This duplication shared a common blood supply with the adjacent sigmoid colon. A colon segmental resection followed by functional end-to-end anastomosis, facilitated by a linear stapler, was performed. Gross examination revealed a blind-end tubular structure of the colon, measuring 33 cm in length and 20 cm in greatest circumference. A polypoid nodule was observed at the end of the duplication, measuring 0.6 cm in greatest dimension (Fig. 1D). Histological analysis demonstrated the full thickness of colonic structures, with three smooth muscle layers (Fig. 2A). The polypoid nodule exhibited characteristics consistent with a hyperplastic epithelial lesion, displaying hyperchromatic and elongated nuclei indicative of a tubular adenoma with low-grade dysplasia (Fig. 2B). Immunohistochemical staining further revealed increased p53 expression (Fig. 2C). The patient was discharged on postoperative day 5 and remained in good health during the 1-month

follow-up.

DISCUSSION

Colonic duplication can be classified into two types: cystic and tubular duplication. Cystic colonic duplication is the most prevalent, constituting approximately 86% of cases, while tubular colonic duplication represents only 14% of occurrences [2]. Unlike cystic duplication, tubular colonic duplication establishes one or more direct communications with the native tract [7]. Typically originating on the mesenteric side of the bowel, this duplication shares a common blood supply with the adjacent native bowel, as observed in the presented case.

Despite the prevalence of nonspecific symptoms such as abdominal pain or constipation, the persistence of colonic duplication can lead to severe complications. In neonates or infants, intussusception and volvulus are frequently reported in cases of gastrointestinal duplication, occurring at rates of 10.9% and

