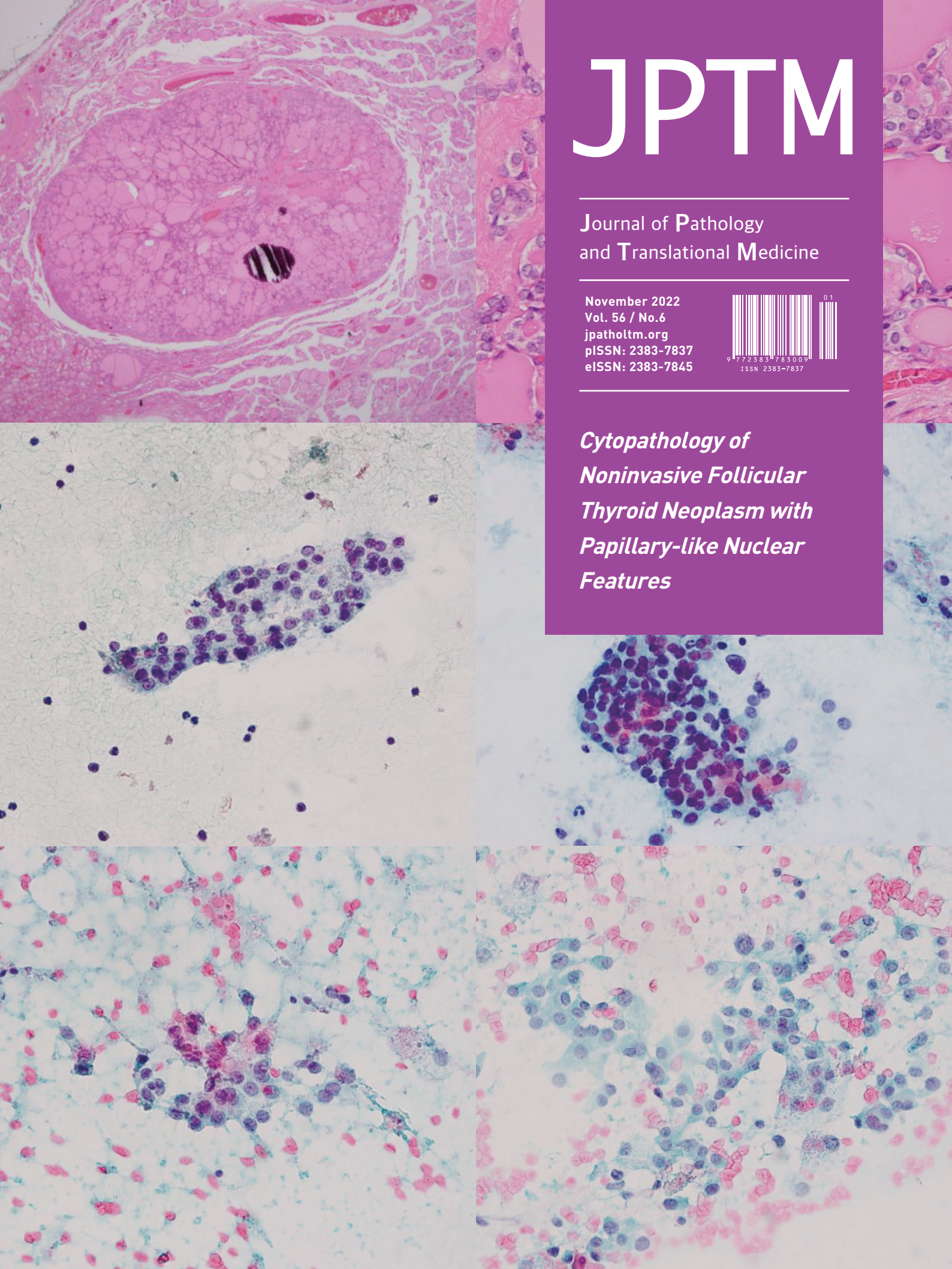


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***Cytopathology of Noninvasive Follicular Thyroid Neoplasm with Papillary-like Nuclear Features***

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Front cover image: Histologic and cytologic features of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (p321).




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REVIEW

# The application of high-throughput proteomics in cytopathology

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High-throughput genomics and transcriptomics are often applied in routine pathology practice to facilitate cancer diagnosis, assess prognosis, and predict response to therapy. However, the proteins rather than nucleic acids are the functional molecules defining the cellular phenotype in health and disease, whereas genomic profiling cannot evaluate processes such as the RNA splicing or post-translational modifications and gene expression does not necessarily correlate with protein expression. Proteomic applications have recently advanced, overcoming the issue of low depth, inconsistency, and suboptimal accuracy, also enabling the use of minimal patient-derived specimens. This review aims to present the recent evidence regarding the use of high-throughput proteomics in both exfoliative and fine-needle aspiration cytology. Most studies used mass spectrometry, as this is associated with high depth, sensitivity, and specificity, and aimed to complement the traditional cytomorphologic diagnosis, in addition to identify novel cancer biomarkers. Examples of diagnostic dilemmas subjected to proteomic analysis included the evaluation of indeterminate thyroid nodules or prediction of lymph node metastasis from thyroid cancer, also the differentiation between benign and malignant serous effusions, pancreatic cancer from autoimmune pancreatitis, non-neoplastic from malignant biliary strictures, and benign from malignant salivary gland tumors. A few cancer biomarkers—related to diverse cancers involving the breast, thyroid, bladder, lung, serous cavities, salivary glands, and bone marrow—were also discovered. Notably, residual liquid-based cytology samples were suitable for satisfactory and reproducible proteomic analysis. Proteomics could become another routine pathology platform in the near future, potentially by using validated multi-omics protocols.

**Key Words:** Cytology; Fine-needle aspiration; Mass spectrometry; Cancer biomarker; Proteomics

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Since next-generation sequencing (NGS) technologies were introduced, sequencing data output significantly increased and brought unprecedented revolution into cancer genomic profiling [1,2]. In addition, the affordable cost of NGS technologies has made their clinical application feasible, as well as their use in the research setting [2,3]. Comprehensive genetic profiling of tumor samples has driven the construction of The Cancer Genome Atlas (TCGA), comprising enormous genomic landscapes across various cancer types. Notably, NGS-based gene panel tests have put genomic sequencing into routine clinical practice as diagnostic tools enabling precision medicine [4]. In addition to surgical pathology, NGS has been extensively used in the field of cytology, utilizing both exfoliative and fine-needle aspiration (FNA) samples [5-9].

However, the number of transcripts does not necessarily correlate with that of the translated proteins, which are the actual functional molecules defining the cellular phenotype in health and disease. Multiple splicing variants could be formed from each transcript during RNA maturation [10-12], while more than 400 different types of post-translational modifications such as acetylation, phosphorylation, glycosylation, methylation, and peptide cleavage might change the properties of the final protein product [12-14]. Furthermore, it may be difficult to define which mutations are the driver and passenger ones while analyzing nucleic acids. All these may limit our understanding of the complexity of cancer and our quest for optimal diagnostic, prognostic, and therapeutic biomarkers, especially when counting solely on data derived from genomics and/or transcriptomics [15]. Thus, the integration of multi-omic approaches, including genomics, epigenomics, transcriptomics, proteomics, and/or metabolomics,

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could combine the strengths of each high-throughput application, enhancing cancer diagnosis, prognosis, and therapy [16,17].

In the past, classic analytical methods to detect proteins struggled due to the structural instability of proteins, which are sensitive to degradation by proteases [12,18]. Proteins cannot be amplified, similar to the nucleic acids via the polymerase chain reaction. Thus, analyzing small amounts of proteins was challenging and a large amount of proteins per sample was needed for quality assurance and successful proteomic analysis [12]. However, since mass spectrometry (MS) has been established as the modern technology of choice for proteomics, it has provided researchers with high depth, improved accuracy, and unbiased quality [15,19]. Recent technological improvements have allowed the analysis of large-scale proteomes and improved the speed of analysis with short turnaround times [19]. Such technical advances have succeeded in the detection of almost entire proteomes in clinical as well as research samples [20,21]. Furthermore, the enhanced sensitivity and specificity of mass spectrometry, enabling the measurement of minute amount of proteins, has allowed the consideration of proteomics application into future routine clinical practice [22,23].

## BASIC PRINCIPLES OF PROTEOMICS

The general aims of proteomic approaches are as follows: (1) identification of specific proteome groups, (2) analysis (e.g., expression levels) of differentially expressed protein signatures from two or more samples, (3) bioinformatic analysis, including the study of protein-protein interactions and gene set enrichment, and (4) study of post-translational modifications in a variety of samples including cell lines, tissue biopsies, and cytology [24,25]. There are two types of proteomic approaches based on the analytic platform used, the protein microarrays and MS-based techniques [26-28]. Regarding the former, there are three types of protein arrays: the analytic microarrays, functional microarrays, and reverse-phase protein microarrays [29]. These arrays have been used to detect differentially expressed protein landscapes, identifying the presence of altered proteins or molecular interactions in certain diseases [30]. However, the restricted number of suitable antibodies needed for such analysis, which could also result in non-specific antigen-antibody interactions, is considered as their main limitation for its use in research or the clinical laboratories [18,28].

During the last years, MS has been significantly improved and emerged as the next generation technology of proteomics, due to its capacity to analyze large-scale proteomes with high sensitivity and specificity [19]. This advanced technique has made protein sequencing possible through three major steps; protein ionization, separation of the ionized analytes based on their own m/z (mass-to-charge) ratio, and detection of the analytes. Finally, the mass spectrum displays the relative abundance of charged analytes vs. their m/z ratios [31,32]. Due to the aforementioned highly accurate and unbiased proteomic analysis through MS, a recent typical proteomic workflow is a mass spectrometry-based one.

## THE HISTORY OF PROTEOMIC APPLICATION IN CYTOLOGY

Since the 2000s, numerous studies have utilized high-throughput proteomics in cytology, most of which have been conducted on breast and thyroid specimens (Table 1). In the early days, the two-dimensional gel electrophoresis (2D-GE) was being used for proteomics analysis [33,34], yet this lacked the reproducibility and accuracy of the newer proteomic applications [18]. In this technique, the proteins are initially separated based on their charge and molecular weight with gel electrophoresis. Subsequently, the areas containing the target proteins are excised from the gel and then identified with MS [35]. Through the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), the cytologic samples are mixed with the substrates, followed by their crystallization within the matrix on a metal plate. Then, the laser energy is absorbed in the matrix generating analyte ions, which are then accelerated into a mass spectrometer [36,37]. In the surface-enhanced laser desorption/ionization time-of-flight mass spectrometry, which is considered as an extended technique of the MALDI-TOF-MS method, the ionized proteins can be directly identified in an electric field by mass spectrometry, without involving protein separation on a 2D gel [38,39]. Over the last decade, electrospray ionization tandem mass spectrometry analysis has become one of the most advanced analytical proteomics methods [40] and has also been applied in cytologic specimens [41].

Regarding breast cancer, most published cytology-based proteomics studies utilized nipple aspirate fluid (NAF), whereas a smaller number FNA samples (Table 1). A few reported significant proteomic profile differences between the NAF of patients with breast cancer compared to non-malignant controls [39,42-44]. In a breast FNA-based study performed by Franzen et al. [45], expression levels of several immune-related proteins differed between cancer and controls, while a few were associated with estrogen receptor, Ki-67 status, and tumor grading. Of interest, liquid-based cytology samples, stored in the methanol-based Pre-

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**Table 1.** Studies utilizing high-throughput proteomics on cytology samples received from various organ sites

| Study | Sample type | No. of samples | High-throughput proteomics approach | Key findings |
|---|---|---|---|---|
| Breast | | | | |
| Pawlik et al. (2006) [62] | NAF | 18 from breast cancer (stages I and II); 4 controls | ICAT LC-MS/MS | Vitamin D binding protein precursor was overexpressed in the NAF of patients with early-stage breast cancer compared to controls |
| Pawlik et al. (2005) [42] | NAF | 23 from breast cancer (stages I and II); 5 controls | SELDI-MS | Significant proteomic profile differences were found in the NAF of patients with early-stage breast cancer compared to controls |
| Sauter et al. (2005) [39] | NAF | 27 from breast cancer; 87 controls | SELDI-MS | Proteomic profile differences were found in the NAF of patients with DCIS compared to controls, and invasive cancer compared to DCIS |
| Alexander et al. (2004) [33] | NAF | 52 from DCIS and invasive cancer; 53 controls | 2D PAGE and MALDI-MS | GCDFP-15 was significantly underexpressed and AAG overexpressed in the breast cancer samples tested |
| Sauter et al. (2002) [43] | NAF | 20 from breast cancer; 13 controls | SELDI-MS | Proteomic profile differences (5 proteins) were found in the NAF of patients with cancer compared to controls |
| George et al. (2021) [44] | NAF | 9 from breast cancer; 4 controls | LC-MS/MS | Proteomic profile differences (40 proteins) were found in the NAF of patients with cancer compared to controls |
| Pavlou et al. (2010) [63] | NAF | 3 from breast cancer; 3 controls | LC-MS/MS | More than 800 proteins were discovered, as part of the NAF proteome |
| Noble et al. (2007) [64] | NAF | Paired samples from 21 patients with breast cancer; paired and unilateral samples from 44 controls | SELDI-MS | Whereas no proteomic profile differences were found in the NAF received from the breast with cancer compared to the contralateral healthy one, significant differences were identified between women with cancer (in both cancerous and healthy breasts) and healthy controls |
| Fowler et al. (2004) [46] | FNA | 24 (benign and malignant lesions) | SELDI-MS | Liquid-based cytology samples, stored in the methanol-based PreservCyt, were suitable for satisfactory and reproducible proteomic analysis |
| Franzen et al. (2019) [45] | FNA | 25 from breast cancer, 32 controls | PEA | Expression levels of several immune-related proteins differed between cancer and controls, while a few were associated with ER, Ki-67 status, and tumor grading |
| Rapkiewicz et al. (2007) [47] | FNA | 63 (50 with cancer) from 21 patients | RPPM | The RPPM technology successfully identified and quantified selected proteins in FNA samples |
| Thyroid | | | | |
| Pagni et al. (2015) [48] | FNA | Samples from 6 patients (3 non-neoplastic, 1 Hurthle cell adenoma, 1 PTC, 1 MTC) | MALDI-MSI | Proteomic profile differences were identified between diverse thyroid lesions sampled with FNA |
| Mainini et al. (2013) [49] | FNA | Samples from 7 patients (non-neoplastic and neoplastic) | MALDI-MSI | In situ proteomic analysis could differentiate between non-neoplastic and malignant lesions, identify PTC, also distinguish PTC cases carrying the *BRAF* V600E mutation |
| Capitoli et al. (2020) [50] | FNA | Samples from 43 patients (non-neoplastic and neoplastic; training and validation cohorts) | MALDI-MSI | In situ proteomic analysis distinguished Hashimoto thyroiditis from hyperplastic nodules and PTC |
| Pagni et al. (2016) [51] | FNA | 36 (13 benign, 10 indeterminate, 13 PTCs) | MALDI-MSI | In situ proteomic analysis distinguished benign thyroid lesions from PTCs and correctly triaged indeterminate FNA lesions as either benign or malignant |
| Giusti et al. (2007) [65] | FNA | 17 suspicious and malignant thyroid lesions | 2D-GE and MALDI-MS | Several proteins were identified, involved in various cell processes (e.g., metabolism, apoptosis, motility) |
| Giusti et al. (2008) [66] | FNA | 13 PTCs | 2D-GE and MALDI-MS | 17 proteins were overexpressed in thyroid cancer patients compared to controls; proteomic profile differences were also identified between classic and tall cell PTC variants |
| Capitoli et al. (2022) [52] | FNA | 240 (internal and external validation cohorts) | MALDI-MSI | Whereas the diagnostic accuracy of the in situ proteomics-based classification model was inferior in the external than internal validation cohort, this was improved when sample cellularity was adequate |
| Ciregia et al. (2016) [67] | FNA | 212 (benign, intermediate, suspicious for malignancy, and malignant) | 2D-GE and LC-ESI-MS/MS | Proteomic profile differences (25 proteins) were found between benign and malignant lesions; ROC curve analysis showed the combination of ENO1, ANXA1, DJ1, SOD, CRNN protein levels had the best discriminatory capacity |
| Ucal et al. (2017) [68] | FNA | 18 (12 PTCs, 6 benign) | LC-MS/MS | Several actin cytoskeleton proteins (e.g., Arp 2/3 complex overexpression) were altered in PTC; IQGAP1 was upregulated in CV-PTC, while IQGAP2 in FV-PTC, at significant levels, respectively |
| Capitoli et al. (2019) [53] | FNA | 28 (benign, intermediate, and malignant; training and validation cohorts) | MALDI-MSI | The in situ proteomics-based model was able to predict the classification derived from the FNA morphologic evaluation of the thyroid lesions |
| Lin et al. (2019) [69] | FNA | 120 PTMCs (60 with LN metastasis, and 60 without) | TMT and LC-MS/MS | ISG15 levels distinguished PTMC patients developing LN metastasis from the ones that did not |

*(Continued to the next page)*

**Table 1.** Continued

| Study | Sample type | No. of samples | High-throughput proteomics approach | Key findings |
|---|---|---|---|---|
| Urine | | | | |
| Park et al. (2020) [70] | Urine (LBC cytology) | 16 (6 NIBUC, 5 SIBUC, and 5 MIBUC) | LC-MS/MS | Proteomic analysis of LBC samples revealed moesin as a biomarker predicting bladder urothelial cancer invasion |
| Yang et al. (2011) [71] | Urine | 54 cancer, and 46 controls | LC-MS/MS | Overexpression of A1AT was associated with the presence of bladder urothelial cancer, at a significant level |
| Theodorescu et al. (2006) [72] | Urine | 655 (non-malignant and malignant) | CE-MS | The model predicted the presence of urothelial cancer in urine samples with high diagnostic accuracy |
| Lee et al. (2018) [73] | Urine (LBC cytology) | 20 (10 bladder cancer; 10 controls) | LC-MS/MS | Proteomic analysis revealed AHNAK as a biomarker differentiating bladder cancer from controls in LBC cytology samples |
| Pap test | | | | |
| Schwamborn et al. (2011) [54] | Pap test | 32 (18 with LSIL or higher; 14 NILM) | MALDI-MSI | In situ proteomics analysis was able to correctly assign most lesions into their original cytologic classification group |
| Boylan et al. (2014) [74] | Pap test | 100, all with normal cytology | 1D PAGE and LC-MS/MS | The core proteome of normal Pap test, comprising 153 proteins, was created by proteomics analysis of residual LBC samples |
| Boylan et al. (2021) [75] | Pap test | One patient with serous ovarian cancer | LC/MS/MS | LBC is suitable for high-throughput proteomic analysis to identify ovarian cancer biomarkers |
| Effusions | | | | |
| Schwamborn et al. (2019) [55] | Pleural and peritoneal effusions | 24 with serous ovarian cancer, 19 with non-ovarian cancers | MALDI-MSI | In situ proteomic analysis was able to differentiate among diverse cancer types in effusions |
| Perzanowska et al. (2018) [56] | Pleural effusion | 69 malignant, 49 benign (controls) | LC/MRM-MS | Multiplex proteomic analysis was able to differentiate between benign and malignant effusions, besides among lung cancer histologic subtypes (SCC, AC, SqCC) |
| Li et al. (2016) [57] | Pleural effusion | 83 malignant (lung ACs), 60 benign (training and validation cohorts) | MALDI-MS | The model was able to differentiate between benign and malignant effusions with high diagnostic accuracy; CARD9 was downregulated in malignant effusions |
| Liu et al. (2015) [76] | Pleural effusion | 405 malignant and benign effusions (discovery and validation cohorts) | 1D-PAGE and LC-MS/MS | Overexpression of MET, DPP4, and PTPRF identified metastatic lung adenocarcinomas in effusion samples with high diagnostic accuracy |
| Li et al. (2015) [77] | Pleural effusion | 6 (3 NSCLC, 3 TB) | 1D-PAGE and LC/MS/MS | Proteomic analysis was able to differentiate NSCLC from TB effusions; IL1A was overexpressed in NSCLC compared to TB effusions |
| Hegmans et al. (2009) [78] | Pleural effusion | 89 (mesothelioma, metastatic carcinoma, benign effusions) | SELDI-MS | SMRP was identified as a diagnostic biomarker of mesothelioma in pleural effusions |
| Pancreatobiliary | | | | |
| Inoue et al. (2022) [58] | EUS-FNA | 40 PDAC, 6 AIP | LC-MS/MS | Expression of several EV proteins differed between PDAC and AIP patients |
| Lee et al. (2012) [59] | EUS-FNA | 5 BD-IPMNs, 5 inflammatory cysts | Cytokine microarray | HGF and GM-CSF differentiated inflammatory cysts from BD-IPMNs |
| Navaneethan et al. (2015) [60] | Bile | 24 (PDAC, CCA, PSC, other non-neoplastic) | SDS-PAGE and LC-MS/MS | Expression of several proteins differed between malignant and non-neoplastic biliary strictures |
| Salivary | | | | |
| Seccia et al. (2020) [61] | FNA | 20 MSGTs, 37 PAs, 14 WTs | 2D-GE and LC-ESI-MS/MS | Overexpression of 4 proteins (annexin-5, cofilin-1, peptidyl-prolyl-cis–trans-isomerase-A, and F-actin-capping-alpha-1) differentiated MSGTs from benign aspirates |
| Bone marrow | | | | |
| Chen et al. (2021) [41] | Bone marrow aspirate | 5 RRMM, 5 NDMM | TMT-MS/MS | Overexpression of the biomarker SERPINB9 was found in RRMM, compared to NDMM |

NAF, nipple aspirate fluid; ICAT, isotope-coded affinity tag; LC-MS/MS, liquid chromatography-tandem mass spectrometry; SELDI-MS, surface-enhanced laser desorption/ionization-mass spectrometry; DCIS, ductal carcinoma in situ; 2D PAGE, two-dimensional polyacrylamide gel electrophoresis; MALDI, matrix-assisted laser desorption ionization; GCDFP, gross cystic disease fluid protein; AAG, alpha1-acid glycoprotein; FNA, fine-needle aspiration; PEA, proximity extension assay; ER, estrogen receptor; RPPM, reverse-phase protein microarrays; PTC, papillary thyroid carcinoma; MTC, medullary thyroid carcinoma; MSI, mass spectrometry imaging; 2D-GE, two-dimensional gel electrophoresis; LC-ESI-MS/MS, liquid chromatography electrospray ionization tandem mass spectrometry; ROC, receiver operating characteristic; ENO1, enolase 1; ANXAI, annexin A1; DJ1, protein DJ-1; SOD, superoxide dismutase; CV-PTC, classic variant PTC; FV-PTC, follicular variant PTC; PTMC, papillary thyroid microcarcinoma; LN, lymph node; TMT, tandem mass tags; ISG15, interferon-stimulated gene 15 protein; LBC, liquid-based cytology; NIBUC, non-invasive bladder urothelial carcinoma; SIBUC, stromal-invasive bladder urothelial carcinoma; MIBUC, muscle-invasive bladder urothelial carcinoma; A1AT, alpha 1 antitrypsin; CE-MS, capillary electrophoresis coupled to mass spectrometry; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesion or malignancy; Pap, Papanicolaou; MRM, multiple reaction monitoring; SCC, small cell carcinoma; AC, adenocarcinoma; SqCC, squamous cell carcinoma; CARD9, caspase recruitment domain family member 9; DPP4, dipeptidyl peptidase-4; PTPRF, protein tyrosine phosphatase receptor type F; NSCLC, non–small cell lung cancer; TB, tuberculosis; SMRP, soluble mesothelin-related protein; EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; PDAC, pancreatic adenocarcinoma; AIP, autoimmune pancreatitis; EV, extracellular vesicles; BD-IPMNs, branch duct intraductal papillary mucinous neoplasms; HGF, hepatocyte growth factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; CCA, cholangiocarcinoma, PSC, primary sclerosing cholangitis; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; MSGTs, malignant salivary gland tumors; PAs, pleomorphic adenomas; WTs, Warthin tumors; RRMM, recurrent and relapsed multiple myeloma; NDMM, newly diagnosed multiple myeloma; TMT-MS, tandem mass tag-mass spectrometry.


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servCyt, were suitable for satisfactory and reproducible proteomic analysis [46], whereas the reverse-phase protein microarrays technology was also applied successfully in breast FNA-based material [47].

To complement the morphologic evaluation of FNA in the evaluation of thyroid lesions, especially the ones with indeterminate interpretations, a few studies utilized in situ proteomics, more specifically the MALDI–mass spectrometry imaging (MSI) technique [48-53]. For instance, MALDI-MSI distinguished benign thyroid lesions from papillary thyroid carcinomas (PTCs) and correctly triaged indeterminate FNA lesions as either benign or malignant [51], while it also distinguished Hashimoto thyroiditis from hyperplastic nodules and PTC in another study [50]. Notably, except differentiating between non-neoplastic lesions from PTC, MALDI-MSI was also able to identify PTC cases carrying the *BRAF* V600E mutation [49]. Furthermore, Schwamborn et al. applied MALDI-MSI aiming to facilitate Papanicolaou (Pap) test and serous effusion cytologic diagnoses; in situ proteomics was able to correctly assign most lesions into their original cervical cytology classification group and differentiate among diverse cancer types in serous effusions, respectively [54,55].

Apart from breast and thyroid cytology, high-throughput proteomics have additionally been applied in urine cytology, Pap tests, serous effusions, pancreatobiliary samples, salivary FNAs, and bone marrow aspirates (Table 1) with the goal to either improve morphologic diagnosis or identify novel cancer biomarkers. Diagnostic dilemmas in cytology subjected to proteomic analysis have been the differentiation between benign and malignant serous effusions [56,57], pancreatic cancer from autoimmune pancreatitis in FNAs of solid pancreatic lesions [58], inflammatory pancreatic cysts from branch duct intraductal papillary mucinous neoplasms while evaluating cystic pancreatic lesions (BD-IPMNs) [59], non-neoplastic from malignant biliary strictures [60], and benign from malignant salivary gland FNAs [61].

## BIOMARKERS DISCOVERED USING CYTOLOGY SPECIMENS THROUGH HIGH-THROUGHPUT PROTEOMICS

Fig. 1 gives a general proteomic workflow used to discover a successful cancer biomarker with cytologic specimens. With the recent advances of MS-based proteomics, even small protein amounts are detectable, while the discovery of biomarker candidates via proteomics has been presented in several studies using cytologic material (Table 2).

Regarding breast cancer, NAF has mainly been used to identify potential breast cancer biomarkers, besides suggesting several proteomic profiles that might have value in assessing the risk of breast cancer (Tables 1, 2). Alexander et al. [33] identified 41 different proteins through 2D-GE and MALDI-MS and suggested two candidate biomarkers, gross cystic disease fluid protein (GCDFP)-15 and alpha1-acid glycoprotein (AAG), testing 52 NAFs from breast cancer patients (in situ and invasive) and 53 controls. GCDFP-15 was found significantly underexpressed, whereas AAG overexpressed in the breast cancer samples [33]. In another study, Pawlik et al. [62] reported that vitamin D binding

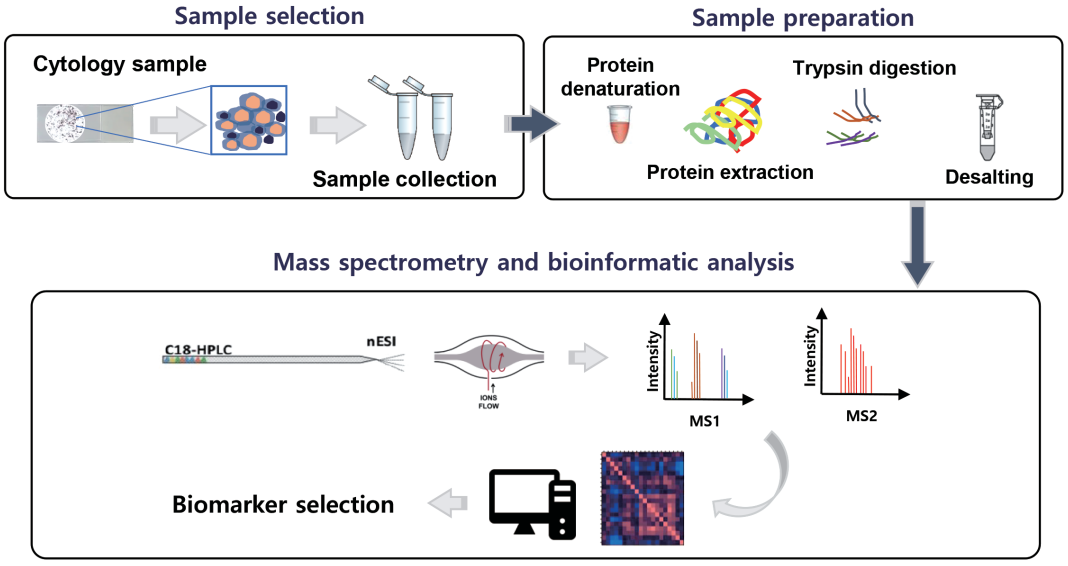


**Fig. 1.** Example of a proteomic analysis workflow utilizing cytology specimens. HPLC, high-performance liquid chromatography; ESI, electrospray ionization.

protein precursor was overexpressed in the NAF of patients with early-stage breast cancer compared to controls.