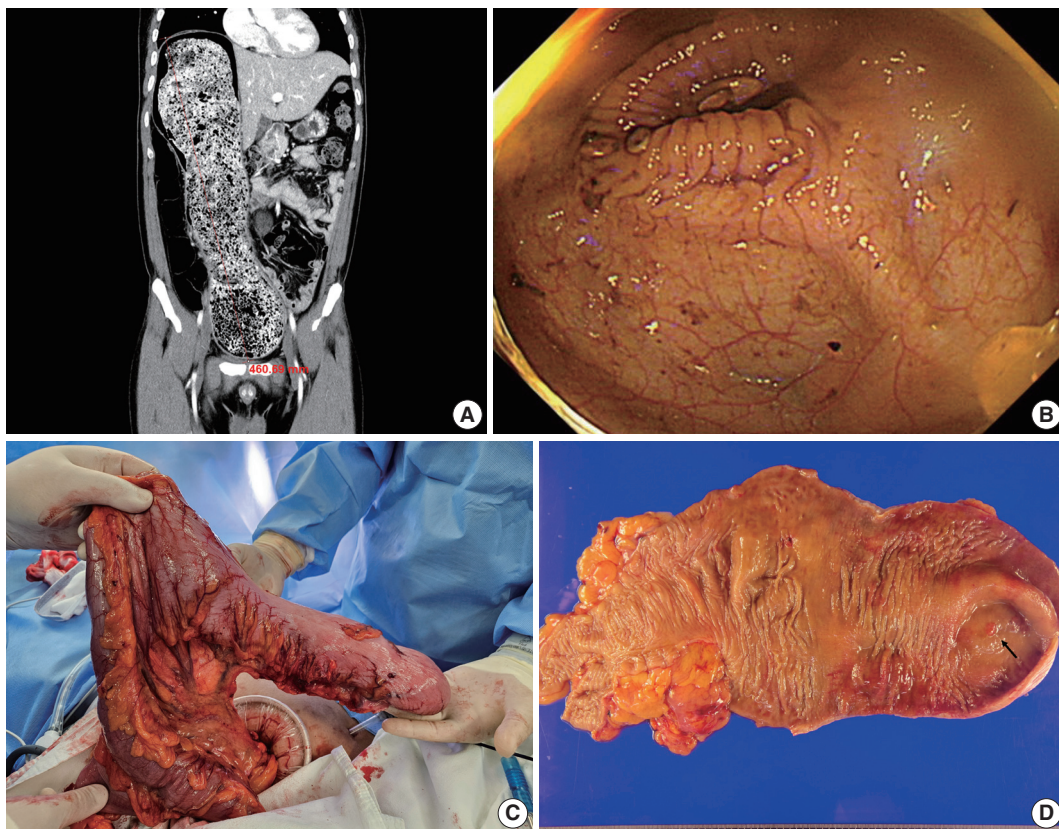


Fig. 1. Radiologic, endoscopic, and gross findings. (A) Abdominal computed tomography shows a large tubular colonic structure, suggesting a duplication cyst connected to the sigmoid colon. (B) Endoscopy image shows a bifurcation leading to the duplication cyst in the sigmoid colon. (C) Intraoperative photograph shows the tubular bowel structure with a dead end, communicating with the sigmoid colon. (D) Gross appearance of tubular colonic duplication. The lesion measures 33 cm in length and 20 cm in greatest circumference. A polypoid nodule at the blind end is highlighted (arrow).

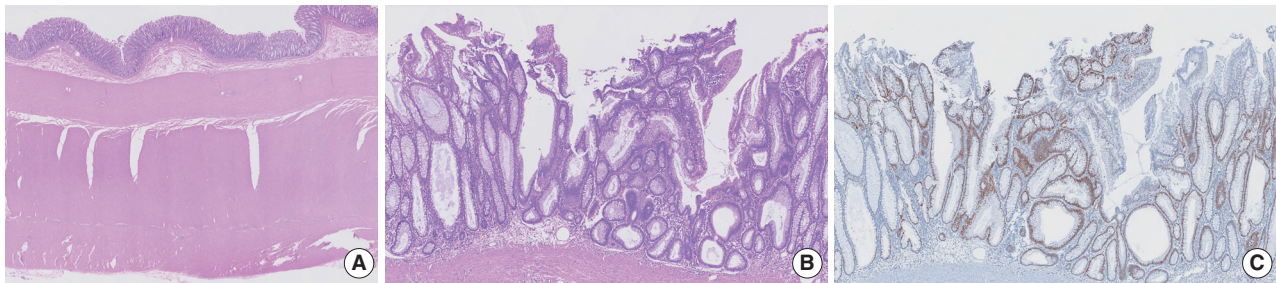


Fig. 2. Microscopic findings. (A) A low-power view of the wall reveals normal colonic epithelial lining and three layers of relatively thickened smooth muscle. (B) A medium-power view of the polypoid nodule shows increased nuclear density with hyperchromasia, consistent with low-grade dysplasia. (C) Dysplastic epithelial cells show increased p53 positivity compared with adjacent normal mucosa.

23.8%, respectively [2]. Some instances of colonic duplication may mimic Crohn's disease, possibly attributed to inflammation or ulceration within the adjacent bowel or the duplication itself [2]. In severe cases, there is a risk of spontaneous bowel perforation due to pressure-induced bowel ischemia [8].

A hypothesis exists suggesting that colonic duplication may harbor malignant potential [5]. These duplications are lined by colonic epithelial cells, and the occurrence of dysplasia or cancerization is plausible, akin to colonic mucosa. Given the tubular adenoma observed in our case and the documented cases of adenocarcinoma arising in colonic duplication [6,9], a comprehensive histological examination is imperative to exclude abnormal epithelial lesions obscured by the colonic duplication and to properly manage the disease.

Colonic duplication in adults is rare and presents diagnostic challenges without surgical intervention. Radiologic evaluations revealing a tubular or cystic structure filled with large stool-like materials communicating with the normal bowel should prompt consideration of colonic duplication. Post-surgery, meticulous gross and histological examinations are warranted, considering the potential presence of associated neoplasms such as tubular adenoma or adenocarcinoma arising within the colonic duplication.

Ethics Statement

Formal written informed consent was not required with a waiver by the appropriate institutional review board (Asan Medical Center IRB No. 2024-0404).

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

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

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What's new in adrenal gland pathology: WHO 5th edition for adrenal cortex

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Abstract

The 5th edition of WHO Classification of Endocrine and Neuroendocrine Tumors (2022) introduced many significant changes relevant to endocrine daily practice. In this newsletter, we summarize the notable changes to the adrenal cortex based on the 5th edition of the WHO classification [1].

NEW CHAPTERS

Adrenal ectopia

- Benign adrenal tissue in an aberrant location (Fig. 1).
- Includes “adrenal rests,” which are aligned with normal embryogenesis (e.g. kidneys or gonads), and “adrenal cortical choristoma,” which are in locations not aligned with embryogenesis.
- The vast majority are incidental findings, contain only adrenocortical cells, and express SF1.
- Rarely, adrenal cortical neoplasms may arise

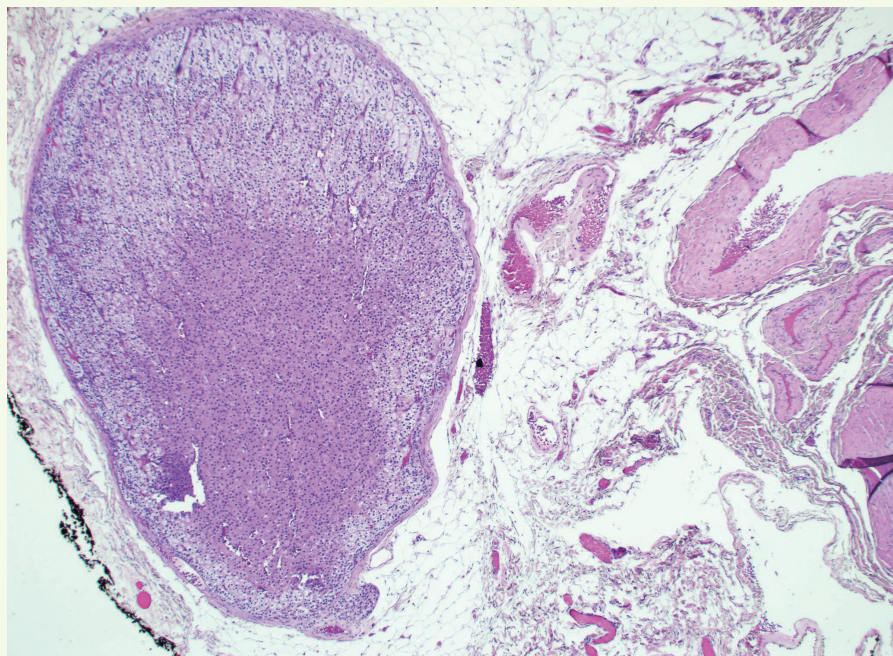


Fig. 1. Adrenal ectopia: benign adrenocortical tissue within the spermatic cord.

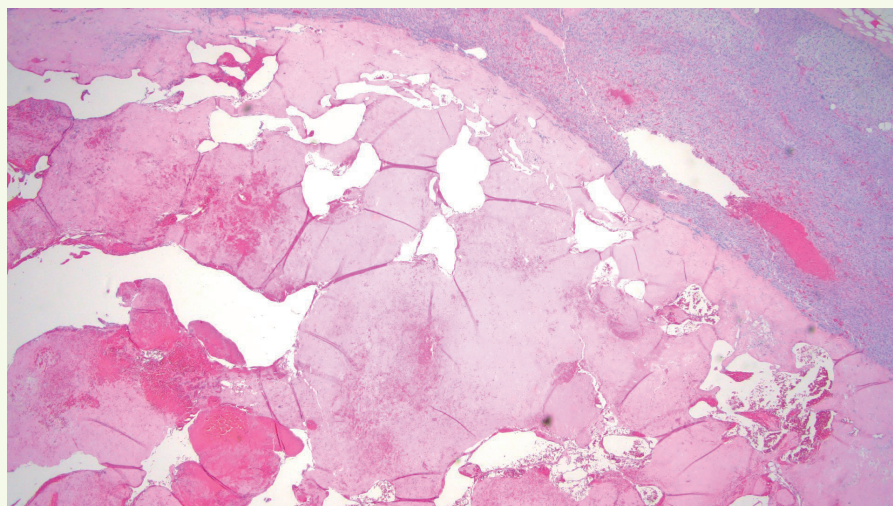


Fig. 2. Adrenal pseudocyst: adrenal cyst without a discrete lining.

from ectopic adrenal tissue.