Thyroid FNAs have often been the subject of proteomics investigation with the goal to solve common diagnostic problems of thyroid cytopathology, for instance the presence of indeterminate thyroid nodules, avoiding unnecessary surgeries (Tables 1, 2). In general, three types of proteomics-based studies using thyroid FNAs have so far been published, aiming to (1) distinguish thyroid cancer from other thyroid lesions [51,53,67], (2) predict lymph node metastasis [69], and (3) predict different PTC variants, currently identified by their histologic characteristics only [66,79]. For example, in a study by Giusti et al. [66], the protein profiles of PTC included several upregulated proteins including transthyretin, ferritin light chain, proteasome activator complex subunit 1 and 2, alpha-1-antitrypsin precursor, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase chain B, apolipoprotein A1 precursor, annexin A1, DJ-1 protein, and cofilin-1. Ucal et al. [68] reported that several actin cytoskeleton proteins (e.g., Arp 2/3 complex overexpression) were altered in PTC, while IQ motif containing GTPase activating protein 1 (IQGAP1) was upregulated in the classic and IQGAP2 in the follicular variant of PTC, at significant levels, respectively. Torres-Cabala et al. [80] also identified a few thyroid cancer-specific spots using 2D-GE and validated their findings by performing immunocytochemistry on thyroid FNAs, identifying galectin-1, galectin-3, S100C, and voltage-dependent anion channel 1 as candidate tumor biomarkers. Notably, authors in another study—utilizing quantitative proteomics with the quest to identify biomarkers predicting lymph node metastasis—identified 3,793 protein groups, while the interferon-stimulated gene 15 protein was finally selected as a potential biomarker related to lymph node metastasis. Authors also suggested that differentially expressed proteins obtained from cytology samples could be important datasets for the development of new biomarkers [69].

Along with FNA cytology, there have been a few published studies where high-throughput proteomics were utilized on exfoliative cytologic specimens, such as Pap tests [74], serous effusions [57,76,77], bile [60], and urine cytology [70,73]. Boylan et al. [74] showed the residual liquid-based Pap test cytology fixative (SurePath) is a suitable source of protein for MS-based proteomics,

**Table 2.** Examples of novel cancer biomarkers discovered by utilizing high-throughput proteomics on cytology samples

| Study | Cancer type/sample type | Novel biomarker(s) | Expression status in cancer |
|---|---|---|---|
| Pawlik et al. (2006) [62] | Breast/NAF | Vitamin D-binding protein precursor | Vitamin D-binding protein precursor: ↑ in breast cancer |
| Alexander et al. (2004) [33] | Breast/NAF | GCDFP-15, AAG | AAG: ↑ in breast cancer<br>GCDFP-15: ↓ in breast cancer |
| Ciregia et al. (2016) [67] | Thyroid/Thyroid FNA, serum, saliva | ANXA1 | ANXA1: ↑ in thyroid cancer |
| Ucal et al. (2017) [68] | Thyroid/FNA | IQGAP1, IQGAP2 | IQGAP1: ↑ in CV-PTC<br>IQGAP2: ↑ in FV-PTC |
| Lin et al. (2019) [69] | Thyroid/FNA | ISG15 | ISG15: ↑ in PTMC patients with metastasis to cervical lymph nodes (prognostic biomarker) |
| Giusti et al. (2008) [66] | Thyroid/FNA | TTR, FLC, proteasome activator complex subunit 1 and 2, alpha-1-antitrypsin precursor, GAPDH, LDH-B, Apo-A1, annexin A1, DJ-1 protein and cofilin-1 | TTR, FLC, proteasome activator complex subunit 1 and 2, alpha-1-antitrypsin precursor, GAPDH, LDH-B, Apo-A1, annexin A1, DJ-1 protein and cofilin-1: ↑ in PTC |
| Park et al. (2020) [70] | Bladder/Urine | Moesin | Moesin: ↑ in invasive bladder cancer |
| Yang et al. (2011) [71] | Bladder/Urine | A1AT | A1AT: ↑ in bladder cancer |
| Lee et al. (2018) [73] | Bladder/Urine | AHNAK | AHNAK: ↑ in bladder cancer |
| Li et al. (2016) [57] | Lung/Effusions | CARD9 | CARD9: ↓ in malignant effusions |
| Liu et al. (2015) [76] | Lung/Effusions | MET, DPP4, and PTPRF | MET, DPP4, and PTPRF: ↑ in malignant effusions |
| Li et al. (2015) [77] | Lung/Effusions | IL1A | IL1A: ↑ in malignant effusions |
| Hegmans et al. (2009) [78] | Mesothelioma/Effusions | SMRP | SMRP: ↑ in mesothelioma |
| Seccia et al. (2020) [61] | MSGTs/FNA | Annexin-5, cofilin-1, peptidyl-prolyl-cis–trans-isomerase-A and F-actin-capping-alpha-1 | Annexin-5, cofilin-1, peptidyl-prolyl-cis–trans-isomerase-A and F-actin-capping-alpha-1: ↑ in MSGTs |
| Chen et al. (2021) [41] | MM/Bone marrow aspirate | SERPINB9 | SERPINB9: ↑ in RRMM (prognostic biomarker) |

NAF, nipple aspirate fluid; GCDFP, gross cystic disease fluid protein; AAG, alpha1-acid glycoprotein; FNA, fine-needle aspiration; ANXA1, annexin A1; IQGAP1, IQ motif containing GTPase activating protein 1; CV-PTC, classic variant PTC; FV-PTC, follicular variant PTC; ISG15, interferon-stimulated gene 15 protein; PTMC, papillary thyroid microcarcinoma; TTR, transthyretin; FLC, ferritin light chain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LDH-B, lactate dehydrogenase chain B; Apo-A1, apolipoprotein A1 precursor; A1AT, alpha 1 antitrypsin; CARD9, caspase recruitment domain family member 9; DPP4, dipeptidyl peptidase-4; PTPRF, protein tyrosine phosphatase receptor type F; IL1A, interleukin 1A; SMRP, soluble mesothelin-related protein; MSGTs, malignant salivary gland tumors; MM, multiple myeloma; RRMM, recurrent and relapsed multiple myeloma.


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reporting the proteome of normal cervical cytology, which was composed of 153 proteins. Regarding serous effusions, caspase recruitment domain family member 9 was found downregulated in malignant effusions [57], overexpression of MET, dipeptidyl peptidase-4, and protein tyrosine phosphatase receptor type F identified metastatic lung adenocarcinomas [76], interleukin 1A was overexpressed in non–small cell lung cancer compared to tuberculosis effusions [77], and serum soluble mesothelin-related protein was identified as a diagnostic biomarker of mesothelioma in pleural effusions [78]. Notably, hepatocyte growth factor and granulocyte-macrophage colony-stimulating factor differentiated inflammatory cysts from BD-IPMNs [59], whereas the overexpression of four proteins (annexin-5, cofilin-1, peptidyl-prolyl-cis–trans-isomerase-A, and F-actin-capping-alpha-1) differentiated malignant from benign salivary gland FNAs [61].

In two recent studies, our group applied MS-based proteomics on liquid-based urine cytology specimens obtained from urothelial carcinoma patients, and reported potential diagnostic and predictive biomarkers through several validation test layers. The latter included cross validation with TCGA, tumor cell lines with gene editing techniques, and immunocytochemistry in independent patient cohorts [70,73]. Lee et al. [73] selected 112 differentially expressed proteins altered in urothelial carcinoma and validated neuroblast differentiation-associated protein AHNAK (AHNAK) as a new cancer biomarker, able to differentiate between urothelial carcinoma and benign urothelial cytology. TCGA also identified AHNAK as a candidate biomarker along with EPPK1, MYH14, and OLFM4. Furthermore, Park et al. [70] found moesin (MSN) as a potential biomarker predicting the presence of invasive urothelial carcinoma in urine cytology. Of interest, MSN knockdown using siRNA led to inhibition of tumor invasion in urothelial carcinoma cell lines. Also, immunocytochemistry consistently confirmed that MSN is a crucial biomarker predicting invasion when applied in urine cytology [70].

## PERSPECTIVES

High-throughput proteomic applications have recently advanced, enabling the use of minimal patient-derived specimens and overcoming the issue of low depth, inconsistency, and suboptimal accuracy. These technical advances are applicable to cytology samples, especially the ones processed with liquid-based cytology, providing reproducible results and revealing a few candidate biomarkers of diagnostic, prognostic, and therapeutic value (Table 2). Most published studies have utilized breast and thyroid cytology samples, showing the potential to help pathologists solve various diagnostic dilemmas and avoid common pitfalls. Such dilemmas comprise the evaluation of indeterminate thyroid nodules while examining thyroid FNAs, the detection of malignant serous effusions, also the differential diagnosis of a few entities in the challenging field of pancreatobiliary cytology, including pancreatic cancer from autoimmune pancreatitis, non-neoplastic from neoplastic pancreatic cysts, and non-neoplastic from malignant biliary strictures. Proteomic profiling of NAF breast samples may identify early-stage breast cancers, also differentiate between in situ and invasive breast cancers and provide information related to prognosis and therapy. Notably, according to the literature, in situ proteomics has exhibited the capacity to triage indeterminate thyroid FNAs thus prevent unnecessary surgeries and reduce healthcare costs, besides provide prognostic information through identifying PTCs carrying the *BRAF* V600E mutation and predicting the presence of lymph node metastasis or PTC histology associated with a more aggressive behavior (e.g., the tall cell variant) (Table 1). Indeed, proteomic profiling could complement traditional morphologic evaluation and ancillary testing used to examine various exfoliative and FNA cytopathology samples in routine practice or even constitute a stand-alone diagnostic modality in specific settings. However, evidence is still primitive, mostly resulting from studies with small sample size. Apart from the shortage of high-quality evidence, the demands of highly-skilled laboratory personnel, also the cost of analytic equipment, have prohibited the routine application of such approaches and limited them in the research setting. To implement high-throughput proteomics into everyday clinical practice, well-designed prospective studies and randomized controlled trials involving large patient cohorts should be used, aiming to evaluate the proteomics benefits and limitations compared to already established cytomorphologic and ancillary approaches, also their potential implementation in diagnostic algorithms used in cytopathology. Most importantly, cytopathologists and researchers should validate these methods in different sample preparations, and assess their clinical utility in diverse diagnostic scenarios. In conclusion, proteomics could become another diagnostic platform—along with genomics, transcriptomics and/or metabolomics—in the near future, potentially by using validated multi-omics approaches.

### 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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Conceptualization: HSR. Project administration: HSR. Supervision: HSR. Writing—original draft: IPN, HSR. Writing—review & editing: IPN, HSR. Approval of final manuscript: all authors.

## Conflicts of Interest

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

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## References

1. Mardis ER. Next-generation sequencing platforms. Annu Rev Anal Chem (Palo Alto Calif) 2013; 6: 287-303.
2. Hou YC, Neidich JA, Duncavage EJ, Spencer DH, Schroeder MC. Clinical whole-genome sequencing in cancer diagnosis. Hum Mutat 2022; 43: 1519-30.
3. Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: an overview. Hum Immunol 2021; 82: 801-11.
4. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med 2015; 372: 793-5.
5. Esagian SM, Grigoriadou G, Nikas IP, et al. Comparison of liquid-based to tissue-based biopsy analysis by targeted next generation sequencing in advanced non-small cell lung cancer: a comprehensive systematic review. J Cancer Res Clin Oncol 2020; 146: 2051-66.
6. Grigoriadou G, Esagian SM, Ryu HS, Nikas IP. Molecular profiling of malignant pleural effusions with next generation sequencing (NGS): evidence that supports its role in cancer management. J Pers Med 2020; 10: 206.
7. Nikas IP, Mountzios G, Sydney GI, Ioakim KJ, Won JK, Papageorgis P. Evaluating pancreatic and biliary neoplasms with small biopsy-based next generation sequencing (NGS): doing more with less. Cancers (Basel) 2022; 14: 397.
8. Tsamis KI, Sakkas H, Giannakis A, Ryu HS, Gartzonika C, Nikas IP. Evaluating infectious, neoplastic, immunological, and degenerative diseases of the central nervous system with cerebrospinal fluid-based next-generation sequencing. Mol Diagn Ther 2021; 25: 207-29.
9. Roy-Chowdhuri S, Pisapia P, Salto-Tellez M, et al. Invited review-next-generation sequencing: a modern tool in cytopathology. Virchows Arch 2019; 475: 3-11.
10. Liu Y, Beyer A, Aebersold R. On the dependency of cellular protein levels on mRNA abundance. Cell 2016; 165: 535-50.
11. Nagaraj N, Wisniewski JR, Geiger T, et al. Deep proteome and transcriptome mapping of a human cancer cell line. Mol Syst Biol 2011; 7: 548.
12. Kageyama S, Isono T, Iwaki H, et al. Proteome research in urothelial carcinoma. Int J Urol 2015; 22: 621-8.
13. Banks RE, Dunn MJ, Hochstrasser DF, et al. Proteomics: new perspectives, new biomedical opportunities. Lancet 2000; 356: 1749-56.
14. Ramazi S, Allahverdi A, Zahiri J. Evaluation of post-translational modifications in histone proteins: a review on histone modification defects in developmental and neurological disorders. J Biosci 2020; 45: 135.
15. Doll S, Gnad F, Mann M. The case for proteomics and phosphoproteomics in personalized cancer medicine. Proteomics Clin Appl 2019; 13: e1800113.
16. Menyhart O, Gyorffy B. Multi-omics approaches in cancer research with applications in tumor subtyping, prognosis, and diagnosis. Comput Struct Biotechnol J 2021; 19: 949-60.
17. Singla D, Sangha MK. Multi-omic approaches to improve cancer diagnosis, prognosis, and therapeutics. In: Raza K, ed. Computational intelligence in oncology: applications in diagnosis, prognosis and therapeutics of cancers. Singapore: Springer Singapore, 2022; 411-33.
18. Chandramouli K, Qian PY. Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Hum Genomics Proteomics 2009; 2009: 239204.
19. Aebersold R, Mann M. Mass-spectrometric exploration of proteome structure and function. Nature 2016; 537: 347-55.
20. Choudhary C, Weinert BT, Nishida Y, Verdin E, Mann M. The growing landscape of lysine acetylation links metabolism and cell signalling. Nat Rev Mol Cell Biol 2014; 15: 536-50.
21. Bekker-Jensen DB, Kelstrup CD, Batth TS, et al. An optimized shotgun strategy for the rapid generation of comprehensive human proteomes. Cell Syst 2017; 4: 587-99.
22. Zhu Y, Piehowski PD, Zhao R, et al. Nanodroplet processing platform for deep and quantitative proteome profiling of 10-100 mammalian cells. Nat Commun 2018; 9: 882.
23. Altelaar AF, Heck AJ. Trends in ultrasensitive proteomics. Curr Opin Chem Biol 2012; 16: 206-13.
24. Anderson NL, Anderson NG. Proteome and proteomics: new technologies, new concepts, and new words. Electrophoresis 1998; 19: 1853-61.
25. Blackstock WP, Weir MP. Proteomics: quantitative and physical mapping of cellular proteins. Trends Biotechnol 1999; 17: 121-7.
26. Melton L. Protein arrays: proteomics in multiplex. Nature 2004; 429: 101-7.
27. Mueller LN, Rinner O, Schmidt A, et al. SuperHirn-a novel tool for high resolution LC-MS-based peptide/protein profiling. Proteomics 2007; 7: 3470-80.
28. Ghose A, Gullapalli SV, Chohan N, et al. Applications of proteomics in ovarian cancer: dawn of a new era. Proteomes 2022; 10: 16.
29. Hall DA, Ptacek J, Snyder M. Protein microarray technology. Mech Ageing Dev 2007; 128: 161-7.
30. Sreekumar A, Nyati MK, Varambally S, et al. Profiling of cancer cells using protein microarrays: discovery of novel radiation-regulated proteins. Cancer Res 2001; 61: 7585-93.
31. Domon B, Aebersold R. Mass spectrometry and protein analysis. Science 2006; 312: 212-7.
32. Mann M, Hendrickson RC, Pandey A. Analysis of proteins and proteomes by mass spectrometry. Annu Rev Biochem 2001; 70: 437-73.
33. Alexander H, Stegner AL, Wagner-Mann C, Du Bois GC, Alexander S, Sauter ER. Proteomic analysis to identify breast cancer biomarkers in nipple aspirate fluid. Clin Cancer Res 2004; 10: 7500-10.
34. Gygi SP, Corthals GL, Zhang Y, Rochon Y, Aebersold R. Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. Proc Natl Acad Sci U S A 2000; 97: 9390-5.
35. Krause K, Jessnitzer B, Fuhrer D. Proteomics in thyroid tumor re-

search. J Clin Endocrinol Metab 2009; 94: 2717-24.
36. Duncan M, DeMarco ML. MALDI-MS: Emerging roles in pathology and laboratory medicine. Clin Mass Spectrom 2019; 13: 1-4.
37. Kriegsmann J, Kriegsmann M, Casadonte R. MALDI TOF imaging mass spectrometry in clinical pathology: a valuable tool for cancer diagnostics (review). Int J Oncol 2015; 46: 893-906.
38. Wright GL Jr. SELDI proteinchip MS: a platform for biomarker discovery and cancer diagnosis. Expert Rev Mol Diagn 2002; 2: 549-63.
39. Sauter ER, Shan S, Hewett JE, Speckman P, Du Bois GC. Proteomic analysis of nipple aspirate fluid using SELDI-TOF-MS. Int J Cancer 2005; 114: 791-6.
40. Leito I, Herodes K, Huopolainen M, et al. Towards the electrospray ionization mass spectrometry ionization efficiency scale of organic compounds. Rapid Commun Mass Spectrom 2008; 22: 379-84.
41. Chen Y, Quan L, Jia C, et al. Proteomics-based approach reveals the involvement of SERPINB9 in recurrent and relapsed multiple myeloma. J Proteome Res 2021; 20: 2673-86.
42. Pawlik TM, Fritsche H, Coombes KR, et al. Significant differences in nipple aspirate fluid protein expression between healthy women and those with breast cancer demonstrated by time-of-flight mass spectrometry. Breast Cancer Res Treat 2005; 89: 149-57.
43. Sauter ER, Zhu W, Fan XJ, Wassell RP, Chervoneva I, Du Bois GC. Proteomic analysis of nipple aspirate fluid to detect biologic markers of breast cancer. Br J Cancer 2002; 86: 1440-3.
44. George AL, Shaheed SU, Sutton CW. High-throughput proteomic profiling of nipple aspirate fluid from breast cancer patients compared with non-cancer controls: a step closer to clinical feasibility. J Clin Med 2021; 10: 2243.
45. Franzen B, Alexeyenko A, Kamali-Moghaddam M, et al. Protein profiling of fine-needle aspirates reveals subtype-associated immune signatures and involvement of chemokines in breast cancer. Mol Oncol 2019; 13: 376-91.
46. Fowler LJ, Lovell MO, Izbicka E. Fine-needle aspiration in PreservCyt: a novel and reproducible method for possible ancillary proteomic pattern expression of breast neoplasms by SELDI-TOF. Mod Pathol 2004; 17: 1012-20.
47. Rapkiewicz A, Espina V, Zujewski JA, et al. The needle in the haystack: application of breast fine-needle aspirate samples to quantitative protein microarray technology. Cancer 2007; 111: 173-84.
48. Pagni F, Mainini V, Garancini M, et al. Proteomics for the diagnosis of thyroid lesions: preliminary report. Cytopathology 2015; 26: 318-24.
49. Mainini V, Pagni F, Garancini M, et al. An alternative approach in endocrine pathology research: MALDI-IMS in papillary thyroid carcinoma. Endocr Pathol 2013; 24: 250-3.
50. Capitoli G, Piga I, Clerici F, et al. Analysis of Hashimoto's thyroiditis on fine needle aspiration samples by MALDI-imaging. Biochim Biophys Acta Proteins Proteom 2020; 1868: 140481.
51. Pagni F, De Sio G, Garancini M, et al. Proteomics in thyroid cytopathology: relevance of MALDI-imaging in distinguishing malignant from benign lesions. Proteomics 2016; 16: 1775-84.
52. Capitoli G, Piga I, L'Imperio V, et al. Cytomolecular classification of thyroid nodules using fine-needle washes aspiration biopsies. Int J Mol Sci 2022; 23: 4156.
53. Capitoli G, Piga I, Galimberti S, et al. MALDI-MSI as a Complementary diagnostic tool in cytopathology: a pilot study for the characterization of thyroid nodules. Cancers (Basel) 2019; 11: 1377.
54. Schwamborn K, Krieg RC, Uhlig S, Ikenberg H, Wellmann A. MALDI imaging as a specific diagnostic tool for routine cervical cytology specimens. Int J Mol Med 2011; 27: 417-21.
55. Schwamborn K, Weirich G, Steiger K, et al. Discerning the primary carcinoma in malignant peritoneal and pleural effusions using imaging mass spectrometry: a deasibility study. Proteomics Clin Appl 2019; 13: e1800064.
56. Perzanowska A, Fatalska A, Wojtas G, et al. An MRM-based cytokeratin marker assay as a tool for cancer studies: application to lung cancer pleural effusions. Proteomics Clin Appl 2018; 12: 170084.
57. Li H, Tang Z, Zhu H, Ge H, Cui S, Jiang W. Proteomic study of benign and malignant pleural effusion. J Cancer Res Clin Oncol 2016; 142: 1191-200.
58. Inoue H, Eguchi A, Kobayashi Y, et al. Extracellular vesicles from pancreatic ductal adenocarcinoma endoscopic ultrasound-fine needle aspiration samples contain a protein barcode. J Hepatobiliary Pancreat Sci 2022; 29: 394-403.
59. Lee LS, Banks PA, Bellizzi AM, et al. Inflammatory protein profiling of pancreatic cyst fluid using EUS-FNA in tandem with cytokine microarray differentiates between branch duct IPMN and inflammatory cysts. J Immunol Methods 2012; 382: 142-9.
60. Navaneethan U, Lourdusamy V, Gk Venkatesh P, Willard B, Sanaka MR, Parsi MA. Bile proteomics for differentiation of malignant from benign biliary strictures: a pilot study. Gastroenterol Rep (Oxf) 2015; 3: 136-43.
61. Seccia V, Navari E, Donadio E, et al. Proteomic investigation of malignant major salivary gland tumors. Head Neck Pathol 2020; 14: 362-73.
62. Pawlik TM, Hawke DH, Liu Y, et al. Proteomic analysis of nipple aspirate fluid from women with early-stage breast cancer using isotope-coded affinity tags and tandem mass spectrometry reveals differential expression of vitamin D binding protein. BMC Cancer 2006; 6: 68.
63. Pavlou MP, Kulasingam V, Sauter ER, Kliethermes B, Diamandis EP. Nipple aspirate fluid proteome of healthy females and patients with breast cancer. Clin Chem 2010; 56: 848-55.
64. Noble JL, Dua RS, Coulton GR, Isacke CM, Gui GP. A comparative proteinomic analysis of nipple aspiration fluid from healthy women and women with breast cancer. Eur J Cancer 2007; 43: 2315-20.
65. Giusti L, Iacconi P, Ciregia F, et al. Proteomic analysis of human thyroid fine needle aspiration fluid. J Endocrinol Invest 2007; 30: 865-9.
66. Giusti L, Iacconi P, Ciregia F, et al. Fine-needle aspiration of thyroid nodules: proteomic analysis to identify cancer biomarkers. J Proteome Res 2008; 7: 4079-88.
67. Ciregia F, Giusti L, Molinaro A, et al. Proteomic analysis of fine-needle aspiration in differential diagnosis of thyroid nodules. Transl Res 2016; 176: 81-94.
68. Ucal Y, Eravci M, Tokat F, Duren M, Ince U, Ozpinar A. Proteomic analysis reveals differential protein expression in variants of papillary thyroid carcinoma. EuPA Open Proteom 2017; 17: 1-6.
69. Lin P, Yao Z, Sun Y, et al. Deciphering novel biomarkers of lymph node metastasis of thyroid papillary microcarcinoma using proteomic analysis of ultrasound-guided fine-needle aspiration biopsy samples. J Proteomics 2019; 204: 103414.
70. Park JH, Lee C, Han D, et al. Moesin (MSN) as a novel proteome-based diagnostic marker for early detection of invasive bladder urothelial carcinoma in liquid-based cytology. Cancers (Basel) 2020; 12: 1018.
71. Yang N, Feng S, Shedden K, et al. Urinary glycoprotein biomarker

discovery for bladder cancer detection using LC/MS-MS and label-free quantification. Clin Cancer Res 2011; 17: 3349-59.
72. Theodorescu D, Wittke S, Ross MM, et al. Discovery and validation of new protein biomarkers for urothelial cancer: a prospective analysis. Lancet Oncol 2006; 7: 230-40.
73. Lee H, Kim K, Woo J, et al. Quantitative proteomic analysis identifies AHNAK (neuroblast differentiation-associated protein AHNAK) as a novel candidate biomarker for bladder urothelial carcinoma diagnosis by liquid-based cytology. Mol Cell Proteomics 2018; 17: 1788-802.
74. Boylan KL, Afiuni-Zadeh S, Geller MA, et al. A feasibility study to identify proteins in the residual Pap test fluid of women with normal cytology by mass spectrometry-based proteomics. Clin Proteomics 2014; 11: 30.
75. Boylan KL, Afiuni-Zadeh S, Geller MA, Argenta PA, Griffin TJ, Skubitz APN. Evaluation of the potential of Pap test fluid and cervical swabs to serve as clinical diagnostic biospecimens for the detection of ovarian cancer by mass spectrometry-based proteomics. Clin Proteomics 2021; 18: 4.
76. Liu PJ, Chen CD, Wang CL, et al. In-depth proteomic analysis of six types of exudative pleural effusions for nonsmall cell lung cancer biomarker discovery. Mol Cell Proteomics 2015; 14: 917-32.
77. Li Y, Lian H, Jia Q, Wan Y. Proteome screening of pleural effusions identifies IL1A as a diagnostic biomarker for non-small cell lung cancer. Biochem Biophys Res Commun 2015; 457: 177-82.
78. Hegmans JP, Veltman JD, Fung ET, et al. Protein profiling of pleural effusions to identify malignant pleural mesothelioma using SELDI-TOF MS. Technol Cancer Res Treat 2009; 8: 323-32.
79. Ucal Y, Tokat F, Duren M, Ince U, Ozpinar A. Peptide profile differences of noninvasive follicular thyroid neoplasm with papillary-like nuclear features, encapsulated follicular variant, and classical papillary thyroid carcinoma: an application of matrix-assisted laser desorption/ionization mass spectrometry imaging. Thyroid 2019; 29: 1125-37.
80. Torres-Cabala C, Bibbo M, Panizo-Santos A, et al. Proteomic identification of new biomarkers and application in thyroid cytology. Acta Cytol 2006; 50: 518-28.

 https://doi.org/10.4132/jptm.2022.08.30

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REVIEW

# Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: its updated diagnostic criteria, preoperative cytologic diagnoses and impact on the risk of malignancy

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Due to the extremely indolent behavior, a subset of noninvasive encapsulated follicular variant papillary thyroid carcinomas has been classified as "noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)" since 2016 and is no longer considered carcinoma. Since the introduction of this new terminology, changes and refinements have been made in diagnostic criteria. Initially, the incidence of NIFTP was estimated substantial. However, the reported incidence of NIFTP varies greatly among studies and regions, with higher incidence in North American and European countries than in Asian countries. Thus, the changes in the risk of malignancy (ROM) in the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) differ inevitably among regions. Because more conservative surgery is recommended for NIFTPs, distinguishing NIFTPs from papillary thyroid carcinomas in preoperative fine-needle aspiration cytology became one of the major concerns. This review will provide comprehensive overview of updates on diagnostic criteria, actual incidence and preoperative cytologic diagnoses of NIFTP, and its impact on the ROM in TBSRTC.

**Key Words:** Thyroid; Noninvasive follicular thyroid neoplasm with papillary-like nuclear features; Fine-needle aspiration cytology; Risk of malignancy

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As nuclear features of papillary thyroid carcinoma (PTC) have been increasingly recognized, the incidence of follicular variant PTC (FVPTC), especially encapsulated subtype, rose 2- to 3-fold over the past decade in Europe and North America [1]. Because of the indolent behavior reported in a subset of noninvasive encapsulated FVPTCs (noninvasive EFVPTCs), the terminology "noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)" was proposed in 2016 by the Endocrine Pathology Society working group [2] to avoid overdiagnosis and unnecessary psychological and financial burden on both clinicians and patients.

The advent of this new terminology has brought up certain issues including the proper diagnostic criteria, actual incidence, preoperative fine-needle aspiration cytology (FNAC) diagnosis, and clinical impact of this neoplasm. This review highlights on the changes in diagnostic criteria of NIFTP, actual incidence in different regions, cytologic features, preoperative FNAC diagnostic categories, and its impact on the risk of malignancy in the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC).

## UPDATES ON DIAGNOSTIC CRITERIA OF NONINVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES

The initial diagnostic criteria proposed by Nikiforov et al. in 2016 [2], the revised criteria suggested in 2018 [3], and the most recent criteria in the 2022 World Health Organization (WHO) classification [4] are summarized in Table 1. In initial criteria, noninvasive EFVPTCs with < 1% papillae, no psammoma bodies and < 30% solid/trabecular/insular growth pattern were renamed as NIFTP in the absence of necrosis and high mitotic activity [2]

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**Table 1.** Diagnostic criteria for noninvasive follicular thyroid neoplasm with papillary-like nuclear features

| Initial criteria [2] | Revised criteria [3] | The 2022 WHO classification [4] |
|---|---|---|
| 1. Encapsulation or clear demarcation | 1. Primary | Essential |
| 2. Follicular growth pattern with | Encapsulation or clear demarcation | 1. Encapsulation or clear demarcation |
| <1% papillae | Follicular growth pattern with no papillae | 2. Follicular growth pattern with |
| No psammoma bodies | No psammoma bodies | <1% true papillae |
| <30% solid/trabecular/insular growth pattern | <30% solid/trabecular/insular growth pattern | No psammoma bodies |
| 3. Nuclear score 2–3 | Nuclear score of 2–3 | <30% solid/trabecular/insular growth |
| 4. No vascular or capsular invasion | No vascular or capsular invasion | 3. Nuclear score of 2–3 |
| 5. No tumor necrosis | No tumor necrosis or high mitotic activity (3 or more mitoses per 10 high power fields) | 4. No vascular or capsular invasion |
| 6. No high mitotic activity | 2. Secondary | 5. No tumor necrosis |
| | Lack of *BRAF* V600E mutation detected by molecular assays or immunohistochemistry | 6. Low mitotic count (<3 mitoses/2 $mm^2$) |
| | Lack of *BRAF* V600E-like mutations or other high-risk mutations (*TERT*, *TP53*) | 7. Lack of cytoarchitectural features of papillary carcinoma variants other than follicular variant |
| | | Desirable |
| | | Immunohistochemistry or molecular testing for *BRAF* and *NRAS* mutation |

WHO, World Health Organization.

(Fig. 1). Among these histologic criteria, the proportion of papillae has become the center of controversy. Above all, it is important to acknowledge that the authors intended to count the proportion of "true papillae", not rudimentary or hyperplastic type papillae. Although Nikiforov et al. [2] have reported no adverse events in cases with NIFTPs in the initial study, lymph node metastases and even distant metastases of NIFTPs were reported by other researchers [5]. Moreover, some of these cases harbored *BRAF* V600E mutation, which is rather a hallmark of conventional PTC [5]. These findings led to the revised criteria which restricted the diagnosis of NIFTP to cases without any well-formed papillae (true papillae) [3]. Also, absence of *BRAF* V600E or other high-risk mutations involving *TP53* or *TERT* promoter, were additionally described as helpful but not required features of NIFTP [3]. However, larger number of studies demonstrated lack of metastasis and disease recurrence in cases harboring <1% papillae [6-9]. In the study by Xu et al. [8], lymph node metastasis was only observed in cases with >10% papillae. The 2022 WHO classification endorses the original criteria allowing <1% papillae based on these studies [4]. However, lymph node metastases [5,10], and even distant metastases [10] were found in NIFTPs with 0% papillae, despite thorough microscopic examination. Authors of these studies underscored the low-risk malignant nature of NIFTP and the necessity of including NIFTP in cancer registry [5,10]. Although cases less than 1cm or showing oncocytic features that are otherwise consistent with NIFTP were not included in the initial study [2], the new WHO classification will also include these scenarios because previous studies have confirmed similar behavior as NIFTPs without these features [9,11].

## ACTUAL INCIDENCE OF NONINVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES

Although it was initially suspected that a substantial proportion of EFVPTCs would be regrouped as NIFTPs, the actual incidence varies greatly according to geographic regions as depicted in recent meta-analyses [12]. The reported incidences of NIFTP range from 1.3% to 23.4% in North America [13-15], from 0.7% to 34.9% in Europe [16-18], and from 0.4% to 29.4% in Asia [19-21]. The pooled incidence of NIFTP was 9.3% and 9.6% in North American and Europe, which was far higher than in Asia with 2.1%, indicating the geographical or ethnic differences [12]. In Korea, the incidence of NIFTP investigated by a multi-institutional study was 0.8%, even lower than Asian average [22]. Of note, the worldwide incidence of NIFTP was 6.0% in the same meta-analysis, suggesting that the impact of NIFTP would not be as considerable as initially estimated [12]. Interestingly, different institutes in the same region also reported widely varying incidences of NIFTP [10,13,15,23], suggesting that interpreting nuclear atypia still lays in subjective area despite the effort to objectifying the nuclear features into three-tiered score.

## PREOPERATIVE CYTOLOGIC DIAGNOSES OF NONINVASIVE FOLLICULAR THYROID NEOPLASM WITH PAPILLARY-LIKE NUCLEAR FEATURES

More conservative management is considered for NIFTPs


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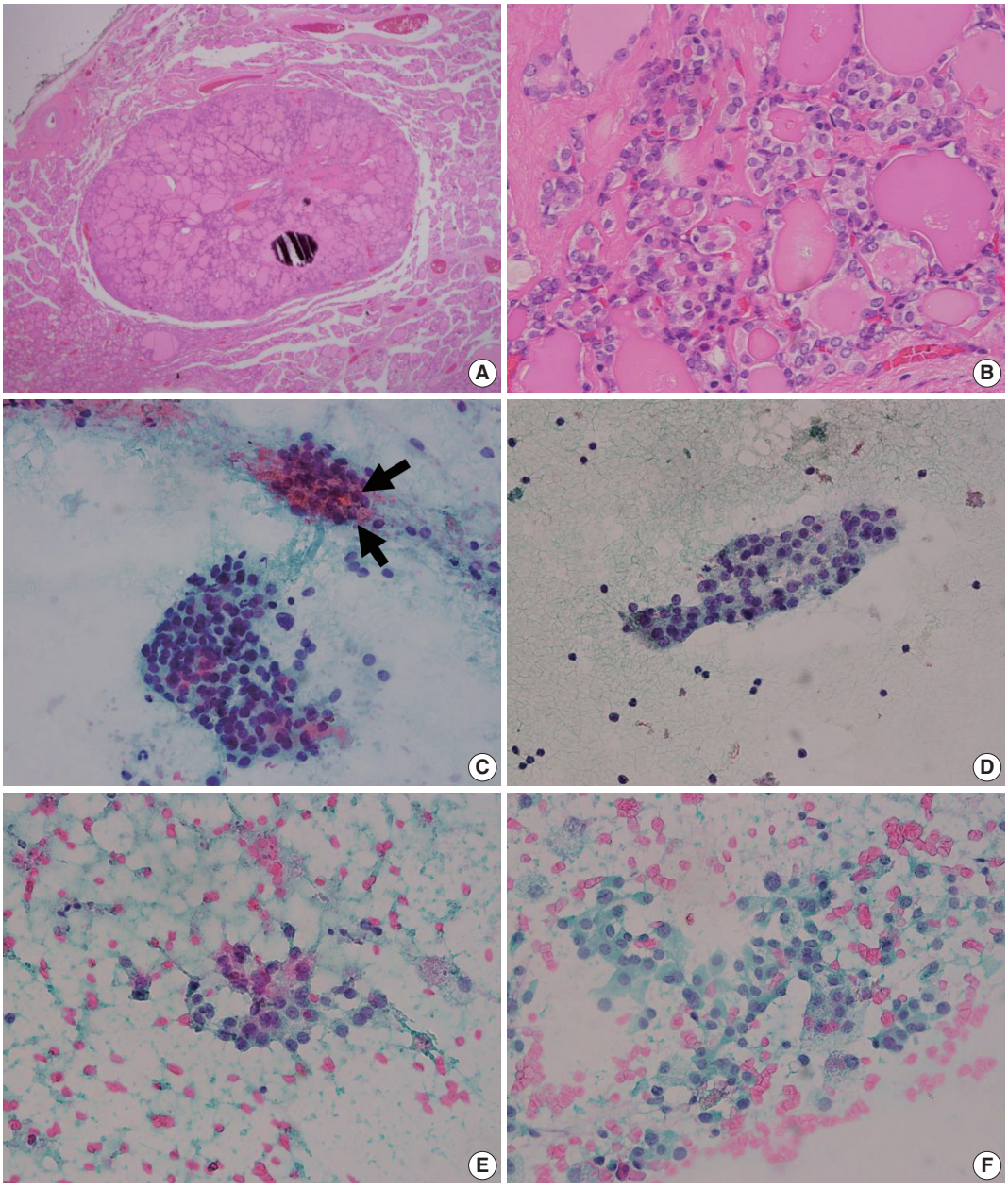


**Fig. 1.** Histologic and cytologic features of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). (A) A well-demarcated mass composed of variable-sized neoplastic follicles is observed in scan view. (B) In high power view, mild nuclear atypia including chromatin clearing, and occasional nuclear grooves which corresponds to "nuclear score 2" is seen (B). (C, D) Fine-needle aspiration cytology of NIFTP generally shows syncytial cell clusters containing microfollicles. Thick colloid can be observed in the microfollicles (arrows). (E, F) Pale chromatin, occasional nuclear grooves, marginal nucleoli are seen. The nuclear atypia is typically mild and patchy. Intranuclear pseudoinclusions, psammomatous calcifications are absent in the presented cases.

compared with conventional PTCs (cPTCs) which require lymph node dissection and radioactive iodine treatment if indicated. Therefore, it has become a major interest whether NIFTP can be diagnosed preoperatively or not.

Upon the introduction of NIFTP terminology, researchers have investigated the differences in cytologic features of NIFTP and other related lesions such as benign follicular lesion, follicular thyroid adenoma, FVPTC, and cPTCs. The results of studies comparing cytologic features of NIFTP and other lesions are summarized in Table 2. In FNAC, NIFTPs generally show crowded syncytial-like fragments containing microfollicles (Fig. 1) [24,25]. Compared with cPTCs, NIFTPs are more commonly associated

**Table 2.** Comparison of cytologic features of NIFTP with other lesions

| Reference | NIFTP vs. benign/FTA | NIFTP vs. FVPTC | NIFTP vs. cPTC | NIFTP/FVPTC vs. cPTC |
|---|---|---|---|---|
| Legesse et al. [14] | - | Less frequent PIs, marginal micronucleoli, irregular branching sheets, and linear arrangement in NIFTP | Absence of PIs/Less frequent MNGs in NIFTP | - |
| Bizzarro et al. [26] | More frequent scant cytoplasm, NE, nuclear elongation, chromatin clearing, grooves, and membrane irregularities in NIFTP | Less frequent grooves in NIFTP | Less frequent papillae, NE, PIs, grooves, and membrane irregularities in NIFTP | - |
| Brandler et al. [27] | More frequent chromatin clearing, crowding, and NE in NIFTP | - | Less frequent PIs, papillae, crowding, NE, membrane irregularities, chromatin clearing, calcifications, and MNGs in NIFTP | - |
| Chandler et al. [28] | - | More frequent MF predominance/ Less frequent nuclear elongation, grooves, and PIs in NIFTP | | - |
| Diaz Del Arco et al. [29] | - | Less frequent nuclear folds in NIFTP | More frequent bidimensional groups and MFs/Less frequent papillary or pseudopapillary architecture, tridimensionality, MNGs, and nuclear folds in NIFTP | - |
| Koshkikawa et al. [30] | - | No differences | - | More frequent MFs, and dense globules of colloids/less frequent PIs, true papillary cell clusters, monolayered cell sheets, ropy colloids, MNGs, psammoma bodies, and cystic background in NIFTP and FVPTC |
| Howitt et al. [31] | - | - | More frequent MFs/Less frequent sheet pattern in NIFTP | - |
| Mahajan et al. [32] | - | No differences in nuclear features/ Less frequent 3-dimensional fragments in NIFTP | Less frequent PIs, nuclear score of 3, and diffuse nuclear change in NIFTP | - |
| Selvaggi et al. [34] | - | Less frequent MNGs in NIFTP | - | - |
| Maletta et al. [35] | More frequent NE, membrane irregularities, chromatin clearing, and nuclear molding in NIFTP | No differences | - | - |
| Strickland et al. [33] | - | - | - | MF predominance without papillae, PIs or psammoma bodies in NIFTP and FVPTC |

NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features; FTA, follicular thyroid adenoma; FVPTC, follicular variant papillary thyroid carcinoma; cPTC, conventional papillary thyroid carcinoma; PI, pseudoinclusion; MNG, multinucleated giant cell; NE, nucelar enlargement; MF, microfollicle.

with predominant microfollicular pattern in bidimensional clusters and show absent or rare papillary structure [26-30]. Monolayered sheet pattern and tridimensional clusters are more frequent in cPTCs [29,30]. Papillary-like nuclear features, including nuclear enlargement, nuclear elongation, chromatin clearing, intranuclear pseudoinclusion are usually mild and patchy (Fig. 1) [14,26,27,29-32]. Diffuse nuclear change and presence of nuclear score 3 can be observed but are reported to be less frequent than in cPTCs [32]. Regarding the nature of colloid, NIFTPs are associated with thick, dense colloid found both in and out of the microfollicles, while cPTCs tend to show ropy colloid [30]. Psammoma bodies and multinucleated giant cells are also absent or infrequent in NIFTPs compared with cPTCs [14,30]. Indeed, Strickland et al. [33] have demonstrated that NIFTPs and invasive FVPTCs can be efficiently separated from cPTCs in preoperative FNAC when diagnosed according to the criteria as following: (1) cPTC: presence of papillae, pseudoinclusions, or psammomatous calcifications; (2) NIFTP and invasive FVPTC:


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microfollicle predominance without papillae, pseudoinclusions, or psammomatous calcifications. These criteria surely are not perfect because papillae or pseudoinclusions, by definition, can also be observed in NIFTPs albeit low frequency. Nevertheless, the criteria itself with some additional cytologic features mentioned above appear to be helpful in distinguishing NIFTPs from cPTCs. In addition, some researchers reported that marginal micronucleoli, nuclear grooves, pseudoinclusions, irregular branching sheet and multinucleated giant cells were more common in invasive FVPTCs than in NIFTPs [14,26,28,29,34]. However, these findings are inconsistent among different studies, and others have failed to reveal significant differences between NIFTPs and invasive FVPTCs [32,35]. Pathologists should be aware of the fact that the diagnosis of NIFTP is determined only after the histopathologic examination of surgical specimen, although certain cytologic features are more or less often associated with NIFTP than other lesions.

In FNACs, NIFTPs are usually diagnosed as indeterminate categories including atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), follicular neoplasm/suspicious for a follicular neoplasm (FN/SFN), and suspicious for malignancy categories due to microfollicular-predominant architectural pattern and subtle nuclear changes. According to previous studies, distribution of TBSRTC diagnostic categories among NIFTPs range from 0% to 25% in nondiagnostic [12,21,26,36], 0% to 35% in benign [12,26,36,37], 0% to 66.7% in AUS/FLUS [27,29,36,38], 0% to 61.9% in FN/SFN [26,29,36,39], 0% to 83.3% in suspicious for malignancy [12, 27,29,36], 0% to 65.9% in malignant category [29,36,39,40]. A recent meta-analysis showed the pooled distribution of NIFTP cases in FNAC diagnostic categories as follows; 1.3% (95% confidence interval [CI], 0.8 to 1.7) in nondiagnostic, 8.9% (95% CI, 6.9 to 10.8) in benign, 29.2% (95% CI, 25.0 to 33.4) in AUS/FLUS, 24.2% (95% CI, 19.6 to 28.9) in FN/SFN, 19.5% (95% CI, 16.1 to 22.9) in suspicious for malignancy, and 6.9% (95% CI, 5.2 to 8.7) in malignant diagnostic category, respectively (Table 3) [41].

## IMPACT OF NIFTP ON THE RISK OF MALIGNANCY IN THE BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY

The impact of NIFTP on the risk of malignancy (ROM) in each TBSRTC diagnostic categories largely depend on distribution of diagnostic categories of NIFTP cases. Therefore, decrease in ROM are more prominent in AUS/FLUS, FN/SFN, and suspicious for malignancy categories compared with other categories. Reported changes in ROM in literature range from 0% to 20.0% in nondiagnostic [12,36,42,43], 0% to 27.6% in benign [19,36, 43,44], 0% to 20.0% in AUS/FLUS [20,36,42,44], 0.2% to 30.8% in FN/SFN [19,36,42,44], 0% to 41.5% in suspicious for malignancy [20,36,42,43], and 0% to 12.8% in malignant diagnostic categories [20,36,44,45]. A recent meta-analysis revealed that the decrease of ROM was 2.4%, 2.7%, 8.2%, 8.2%, 7.3%, and 1.1% in nondiagnostic, benign, AUS/FLUS, FN/SFN, suspicious for malignancy, and malignant diagnostic categories, respectively (Table 3) [41]. While the impact of NIFTP was suspected considerable in European and North American countries due to the high incidence of NIFTP, it does not seem to be the same in Asian counterparts. Compared with European and North American countries, Asian countries generally have reported lower incidence of NIFTPs [22,46,47]. Indeed, results from studies including Asian multi-institutional study performed by Bychkov et al. [48] indicate that magnitude of ROM decrease was slight and not significant [47]. A meta-analysis performed by Vuong et al. [49] compared the ROM decrease in Asian regions to Western counterparts and found that the decrease in ROM in each category is generally lower in Asian countries, with the greatest difference in SM category (5% vs. 18%), followed by AUS/FLUS category (8% vs. 10%).

**Table 3.** Distribution of diagnostic categories of noninvasive follicular thyroid neoplasm with papillary-like nuclear features and its impact on the risk of malignancy from recent meta-analysis [40]

| Diagnostic category | Proportion (%) (95% CI) | Change in ROM (%) (95% CI) |
|---|---|---|
| I. Nondiagnostic | 1.3 (0.8 to 1.7) | –2.4 (–7.5 to 2.7) |
| II. Benign | 8.9 (6.9 to 10.8) | –2.7 (–4.1 to –1.3) |
| III. AUS/FLUS | 29.2 (25.0 to 33.4) | –8.2 (–11.2 to –5.1) |
| IV. FN/SFN | 24.2 (19.6 to 28.9) | –8.2 (–12.3 to –4.1) |
| V. Suspicious for malignancy | 19.5 (16.1 to 22.9) | –7.3 (–9.6 to –5.1) |
| VI. Malignant | 6.9 (5.2 to 8.7) | –1.1 (–1.6 to –0.5) |

CI, confidence interval; ROM, risk of malignancy; AUS/FLUS, atypia of undetermined significance/follicular lesion of undetermined significance; FN/SFN, follicular neoplasm/suspicious for a follicular neoplasm.

## CONCLUSION

NIFTPs are a group of neoplasm that have been renamed due to the indolent behavior. Although there was a change regarding the amount of papillae in the revised criteria, the initial < 1% cutoff is maintained in the 2022 WHO classification based on the recent studies. Diagnosis of NIFTP can only be made according to the strict criteria after thorough pathological examination in surgical specimen. Still, it is important to be aware that some

cytological features can be helpful in distinguishing NIFTPs from cPTCs. The impact of NIFTP in cytologic diagnostic categories varies among studies, and regions which hold different incidences.

## 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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## Author Contributions

Conceptualization: HYN, SYP. Project administration: SYP. Supervision: SYP. Writing—original draft: HYN. Writing—review & editing: HYN, SYP. Approval of final manuscript: all authors.

## Conflicts of Interest

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## References

1. Jung CK, Little MP, Lubin JH, et al. The increase in thyroid cancer incidence during the last four decades is accompanied by a high frequency of *BRAF* mutations and a sharp increase in RAS mutations. J Clin Endocrinol Metab 2014; 99: E276-85.
2. Nikiforov YE, Seethala RR, Tallini G, et al. Nomenclature revision for encapsulated follicular variant of papillary thyroid carcinoma: a paradigm shift to reduce overtreatment of indolent tumors. JAMA Oncol 2016; 2: 1023-9.
3. Nikiforov YE, Baloch ZW, Hodak SP, et al. Change in diagnostic criteria for noninvasive follicular thyroid neoplasm with papillary-like nuclear features. JAMA Oncol 2018; 4: 1125-6.
4. Baloch ZW, Asa SL, Barletta JA, et al. Overview of the 2022 WHO classification of thyroid neoplasms. Endocr Pathol 2022; 33: 27-63.
5. Cho U, Mete O, Kim MH, Bae JS, Jung CK. Molecular correlates and rate of lymph node metastasis of non-invasive follicular thyroid neoplasm with papillary-like nuclear features and invasive follicular variant papillary thyroid carcinoma: the impact of rigid criteria to distinguish non-invasive follicular thyroid neoplasm with papillary-like nuclear features. Mod Pathol 2017; 30: 810-25.
6. Rosario PW, Mourao GF, Nunes MB, Nunes MS, Calsolari MR. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features. Endocr Relat Cancer 2016; 23: 893-7.
7. Thompson LD. Ninety-four cases of encapsulated follicular variant of papillary thyroid carcinoma: a name change to noninvasive follicular thyroid neoplasm with papillary-like nuclear features would help prevent overtreatment. Mod Pathol 2016; 29: 698-707.
8. Xu B, Serrette R, Tuttle RM, et al. How many papillae in conventional papillary carcinoma? A clinical evidence-based pathology study of 235 unifocal encapsulated papillary thyroid carcinomas, with emphasis on the diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features. Thyroid 2019; 29: 1792-803.
9. Xu B, Tallini G, Scognamiglio T, Roman BR, Tuttle RM, Ghossein RA. Outcome of large noninvasive follicular thyroid neoplasm with papillary-like nuclear features. Thyroid 2017; 27: 512-7.
10. Parente DN, Kluijfhout WP, Bongers PJ, et al. Clinical safety of renaming encapsulated follicular variant of papillary thyroid carcinoma: is NIFTP truly benign? World J Surg 2018; 42: 321-6.
11. Xu B, Farhat N, Barletta JA, et al. Should subcentimeter non-invasive encapsulated, follicular variant of papillary thyroid carcinoma be included in the noninvasive follicular thyroid neoplasm with papillary-like nuclear features category? Endocrine 2018; 59: 143-50.
12. Rana C, Vuong HG, Nguyen TQ, et al. The incidence of noninvasive follicular thyroid neoplasm with papillary-like nuclear features: a meta-analysis assessing worldwide impact of the reclassification. Thyroid 2021; 31: 1502-13.
13. Cubero Rego D, Lee H, Boguniewicz A, Jennings TA. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) is rare, benign lesion using modified stringent diagnostic criteria: reclassification and outcome study. Ann Diagn Pathol 2020; 44: 151439.
14. Legesse T, Parker L, Heath J, Staats PN. Distinguishing non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) from classic and invasive follicular-variant papillary thyroid carcinomas based on cytologic features. J Am Soc Cytopathol 2019; 8: 11-7.
15. Sung S, Margolskee E, Chen D, Tiscornia-Wasserman P. Incidence of noninvasive follicular thyroid neoplasm with papillary-like nuclear features and change in risk of malignancy for "The Bethesda System for Reporting Thyroid Cytology". J Am Soc Cytopathol 2019; 8: 133-40.
16. Borda A, Zahan AE, Piciu D, Barbus E, Berger N, Nechifor-Boila A. A 15 year institutional experience of well-differentiated follicular cell-derived thyroid carcinomas; impact of the new 2017 TNM and WHO Classifications of Tumors of Endocrine Organs on the epidemiological trends and pathological characteristics. Endocrine 2020; 67: 630-42.
17. Boursier L, Clerc Urmes I, Garon J, Klein M, Demarquet L. Ultrasound and cytological characteristics of non-invasive follicular thyroid neoplasm with papillary-like nuclear features compared to papillary carcinomas. Ann Endocrinol (Paris) 2020; 81: 28-33.
18. Reinke RH, Larsen SR, Mathiesen JS, Godballe C, Londero SC. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features is rare: a population based study of incidence. Head Neck Pathol 2020; 14: 144-9.
19. Ke J, Jianyong L, Ying L, et al. The use of The Bethesda System for Reporting Thyroid Cytopathology in a Chinese population: an analysis of 13 351 specimens. Diagn Cytopathol 2019; 47: 876-80.
20. Rana C, Manjunath S, Ramakant P, Singh K, Babu S, Mishra A. Noninvasive follicular neoplasm with papillary like nuclear features: a comprehensive analysis with a diagnostic algorithm. Diagn Cytopathol 2020; 48: 330-41.
21. Zhu Y, Song Y, Xu G, Fan Z, Ren W. The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC): a report of 2,781 cases in