Adrenal cysts

- Benign, circumscribed, fluid-containing masses, which are divided into 4 subtypes:
 - Pseudocysts: most common (>60% of adrenal cysts), do not have a cell lining, occur following trauma, hemorrhage, or infection including COVID-19 (Fig. 2).
 - Endothelial (vascular) cysts: lined by endothelium, occur as a malformation or as part of recanalization (Fig. 3).
 - Epithelial cysts: lined by mesothelium, occur as an inclusion cyst of embryologic remnants.
 - Parasitic cysts: rare, uni/multi-loculated cysts with a fibrous wall and clear contents.
- A broad differential ought to be considered since cystic changes can occur in adrenocortical adenoma/carcinoma, pheochromocytoma, hemangioma, renal cell carcinoma, metastases, etc.

Myelolipoma

- Benign asymptomatic tumor composed of mature adipocytes and trilineage hematopoiesis.
- Often coexist with adrenocortical nodular disease, hyperplasia, or neoplasms (Fig. 4).

UPDATED CLASSIFICATION OF ADRENAL CORTICAL PROLIFERATIONS

Adrenal cortical hyperplasia

- Cortical zonation is intact as this is a physiologic response, rather than a clonal proliferation.
- Three types of true hyperplasia:
 - Congenital adrenal hyperplasia (CAH): caused by mutations in genes encoding enzymes of steroid production.
 - ACTH/CRH dependent diffuse hyperplasia: caused by Cushing's disease, ectopic secretion, chronic stress.
 - Diffuse zona glomerulosa hyperplasia: idiopathic.

Adrenal cortical nodular disease

- Group of sporadic or germline nodular clonal proliferations:
 - Sporadic nodular adrenocortical disease:
 - Formerly known as "nodular adrenal cortical hyperplasia".
 - <10 mm non-functional adrenocortical nodules.
 - Bilateral micronodular adrenocortical disease:
 - Occurs more commonly in children and young adults (<30 years old).
 - <10 mm bilateral adrenocortical nodules, could be pigmented.

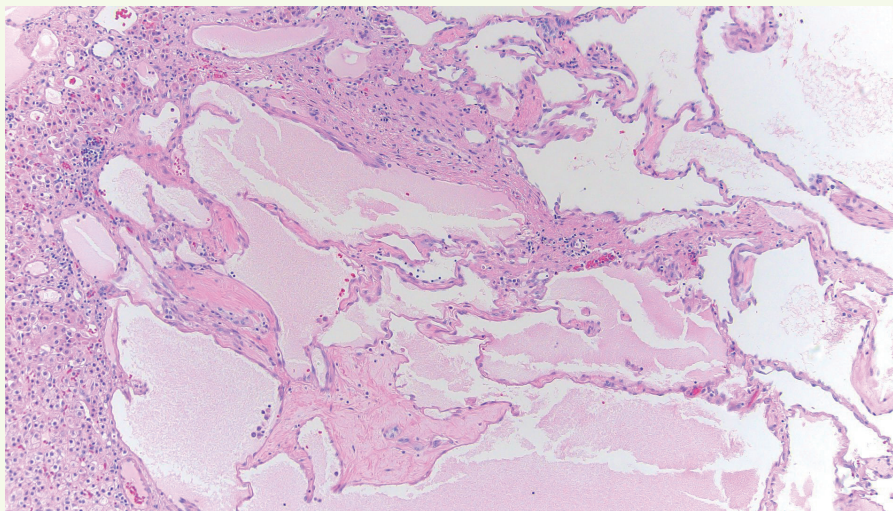


Fig. 3. Adrenal vascular cyst: adrenal cyst with lymphangitic endothelial lining (D2-40 positive).