a Chinese population. Chin J Cancer Res 2020; 32: 140-8.
22. Seo JY, Park JH, Pyo JY, et al. A multi-institutional study of prevalence and clinicopathologic features of non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) in Korea. J Pathol Transl Med 2019; 53: 378-85.
23. Eskander A, Hall SF, Manduch M, Griffiths R, Irish JC. A population-based study on NIFTP incidence and survival: is NIFTP really a "benign" disease? Ann Surg Oncol 2019; 26: 1376-84.
24. Ali SZ, Cibas ES. The Bethesda System for Reporting Thyroid Cytopathology. 2nd ed. New York: Springer, 2018.
25. Yan L, Sethi S, Park JW. Cytologic and clinical features of NIFTP: can we diagnose based on preoperative fine-needle aspiration. Diagn Cytopathol 2019; 47: 1259-66.
26. Bizzarro T, Martini M, Capodimonti S, et al. Young investigator challenge: the morphologic analysis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features on liquid-based cytology: Some insights into their identification. Cancer Cytopathol 2016; 124: 699-710.
27. Brandler TC, Zhou F, Liu CZ, et al. Can noninvasive follicular thyroid neoplasm with papillary-like nuclear features be distinguished from classic papillary thyroid carcinoma and follicular adenomas by fine-needle aspiration? Cancer Cytopathol 2017; 125: 378-88.
28. Chandler JB, Colunga M, Prasad ML, et al. Identification of distinct cytomorphologic features in the diagnosis of NIFTP at the time of preoperative FNA: Implications for patient management. Cancer Cytopathol 2017; 125: 865-75.
29. Diaz Del Arco C, Fernandez Acenero MJ. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: can cytology face the challenge of diagnosis in the light of the new classification? Acta Cytol 2018; 62: 265-72.
30. Koshikawa T, Fujita N, Ueda N, et al. Important cytological findings for distinction between follicular variant and conventional papillary thyroid carcinoma, including noninvasive follicular thyroid tumors with papillary-like nuclear features. Endocr J 2019; 66: 475-83.
31. Howitt BE, Chang S, Eszlinger M, et al. Fine-needle aspiration diagnoses of noninvasive follicular variant of papillary thyroid carcinoma. Am J Clin Pathol 2015; 144: 850-7.
32. Mahajan S, Agarwal S, Kocheri N, Jain D, Mathur SR, Iyer VK. Cytopathology of non-invasive follicular thyroid neoplasm with papillary-like nuclear features: a comparative study with similar patterned papillary thyroid carcinoma variants. Cytopathology 2018; 29: 233-40.
33. Strickland KC, Vivero M, Jo VY, et al. Preoperative cytologic diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features: a prospective analysis. Thyroid 2016; 26: 1466-71.
34. Selvaggi SM. The presence of multinucleated giant cells: noninvasive follicular thyroid neoplasm with papillary-like nuclear features vs the follicular variant of papillary thyroid carcinoma. Diagn Cytopathol 2019; 47: 1007-10.
35. Maletta F, Massa F, Torregrossa L, et al. Cytological features of "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" and their correlation with tumor histology. Hum Pathol 2016; 54: 134-42.
36. Kim M, Kim JE, Kim HJ, et al. Cytologic diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclear features and its impact on the risk of malignancy in the bethesda system for reporting thyroid cytopathology: an institutional experience. J Pathol Transl Med 2018; 52: 171-8.
37. Ibrahim AA, Wu HH. Fine-needle aspiration cytology of noninvasive follicular variant of papillary thyroid carcinoma is cytomorphologically distinct from the invasive counterpart. Am J Clin Pathol 2016; 146: 373-7.
38. Ventura M, Melo M, Fernandes G, Carrilho F. Risk of malignancy in thyroid cytology: the impact of the reclassification of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (Niftp). Endocr Pract 2019; 25: 642-7.
39. Celik M, Bulbul BY, Can N, et al. Comparison of clinicopathological features in patients with noninvasive follicular thyroid neoplasm with papillary-like nuclear features and follicular variant papillary thyroid cancer. Pol Arch Intern Med 2020; 130: 100-5.
40. Hirokawa M, Higuchi M, Suzuki A, Hayashi T, Kuma S, Miyauchi A. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: a single-institutional experience in Japan. Endocr J 2017; 64: 1149-55.
41. Haaga E, Kalfert D, Ludvikova M, Kholova I. Non-invasive follicular thyroid neoplasm with papillary-like nuclear features is not a cytological diagnosis, but it influences cytological diagnosis outcomes: a systematic review and meta-analysis. Acta Cytol 2022; 66: 85-105.
42. Lau RP, Paulsen JD, Brandler TC, Liu CZ, Simsir A, Zhou F. Impact of the reclassification of "noninvasive encapsulated follicular variant of papillary thyroid carcinoma" to "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" on the Bethesda System for Reporting Thyroid Cytopathology: a large academic institution's experience. Am J Clin Pathol 2017; 149: 50-4.
43. Strickland KC, Howitt BE, Marqusee E, et al. The impact of noninvasive follicular variant of papillary thyroid carcinoma on rates of malignancy for fine-needle aspiration diagnostic categories. Thyroid 2015; 25: 987-92.
44. Mito JK, Alexander EK, Angell TE, et al. A modified reporting approach for thyroid FNA in the NIFTP era: a 1-year institutional experience. Cancer Cytopathol 2017; 125: 854-64.
45. Layfield LJ, Baloch ZW, Esebua M, Kannuswamy R, Schmidt RL. Impact of the reclassification of the non-invasive follicular variant of papillary carcinoma as benign on the malignancy risk of the Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis study. Acta Cytol 2017; 61: 187-93.
46. Bychkov A, Hirokawa M, Jung CK, et al. Low rate of noninvasive follicular thyroid neoplasm with papillary-like nuclear features in Asian practice. Thyroid 2017; 27: 983-4.
47. Chen CC, Hang JF, Liu CY, Wang YH, Lai CR. Thyroid fine-needle aspiration cytology in Taiwan: a nationwide survey and literature update. J Pathol Transl Med 2020; 54: 361-6.
48. Bychkov A, Keelawat S, Agarwal S, et al. Impact of non-invasive follicular thyroid neoplasm with papillary-like nuclear features on the Bethesda System for Reporting Thyroid Cytopathology: a multi-institutional study in five Asian countries. Pathology 2018; 50: 411-7.
49. Vuong HG, Tran TTK, Bychkov A, et al. Clinical impact of non-invasive follicular thyroid neoplasm with papillary-like nuclear features on the risk of malignancy in the Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis of 14,153 resected thyroid nodules. Endocr Pract 2019; 25: 491-502.

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REVIEW

# Biomarker testing of cytology specimens in personalized medicine for lung cancer patients

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Every patient with advanced non–small cell lung cancer (NSCLC) should be tested for targetable driver mutations and gene arrangements that may open avenues for targeted therapy. As most patients with NSCLC in the advanced stage of the disease are not candidates for surgery, these tests have to be performed on small biopsies or cytology samples. A growing number of other genetic changes with targetable mutations may be treatable in the near future. To identify patients who might benefit from novel targeted therapy, relevant markers should be tested in an appropriate context. In addition, immunotherapy of lung cancer is guided by the status of programmed death-ligand 1 expression in tumor cells. The variety and versatility of cytological specimen preparations offer significant advantages for molecular testing; however, they frequently remain underused. Therefore, evaluating the utility and adequacy of cytologic specimens is important, not only from a lung cancer diagnosis, but also for the large number of ancillary studies that are necessary to provide appropriate clinical management. A large proportion of lung cancers is diagnosed by aspiration or exfoliative cytology specimens; thus, optimizing strategies to triage and best use the tissue for diagnosis and biomarker studies forms a critical component of lung cancer management. In this review, we discuss the opportunities and challenges of using cytologic specimens for biomarker testing of lung cancer and the role of cytopathology in the molecular era.

**Key Words:** Lung neoplasms; Cytology; Molecular testing; Biomarkers; Precision medicine

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The genomic landscape of non–small cell lung carcinoma (NSCLC) is constantly evolving, with the discovery of a growing number of molecular alterations and associated targeted therapies that have a huge impact on patient care. The College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology (CAP/IASLC/AMP) issued a guideline in 2013 to provide a roadmap for molecular testing to select patients for treatment with targeted tyrosine kinase inhibitors [1]. However, since 2013, many new emerging target molecules have been identified, including mutations in *BRAF*, *ERBB2*, and in *MET* exon 14, and rearrangements in RET. The guidelines were thus updated in 2018 and endorsed by the American Society of Clinical Oncology [2]. The latest version of the molecular testing guidelines for NSCLC recommends that molecular studies be performed before any systemic therapy is administered to assess a minimum of epidermal growth factor receptor (*EGFR*) mutations, anaplastic lymphoma kinase (*ALK*) and c-ros oncogene 1 (*ROS1*) rearrangements, and *BRAF* mutations [2]. In addition, with the advent of immunotherapy, evaluating the expression level of programmed death-ligand 1 (PD-L1) has been recommended for the identification of patients who respond to immune checkpoint inhibitors [3].

Despite the rapid increase in the number of clinically relevant biomarkers for advanced-stage NSCLC, limited availability of tissue samples for molecular analysis remains a major challenge. Undoubtedly, both surgical and biopsy samples still represent the "gold standard" of the starting material for molecular purposes. This is mainly because formalin-fixed and paraffin-embedded (FFPE) histological specimens have the advantage of enabling morphological evaluation, and do not require additional molecular validation. However, in real-world clinical practice, obtaining sufficient tissue specimens from advanced-stage NSCLC




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patients is highly impractical. In this setting, cytological samples may be an excellent alternative to histological samples. The updated CAP/IASLC/AMP guidelines recommend the adoption of cytological smears for molecular analysis of advanced-stage NSCLC patients [2].

Here, we critically reviewed the molecular cytopathology of NSCLC, including (1) the various types of lung cytology specimens, preparation methods, and pre-analytic factors affecting nucleic acid yield and downstream biomarker testing; (2) the variety of molecular techniques applied to cytology samples; and (3) the opportunities and challenges in biomarker testing of cytological specimens.

## WHICH CYTOLOGICAL SPECIMENS CAN BE USED?

The most common cytologic sampling methods in NSCLC cancer patients are fine needle aspiration of computed tomography–guided or electromagnetic navigation bronchoscopy–guided lung lesions and endobronchial ultrasound–guided lymph nodes and collection of exfoliative samples such as body fluid/effusions, bronchial brushing/washings, bronchoalveolar lavages, and sputum. Occasionally, minimally invasive aspiration samples from distant, deep-seated, or superficial metastatic lesions are also included. Cytological preparations that can be used for molecular studies include cell blocks (CBs), needle rinses, direct smears, cytospins, and liquid-based preparations (LBPs). To provide the best material for biomarker testing, the correct choice among different cytological preparations of the same sample should be considered. Representative microscopic images and advantages/disadvantages of different cytological preparations are shown in Table 1 and Fig. 1, respectively.

CBs are most commonly used for molecular diagnostic testing because they closely recapitulate FFPE specimens and generally do not require further validation; in addition, it is relatively easy to acquire multiple serial sections to perform immunocytochemical and molecular diagnostic assays [4]. However, on-site adequacy evaluation cannot be performed on CB, which leads to unpredictable results of cellularity and sometimes renders the CB paucicellular. Additionally, tumor cells are often widely spaced, resulting in low tumor cellularity per section area. In addition, the standard 4–5-μm CB sections do not represent the entire nuclei from the cell and are likely to have lower nucleic acid yields for molecular testing per cell than the whole cells obtained from other non–formalin-fixed cytologic preparations. To increase nucleic acid yield, not only providing more sections, but also macrodissecting the regions of highest tumor cellularity may be an option [5].

Direct smears and cytospins that are either air-dried or ethanol-fixed are not formalin-fixed preparations, which have the obvious advantage of obtaining an excellent quality material with a higher nucleic acid yield than CBs [6,7]. Besides being suitable for DNA-based next-generation sequencing (NGS) analysis, direct smears may also be appropriate for RNA-based NGS testing [8]. In addition, they offer the advantage of on-site adequacy assessment and better triaging of the sample for diagnosis and ancillary studies. In cases in which all or most of the diagnostic material is on a single smear/cytospin preparation that will be used for biomarker testing, CAP guidelines allow for the sacrifice of diagnostic material when medically necessary; the diagnostic slide can be digitally scanned for the archives to mitigate the medicolegal constraints [9].

Finally, LBPs represent a valuable alternative to conventional preparations to avoid inadequate management of the achieved material. The advantages of liquid-based cytology (LBC) speci-

**Table 1.** Advantages and disadvantages of different cytological preparations for molecular testing

| | Advantage | Disadvantage |
|---|---|---|
| Cell-block | Does not require additional validation for molecular assays (guideline recommendation)<br>Serial sections for downstream testing → diagnostic smear can preserve | Formalin artifacts in nucleic acids may affect quality of extracted DNA → relatively poor DNA quality<br>Inability to perform on-site adequacy assessment → cannot predict cellularity<br>4- to 5-μm sections are not representative of the entire nucleus |
| Direct smear | High-quality nucleic acids (non–formalin fixed) and acquisition of whole cells<br>Direct assessment of adequacy and cellularity | Sacrificing of slides from the patient archival material<br>Obtained tissue may be low volume to proceed with downstream processes |
| Cytospin | High-quality nucleic acids (non–formalin fixed) and acquisition of whole cells<br>Direct assessment of adequacy and cellularity<br>Direct extraction → no preanalytic factors associated with scraping/cell lifting (Pellet only) | Sacrificing of slides from the patient archival material<br>Obtained tissue may be low volume to proceed with downstream processes<br>Inability to assess presence of tumor and tumor fraction (Pellet only) |
| Liquid-based preparations | Optimal preservation of cells → recovery of good quality of DNA | Different preservatives (CytoLyt vs. CytoRich Red) can have quantitative/qualitative differences in nucleic acid yields |

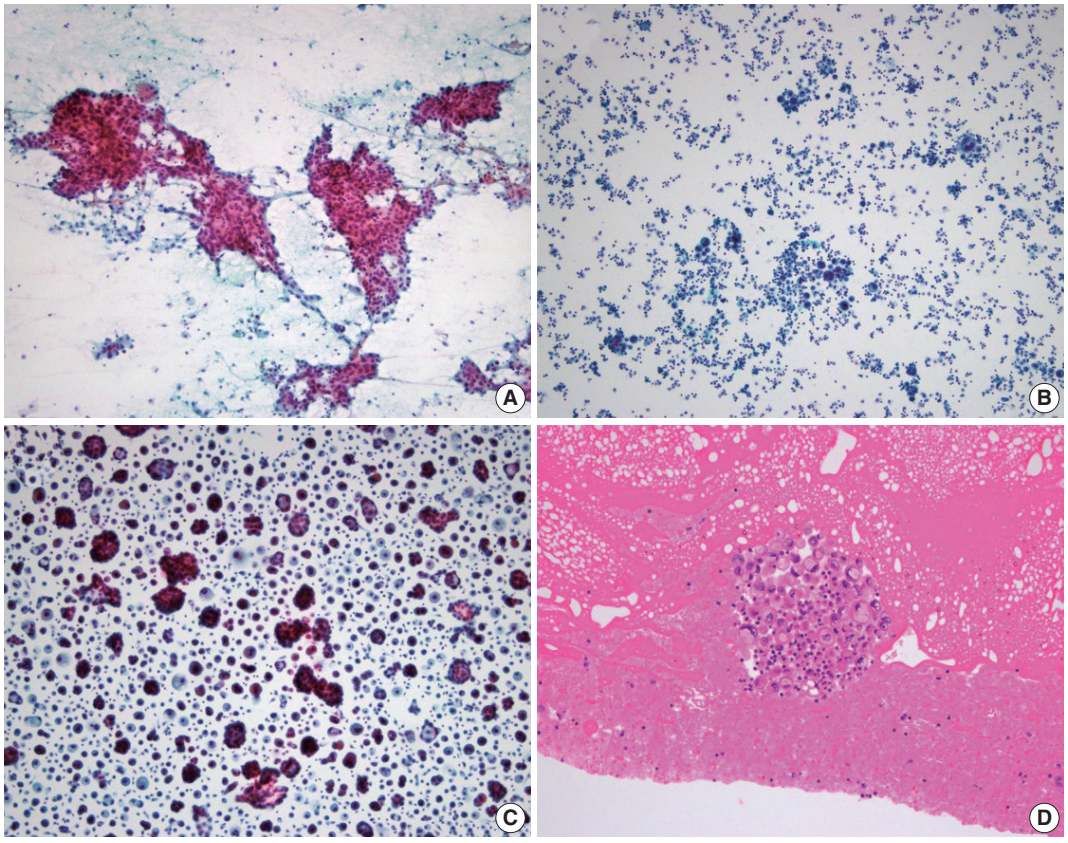


**Fig. 1.** Reperesentatvie microscopic findings according to cytologic preparations diagnosed as metastatic non-small cell carcinoma. (A) Direct smear of endobronchial ultrasound–guided fine needle aspiration samples from mediastinal lymph nodes (Papanicolaou stain). (B, C) Cytospin and liquid based preparation of pleural fluids from advanced lung cancer patients, respectively (Papanicolaou stain). (D) Cell block from (B).

mens include optimal cell preservation, easy specimen transportation because of the stability of cells at room temperature, and minimal background debris and blood on slides [10-12]. Nucleic acid can be extracted from both rinse solutions, and cells can be scraped off the sides [13-15]. Of note, the properties of the different preservative solutions used in LBC may affect downstream molecular analysis. Some studies have indicated that cells preserved in CytoLyt (Cytyc Corp., Boxborough, MA, USA) solution provide higher DNA yields than those preserved in CytoRich Red fluid [16]. One study comparing cellularity and DNA yield between ThinPrep (Cytyc Corp.) slides (CytoLyt LBC) and direct smears reported greater cellularity and significantly higher average DNA yields in the latter [13], whereas a more recent study reported issues with long-term DNA stability and accelerated DNA degradation in LBC samples when compared with conventional smears [17].

## WHAT TYPES OF BIOMARKER TESTING CAN BE PERFORMED ON CYTOLOGY SPECIMENS?

### Polymerase chain reaction–based tests

Molecular testing for genetic mutations, such as in *EGFR*, in cytologic specimens has been described using a variety of polymerase chain reaction (PCR)–based techniques, including direct sequencing, real-time PCR, pyrosequencing, and peptide nucleic acid–locked nucleic acid [14,18-21]. Different techniques have different limits of detection and reference ranges, and the choice of platform used for the detection of mutations remains a decision of the individual molecular laboratories performing the assay (Table 2). Although the CAP/IASLC/AMP guidelines recommend a technique used to detect mutations in specimens with > 50% tumor fraction [2], more sensitive platforms capable of detecting mutations in specimens with < 10% tumor are strongly encouraged. The adequacy of cytological samples for mutational analysis is another important factor that is assessed according to tumor cellularity and viability. The CAP/IASLC/AMP guide-


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lines recommend testing from samples with as little as 20% tumor cellularity because current mutation testing uses PCR-based methods that are more sensitive than unmodified Sanger sequencing [2]. In our study for the detection of *EGFR* mutation using the cytologic samples, the following parameters were correlated with the most reliable *EGFR* mutation results using the pyrosequencing method (100% concordance with the corresponding histologic specimens) in cytologic samples: a DNA concentration > 25 ng/μL, content of > 30 tumor cells, or a tumor percentage > 30% [22].

### Fluorescence in situ hybridization

To detect gene rearrangements such as in *ALK* and *ROS1*, fluorescence in situ hybridization (FISH), which was verified as a break-apart probe in a clinical trial, was first certified as a companion diagnostic test [23]. Previously, FISH testing was recommended only for CBs, but the 2018 CAP/IASLC/AMP guidelines recommend the use of conventional cytologic preparations for FISH [2]. Several groups have reported the potential and usefulness of the probe in non-formalin cytological preparations, including Diff-Quik and Papanicolaou-stained smears, as well as LBC ThinPrep slides; some report better performance than that seen with CB sections [24-26]. The advantage of using smears or LBCs is that whole-cell nuclei are analyzed to eliminate signal loss due to truncating artifacts, as seen in FFPE sections, but the disadvantage is that thresholds for positive and negative cutoffs are established using FFPE histological materials [27]. Therefore, independent standardization and validation of each sample type are required.

**Table 2.** Commonly used mutation detection assays in lung cancer cytology samples

| Assay | Type | Limit of detection (%) |
|---|---|---|
| Sanger sequencing | General (within analyzed gene regions) | 10–20 |
| Pyrosequencing | Targeted | 5–10 |
| Real-time PCR | Targeted | 0.5–5 |
| Digital PCR | Targeted | 0.1–1 |
| NGS | General (within analyzed gene regions) | 0.01–5 |

PCR, polymerase chain reaction; NGS, next generation sequencing.

### Immunocytochemistry

After the ALK D5F3 CDx Assay (Ventana Medical Systems, Tucson, AZ, USA) was approved by the Food and Drug Administration (FDA), immunohistochemistry (IHC) has been established as a confirmatory diagnostic test rather than screening, supplementing the shortcomings of FISH in detecting *ALK* rearrangement. The 2018 CAP/IASLC/AMP guidelines recommend ALK IHC as a valid alternative to the FISH (Fig. 2) [2]. The FDA has approved the assay only for "routinely processed, paraffin-embedded specimens fixed in neutral-buffered formalin." However, several studies have demonstrated the feasibility of ALK immunocytochemistry (ICC) for direct smears and LBPs [28,29]. The updated guidelines recommend using ROS1 IHC with D4D6 (Cell Signaling Technology, Danvers, MA, USA) only as a screening test that requires confirmation by a molecular or cytogenetic method [2]. A limited number of studies using *ROS1* FISH in cytological specimens are available [30,31]. Studies on the use of ROS1 ICC in cytology preparations are currently lim-

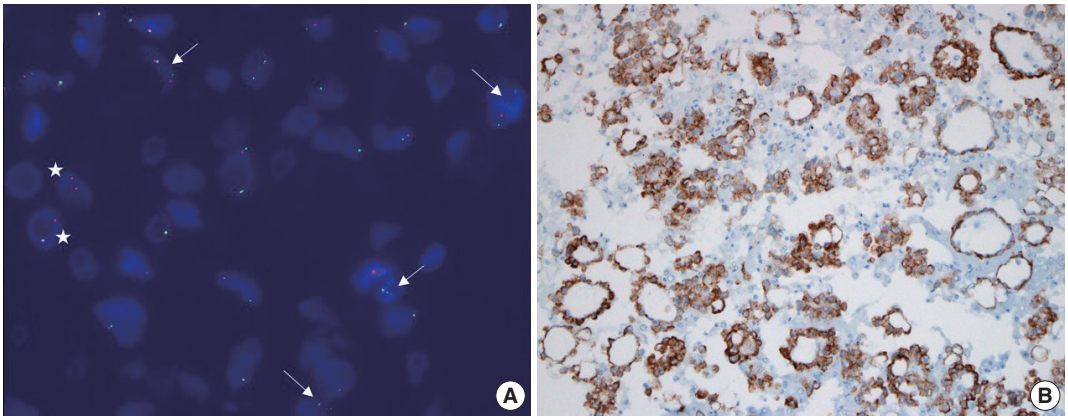


**Fig. 2.** Fluorescence in situ hybridization using an LSI anaplastic lymphoma kinase (ALK) dual-color break-apart probe (A) and immunocytochemical staining using ALK D5F3 clone (B) on cytologic blocks of lung adenocarcinoma. (A) Two distinct red and green (break apart) signals with one intact fusion signal patterns (arrows) and an isolated red signal (IRS) with one intact fusion signal pattens (asterisks) were observed in > 50% of tumor cells. (B) Tumor cells exhibited strong, granular, and diffuse cytoplasmic signal, indicating aberrant ALK protein expression generated by gene fusion.

ited in the literature [32,33].

However, application of these assays to cytologic specimens requires meticulous validation because these assays are validated primarily on FFPE histological tissue samples. The lack of standardized processing protocols in cytology lead to a variety of preanalytic variables that can affect the antigenicity of antibodies used for predictive biomarker testing. CBs are most widely used for ICC; however, there is no standardized protocol for the type of collection media, prefixation, and processing techniques, and there is wide variation among pathology laboratories. Several recent studies have highlighted issues with immunostaining of specific markers that demonstrate reduced antigenicity and false-negative results, mostly related to ethanol or methanol-based fixatives used prior to CB preparation [34,35]. Non-CB cytological preparations present an even greater challenge for ICC validation. Immunostaining of ethanol-fixed smears or cytospins is used more frequently, with prior Papanicolau staining that can identify areas or cells of interest, or air-dried unfixed extra slides that can be used for ICC, usually after post-fixation step involving formalin or acetone [36,37]. In a recent meta-analysis of ALK ICC, the smear showed a slightly lower sensitivity than that of CB. These results are interpreted to indicate that the expression intensity of the antibody is low in alcohol-fixed smear slides because the expression of the antibody is optimized in FFPE [37].

Unfortunately, guidelines for PD-L1 testing have not yet been provided even in updated guideline [2,38]. Although cytology specimens were not included in the initial clinical validation studies for PD-L1, several groups have evaluated the feasibility of PD-L1 in cytology specimens and have demonstrated results that are comparable to those of paired histologic samples [39,40]. Fig. 3 shows representative microscopic findings of strong positive expression of PD-L1 stained in CB of a patient diagnosed with metastatic adenocarcinoma in pericardial fluid. Lozano et al. reported the variation in patterns of PD-L1 expression on cytological specimens; because entire cells were present on direct smear, tumor cells often demonstrated a folded cell membrane, demonstrating a thick and strong membranous positivity [41]. Taken together, ICC in cytologic specimens remains a number of challenges to be solved throughout the standardization of protocols that can control preanalytical variables, rigorous validation of staining results, and systematic training for interpretation.

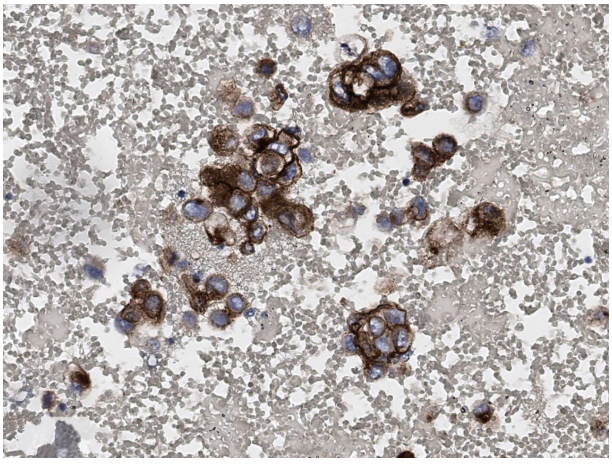

**Fig. 3.** Programmed death–ligand 1 (PD-L1) expression on a cytological cell block of pericardial fluid with advanced lung adenocarcinoma patient using the PD-L1 22C3 PharmDx assay.

## Next-generation sequencing

Next-generation sequencing (NGS) is a fascinating tool that can analyze multiple genetic alterations simultaneously, even when applied to cytological samples with low DNA/RNA yields. The advantages of using cytology specimens for NGS include quicker fixation or, if the platform is validated, minimal/no fixation, improving the quality of the input nucleic acids. Several studies using cytological material, including CBs as well as non-FFPE substrates, have shown them to be equally effective in the genomic profiling of NSCLC by NGS analysis [42-46]. In fact, some studies have indicated better quality metrics when comparing NGS analysis in non-FFPE cytologic substrates versus FFPE materials [6,47]. However, studies of the application of NGS to cytology specimens generally have a retrospective design, and only samples characterized by at least 20% of tumor cells, which may not fully reflect current practice, were selected. Therefore, it is crucial to establish the minimum number of cells needed to allow an NGS approach from cytology sample in routine practice. In any case, sample requirement depends on target capture, gene panel, and platform types. Illumina NGS usually requires more cells and/or higher DNA input than Ion Torrent NGS; thus, the latter seems to be more efficient with the cytopathologist specimens [5]. Recently, it was shown that lowering the input DNA concentration below the manufacturer's recommended threshold of 10 ng (> 0.8 ng/μL) is feasible leading to a marked increase in the NGS success rate from 58.6% to 89.8% [5,48]. More important than DNA input is the percentage of neoplastic cells. The preferential amplification of a small number of DNA in a small amount of cancer cells may only be representative of non-neoplastic components, which may lead to false-negative results. Macrodissection or microdissection are especially important for enrichment of viable tumor cells [5,49].


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## CHALLENGES AND FUTURE DIRECTIONS

In this era of personalized medicine, biomarker testing of cytology preparations is a relatively new and rapidly developing field with great potential, especially in patients with advanced NSCLC. However, cytological specimens continue to be excluded from most biomarker-driven clinical trials, primarily because of the failure to exploit the variety of different specimen preparations and the lack of validation for different assays. The lack of standardization of specimen processing among laboratories is major limitation. Therefore, the implementation of strategies to optimize and standardize procedures for specimen acquisition, processing, and tissue extraction is critical to maximize the use of cytological samples for ancillary studies and to provide relevant information for inclusion in clinical trial design. At minimum, confirmation of validation for cytology preparations and close check of quantity and quality of submitted material is also expected.

## CONCLUSION

In conclusion, biomarker testing can be used for a variety of cytologic specimen types and preparations. This is of utmost importance for NSCLC patients, where the cytology specimen may be the only sample available for diagnosis and ancillary studies. Therefore, a thorough understanding of the potential and the limitations of these substrates is required to properly classify and use them for molecular studies that can guide patient management.

### Ethics Statement

Not applicable.

### 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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### Author Contributions

Conceptualization: HK, JHC. Writing—original draft: HK. Writing—review & editing: HK, JHC. Approval of final manuscript: all authors.

### Conflicts of Interest

JHC, a contributing editor of the *Journal of Pathology and Translational Medicine*, was not involved in the editorial evaluation or decision to publish this article. Remaining author has declared no conflicts of interest.