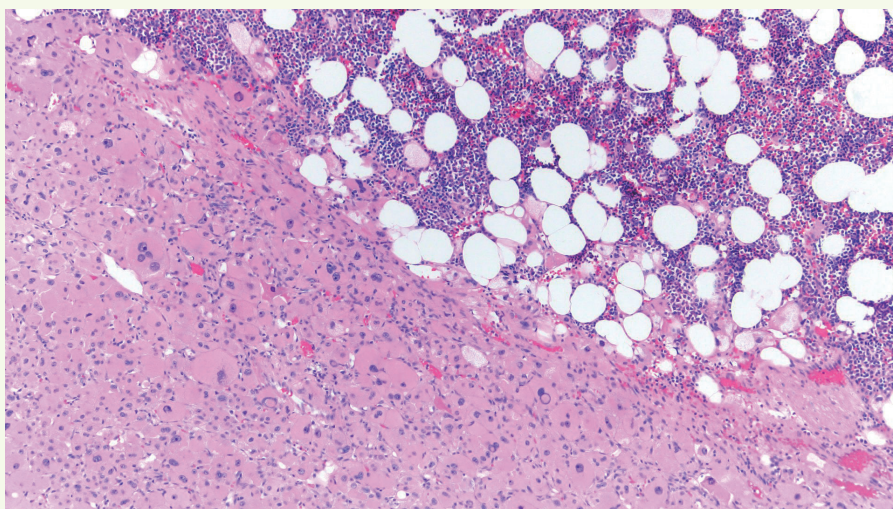


Fig. 4. Myelolipoma coexisting with adrenal cortical carcinoma (oncocyte variant).

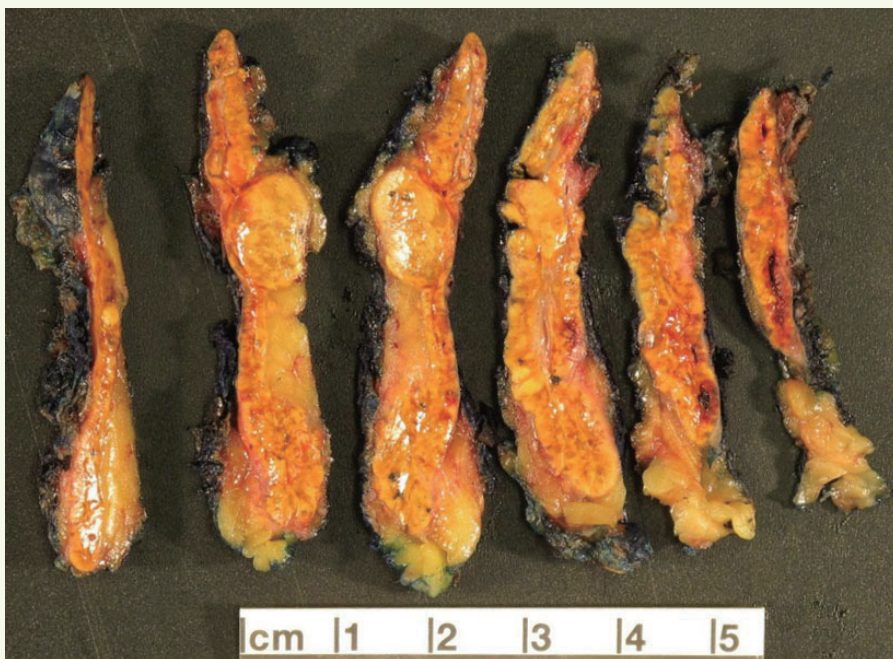


Fig. 5. Bilateral macronodular (>10 mm) adrenocortical disease: representative gross sections.

- Bilateral macronodular adrenocortical disease:
 - More frequent in adults.
 - >10 mm bilateral nodules (Fig. 5).
 - Formerly known as “primary bilateral macronodular adrenal cortical hyperplasia” or “ACTH-independent macronodular adrenocortical hyperplasia”.
- Bilateral micronodular and macronodular forms of adrenocortical nodular disease typically contribute to hypercortisolism and are often associated with germline variants in specific susceptibility genes (*PRKAR1A*, *PRKACA*, *PDE11A*, *PDE8B*, *ARMC5*, *MEN1*, etc).
- The former designation “hyperplasia” is discouraged as it is a physiological reaction

to elevated ACTH levels. The term should not be used to describe multifocal nodules resulting from clonal expansions.

Primary aldosteronism (PA)

- Primary aldosteronism (Conn syndrome) is a leading cause of secondary hypertension characterized by aldosterone overproduction and suppression of the renin-angiotensin system.
- HISTALDO Classification combines CYP11B2 (aldosterone synthase) immunohistochemistry and morphologic features to predict the risk of biochemical recurrence.
- “Classic” histology: 5% recurrence risk
 - Aldosterone-producing adrenal cortical carcinoma (APACC)
 - Aldosterone-producing adrenal cortical

adenoma (APA) (Fig. 6): solitary, > 10 mm, CYP11B2 diffuse reactivity.