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### References

1. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. J Thorac Oncol 2013; 8: 823-59.
2. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol 2018; 13: 323-58.
3. Camidge DR, Doebele RC, Kerr KM. Comparing and contrasting predictive biomarkers for immunotherapy and targeted therapy of NSCLC. Nat Rev Clin Oncol 2019; 16: 341-55.
4. Roh MH. The Utilization of cytologic fine-needle aspirates of lung cancer for molecular diagnostic testing. J Pathol Transl Med 2015; 49: 300-9.
5. Roy-Chowdhuri S, Stewart J. Preanalytic variables in cytology: lessons learned from next-generation sequencing: the MD Anderson experience. Arch Pathol Lab Med 2016; 140: 1191-9.
6. Hwang DH, Garcia EP, Ducar MD, Cibas ES, Sholl LM. Next-generation sequencing of cytologic preparations: an analysis of quality metrics. Cancer Cytopathol 2017; 125: 786-94.
7. Harada S, Agosto-Arroyo E, Levesque JA, et al. Poor cell block adequacy rate for molecular testing improved with the addition of Diff-Quik-stained smears: need for better cell block processing. Cancer Cytopathol 2015; 123: 480-7.
8. Velizheva NP, Rechsteiner MP, Wong CE, et al. Cytology smears as excellent starting material for next-generation sequencing-based molecular testing of patients with adenocarcinoma of the lung. Cancer Cytopathol 2017; 125: 30-40.
9. Huang M, Wei S. Overview of molecular testing of cytology specimens. Acta Cytol 2020; 64: 136-46.
10. Petriella D, Galetta D, Rubini V, et al. Molecular profiling of thinprep FNA samples in assisting clinical management of non-small-cell lung cancer. Mol Biotechnol 2013; 54: 913-9.
11. Abedi-Ardekani B, Vielh P. Is liquid-based cytology the magic bullet for performing molecular techniques? Acta Cytol 2014; 58: 574-81.
12. Zeppa P. Liquid-based cytology: a 25-year bridge between the pap smear and molecular cytopathology. Acta Cytol 2014; 58: 519-21.
13. Bellevicine C, Malapelle U, Vigliar E, de Luca C, Troncone G. Epidermal growth factor receptor test performed on liquid-based cytology lung samples: experience of an academic referral center. Acta Cytol 2014; 58: 589-94.
14. Malapelle U, de Rosa N, Bellevicine C, et al. *EGFR* mutations detection on liquid-based cytology: is microscopy still necessary? J Clin Pathol 2012; 65: 561-4.
15. Reynolds JP, Tubbs RR, Minca EC, et al. *EGFR* mutational genotyping of liquid based cytology samples obtained via fine needle aspiration (FNA) at endobronchial ultrasound of non-small cell lung cancer (NSCLC). Lung Cancer 2014; 86: 158-63.

16. Dejmek A, Zendehrokh N, Tomaszewska M, Edsjo A. Preparation of DNA from cytological material: effects of fixation, staining, and mounting medium on DNA yield and quality. Cancer Cytopathol 2013; 121: 344-53.
17. Kim WY, Oh SY, Kim H, Hwang TS. DNA degradation in liquid-based cytology and its comparison with conventional smear. Diagn Cytopathol 2016; 44: 450-8.
18. Lozano MD, Zulueta JJ, Echeveste JI, et al. Assessment of epidermal growth factor receptor and K-ras mutation status in cytological stained smears of non-small cell lung cancer patients: correlation with clinical outcomes. Oncologist 2011; 16: 877-85.
19. Billah S, Stewart J, Staerkel G, Chen S, Gong Y, Guo M. *EGFR* and *KRAS* mutations in lung carcinoma: molecular testing by using cytology specimens. Cancer Cytopathol 2011; 119: 111-7.
20. Malapelle U, Bellevicine C, De Luca C, et al. *EGFR* mutations detected on cytology samples by a centralized laboratory reliably predict response to gefitinib in non-small cell lung carcinoma patients. Cancer Cytopathol 2013; 121: 552-60.
21. Rekhtman N, Brandt SM, Sigel CS, et al. Suitability of thoracic cytology for new therapeutic paradigms in non-small cell lung carcinoma: high accuracy of tumor subtyping and feasibility of EGFR and KRAS molecular testing. J Thorac Oncol 2011; 6: 451-8.
22. Sun PL, Jin Y, Kim H, Lee CT, Jheon S, Chung JH. High concordance of *EGFR* mutation status between histologic and corresponding cytologic specimens of lung adenocarcinomas. Cancer Cytopathol 2013; 121: 311-9.
23. Lindeman NI, Cagle PT, Beasley MB, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Arch Pathol Lab Med 2013; 137: 828-60.
24. Minca EC, Lanigan CP, Reynolds JP, et al. ALK status testing in non-small-cell lung carcinoma by FISH on ThinPrep slides with cytology material. J Thorac Oncol 2014; 9: 464-8.
25. Betz BL, Dixon CA, Weigelin HC, Knoepp SM, Roh MH. The use of stained cytologic direct smears for *ALK* gene rearrangement analysis of lung adenocarcinoma. Cancer Cytopathol 2013; 121: 489-99.
26. Proietti A, Ali G, Pelliccioni S, et al. Anaplastic lymphoma kinase gene rearrangements in cytological samples of non-small cell lung cancer: comparison with histological assessment. Cancer Cytopathol 2014; 122: 445-53.
27. Roy-Chowdhuri S, Aisner DL, Allen TC, et al. Biomarker testing in lung carcinoma cytology specimens: a perspective from members of the Pulmonary Pathology Society. Arch Pathol Lab Med 2016; 140: 1267-72.
28. Savic S, Bode B, Diebold J, et al. Detection of ALK-positive non-small-cell lung cancers on cytological specimens: high accuracy of immunocytochemistry with the 5A4 clone. J Thorac Oncol 2013; 8: 1004-11.
29. Rosenblum F, Hutchinson LM, Garver J, Woda B, Cosar E, Kurian EM. Cytology specimens offer an effective alternative to formalin-fixed tissue as demonstrated by novel automated detection for ALK break-apart FISH testing and immunohistochemistry in lung adenocarcinoma. Cancer Cytopathol 2014; 122: 810-21.
30. Bozzetti C, Nizzoli R, Tiseo M, et al. *ALK* and *ROS1* rearrangements tested by fluorescence in situ hybridization in cytological smears from advanced non-small cell lung cancer patients. Diagn Cytopathol 2015; 43: 941-6.
31. Fernandez-Bussy S, Labarca G, Pires Y, Caviedes I, Burotto M. Molecular testing of EGFR, EGFR resistance mutation, *ALK* and *ROS1* achieved by EBUS-TBNA in Chile. Arch Bronconeumol 2017; 53: 172-4.
32. Vlajnic T, Savic S, Barascud A, et al. Detection of ROS1-positive non-small cell lung cancer on cytological specimens using immunocytochemistry. Cancer Cytopathol 2018; 126: 421-9.
33. Conde E, Hernandez S, Martinez R, et al. Assessment of a new ROS1 immunohistochemistry clone (SP384) for the identification of *ROS1* rearrangements in patients with non-small cell lung carcinoma: the ROSING study. J Thorac Oncol 2019; 14: 2120-32.
34. Sauter JL, Grogg KL, Vrana JA, Law ME, Halvorson JL, Henry MR. Young investigator challenge: validation and optimization of immunohistochemistry protocols for use on cellient cell block specimens. Cancer Cytopathol 2016; 124: 89-100.
35. Gruchy JR, Barnes PJ, Dakin Hache KA. CytoLyt(R) fixation and decalcification pretreatments alter antigenicity in normal tissues compared with standard formalin fixation. Appl Immunohistochem Mol Morphol 2015; 23: 297-302.
36. Shidham VB, Chang CC, Rao RN, Komorowski R, Chivukula M. Immunostaining of cytology smears: a comparative study to identify the most suitable method of smear preparation and fixation with reference to commonly used immunomarkers. Diagn Cytopathol 2003; 29: 217-21.
37. Roy-Chowdhuri S. Immunocytochemistry of cytology specimens for predictive biomarkers in lung cancer. Transl Lung Cancer Res 2020; 9: 898-905.
38. Chang S, Shim HS, Kim TJ, et al. Molecular biomarker testing for non-small cell lung cancer: consensus statement of the Korean Cardiopulmonary Pathology Study Group. J Pathol Transl Med 2021; 55: 181-91.
39. Skov BG, Skov T. Paired comparison of PD-L1 expression on cytologic and histologic specimens from malignancies in the lung assessed with PD-L1 IHC 28-8pharmDx and PD-L1 IHC 22C3pharmDx. Appl Immunohistochem Mol Morphol 2017; 25: 453-9.
40. Noll B, Wang WL, Gong Y, et al. Programmed death ligand 1 testing in non-small cell lung carcinoma cytology cell block and aspirate smear preparations. Cancer Cytopathol 2018; 126: 342-52.
41. Lozano MD, Abengozar-Muela M, Echeveste JI, et al. Programmed death-ligand 1 expression on direct Pap-stained cytology smears from non-small cell lung cancer: comparison with cell blocks and surgical resection specimens. Cancer Cytopathol 2019; 127: 470-80.
42. Karnes HE, Duncavage EJ, Bernadt CT. Targeted next-generation sequencing using fine-needle aspirates from adenocarcinomas of the lung. Cancer Cytopathol 2014; 122: 104-13.
43. Baum JE, Zhang P, Hoda RS, et al. Accuracy of next-generation sequencing for the identification of clinically relevant variants in cytology smears in lung adenocarcinoma. Cancer Cytopathol 2017; 125: 398-406.
44. Buttitta F, Felicioni L, Del Grammastro M, et al. Effective assessment of egfr mutation status in bronchoalveolar lavage and pleural fluids by next-generation sequencing. Clin Cancer Res 2013; 19: 691-8.
45. Reynolds JP, Zhou Y, Jakubowski MA, et al. Next-generation sequencing of liquid-based cytology non-small cell lung cancer samples. Cancer Cytopathol 2017; 125: 178-87.
46. Treece AL, Montgomery ND, Patel NM, et al. FNA smears as a potential source of DNA for targeted next-generation sequencing of

 https://doi.org/10.4132/jptm.2022.10.17

lung adenocarcinomas. Cancer Cytopathol 2016; 124: 406-14.
47. Roy-Chowdhuri S, Chen H, Singh RR, et al. Concurrent fine needle aspirations and core needle biopsies: a comparative study of substrates for next-generation sequencing in solid organ malignancies. Mod Pathol 2017; 30: 499-508.
48. Roy-Chowdhuri S, Goswami RS, Chen H, et al. Factors affecting the success of next-generation sequencing in cytology specimens. Cancer Cytopathol 2015; 123: 659-68.
49. Bellevicine C, Malapelle U, Vigliar E, Pisapia P, Vita G, Troncone G. How to prepare cytological samples for molecular testing. J Clin Pathol 2017; 70: 819-26.

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ORIGINAL ARTICLE

# Usefulness of BRAF VE1 immunohistochemistry in non–small cell lung cancers: a multi-institutional study by 15 pathologists in Korea

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**Background:** Next-generation sequencing (NGS) is an approved test to select patients for *BRAF* V600E targeted therapy in Korea. However, the high cost, long turnaround times, and the need for sophisticated equipment and skilled personnel limit the use of NGS in daily practice. Immunohistochemistry (IHC) is a rapid and relatively inexpensive assay available in most laboratories. Therefore, in this study, we evaluate the usefulness of BRAF VE1 IHC in terms of predictive value and interobserver agreement in non–small cell lung cancers (NSCLCs). **Methods:** A total of 30 cases with known *BRAF* mutation status were selected, including 20 cases of lung adenocarcinomas, six cases of colorectal adenocarcinomas, and four cases of papillary thyroid carcinomas. IHC for BRAF V600E was carried out using the VE1 antibody. Fifteen pathologists independently scored both the staining intensity and the percentage of tumor cell staining on whole slide images. **Results:** In the lung adenocarcinoma subset, interobserver agreement for the percentage of tumor cell staining and staining intensity was good (percentage of tumor cell staining, intraclass correlation coefficient = 0.869; staining intensity, kappa = 0.849). The interobserver agreement for the interpretation using the cutoff of 40% was almost perfect in the entire study group and the lung adenocarcinoma subset (kappa = 0.815). Sensitivity, specificity, positive predictive value, and negative predictive value of BRAF VE1 IHC were 80.0%, 90.0%, 88.9%, and 81.8%, respectively. **Conclusions:** BRAF VE1 IHC could be a screening test for the detection of *BRAF* V600E mutation in NSCLC. However, further studies are needed to optimize the protocol and to establish and validate interpretation criteria for BRAF VE1 IHC.

**Key Words:** *BRAF* V600E; Observer variation; Predictive value of tests; Immunohistochemistry; Adenocarcinoma of lung

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Dabrafenib plus trametinib combination treatment received approval and reimbursement for patients with serine/threonine-protein kinase B-raf (*BRAF*) V600E–mutant advanced non–small cell lung cancers (NSCLCs) in Korea [1]. Next-generation sequencing (NGS) is an approved test to select patients for *BRAF* V600E targeted therapy in Korea. The advantage of NGS is that it can detect low-frequency variants due to its high sensitivity and can perform comprehensive genome-wide testing [1,2]. However, the high cost, long turnaround times, and the need for sophisticated equipment and skilled personnel limit the use of NGS in daily practice. Although the PNAClamp BRAF mutation detection kit (Panagene, Daejeon, Korea) is approved in Korea, the test is currently not covered by the Korean health insurance system and detects the *BRAF* V600 mutation, so additional sanger sequencing or NGS should be performed to confirm V600E.

Immunohistochemistry (IHC) is a rapid and relatively inexpensive assay available in most laboratories. Anaplastic lymphoma kinase (ALK) D5F3 CDx assay (Ventana Medical Systems) is an approved IHC-based assay as a companion diagnostic for the identification of patients for treatment with ALK inhibitor [1]. IHC is




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used as a screening test for *NTRK* and *ROS1* fusions. *BRAF* V600E mutation-specific antibodies, VE1, are commercially available. VE1 antibody is mouse monoclonal and detects BRAF V600E–mutant amino acid sequence between codon amino acid 596 to 606 (GLATEKSRWSG) [3,4]. The BRAF VE1 IHC can differentiate the V600E mutation from the wild-type and non-V600E mutation in the BRAF protein [4-8].

The BRAF VE1 IHC was known to have a sensitivity of 86%–100% and a specificity of 93–100% in detecting *BRAF* V600E mutation in papillary thyroid carcinomas (PTCs), melanomas, and colorectal cancers (CRCs) with moderate to perfect interobserver agreements [3,9-20]. Hence, National Comprehensive Cancer Network guidelines recommend the BRAF VE1 IHC as a screening test for the assessment of *BRAF* V600E status in melanomas and CRCs. In spite of this, there have been few studies on BRAF VE1 IHC in NSCLCs, which reported a sensitivity of 90%–100% and specificity of 92.3%–100% (Table 1) [7,8,21,22]. In addition, there is only one study for the interobserver agreement of BRAF VE1 IHC in NSCLC [22]. The use of BRAF VE1 IHC as a screening test in NSCLC requires further validation of sensitivity, specificity, and interobserver reproducibility. Therefore, in this study, we evaluate the usefulness of BRAF VE1 IHC in terms of predictive value and interobserver agreement in NSCLCs.

## MATERIALS AND METHODS

### Materials

A total of 30 cases with known *BRAF* mutation status were selected from the archives of the Department of Pathology of Samsung Medical Center. Of these, 20 cases were lung adenocarcinomas (15 resections, 3 endobronchial ultrasound [EBUS]–guided biopsies, one needle biopsy, and one bronchoscopic biopsy), and six were colon adenocarcinomas, and four were PTCs (Table 2). The *BRAF* V600E mutation status was examined by real-time polymerase chain reaction using the Real-Q *BRAF* V600E detection kit (Biosewoom, Seoul, Korea) and a BRAF probe and primer mixture according to the manufacturer's protocol. Twenty of 30 cases had *BRAF* V600E mutation. Of 20 cases of lung adenocarcinoma, 10 cases were positive for *BRAF* V600E mutation, and others were negative. All six colon adenocarcinomas and four PTCs were positive for *BRAF* V600E mutation.

### Immunohistochemical staining and scoring

IHC for BRAF V600E was carried out using Ventana BenchMark ULTRA IHC/ISH (Ventana Medical Systems, Tucson, AZ, USA) immunostainer. Unstained slides were prepared by cutting 4-μm-thick sections. Antigen retrieval was performed using ULTRA Cell Conditioning Solution (Ventana Medical Systems). The sections were incubated with the VE1 primary antibody (mouse monoclonal, prediluted, Ventana Medical Systems) for 16 minutes at 36°C. The slides were visualized using OptiView DAB IHC Detection Kit (Ventana Medical Systems), followed by hematoxylin II counterstaining. PTCs with *BRAF* V600E mutation were used as positive controls. To obtain whole slide images, the IHC slides were scanned with DP-200 (Roche Diagnostics, Risch-Rotkreuz, Switzerland).

Fifteen pathologists independently evaluated the whole slide images using Roche uPath enterprise software (Roche Diagnostics).

**Table 1.** Literature review of *BRAF* V600E immunohistochemistry in lung cancers

| Study | No. of cases | Cases with *BRAF* V600E mutation | Manufacturer | Platform | Molecular testing | Positive criteria | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|---|---|---|---|
| Sasaki et al. [5] | 26[a] | 5 | Dako | EnVision FLEX system | RT-PCR | ≥50% of tumor cells, any intensity | 100 | 95.2 |
| Ilie et al. [6] | 450[b] | 21 | Ventana | BenchMark XT | Direct sequencing, pyrosequencing | All tumor cells, strong and homogenous staining | 90.5 | 100 |
| Gow et al. [7] | 99[a] | 29 | Ventana | Benchmark XT | Direct sequencing, RT-PCR | ≥50% of tumor cells, any intensity | 96.55 | 98.57 |
| Karbel et al. [22] | 53[c] | 5 | Bio SB | PolyDetector Detection Systems | SSCP-PCR | ≥50% of tumor cells, any intensity | 97.9 | 100 |
| Seto et al. [4] | 219[d] | 14 | Ventana | Benchmark XT | Luminex GENOSEARCH BRAF, RT-PCR | N/A | 92.9 | 100 |
| Hofman et al. [21] | 1,317[c] | 32 | Ventana | Benchmark ULTRA | NGS, pyrosequencing | ≥80% of tumor cells, strong and homogenous staining | 100 | 100 |
| Hwang et al. [8] | 39[e] | 20 | Ventana | Benchmark ULTRA | NGS | At least weak and focal staining | 90.0 | 92.3 |

RT-PCR, reverse transcription polymerase chain reaction; SSCP, single-stranded conformation polymorphism; N/A, not available; NGS, next-generation sequencing.
[a]Adenocarcinomas; [b]EGFR, KRAS, PI3KCA, Her2, and ALK wild-type non–small cell lung cancers (NSCLCs); [c]NSCLCs; [d]218 NSCLC cases and one small cell lung cancer case; [e]Confirmed *BRAF*-mutated NSCLCs by NGS.

Nine of 15 were pulmonary pathology specialists and six were surgical pathology fellows. Pathologists scored both the staining intensity and the percentage of tumor cell staining of any intensity (0%–100%, 5% increments). Tumor cell staining was defined as any perceptible cytoplasmic staining of viable tumor cells. The intensities were scored as "0" (negative staining), "1+" (weak staining), "2+" (moderate staining), and "3+" (strong staining) (Fig. 1).

### Statistical analysis

Interobserver agreement for the percentage of tumor cell staining was evaluated by the intraclass correlation coefficients (ICCs). Interobserver agreement for staining intensity was evaluated by the Kendall concordance coefficient. ICC and Kendall concordance coefficient are interpreted as follows: < 0.5 indicates poor agreement, between 0.5 and 0.75 indicates moderate agreement, between 0.75 and 0.9 indicates good agreement, and above 0.9 indicates excellent agreement. Differences in the concordance between the specialist group and fellow group were assessed by the Wilcoxon rank sum test.

The receiver operating characteristic (ROC) curve was used to determine the cutoff value for the BRAF VE1 IHC analysis. ROC curves were analyzed based on the average percentage of tumor cell staining of 15 pathologists for each case. The cutoff value or more was interpreted as positive for BRAF VE1 IHC. The sensitivity, specificity, positive predictive value, and negative predictive value for detecting *BRAF* V600E mutation were calculated based on the consistent interpretation results of more than eight of 15 pathologists (Table 2). Interobserver agreement for the interpretation was evaluated by the Fleiss kappa coefficient. A kappa coefficient of < 0.20 indicates poor, 0.21 to 0.40 indicates fair agreement, 0.41 to 0.60 indicates moderate agreement, 0.61 to 0.80 indicates substantial agreement, and greater than 0.80 indicates almost perfect agreement. Statistical analysis was performed with SPSS ver. 25 (IBM Corp., Armonk, NY, USA).

## RESULTS

### Interobserver agreement for IHC scoring

In the entire study group (n = 30), the percentage of tumor cell staining and staining intensity showed good interobserver agree-

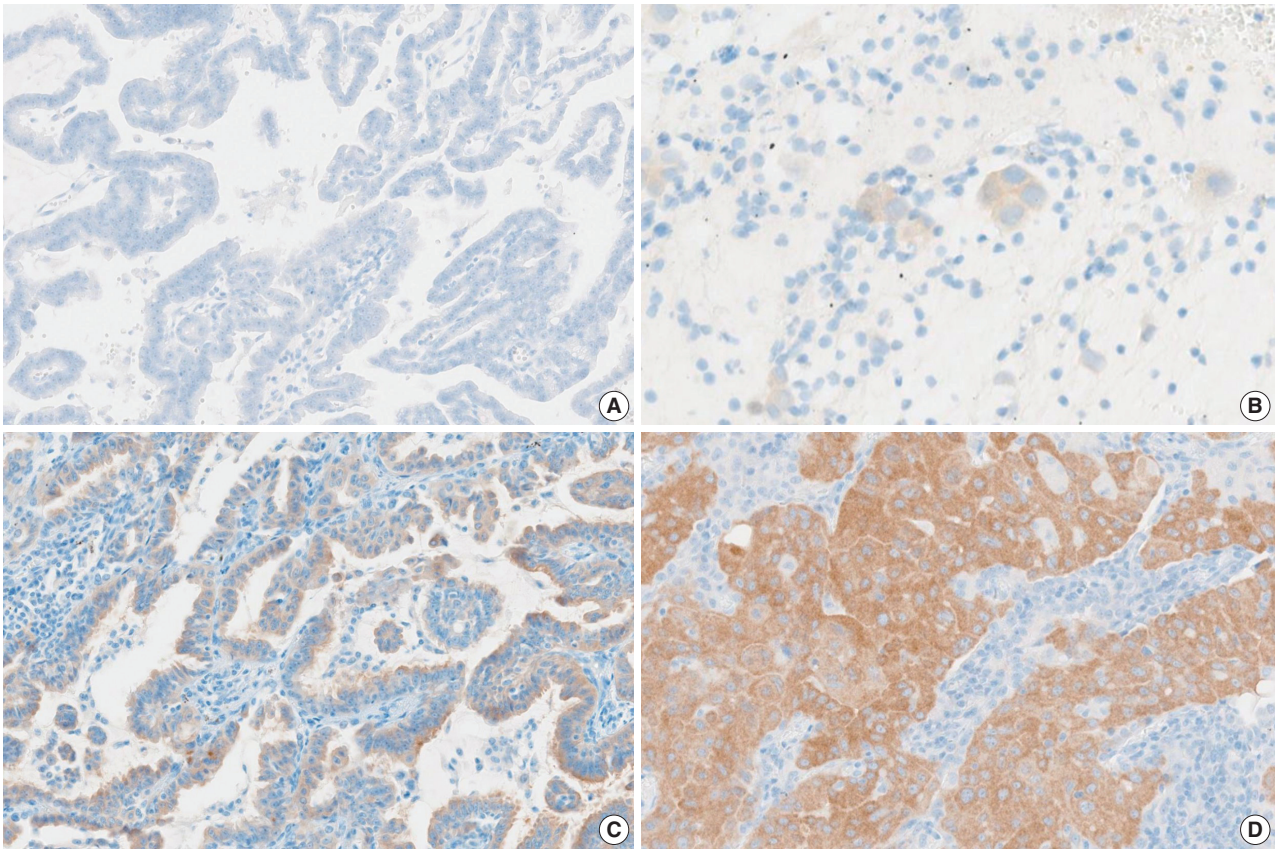


**Fig. 1.** BRAF VE1 expression was observed in the cytoplasm of tumor cells. Negative (A), weak (1+) (B), moderate (2+) (C), and strong (3+) (D) VE1 expression.


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**Table 2.** Summary of IHC interpretation

| | Case No. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Negative (non-V600E) | | | | | | | | | | Positive (V600E) | | | | | | | | | | | | | | | | | | | |
| | Lung ADC | | | | | | | | | | Lung ADC | | | | | | | | | | PTC | | | | Colon ADC | | | | | |
| | 8 | 11 | 15 | 20 | 26 | 28 | 17 | 4[a] | 24 | 22[b] | 14 | 7[c] | 6 | 1 | 5[b] | 9 | 12 | 19 | 29 | 30[b] | 10 | 13 | 21 | 16 | 2 | 3 | 23 | 25 | 27 | 18 |
| IHC | N | N | N | N | N | N | N | N | N | P | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| Specialist | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | N | N | N | N | N | N | N | N | N | P | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 2 | N | N | N | N | N | N | N | N | P | P | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 3 | N | N | N | N | N | N | N | N | P | P | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 4 | N | N | N | N | N | N | N | N | P | P | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 5 | N | N | N | N | N | N | N | N | N | P | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 6 | N | N | N | N | N | N | N | N | P | P | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 7 | N | N | N | N | N | N | N | N | N | P | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 8 | N | N | N | N | N | N | N | P | P | P | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 9 | N | N | N | N | N | N | N | N | N | P | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| Fellow | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | N | N | N | N | N | N | N | N | N | P | N | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 2 | N | N | N | N | N | N | N | N | N | N | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 3 | N | N | N | N | N | N | P | N | P | P | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 4 | N | N | N | N | N | N | N | P | N | P | N | N | N | P | P | P | P | P | P | P | P | P | P | N | P | P | P | P | P | N |
| 5 | N | N | N | N | N | N | N | N | N | P | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |
| 6 | N | N | N | N | N | N | N | N | N | P | N | N | N | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P | P |

IHC, immunohistochemistry; ADC, Adenocarcinoma; PTC, papillary thyroid carcinoma; N, negative; P, positive.
[a]Needle biopsy; [b]Endobronchial ultrasound-guided biopsy; [c]Bronchoscopic biopsy.

ments (percentage of tumor cell staining, ICC = 0.878 [95% confidence interval (CI), 0.813 to 0.930]; staining intensity, kappa = 0.804). The results in the lung adenocarcinoma subset (n = 20) also displayed good interobserver agreements for the percentage of tumor cell staining and staining intensity (percentage of tumor cell staining, ICC = 0.869 [95% CI, 0.786 to 0.935]; staining intensity, kappa = 0.849). Only the staining intensity exhibited a discrepancy between specialists and fellows (p = 0.029). VE1 staining tended to be weaker and more heterogeneous in lung adenocarcinomas than PTCs and colon adenocarcinomas.