- Aldosterone-producing nodule (APN): solitary, < 10 mm, CYP11B2 gradient reactivity, emphasizing increased intensity at the outer part of the nodule.
- “Non-classic” histology: 42% recurrence risk
 - Aldosterone-producing micronodule (APM): < 10 mm, may not be recognized on H&E but is highlighted with CYP11B2 (gradient reactivity).
 - Multifocal aldosterone-producing nodules (APNs).
 - Aldosterone-producing bilateral diffuse hyperplasia (APDH).

Adrenal cortical adenoma

- Adrenocortical neoplasm that lacks morphologic features of malignancy.
- Features worrisome for malignancy: vascular invasion, tumor necrosis, atypical mitotic figures, increased mitotic activity (>5 mitoses per 10 mm²), loss of reticulin framework.
- Usually unilateral and solitary; can be nonfunctional or hormonally active.

CLASSIFICATION OF ADRENAL CORTICAL CARCINOMAS, MAIN SUBTYPES, ANCILLARY STUDIES & GRADING

Adrenal cortical carcinoma

- Malignant adrenocortical neoplasm; can be nonfunctional or hormonally active.
- Adrenal masses associated with virilization or feminization are clinically highly worrisome for malignancy.
- In addition to conventional adrenal cortical carcinoma, three morphologic subtypes exist:
 - Oncocytic: oncocytic cells in >90% of tumor; extensive sampling is required to better quantify the oncocytic component.
 - Myxoid: prominent extracellular mucin deposition; poor prognosis (Fig. 7).
 - Sarcomatoid: resembles sarcomatoid carcinomas of other organs; poor prognosis.
- Vascular invasion is an important diagnostic and prognostic tool in assessment of malignancy. It is assessed at the intersection of tumor and adrenal capsule or beyond the capsule where tumor cells are seen invading through the vessel wall and forming a thrombus/fibrin-tumor complex.
- Many scoring criteria are available for diagnosing adrenal cortical carcinoma and risk stratification; their use depends on morphologic subtype and patient age:
 - Weiss score, modified Weiss, and Helsinki multiparameter scores.
 - Lin-Weiss-Bisceglia system was developed to evaluate oncocytic adrenal cortical

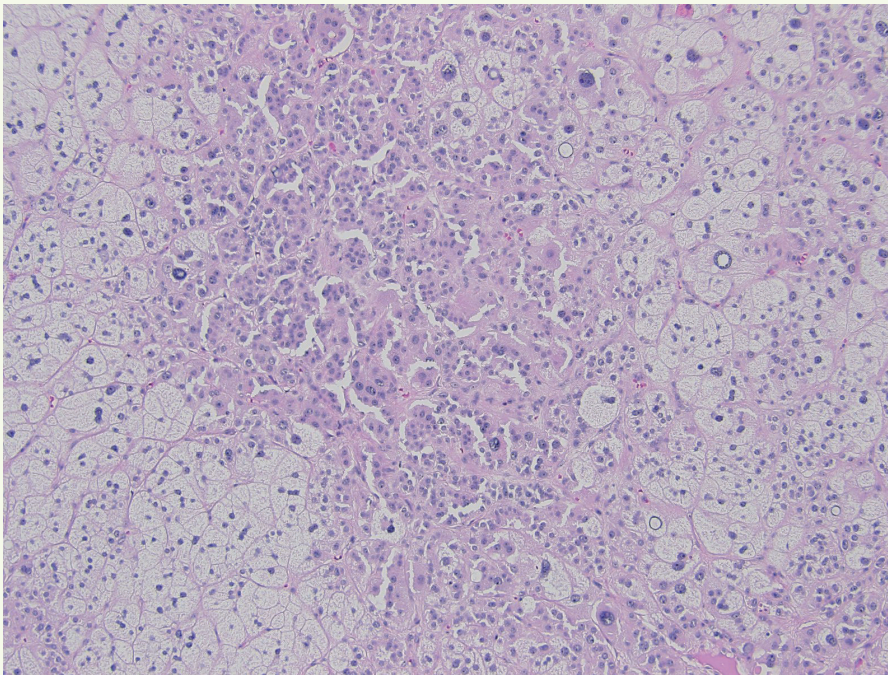


Fig. 6. Functional aldosterone-producing adrenal cortical adenoma with predominantly vacuolated cells and scattered pleomorphism.