## Diagnostic performance of BRAF VE1 IHC

Fig. 2 shows the ROC curve for the estimated diagnostic performance of the percentage of tumor cell staining in detecting *BRAF* V600E mutation. The area under the ROC curve was 0.950 (95% CI, 0.877 to 1.000). The 42.5% cutoff value maximized both sensitivity and specificity for the *BRAF* V600E mutation. Since the percentage of tumor cell staining was measured in increments of 5%, the cutoff value positive for BRAF VE1 IHC was defined as 40%. When 40% or more was considered positive for BRAF VE1 IHC, the sensitivity, specificity, positive predictive value, and negative predictive value of BRAF VE1 IHC were 90.0%, 90.0%, 94.7%, and 81.8%, respectively, for the entire study group. For the lung adenocarcinoma subset, sensitivity, specificity, positive predictive value, and negative predictive value of BRAF VE1 IHC were 80.0%, 90.0%, 88.9%, and 81.8%, respectively. False-negative results for BRAF VE1 IHC were shown in two resected lung adenocarcinoma cases (cases 7 and 14) (Table 2). Case 7 was interpreted as negative by 10 of 15 pathologists (Fig. 3A). Case 14,

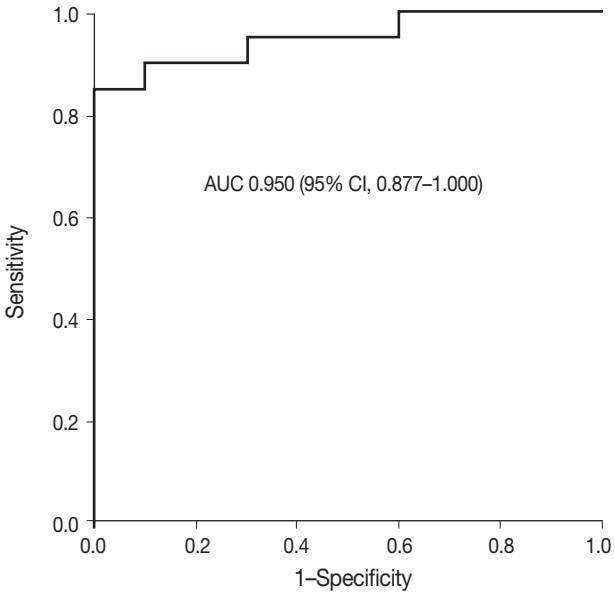


**Fig. 2.** Receiver operating characteristic curve. AUC, area under the curve; CI, confidence interval.

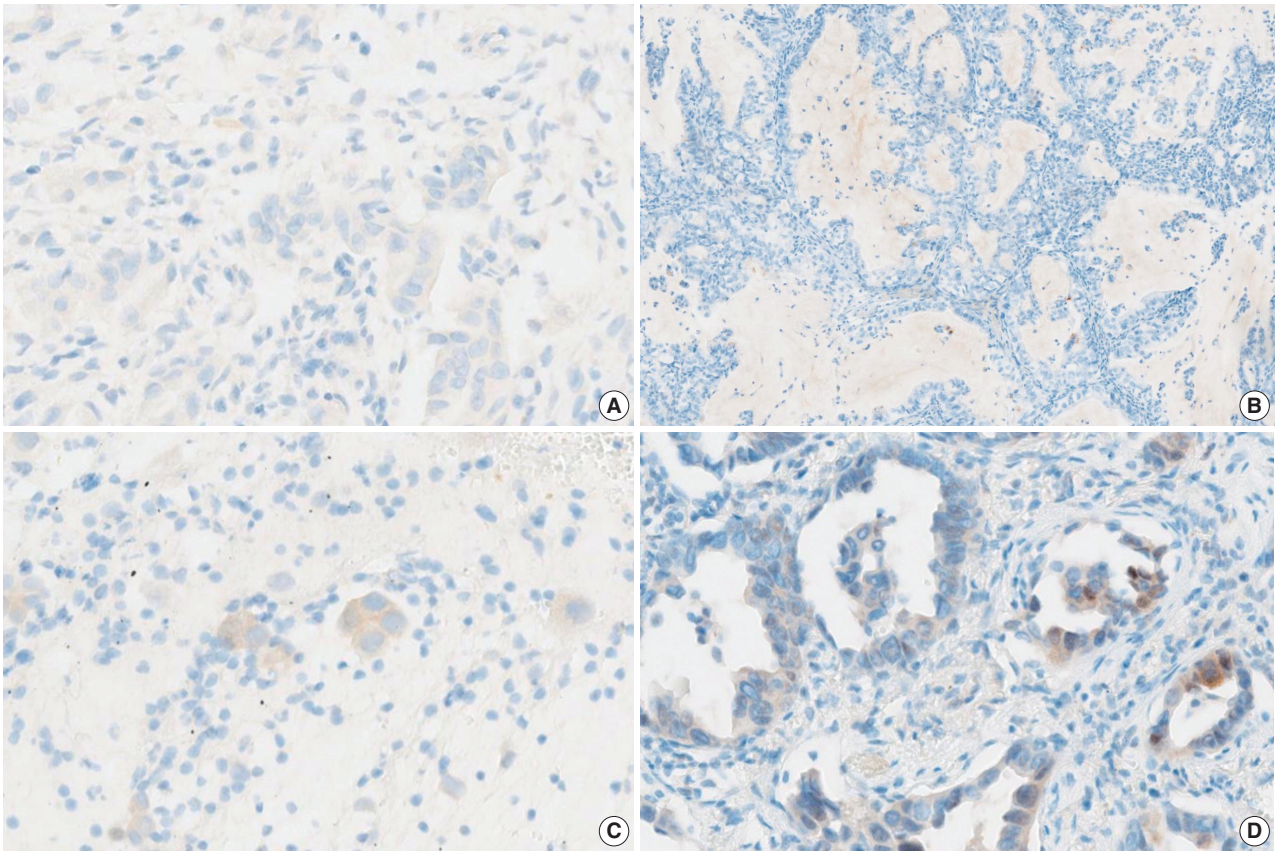


**Fig. 3.** BRAF expression in the discrepant cases. (A) Case 7 showing questionable cytoplasmic staining of faint intensity. (B) Case 14 showing no cytoplasmic staining. (C) Case 22 showing weak but diffuse cytoplasmic staining. (D) Case 24 showing heterogeneous weak to moderate staining within individual glands.

interpreted as negative by all pathologists, showed weak cytoplasmic staining in less than 5% of tumor cells (Fig. 3B). False-positive result for BRAF VE1 IHC was shown in one EBUS-guided biopsy specimen (case 22), which was interpreted as positive by 14 of 15 pathologists. Case 22 showed weak but diffuse cytoplasmic staining, but it turned out to be negative for the *BRAF* V600E mutation test (Fig. 3C).

The interobserver agreement for the interpretation using the cutoff of 40% was almost perfect in the entire study group and the lung adenocarcinoma subset (entire study group, kappa = 0.845 [95% CI, 0.810 to 0.880]; lung adenocarcinoma subset, kappa = 0.815 [95% CI, 0.772 to 0.858]). The interpretations of all 15 observers were consistent in 22 of 30 cases (73%), which includes 16 of 20 *BRAF* V600E–mutant cases (80%) and six of 10 *BRAF* V600E-negative cases (60%). Among the eight discrepant cases, cases 7 and 24 showed the greatest interobserver discrepancy (Table 2). Case 7 showed very faintly, questionable cytoplasmic staining. Thus, the percentage of tumor cell staining varied from 0% to 90% (mean 24%, standard deviation 34), depending on whether the observer considered the faint staining to be significant (Fig. 3A). Case 24, interpreted as negative by nine of 15 pathologists, showed heterogeneous weak to moderate staining within individual glands (Fig. 3D). Thus, the percentage of tumor cell staining was varied from 5% to 50% (mean 25%, standard deviation 20). In case 6, interpreted as positive by 12 of 15 pathologists, the percentage of tumor cell staining varied from 30% to 90% (mean ± standard deviation, 56% ± 20%) due to heterogenous zonal staining with weak to moderate intensity. The other five cases showed discrepancies in one or two observers.

Considering the presence of moderate to strong granular cytoplasmic staining in any tumor cells as positive criteria, sensitivity, specificity, positive predictive value, and negative predictive value were 85.0%, 90.0%, 94.4%, and 75.0% in the entire study group and 70.0%, 90.0%, 87.5%, and 75.0% in the lung adenocarcinomas. Complete agreement for all observers was obtained in four cases of 10 non-*BRAF* V600E mutant cases (40%) and eight of 20 *BRAF* V600E cases including one false-negative case (40%).


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## DISCUSSION

This study shows that interobserver agreement for BRAF VE1 IHC interpretation was almost perfect (kappa = 0.815) in lung adenocarcinoma, similar to the results of previous studies. Karbel et al. [22] reported almost perfect agreement (kappa = 1.0) for BRAF VE1 IHC interpretation with three pathologists in 53 lung cancers. Previous studies on the interobserver agreement for BRAF VE1 IHC interpretation in PTCs, melanomas, and CRCs reported moderate to perfect agreement (kappa=0.554–1.0) [12-20]. But, in most studies, two or three pathologists interpreted the results of IHC. The study by Marin et al. [14] involved the largest number of pathologists and showed almost perfect agreement (kappa=0.81) for seven pathologists in 67 cases of melanoma. Fifteen pathologists were involved in this study, which is the largest number of pathologists to our knowledge. This study also evaluated interobserver agreement for the percentage of tumor cell staining and staining intensity and showed good agreement. The interobserver disagreement resulted from discrepancies in the interpretation of heterogeneous staining patterns and tumor cells showing weak staining intensity.

In this study, the sensitivity and specificity of BRAF VE1 IHC were 80.0% and 90.0% in the lung adenocarcinomas, which are slightly lower than those reported in other studies on lung cancer (Table 1) [7,8,21,22]. This is probably because our study had a smaller sample size with a variety of types of specimens such as resection, biopsy, and EBUS specimens.

The positive criteria for BRAF VE1 IHC have not yet been established. This study used a 40% cutoff for the interpretation of BRAF VE1 IHC irrespective of staining intensity according to the ROC curve. Previous studies reported false-positives in cases with heterogeneous non-diffuse cytoplasmic staining of variable intensity [3,14,16]. Dvorak et al. [3] recommended that cases showing heterogeneous cytoplasmic staining should be interpreted with caution. In melanomas, the unequivocal (≥1+) cytoplasmic staining of most tumor cells was used as positive criteria [10]. In lung cancers, Ilie et al. [6] used strong, homogenous staining as positive criteria. Hofman et al. [21] used at least 80% of tumor cells showing strong and homogenous staining as positive criteria, similar to Ilie et al. [6]. Our wild-type BRAF/VE1 positive case showed weak (1+) but diffuse cytoplasmic staining without nuclear staining. Nevertheless, if weak-stained cases are considered to be negative, the sensitivity decreases from 80.0% to 70.0% in lung adenocarcinoma. In consideration of the role of BRAF VE1 IHC as a screening test, the case with weak but diffuse cytoplasmic staining on BRAF VE1 IHC should be considered to be positive, and recommend further molecular tests for *BRAF* V600E mutation [3,16]. Sasaki et al. [5], Gow et al. [7], and Karbel et al. [22] used at least 50% of tumor cells with positive staining irrespective of staining intensity as positive criteria in lung cancers. In this study, even when the cutoff value was increased from 40% to 50%, the overall sensitivity and specificity were the same in lung adenocarcinoma. But, sensitivity to each pathologist decreased slightly in four out of 15 pathologists. Hwang et al. [8] interpreted cases showing at least weak and focal staining as positive. In this study, If Hwang's criteria [8] are adopted, the sensitivity increases from 80% to 90%, but the specificity decreases from 90% to 50%. Further studies to validate the positive cutoff and/or staining intensity of BRAF VE1 IHC in the sensitive prediction of *BRAF* V600E mutation will be required.

Various pre-analytical and analytical factors may affect the sensitivity and specificity of BRAF VE1 IHC. It is controversial whether there is a difference in sensitivity depending on the platform [3,10,23]. The Ventana platform can produce more optimal staining than the Dako or Leica platforms [16,23-25]. However, Sasaki et al. [5] (Dako platform) and Karbel et al. [22] (BioSB platform) reported similar results to other studies using Ventana platforms in lung cancers (Table 1). Antigen retrieval methods may affect the sensitivity of IHC [3,23]. Acidic buffers such as citrate buffer or Bond Epitope Retrieval Solution 1 (pH 6) may result in suboptimal staining [23,26,27]. Tris or EDTA buffers (pH 8) were recommended for retrieval agents [3,23]. As reported by Hwang et al. [8] in this study, lung adenocarcinomas showed more heterogeneous staining patterns and staining intensity than PTC and colon adenocarcinomas. The NordiQC data for BRAF VE1 IHC recommends the OptiView amplification kit–based protocol based for optimal results [23]. OptiView amplification kits, used to improve the visualization of ALK D5F3 IHC, could be also helpful in BRAF VE1 IHC in NSCLC. Rigorous antibody validation and protocol optimization, and quality control are required for the clinical application of BRAF VE1 IHC [9,16-18].

The cross-reactivity of the VE1 antibody to non-V600E mutations, different *BRAF* point mutations, or unknown epitopes may cause false-negative results. In lung cancer, VE1 positivity has been reported in two of 72 cases with non-V600E mutations across previous studies to date [4-8]. Low tumor cell content may be attributed to false positivity [4,7,14]. Our false-positive case (case 22) was an EBUS biopsy sample with adequate tumor purity (20%). However, the total trimming-out of tumor cell contents from the sections for molecular tests is possible in a small biopsy sample and may affect the false-positive results. Decalcifying agents containing strong acids can affect DNA yield and protein

expression and cause false-positive or false-negative results [1]. Cryofixation may cause false-negative results due to low expression of target proteins from tissue damage [10,11,28]. Our false-positive or -negative cases did not undergo decalcification or cryofixation. Signet ring cell morphology has been reported as common pitfall of VE1 interpretation in CRCs [16,17]. Signet ring cells can cause false-negative results due to minimal cytoplasm, which was almost entirely replaced by mucin [17]. On the other hand, the VE1 antibody could cross-react with intracellular mucin and result in false positivity [18]. This study had no signet ring cell component in false-positive or false-negative cases.

The limitation of our study is the relatively small sample size and potential selection bias. Nevertheless, together with previous reports, this study suggests that BRAF VE1 IHC could be a screening test for further molecular testing of *BRAF* V600E mutation.

BRAF VE1 IHC could be a screening test for the detection of *BRAF* V600E mutation in NSCLC. However, to introduce BRAF VE1 IHC into clinical practice, further studies are needed to optimize the protocol and to establish and validate interpretation criteria for BRAF VE1 IHC.

## Ethics Statement

This study was approved by the Institutional Review Board of Inje University Ilsan Paik Hospital with a waiver of informed consent (2022-01-002).

## 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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Conceptualization: GKL, SYH. Data curation: SC. Formal analysis: SC. Investigation: YLC, GKL, SYH. Methodology: SC, YLC, HSS. Project administration: GKL, SYH. Supervision: GKL, SYH. Writing—original draft preparation: SC. Writing—review & editing: YLC, HSS, GKL, SYH. Approval of final manuscript: all authors.

## Conflicts of Interest

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

## Funding Statement

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## References

1. Chang S, Shim HS, Kim TJ, et al. Molecular biomarker testing for non-small cell lung cancer: consensus statement of the Korean Cardiopulmonary Pathology Study Group. J Pathol Transl Med 2021; 55: 181-91.
2. Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. Arch Pathol Lab Med 2017; 141: 625-57.
3. Dvorak K, Higgins A, Palting J, Cohen M, Brunhoeber P. Immunohistochemistry with anti-BRAF V600E (VE1) mouse monoclonal antibody is a sensitive method for detection of the *BRAF* V600E mutation in colon cancer: evaluation of 120 cases with and without *KRAS* mutation and literature review. Pathol Oncol Res 2019; 25: 349-59.
4. Seto K, Haneda M, Masago K, et al. Negative reactions of *BRAF* mutation-specific immunohistochemistry to non-V600E mutations of BRAF. Pathol Int 2020; 70: 253-61.
5. Sasaki H, Shimizu S, Tani Y, et al. Usefulness of immunohistochemistry for the detection of the *BRAF* V600E mutation in Japanese lung adenocarcinoma. Lung Cancer 2013; 82: 51-4.
6. Ilie M, Long E, Hofman V, et al. Diagnostic value of immunohistochemistry for the detection of the *BRAF*V600E mutation in primary lung adenocarcinoma Caucasian patients. Ann Oncol 2013; 24: 742-8.
7. Gow CH, Hsieh MS, Lin YT, Liu YN, Shih JY. Validation of immunohistochemistry for the detection of *BRAF* V600E-mutated lung adenocarcinomas. Cancers (Basel) 2019; 11: 886.
8. Hwang I, Choi YL, Lee H, et al. Selection strategies and practical application of *BRAF* V600E-mutated non-small cell lung carcinoma. Cancer Res Treat 2022; 54: 782-92.
9. O'Brien O, Lyons T, Murphy S, Feeley L, Power D, Heffron C. *BRAF* V600 mutation detection in melanoma: a comparison of two laboratory testing methods. J Clin Pathol 2017; 70: 935-40.
10. Anwar MA, Murad F, Dawson E, Abd Elmageed ZY, Tsumagari K, Kandil E. Immunohistochemistry as a reliable method for detection of *BRAF*-V600E mutation in melanoma: a systematic review and meta-analysis of current published literature. J Surg Res 2016; 203: 407-15.
11. Pyo JS, Sohn JH, Kang G. BRAF immunohistochemistry using clone VE1 is strongly concordant with *BRAF*(V600E) mutation test in papillary thyroid carcinoma. Endocr Pathol 2015; 26: 211-7.
12. Schafroth C, Galvan JA, Centeno I, et al. VE1 immunohistochemistry predicts *BRAF* V600E mutation status and clinical outcome in colorectal cancer. Oncotarget 2015; 6: 41453-63.
13. Rusu S, Verocq C, Trepant AL, et al. Immunohistochemistry as an accurate tool for the assessment of *BRAF* V600E and *TP53* muta-

tions in primary and metastatic melanoma. Mol Clin Oncol 2021; 15: 270.
14. Marin C, Beauchet A, Capper D, et al. Detection of *BRAF* p.V600E mutations in melanoma by immunohistochemistry has a good interobserver reproducibility. Arch Pathol Lab Med 2014; 138: 71-5.
15. Fisher KE, Cohen C, Siddiqui MT, Palma JF, Lipford EH, 3rd, Longshore JW. Accurate detection of *BRAF* p.V600E mutations in challenging melanoma specimens requires stringent immunohistochemistry scoring criteria or sensitive molecular assays. Hum Pathol 2014; 45: 2281-93.
16. Bledsoe JR, Kamionek M, Mino-Kenudson M. *BRAF* V600E immunohistochemistry is reliable in primary and metastatic colorectal carcinoma regardless of treatment status and shows high intratumoral homogeneity. Am J Surg Pathol 2014; 38: 1418-28.
17. Toon CW, Walsh MD, Chou A, et al. *BRAF*V600E immunohistochemistry facilitates universal screening of colorectal cancers for Lynch syndrome. Am J Surg Pathol 2013; 37: 1592-602.
18. Rossle M, Sigg M, Ruschoff JH, et al. Ultra-deep sequencing confirms immunohistochemistry as a highly sensitive and specific method for detecting *BRAF* V600E mutations in colorectal carcinoma. Virchows Arch 2013; 463: 623-31.
19. Capper D, Voigt A, Bozukova G, et al. *BRAF* V600E-specific immunohistochemistry for the exclusion of Lynch syndrome in MSI-H colorectal cancer. Int J Cancer 2013; 133: 1624-30.
20. Affolter K, Samowitz W, Tripp S, Bronner MP. *BRAF* V600E mutation detection by immunohistochemistry in colorectal carcinoma. Genes Chromosomes Cancer 2013; 52: 748-52.
21. Hofman V, Benzaquen J, Heeke S, et al. Real-world assessment of the *BRAF* status in non-squamous cell lung carcinoma using VE1 immunohistochemistry: a single laboratory experience (LPCE, Nice, France). Lung Cancer 2020; 145: 58-62.
22. Karbel HA, Ejam SS, Naji AZ. Immunohistochemical study using monoclonal VE1 antibody can substitute the molecular tests for apprehension of *BRAF* V600E mutation in patients with non-small-cell lung carcinoma. Anal Cell Pathol (Amst) 2019; 2019: 2315673.
23. NordiQC. Assessment Run 62 2021 BRAF (BRAF V600E) [Internet]. Aalborg: NordiQC, 2021 [cited 2021 Nov 30]. Available from: https://www.nordiqc.org/downloads/assessments/146_113.pdf.
24. Sinicrope FA, Smyrk TC, Tougeron D, et al. Mutation-specific antibody detects mutant *BRAF*V600E protein expression in human colon carcinomas. Cancer 2013; 119: 2765-70.
25. Adackapara CA, Sholl LM, Barletta JA, Hornick JL. Immunohistochemistry using the *BRAF* V600E mutation-specific monoclonal antibody VE1 is not a useful surrogate for genotyping in colorectal adenocarcinoma. Histopathology 2013; 63: 187-93.
26. Kuan SF, Navina S, Cressman KL, Pai RK. Immunohistochemical detection of *BRAF* V600E mutant protein using the VE1 antibody in colorectal carcinoma is highly concordant with molecular testing but requires rigorous antibody optimization. Hum Pathol 2014; 45: 464-72.
27. Lasota J, Kowalik A, Wasag B, et al. Detection of the *BRAF* V600E mutation in colon carcinoma: critical evaluation of the imunohistochemical approach. Am J Surg Pathol 2014; 38: 1235-41.
28. Bullock M, O'Neill C, Chou A, et al. Utilization of a MAB for *BRAF*(V600E) detection in papillary thyroid carcinoma. Endocr Relat Cancer 2012; 19: 779-84.

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ORIGINAL ARTICLE

# A clinicopathologic and immunohistochemical study of primary and secondary breast angiosarcoma

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**Background:** We aimed to study the clinicopathologic and immunohistochemical (IHC) (CD117, c-Myc, and p53) characteristics, and overall survival of primary and secondary breast angiosarcoma (BAS). **Methods:** This was a retrospective study of BAS cases diagnosed between 1997 and 2020 at our institution. Hematoxylin and eosin-stained slides were reviewed for tumor morphology, margin status, and lymph node metastasis. CD117, p53, D2-40, CD31, and c-Myc IHC stains were performed on 11 viable tissue blocks. Additional clinical information was obtained from the electronic medical records. **Results:** Seventeen patients with BAS were identified. Of these, five (29%) were primary and 12 (71%) were secondary BAS, respectively. The median age at diagnosis for primary BAS was 36 years. The median age at diagnosis for secondary BAS was 67 years. The median time to secondary BAS development following radiotherapy was 6.5 years (range, 2 to 12 years). There was no significant difference between primary and secondary BAS in several histopathologic parameters examined, including histologic grade, necrosis, mitotic count, lymph node metastasis, and positive tumor margins. There was also no difference in CD117, p53, D2-40, CD31, and c-Myc expression by IHC between primary and secondary BAS. During a median follow-up of 21 months, primary BAS had two (40%) reported deaths and secondary BAS had three (25%) reported deaths. However, this difference in survival between both groups was not statistically significant (hazard ratio, 0.51; 95% confidence interval, 0.09 to 3.28; p = .450). **Conclusions:** BAS is a rare and aggressive disease. No histologic, IHC (CD117, c-Myc, and p53), or survival differences were identified between primary and secondary BAS in this study.

**Key Words:** Breast angiosarcoma; Primary breast angiosarcoma; Secondary breast angiosarcoma; Immunohistochemistry; Overall survival

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Breast sarcomas are a heterogeneous group of tumors, with an annual incidence of 4.6 cases per million year and account for about 1% of all breast malignancies and < 5% of all soft tissue sarcomas [1]. Within this cohort, angiosarcoma represents the most common histological subtype with high recurrence rates and an overall poor prognosis [2]. Breast angiosarcoma (BAS) can be divided into two main types: primary BAS developing de novo and secondary BAS developing as a consequence of previous breast cancer treatment (e.g., prior postoperative radiotherapy and/or long-lasting lymphedema after treatment for breast cancer known as Stewart-Treves syndrome) [3]. Primary BAS is a malignant vascular neoplasm that arises within the breast parenchyma, while secondary BAS often arises at the site of previous radiotherapy in the skin/cutaneous tissue and might invade the breast parenchyma secondarily [4]. These tumors tend to involve the dermis of the skin and can be misdiagnosed as other benign clinical entities [5]. Primary BAS has been observed in women between the ages of 30–50 years who present with poorly defined palpable masses, fullness or swelling in the breast [6]. Secondary BAS, on the other hand, presents in older women as painless bruising that is frequently multifocal but, can also present with a mass which is often neglected because of its seemingly innocent appearance [6]. Diagnosing BAS on imaging may be problematic as mammogram and ultrasound do not have pathognomonic characteristics as may be seen with adenocarcinoma and other nodules [6]. However, there is some evidence that mammographic findings might raise suspicion for this diagnosis [7] and some studies have demonstrated the ability of magnetic resonance imaging to identify pat-




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terns of malignancy in BAS [6].

The genetic and molecular alterations in BAS are still poorly understood due to its rarity and limited number of cases. Mutation of the *TP53* gene which is a tumor suppressor gene, results in malfunctioning of DNA damage repair pathways, cell-cycle arrest, chromatin remodeling, and apoptosis [8]. *MYC* proto-oncogene—the human cellular homolog of the v-myc oncogene of avian myelocytomatosis retrovirus MC29—which is located on chromosome 8 (8q24.12-q24.13)—is found to act as a strong transcription factor, implicated in the control of cell differentiation and apoptosis [9]. Induction of this transcription factor promotes cell proliferation and transformation [10]. *MYC* amplification is observed frequently in solid malignancies of different histogenetic origin [11-13]. The *KIT* gene encodes for a tyrosine kinase growth factor receptor stem cell factor, c-kit (CD117) protein and is constitutionally expressed in hematopoietic stem cells, mast cells, germ cells, melanocytes, certain basal epithelial cells, luminal epithelium of breast, and the interstitial cells of Cajal of the gastrointestinal tract [14]. Some reports suggest a genetic predisposition leading to the development of post-radiation (secondary) angiosarcoma, such as a mutation in the *TP53*, *ATM*, or *KIT* gene [15-17]. *MYC* amplifications have also been reported in post-radiation (secondary) BAS, but not in primary BAS [18]. However, the limited number of patients in each analysis has made general conclusions difficult [19]. Immunohistochemistry (IHC) as a laboratory technique is used to determine the differential expression of proteins in tissues. c-Myc, p53, and CD117 expression by IHC has been infrequently described in primary and secondary BAS. We, therefore, investigated the expression of these antibodies and their prognostic significance on viable tissue blocks within our patient cohort. Additionally, markers of lymphovascular differentiation including CD31, and D2-40 were also performed.

The primary aim of this study is to describe the clinicopathologic characteristics and results of c-Myc, p53, CD117, CD31, and D2-40 expression by IHC in BAS. The secondary aim of our study is to describe the overall survival (OS) of BAS in our patient population, defined as time from first diagnosis of BAS to death or date of last follow-up.

## MATERIALS AND METHODS

We retrospectively reviewed our database to identify patients diagnosed with primary and secondary BAS between January 1997 and February 2020. Study inclusion criteria included a prior diagnosis of BAS. Cases were categorized as primary BAS if they had no prior history of breast cancer or secondary BAS following post-radiation therapy for a prior breast cancer. Two surgical pathologists with expertise in breast pathology (RAF and SB) and EA reviewed pathology records including histologic slides to confirm the diagnosis of BAS. Additionally, IHC staining for CD117, p53, c-Myc, CD31, and D2-40 was performed on eleven 4-micron thick paraffin-embedded tissue sections. The slides were dried at 62°C and counterstained with Mayer's hematoxylin and bluing reagent. Appropriate positive and negative controls were employed throughout. The antibody incubation and detection were performed on an autostainer (Ventana Bench Mark UltraView, Universal DAB kit, Ventana Medical Systems, Tucson, AZ, USA). Table 1 summarizes the clones, dilutions, incubation times, and sources for the antibodies used. CD117 immunoreactivity was interpreted as positive with the presence of uniform cytoplasmic (or membranous) staining in the tumor cells. For p53, the immunoreactivity scoring was counted as the percentage of nuclear staining per 10 high-power fields (HPF), in several areas, regardless of the staining intensity [20]. A 20% cutoff value for detection of positive nuclear reactivity was selected for p53 antibody as previously described [21,22]. Strong nuclear staining in greater than 50% of the tumor cells was interpreted as positive for c-Myc and a negative result was represented by faint staining in a small percentage of cells (less than 50%) [23]. CD31 and D2-40 were both interpreted as positive with membranous (and cytoplasmic) staining in the tumor cells. Histologic parameters reviewed include tumor size, tumor grade, mitotic count (number of mitoses per 10 HPF, using a 40 × objective and a 10 × ocular lens; field size

**Table 1.** Antibody characteristics

| Antibody | Clone | Dilution | Vendor | Retrieval (HIER) (min) | Antibody incubation (min) |
|---|---|---|---|---|---|
| c-kit (CD117) | Rabbit polyclonal | 1:250 | Dako | CC1: 36 | 60 |
| p53 | DO-7 mouse monoclonal | RTU | Ventana Medical Systems | CC1: 36 | 32 |
| c-Myc | Y69 (rabbit) | RTU | Ventana Medical Systems | CC1: 64 | 32 |
| CD31 | JC70 (mouse) | RTU | Cell Marque | CC1: 36 | 40 |
| D2-40 (podoplanin) | D2-40 (mouse) | RTU | Cell Marque | CC1: 36 | 32 |

HIER, heat-induced epitope retrieval; RTU, ready to use reagent.
CC1 is Ventana Medical Systems retrieval solution, RTU, at pH 8.0.