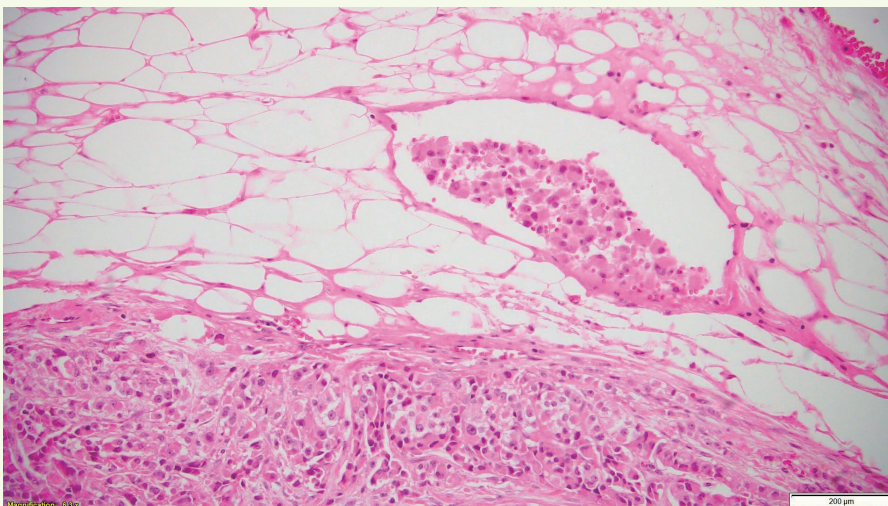


Fig. 7. Adrenal cortical carcinoma (myxoid variant) with lymphovascular invasion.

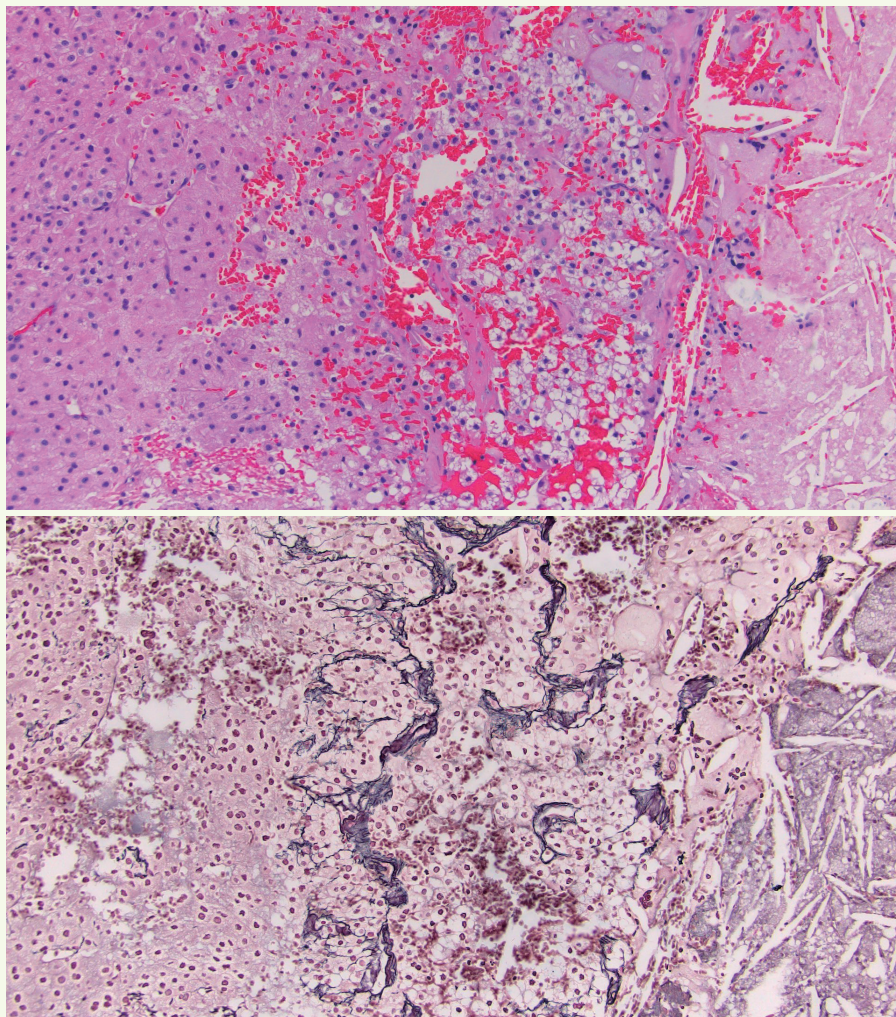


Fig. 8. A: Adrenal cortical carcinoma with tumor necrosis (right of image). B: Adrenal cortical carcinoma with a disrupted reticulin framework.

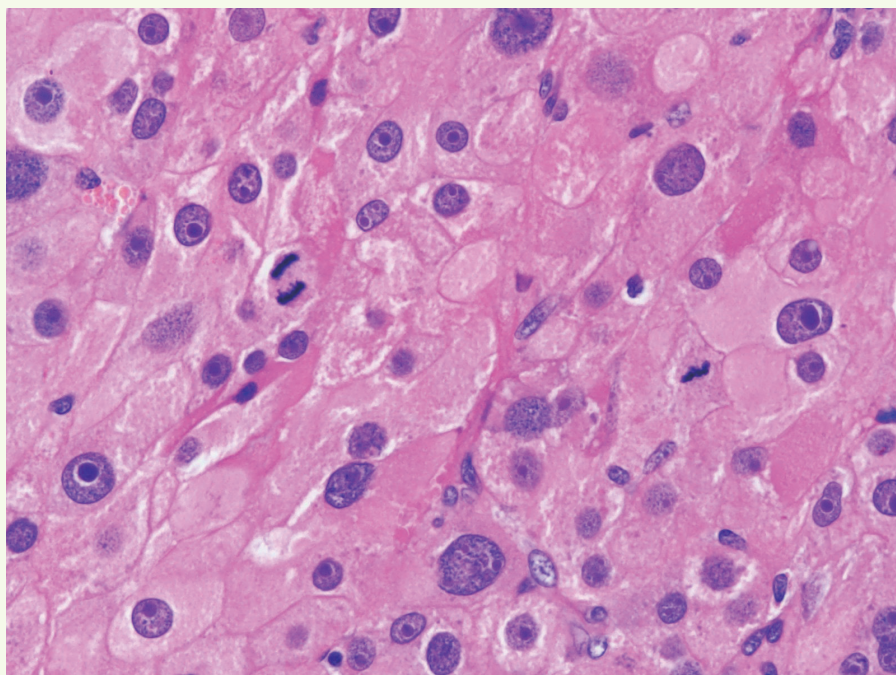


Fig. 9. Adrenal cortical carcinoma with high mitotic activity (2 mitoses in a 60x HPF).

neoplasms.

- Wieneke system is used for assessing pediatric adrenal cortical neoplasms.
- Reticulin algorithm requires an altered reticulin network, in association with any one of the following: increased mitotic rate (>5 mitoses per 50 high-power fields [HPF]), tumor necrosis, or vascular invasion (Fig. 8).
- A simplified approach via the reticulin algorithm has gained popularity given its high reproducibility and applicability to all morphologic subtypes.
- Following use of the reticulin algorithm, a carcinoma is subsequently classified as low-grade or high-grade based on mitotic activity (high grade if >20 mitoses/50 HPF) (Fig. 9).
- Diagnostic and prognostic biomarkers:
 - p53: overexpression or global loss may be identified in high-grade areas.
 - Beta-catenin: nuclear overexpression is a poor prognostic sign.
 - IGF2: used as a diagnostic tool given that paranuclear granular expression is found in $\approx 80\%$ of adrenal cortical carcinomas.
 - Ki67: recommended to specify the Ki67 labeling index for all adrenal cortical carcinomas via manual count or automated image analysis, and to document the methodology used. Carcinomas typically label $>5\%$, and the index matters in terms of prognosis.

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