0.25 $mm^2$), necrosis, lymphovascular invasion, lymph node metastasis, and margin status. Tumors were histologically graded as low, intermediate, or high [24,25]. Low-grade tumors contain open, anastomosing vascular channels that proliferate within dermis, subcutaneous tissue or breast tissue [26]. A single layer of flat endothelial cells which may be hyperchromatic with small nucleoli line these channels, which dissect through the stroma, causing distortion but little destruction of the preexisting lobules and ducts, with the absence of solid/spindle cell foci, blood lakes, and necrosis [26]. Intermediate-grade angiosarcoma differs from low-grade by containing additional cellular foci of papillary formations and/or solid/spindle cell proliferation with slightly increased mitoses [26]. High-grade angiosarcoma contains tumor lined by malignant endothelial cells with prominent cytologic atypia. Endothelial tufting and papillary formations are present, with conspicuous solid and spindle cell areas mostly devoid of vascular formations [26]. In addition, mitoses may be brisk especially in more cellular or solid areas and areas of hemorrhage, known as "blood lakes" and necrosis are also prominent [26]. Additional clinical data, including demography, tumor laterality, time to development of secondary breast angiosarcoma, comorbidities including obesity, diabetes, hypertension, and smoking history, treatment received and OS were obtained from the electronic medical record.

Patient baseline characteristics were summarized by median (range) and frequency (%) for continuous and categorical variables, respectively. Group comparisons were performed by Fisher exact tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. The distribution of OS was graphically described using Kaplan-Meier curve along with a median and 95% confidence interval (CI). The median follow-up time was estimated using the reverse Kaplan-Meier method. A log-rank test was used to compare Kaplan-Meier curves between groups. When a categorical variable has more than two levels, a global log-rank p-value was calculated using likelihood ratio tests. Due to the small sample size, Cox proportional regression analyses were limited to univariable analyses. Firth Cox regression models were used to reduce bias in maximum likelihood estimation caused by rare events. The proportional hazard assumption was verified based on Schoenfeld residuals, and no violation was found except for positive margins and tumor site that were further confirmed using restricted mean survival time.

## RESULTS

Seventeen women with a diagnosis of BAS were identified and of these, 12 (71%) were Caucasians and five (29%) were Black/African Americans (AA). Primary BAS was seen in five cases (29%) and secondary BAS was seen in 12 cases (71%), respectively. In terms of race and its association with disease occurrence, secondary BAS was more common in both Caucasian and Black/AA women. However, the impact of race on disease occurrence was not statistically significant ($p > .99$). The median age at diagnosis for primary BAS was 36 years (range, 23 to 71 years) and the median age at diagnosis for secondary BAS was 67 years (range, 33 to 76 years). However, this difference in age was not statistically significant ($p = .246$).

Right-sided tumors were more common (80%) in women with primary BAS, compared with women with secondary BAS in which left-sided tumors were more common (58%). However, this difference in tumor laterality was not statistically significant ($p = .294$). Multifocal tumors were observed in one case of primary BAS (20%) and three cases of secondary BAS (25%) ($p > .99$). Skin involvement was present in seven secondary BAS cases (58%); however, there was no involvement of the skin in any of the primary BAS cases ($p = .044$). Positive margins were seen in two cases (40%) of primary BAS and six cases (50%) of secondary BAS. However, this difference was not statistically significant ($p > .99$). For the remaining cases with negative tumor margins, the median tumor size for primary BAS was 2 cm (range, 0.5 to 28 cm) and the median tumor size for secondary BAS was 0.95 cm (range, 0.4 to 2.5 cm). However, this difference in tumor size between both groups was not statistically significant ($p = .437$).

The tumors were graded into low (Fig. 1A, B: primary BAS; Fig. 1C, D: secondary BAS), intermediate (Fig. 2A, B: primary BAS) and high grade (Fig. 3A, B: primary BAS; Fig. 3C, D: secondary BAS) based on previously defined histologic criteria [24,25]. However, we found no difference in histologic grade between primary and secondary BAS ($p = .087$). Other histologic parameters examined include presence of tumor necrosis, lymph node metastasis, and mitotic count (categorized into > 10/10 HPF and < 10/10 HPF). There was no difference between primary and secondary BAS with regard to tumor necrosis ($p = .538$), lymph node metastasis ($p = .191$), and mitotic count ($p = .593$).

Additional clinical characteristics evaluated from our patient population include the incidence of obesity (body mass index ≥30), hypertension, smoking, diabetes, and the treatment received for their BAS diagnosis. We observed that patients with secondary BAS appear to have more concurrent clinical comorbidities; however, these findings were not statistically significant when compared with patients with primary BAS (obesity: $p = .117$; hypertension: $p = .102$; smoking: $p > .99$; diabetes: $p = .338$). In line with


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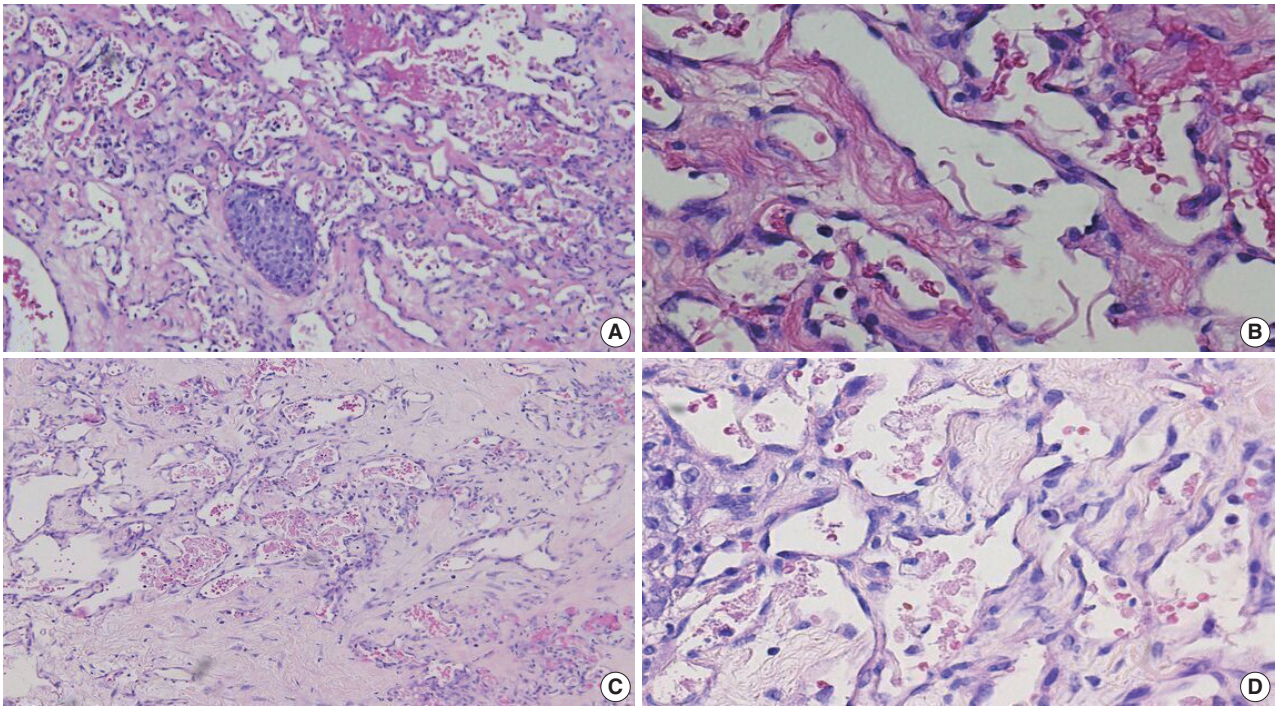


**Fig. 1.** Low-grade breast angiosarcoma (BAS). (A) Primary BAS with dilated and anastomosing vascular channels lined by flat endothelial cells infiltrating into the breast parenchyma. A focus of atypical ductal hyperplasia is also seen. (B) Higher magnification of previous image. (C) Secondary BAS with dilated and anastomosing vascular channels lined by flat endothelial cells. (D) Higher magnification of previous image.

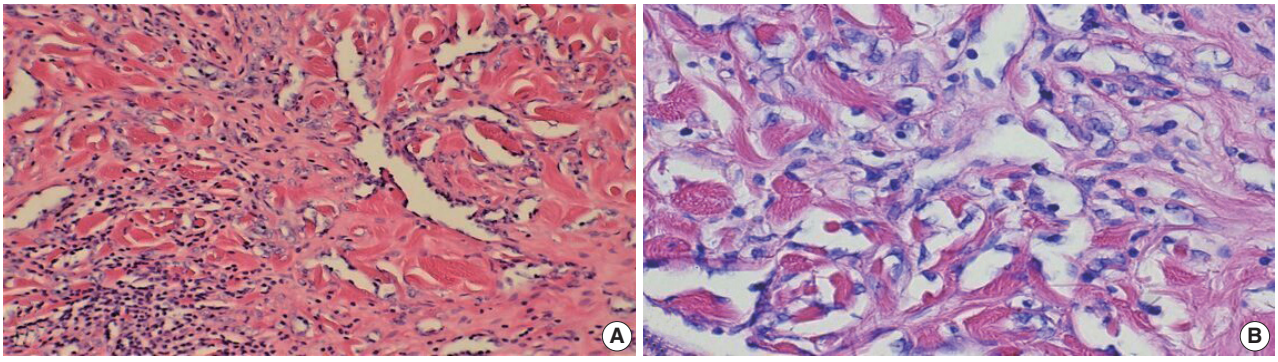


**Fig. 2.** Intermediate-grade primary breast angiosarcoma. (A) Anastomosing vascular channels lined by endothelial cells displaying mild to moderate cytologic atypia, with increased cellularity and tufting. (B) Higher magnification of previous image.

published literature, CD117 (Fig. 4A: primary BAS; Fig. 4B: secondary BAS), p53 (Fig. 5: secondary BAS), c-Myc (Fig. 6A: primary BAS; Fig. 6B: secondary BAS), CD31 (Fig. 7A: primary BAS; Fig. 7B: secondary BAS), and D240 (Fig. 7C: primary BAS; Fig. 7D: secondary BAS) IHC stains were performed on 11 (3 primary and 8 secondary BAS) viable tissue blocks. Although there was no significant difference in the expression of CD117 ($p > .99$), p53 ($p = .236$), D240 ($p > .99$), CD31 ($p > .99$), and c-Myc ($p > .99$) between primary and secondary BAS, it's noteworthy that p53 was only expressed in secondary BAS cases, and lacked expression in the primary BAS cases evaluated. Furthermore, c-Myc showed expression in both primary and secondary BAS cases. Table 2 summarizes the IHC pattern of expression in both primary and secondary BAS. All patients with BAS were managed with wide local excision or mastectomy. Two patients with secondary BAS received additional chemotherapy and one patient with primary BAS received additional chemotherapy and radiotherapy. However, there was no difference between patients who were treated with surgery alone and those who received additional treatments ($p = .353$). Table 3 summarizes the clinicopathologic characteristics, comorbidities, and treatment received by BAS type, respectively.

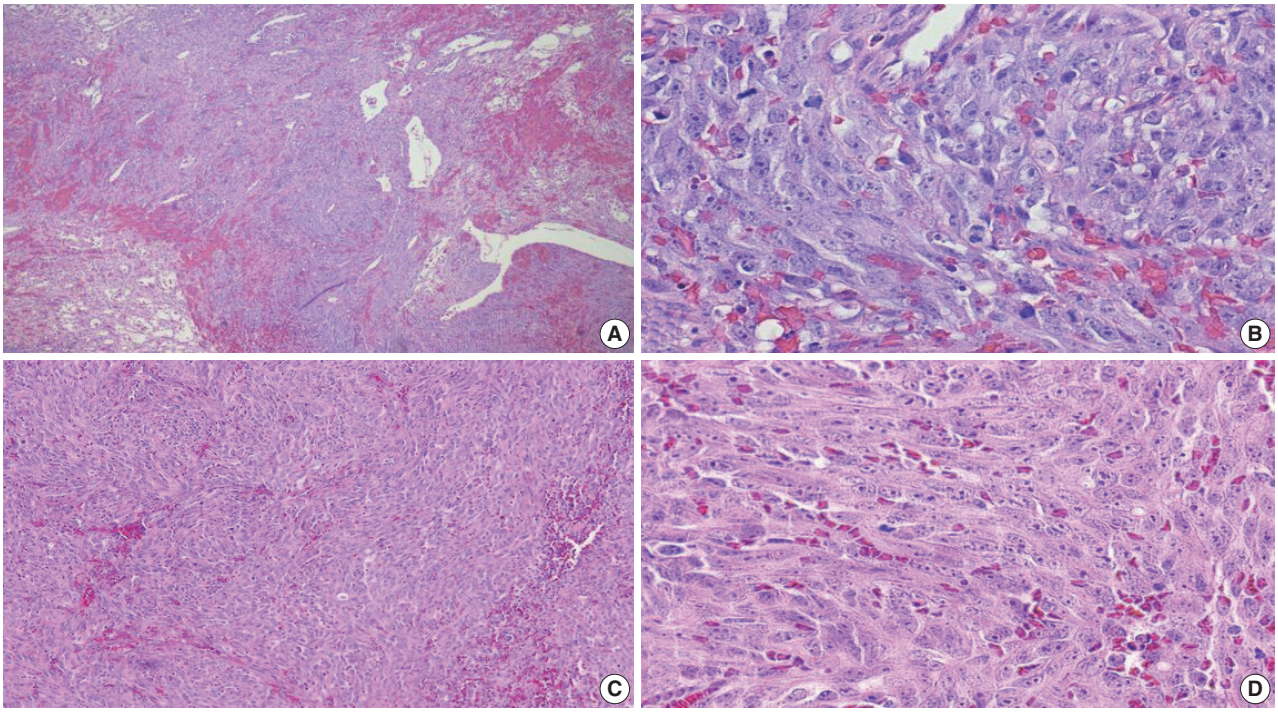


**Fig. 3.** High grade breast angiosarcoma (BAS). (A) Primary BAS showing vascular channels lined by malignant spindled to ovoid cells with prominent cytologic atypia, increased cellularity and solid areas. (B) Higher magnification of previous image. A mitotic figure is also seen. (C) Secondary BAS showing vascular channels with mostly solid spindled to ovoid malignant cells with prominent cytologic atypia and increased cellularity. (D) Higher magnification of previous image.

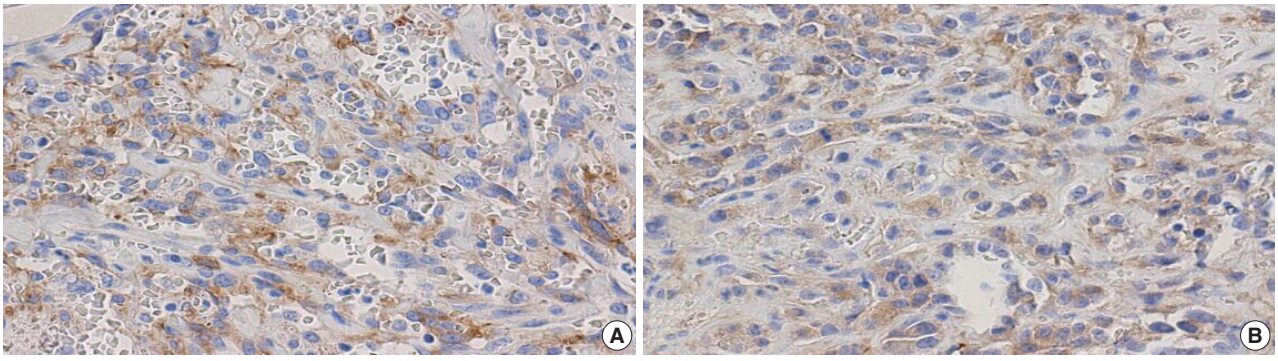


**Fig. 4.** CD117. (A) Primary breast angiosarcoma (BAS) showing CD117 cytoplasmic immunoreactivity on immunohistochemistry (IHC). (B) Secondary BAS showing CD117 cytoplasmic immunoreactivity on IHC.

The median time to development of secondary BAS following radiation therapy was 6.5 years (range, 2 to 12 years). The presence of tumor necrosis had an adverse effect on OS in BAS and this observation was statistically significant (p = .034). However, other histologic variables, including tumor size (p = .307), tumor grade (global p = .638), mitotic count (p = .075), lymph node metastasis (p = .278), and positive margin status (p = .998) had no significant effect on OS in BAS. Factors such as age at diagnosis (p = .845), race (p = .787) and type of BAS primary vs. secondary (p = .450) all had no significant effect on OS. Additionally, comorbid characteristics including obesity (p = .063), hypertension (p = .990), smoking (p = .551), and diabetes (p = .548) also had no significant impact on OS in BAS. There was also no difference in OS between patients who were treated with surgery alone and those who were treated with surgery and chemotherapy or radiotherapy (p = .671). By IHC, CD117 (p = .676), p53 (p = .847), D2-40 (p = .960), and c-Myc (p = .847) all had no significant effect on OS in BAS. The median follow-up period for both primary and secondary BAS was 21 months. The Kaplan-Meier curve of OS suggests that patients with primary BAS may have worse outcomes compared


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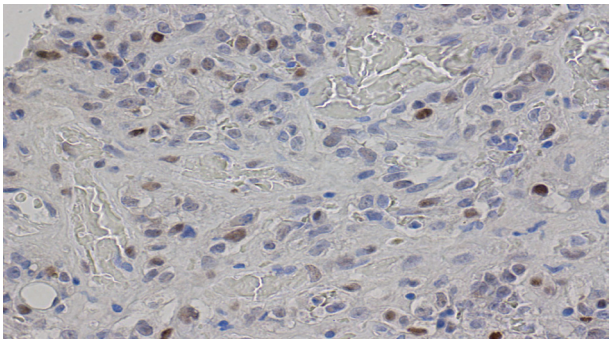

**Fig. 5.** Secondary breast angiosarcoma showing p53 nuclear immunoreactivity on immunohistochemistry.

with patients with secondary BAS; however, this finding was not statistically significant (hazard ratio [HR], 0.51; 95% CI, 0.09 to 3.28; p = .450) (Fig. 8). Kaplan-Meier curve also shows no difference in OS between Caucasian and Black/AA women (Fig. 9). Tumors with mitoses > 10/10 HPF also appear to have worse OS on the Kaplan-Meier curve; however, this finding was not statistically significant (Fig. 10). Additionally, Kaplan-Meier evaluation of OS, also shows worse OS for tumors with positive margins (Fig. 11), necrosis (Fig. 12), and histologic high grade (Fig. 13). However, these observations were all not statistically significant. By IHC, the Kaplan-Meier evaluation of OS shows that

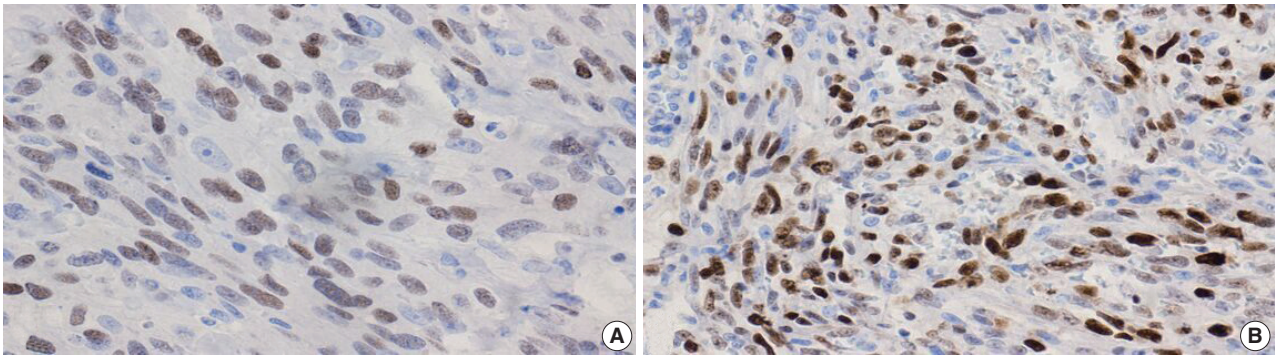


**Fig. 6.** c-Myc immunohistochemistry (IHC). (A) Primary breast angiosarcoma (BAS) showing c-Myc nuclear immunoreactivity on IHC. (B) Secondary BAS showing c-Myc nuclear immunoreactivity on IHC.

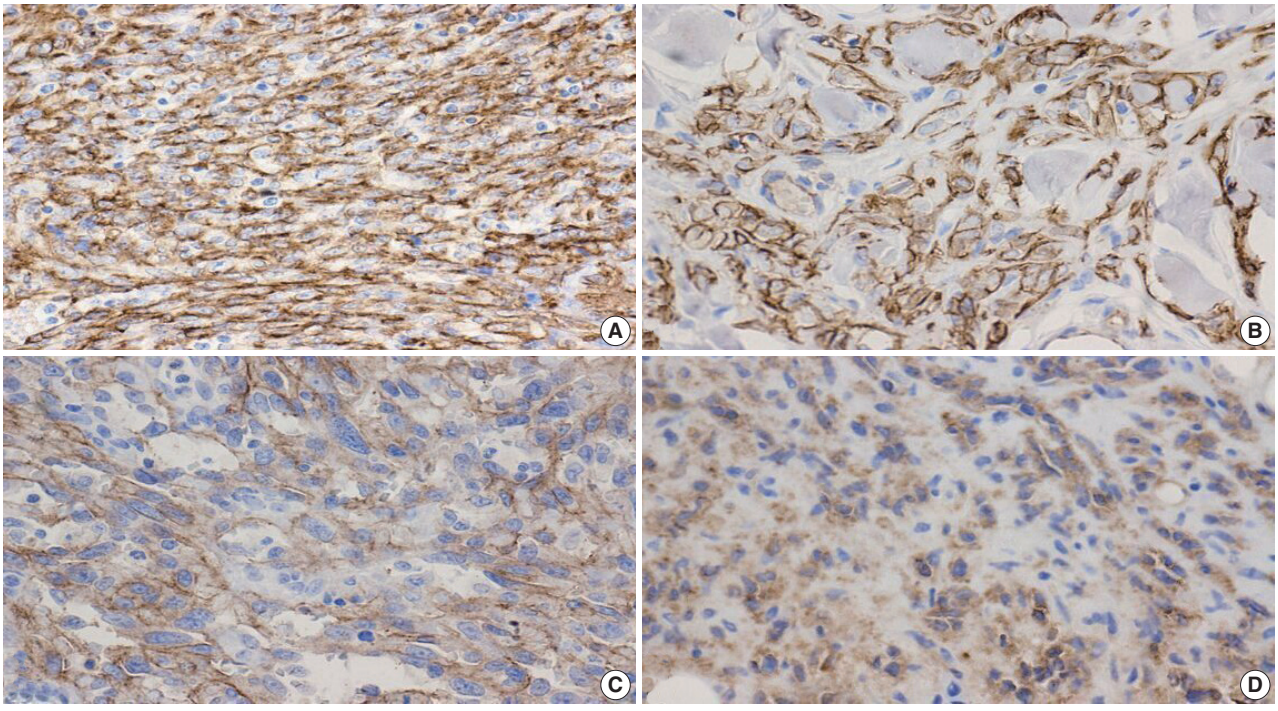


**Fig. 7.** (A) Primary breast angiosarcoma (BAS) showing CD31 membranous immunoreactivity on immunohistochemistry (IHC). (B) Secondary breast angiosarcoma showing CD31 membranous immunoreactivity on IHC. (C) Primary BAS showing D2-40 membranous immunoreactivity on IHC. (D) Secondary BAS showing D2-40 membranous immunoreactivity on IHC.

**Table 2.** Immunohistochemical profile

| Variable | Total (n=11) | Primary BAS (n=3) | Secondary BAS (n=8) | p-value[a] |
|---|---|---|---|---|
| CD117 | | | | >.99 |
| Positive | 7 (63.6) | 2 (66.7) | 5 (62.5) | |
| Negative | 4 (36.4) | 1 (33.3) | 3 (37.5) | |
| p53 | | | | .236 |
| Positive | 4 (36.4) | 0 | 4 (50.0) | |
| Negative | 7 (63.6) | 3 (100) | 4 (50.0) | |
| D-240 | | | | >.99 |
| Positive | 7 (63.6) | 2 (66.7) | 5 (62.5) | |
| Negative | 4 (36.4) | 1 (33.3) | 3 (37.5) | |
| CD31 | | | | >.99 |
| Positive | 11 (100) | 3 (100) | 8 (100) | |
| Negative | 0 | 0 | 0 | |
| C-MYC | | | | >.99 |
| Positive | 8 (72.7) | 2 (66.7) | 6 (75.0) | |
| Negative | 3 (27.3) | 1 (33.3) | 2 (25.0) | |

BAS, breast angiosarcoma.
[a]Fisher exact test.

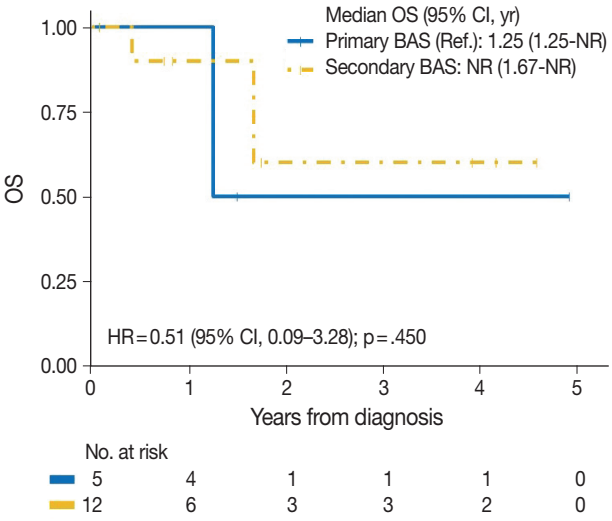


**Fig. 8.** Kaplan-Meier curves of overall survival (OS) by type of breast angiosarcoma (BAS). CI, confidence interval; NR, not reached; HR, hazard ratio.

CD117 positive (Fig. 14) and c-Myc positive (Fig. 15) tumors all behave worse than their negative counterparts, respectively; however, these observations were not statistically significant. Table 4 summarizes the univariable Cox proportional hazard regression analyses of risk factors associated with OS. Additional patient and tumor characteristics are presented in the Supplementary Table S1.

## DISCUSSION

Our study is unique because we describe a single academic med-

**Table 3.** Patient characteristics according to BAS type

| Variable | Total (n=17) | Primary BAS (n=5) | Secondary BAS (n=12) | p-value[a] |
|---|---|---|---|---|
| Age at diagnosis (yr) | 66 (23–76) | 36 (23–71) | 67 (33–76) | .246 |
| Race | | | | >.99 |
| Caucasian | 12 (70.6) | 4 (80.0) | 8 (66.7) | |
| African American | 5 (29.4) | 1 (20.0) | 4 (33.3) | |
| Tumor size (cm) | 1.1 (0.4–28) | 2 (0.5–28) | 0.95 (0.4–2.5) | .437 |
| Missing | 8 | 2 | 6 | |
| Tumor grade | | | | .087 |
| Low | 6 (35.3) | 2 (40.0) | 4 (33.3) | |
| Intermediate | 2 (11.8) | 2 (40.0) | 0 | |
| High | 9 (52.9) | 1 (20.0) | 8 (66.7) | |
| Tumor necrosis | | | | .538 |
| Yes | 4 (23.5) | 2 (40.0) | 2 (16.7) | |
| No | 13 (76.5) | 3 (60.0) | 10 (83.3) | |
| Mitotic count | | | | .593 |
| >10/10 HPF | 7 (41.2) | 3 (60.0) | 4 (33.3) | |
| <10/10 HPF | 10 (58.8) | 2 (40.0) | 8 (66.7) | |
| Lymph node metastasis | | | | .191 |
| Yes | 3 (17.6) | 2 (40.0) | 1 (8.3) | |
| No | 14 (82.4) | 3 (60.0) | 11 (91.7) | |
| Positive margins | | | | >.99 |
| Yes | 8 (47.1) | 2 (40.0) | 6 (50.0) | |
| No | 9 (52.9) | 3 (60.0) | 6 (50.0) | |
| Tumor site | | | | .294 |
| Right | 9 (52.9) | 4 (80.0) | 5 (41.7) | |
| Left | 8 (47.1) | 1 (20.0) | 7 (58.3) | |
| Multifocal tumors | | | | >.99 |
| Yes | 4 (23.5) | 1 (20.0) | 3 (25.0) | |
| No | 13 (76.5) | 4 (80.0) | 9 (75.0) | |
| Skin involved | | | | .044 |
| Yes | 7 (41.2) | 0 | 7 (58.3) | |
| No | 10 (58.8) | 5 (100) | 5 (41.7) | |
| Obesity | | | | .117 |
| Yes | 12 (70.6) | 2 (40.0) | 10 (83.3) | |
| No | 5 (29.4) | 3 (60.0) | 2 (16.7) | |
| Hypertension | | | | .102 |
| Yes | 6 (35.3) | 0 | 6 (50.0) | |
| No | 11 (64.7) | 5 (100) | 6 (50.0) | |
| Smoking | | | | >.99 |
| Yes | 4 (23.5) | 1 (20.0) | 3 (25.0) | |
| No | 13 (76.5) | 4 (80.0) | 9 (75.0) | |
| Diabetes | | | | .338 |
| Yes | 7 (41.2) | 1 (20.0) | 6 (50.0) | |
| No | 10 (58.8) | 4 (80.0) | 6 (50.0) | |
| Treatment received | | | | .353 |
| Surgery only | 14 (82.4) | 4 (80.0) | 10 (83.3) | |
| Surgery+chemotherapy | 2 (11.8) | 0 | 2 (16.7) | |
| Surgery+radiation+chemotherapy | 1 (5.9) | 1 (20.0) | 0 | |

Values are presented as median (range) or number (%).
BAS, breast angiosarcoma; HPF, high-power field.
[a]Fisher exact test or Wilcox rank-sum test as appropriate.


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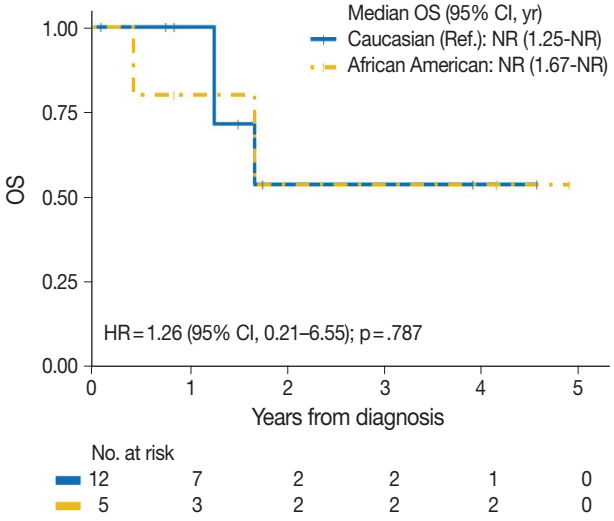


**Fig. 9.** Kaplan-Meier curves of overall survival (OS) by race. CI, confidence interval; NR, not reached; HR, hazard ratio.

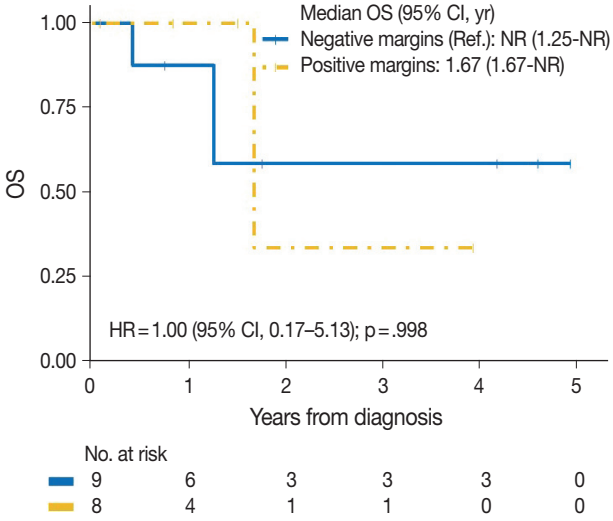


**Fig. 11.** Kaplan-Meier curves of overall survival (OS) by positive margins. CI, confidence interval; NR, not reached; HR, hazard ratio.

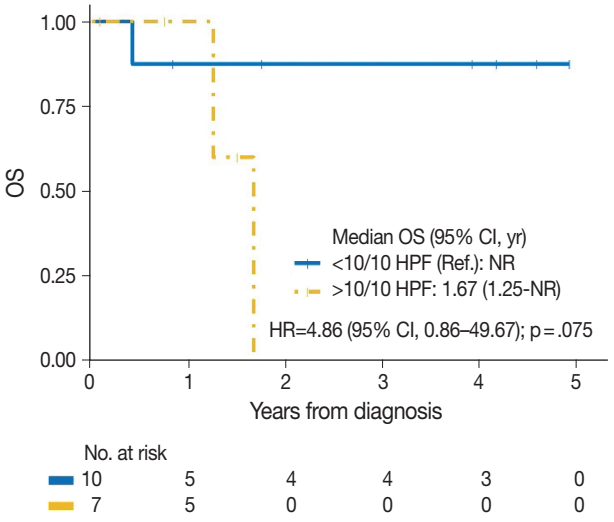


**Fig. 10.** Kaplan-Meier curves of overall survival (OS) by mitotic count. CI, confidence interval; NR, not reached; HPF, high-power field; HR, hazard ratio.

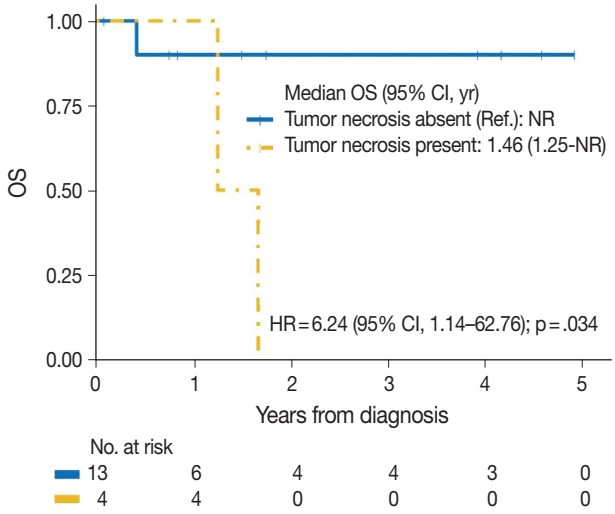


**Fig. 12.** Kaplan-Meier curves of overall survival (OS) by tumor necrosis. CI, confidence interval; NR, not reached; HR, hazard ratio.

ical center's experience with primary and secondary BAS spanning over 2 decades. Our findings emphasize the rarity of these tumors and presents information that describes the similarities and differences between primary and secondary BAS, including clinicopathologic and IHC characteristics, which only very few studies have hitherto described. The rarity of this disease indeed precludes any prospective study and poses significant challenges in its diagnosis, treatment, and research [4]. Results from our patient population shows that secondary BAS occurs at a higher frequency than primary BAS and this finding is in agreement with other studies [1,2,4]. The higher incidence of secondary BAS may be explained by the fact that more women with breast cancer are seeking breast-conserving surgeries with adjuvant radiotherapy, which may put them at risk of developing secondary BAS. Although we found no significant difference in age distribution between both groups of patients, it is remarkable to note that patients with primary BAS are much younger (median age, 36 years) than patients with secondary BAS (median age, 67 years). This observation is important, as breast cancer is known to be more common in older women. Therefore, for younger patients with BAS, this diagnosis may be missed, especially if it is misclassified as other benign skin pathologies. Our findings are similar to what has been

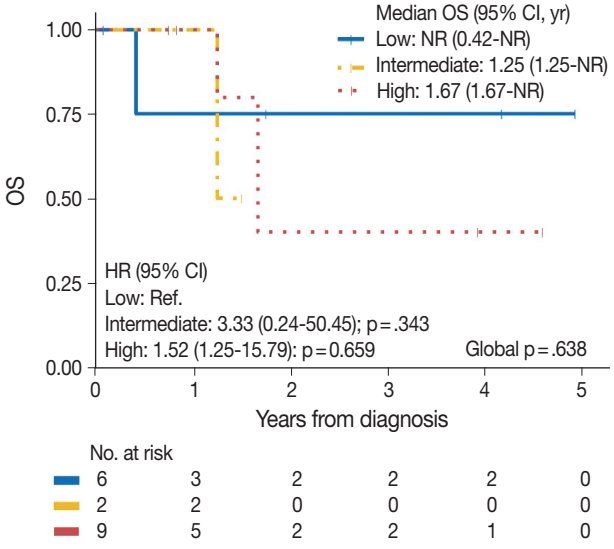


**Fig. 13.** Kaplan-Meier curves of overall survival (OS) by tumor grade. CI, confidence interval; NR, not reached; HR, hazard ratio.

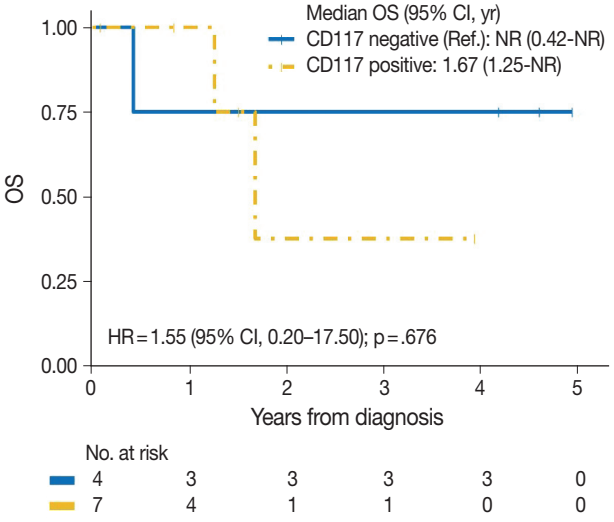


**Fig. 14.** Kaplan-Meier curves of overall survival (OS) by CD117. CI, confidence interval; NR, not reached; HR, hazard ratio.

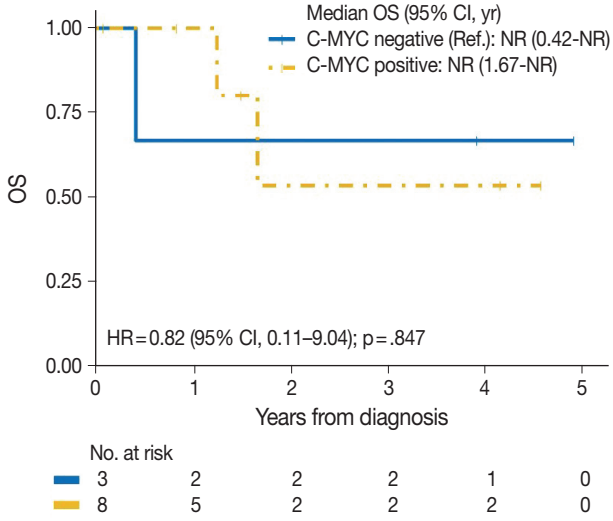


**Fig. 15.** Kaplan-Meier curves of overall survival (OS) by c-Myc. CI, confidence interval; NR, not reached; HR, hazard ratio.

previously described, with primary BAS occurring in women ages 30–50 years [27] and, secondary BAS occurring in older women (median age, 67 to 71 years) following a median of 10.5 years after radiotherapy for breast cancer [27-30]. In our study, however, patients with secondary BAS appeared to develop the disease at shorter latency times with a median time of 6.5 years. This finding may be explained by the small sample size of our patient population, which is not unexpected due to the rarity of this disease. In addition, it is unclear whether the finding of more concurrent comorbidities in patients with secondary BAS in our study had any role to play in terms of disease latency, and thus leaves room for further research. While our study showed that BAS was more common in Caucasian women compared with Black/AA women and is similar to what has been reported in another study [1], it is important to emphasize that incidence by race had no significant impact on the characteristics of BAS of the women in this study.

We studied the histologic similarities and differences between primary and secondary BAS. Following our review, we found no significant difference between histologic parameters examined in primary and secondary BAS. In other words, regardless of whether a patient develops BAS de novo or following radiotherapy for a previous breast cancer diagnosis, the histologic phenotypes are ultimately the same. Similar to what we observed in our study, no difference in tumor characteristics was found between primary and secondary BAS in another study [1]. Of note is that differentiating low-grade angiosarcoma from atypical post-radiation vascular lesions (AVLs) may be difficult because they both represent the low-grade end of the morphologic spectrum of radiation-associated vascular lesions [26]. Nonetheless, AVLs are typically smaller, more superficial, non-infiltrative, and fairly well circumscribed [31].

The immunostains performed as part of this study were selected based on previous research on angiosarcoma in other regions of the body and the breast, which have shown inconsistent findings. Slightly more than half (28 of 50 [56%]) of the angiosarcomas in one series showed CD117 positivity in the neoplastic endothelial cells by IHC, including post-radiation angiosarcoma [14]. CD117 expression by IHC was also seen in 25% of angiosarcoma in another series [32] and showed weak staining by IHC in two of 14 primary BAS cases in another series [33]. Additionally,


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**Table 4.** Univariable Cox proportional hazard regression analyses of risk factors associated with overall survival

| Variable | Event/ No. | HR (95% CI) | p-value |
|---|---|---|---|
| Age at diagnosis | 5/17 | 1.01 (0.96–1.07) | .845 |
| Race | | | |
| Caucasian | 3/12 | Reference | |
| African American | 2/5 | 1.26 (0.21–6.55) | .787 |
| BAS type | | | |
| Primary BAS | 2/5 | Reference | |
| Secondary BAS | 3/12 | 0.51 (0.09–3.28) | .450 |
| Tumor size (cm) | 3/9 | 1.04 (0.95–1.13) | .307 |
| Tumor grade | | | .638[a] |
| Low | 1/6 | Reference | |
| Intermediate | 1/2 | 3.33 (0.24–50.45) | .343 |
| High | 3/9 | 1.52 (0.25–15.79) | .659 |
| Tumor necrosis | | | |
| No | 1/13 | Reference | |
| Yes | 4/4 | 6.24 (1.14–62.76) | .034 |
| Mitotic count | | | |
| <10/10 HPF | 1/10 | Reference | |
| >10/10 HPF | 4/7 | 4.86 (0.86–49.67) | .075 |
| Lymph node metastasis | | | |
| No | 3/14 | Reference | |
| Yes | 2/3 | 2.61 (0.42–14.00) | .278 |
| Positive margins | | | |
| No | 3/9 | Reference | |
| Yes | 2/8 | 1.00 (0.17–5.13) | .998 |
| Tumor site | | | |
| Left | 2/8 | Reference | |
| Right | 3/9 | 1.35 (0.26–8.26) | .719 |
| Multifocal tumors | | | |
| No | 5/13 | Reference | |
| Yes | 0/4 | 0.37 (0.003–3.32) | .439 |
| Skin involved | | | |
| No | 3/10 | Reference | |
| Yes | 2/7 | 0.78 (0.13–4.14) | .773 |
| Obesity | | | |
| No | 3/5 | Reference | |
| Yes | 2/12 | 0.20 (0.03–1.10) | .063 |
| Hypertension | | | |
| No | 4/11 | Reference | |
| Yes | 1/6 | 0.99 (0.10–5.40) | .990 |
| Smoking | | | |
| No | 5/13 | Reference | |
| Yes | 0/4 | 0.45 (0.003–4.04) | .551 |
| Diabetes | | | |
| No | 4/10 | Reference | |
| Yes | 1/7 | 0.58 (0.06–3.17) | .548 |
| Treatment received | | | |
| Surgery only | 4/14 | Reference | |
| Surgery + chemotherapy/radiotherapy | 1/3 | 1.52 (0.15–8.29) | .671 |
| CD117 | | | |
| Negative | 1/4 | Reference | |
| Positive | 2/7 | 1.55 (0.20–17.50) | .676 |
| p53 | | | |
| Negative | 2/7 | Reference | |
| Positive | 1/4 | 1.23 (0.11–9.37) | .847 |
| D2-40 | | | |
| Negative | 1/4 | Reference | |
| Positive | 2/7 | 0.95 (0.12–10.49) | .960 |
| c-Myc | | | |
| Negative | 1/3 | Reference | |
| Positive | 2/8 | 0.82 (0.11–9.04) | .847 |

Event/n, the number of events and patients; HR, hazard ratio; CI, confidence interval; BAS, breast angiosarcoma; HPF, high-power field.
[a]Global p-value calculated by likelihood ratio test.

multiple studies have shown conflicting p53 immunoreactivity by IHC with one study reporting that p53 was expressed in primary angiosarcoma but not in secondary sarcoma [19], and another study reporting no difference in p53 expression between primary and secondary sarcomas [20]. Furthermore, *MYC* amplification and c-Myc overexpression were detected almost exclusively in secondary angiosarcoma, compared with primary angiosarcoma [34]. In contrast, one study reported weak c-Myc staining by IHC in two of nine cases of primary BAS in their series [33]. Following our review, we found no significant difference in expression of CD117 ($p > .99$), p53 ($p = .236$), D240 ($p > .99$), CD31 ($p > .99$), and c-Myc ($p > .99$) between primary and secondary BAS. Our findings may be explained by the limited number of cases in this study but leaves room for future research. It's however, noteworthy that p53 was only expressed in the secondary BAS cases in our cohort, and was not expressed in the primary BAS cases evaluated. In addition, c-Myc showed expression in both primary and secondary BAS cases in our series.

Our study also compared comorbidities (hypertension, obesity, diabetes mellitus, and smoking history) and the treatment received between patients with primary and secondary BAS. Following our review, we found that patients with secondary BAS had more concurrent comorbid conditions compared with patients with primary BAS. However, these findings were not statistically significant. While these comorbid observations in patients with secondary BAS may be explained by the fact that they are older and thus more prone to chronic diseases, these findings may nonetheless have prognostic implications and thus leave room for future research.

The role of histologic characteristics in the prognostication and outcome of BAS has not been fully validated. Our experience shows that the presence of tumor necrosis is associated with worse OS in BAS and this observation was statistically significant ($p =$

.034). We, however found that other histologic parameters, including tumor grade, mitotic count, lymph node metastasis, and positive tumor margins had no significant effect on OS in BAS patients. Additionally, the expression of CD117 (p = .676), p53 (p = .847), D2-40 (p = .960), and c-Myc (p = .847) all had no impact on OS in BAS patients. While these findings are inconclusive due to the limited number of patients in this study, additional studies are needed to characterize the utility of these markers in BAS.

During a median follow-up of 21 months, primary BAS with two (40%) reported deaths appears to have a worse OS compared with secondary BAS with three (25%) reported deaths. However, this difference in survival between primary and secondary BAS was not statistically significant (HR, 0.51; 95% CI, 0.09 to 3.28; p = .450). Our findings are consistent with what has been reported in similar studies [1,35]. However, Yin et al. [4] found a nominal increased death risk in secondary BAS due to advanced clinicopathologic features.

With no consensus management guidelines for BAS, treatment includes a combination of wide local excision or mastectomy, with or without chemotherapy and/or radiotherapy. In this study, all patients with BAS were managed with wide local excision or mastectomy. Two patients with secondary BAS received additional chemotherapy and one patient with primary BAS received additional chemotherapy and radiotherapy. However, there was no difference between patients who were treated with surgery alone and those who received additional treatments (p = .35). One study reported no survival benefit in patients treated with routine radiation therapy in primary and secondary BAS, respectively [36]. Chemotherapy has been reported to be beneficial in high-grade and metastatic settings [36]. Therefore, we suggest that the management of patients with BAS should be optimally selected, based on their clinicopathologic characteristics.

Our study is not devoid of limitations. We recognize that findings from this study are limited and may not be representative of the general population due to the small sample size and its retrospective nature. We also acknowledge that our findings of no statistically significant differences between primary and secondary BAS, are not conclusive due to the limited number of cases in our cohort. However, this is not unusual because BAS remains a rare disease with catastrophic outcomes, which limits the ability to conduct a prospective study and include a larger study population. Additionally, the median follow-up of less than 5 years is obviously inadequate to establish prognosis in this rare disease. However, our follow-up data is similar to the experiences of other studies [26,27,35,37,38] and this is understandable due to the rarity and adverse outcomes of this disease. Despite these limitations, our study highlights our experience from a tertiary healthcare center and provides additional information that is particularly relevant to the natural history of this very rare disease. In addition, the study team has dedicated surgical pathologists with expertise in breast pathology (SB and RAF) whose review of available study materials were invaluable in the conduct of this study.

In summary, BAS is a rare and aggressive disease. On the basis of our results, only the presence of tumor necrosis was associated with worse OS in BAS. However, no histologic, immunohistochemical (CD117, p53, and c-Myc) or survival differences were identified between primary and secondary BAS in this study.

## Supplementary Information

The Data Supplement is available with this article at https://doi.org/10.4132/jptm.2022.08.31.

## Ethics Statement

All procedures performed in the current study were approved by the Wayne State University IRB and/or national research ethics committee (063419M1X; 08/13/2019) in accordance with the 1964 Helsinki declaration and its later amendments. Formal written informed consent was not required with a waiver by the appropriate IRB and/or national research ethics committee.

## Availability of Data and Material

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

## Code Availability

Not applicable.

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## Author Contributions

Conceptualization: EA, RAF. Data curation: EA, HJ, SK. Formal analysis: HJ, SK. Investigation: EA, RAF, SB. Methodology: EA, RAF, SB, SK. Project administration: EA, RAF. Resources: EA, RAF. Supervision: RAF. Visualization: EA, RAF, SB. Writing—original draft preparation: EA. Writing—review and editing: EA, HJ, SK, RAF, SB.

## Conflicts of Interest

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

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## References

1. Abdou Y, Elkhanany A, Attwood K, Ji W, Takabe K, Opyrchal M. Primary and secondary breast angiosarcoma: single center report and a meta-analysis. Breast Cancer Res Treat 2019; 178: 523-33.
2. Brar R, West R, Witten D, Raman B, Jacobs C, Ganjoo K. Breast

angiosarcoma: case series and expression of vascular endothelial growth factor. Case Rep Oncol 2009; 2: 242-50.
3. Kunkiel M, Maczkiewicz M, Jagiello-Gruszfeld A, Nowecki Z. Primary angiosarcoma of the breast-series of 11 consecutive cases: a single-centre experience. Curr Oncol 2018; 25: e50-3.
4. Yin M, Wang W, Drabick JJ, Harold HA. Prognosis and treatment of non-metastatic primary and secondary breast angiosarcoma: a comparative study. BMC Cancer 2017; 17: 295.
5. Torres KE, Ravi V, Kin K, et al. Long-term outcomes in patients with radiation-associated angiosarcomas of the breast following surgery and radiotherapy for breast cancer. Ann Surg Oncol 2013; 20: 1267-74.
6. Arora TK, Terracina KP, Soong J, Idowu MO, Takabe K. Primary and secondary angiosarcoma of the breast. Gland Surg 2014; 3: 28-34.
7. Moore A, Hendon A, Hester M, Samayoa L. Secondary angiosarcoma of the breast: can imaging findings aid in the diagnosis? Breast J 2008; 14: 293-8.
8. Kaur RP, Vasudeva K, Kumar R, Munshi A. Role of p53 gene in breast cancer: focus on mutation spectrum and therapeutic strategies. Curr Pharm Des 2018; 24: 3566-75.
9. Croce CM, Thierfelder W, Erikson J, et al. Transcriptional activation of an unrearranged and untranslocated c-myc oncogene by translocation of a C lambda locus in Burkitt. Proc Natl Acad Sci U S A 1983; 80: 6922-6.
10. Mastronikolis N, Ragos V, Kyrodimos E, et al. Mechanisms of C-myc oncogenic activity in head and neck squamous cell carcinoma. J BUON 2019; 24: 2242-4.
11. Georgakopoulos G, Tsiambas E, Korkolopoulos P, et al. c-MYC and h-TERT co-expression in colon adenocarcinoma: a tissue microarray digitized image analysis. J BUON 2013; 18: 124-30.
12. La Rosa S, Bernasconi B, Vanoli A, et al. c-MYC amplification and c-myc protein expression in pancreatic acinar cell carcinomas. New insights into the molecular signature of these rare cancers. Virchows Arch 2018; 473: 435-41.
13. Tsiambas E, Stamatelopoulos A, Baltayiannis N, et al. Evaluation of combined telomerase and c-myc expression in non-small cell lung carcinomas using tissue microarrays and computerized image analysis. J BUON 2005; 10: 533-9.
14. Miettinen M, Sarlomo-Rikala M, Lasota J. KIT expression in angiosarcomas and fetal endothelial cells: lack of mutations of exon 11 and exon 17 of C-kit. Mod Pathol 2000; 13: 536-41.
15. Billings SD, McKenney JK, Folpe AL, Hardacre MC, Weiss SW. Cutaneous angiosarcoma following breast-conserving surgery and radiation: an analysis of 27 cases. Am J Surg Pathol 2004; 28: 781-8.
16. de Bree E, van Coevorden F, Peterse JL, Russell NS, Rutgers EJ. Bilateral angiosarcoma of the breast after conservative treatment of bilateral invasive carcinoma: genetic predisposition? Eur J Surg Oncol 2002; 28: 392-5.
17. Komdeur R, Hoekstra HJ, Molenaar WM, et al. Clinicopathologic assessment of postradiation sarcomas: KIT as a potential treatment target. Clin Cancer Res 2003; 9: 2926-32.
18. Lae M, Lebel A, Hamel-Viard F, et al. Can c-myc amplification reliably discriminate postradiation from primary angiosarcoma of the breast? Cancer Radiother 2015; 19: 168-74.
19. Hung J, Hiniker SM, Lucas DR, et al. Sporadic versus radiation-associated angiosarcoma: a comparative clinicopathologic and molecular analysis of 48 cases. Sarcoma 2013; 2013: 798403.
20. Italiano A, Chen CL, Thomas R, et al. Alterations of the p53 and PIK3CA/AKT/mTOR pathways in angiosarcomas: a pattern distinct from other sarcomas with complex genomics. Cancer 2012; 118: 5878-87.
21. Cordon-Cardo C, Reuter VE. Alterations of tumor suppressor genes in bladder cancer. Semin Diagn Pathol 1997; 14: 123-32.
22. Mallofre C, Castillo M, Morente V, Sole M. Immunohistochemical expression of CK20, p53, and Ki-67 as objective markers of urothelial dysplasia. Mod Pathol 2003; 16: 187-91.
23. Nwanze J, Siddiqui MT, Stevens KA, Saxe D, Cohen C. MYC immunohistochemistry Predicts MYC Rearrangements by FISH. Front Oncol 2017; 7: 209.
24. Donnell RM, Rosen PP, Lieberman PH, et al. Angiosarcoma and other vascular tumors of the breast. Am J Surg Pathol 1981; 5: 629-42.
25. Rosen PP, Kimmel M, Ernsberger D. Mammary angiosarcoma: the prognostic significance of tumor differentiation. Cancer 1988; 62: 2145-51.
26. Wang XY, Jakowski J, Tawfik OW, Thomas PA, Fan F. Angiosarcoma of the breast: a clinicopathologic analysis of cases from the last 10 years. Ann Diagn Pathol 2009; 13: 147-50.
27. Hui A, Henderson M, Speakman D, Skandarajah A. Angiosarcoma of the breast: a difficult surgical challenge. Breast 2012; 21: 584-9.
28. Seinen JM, Styring E, Verstappen V, et al. Radiation-associated angiosarcoma after breast cancer: high recurrence rate and poor survival despite surgical treatment with R0 resection. Ann Surg Oncol 2012; 19: 2700-6.
29. Jallali N, James S, Searle A, Ghattaura A, Hayes A, Harris P. Surgical management of radiation-induced angiosarcoma after breast conservation therapy. Am J Surg 2012; 203: 156-61.
30. Morgan EA, Kozono DE, Wang Q, et al. Cutaneous radiation-associated angiosarcoma of the breast: poor prognosis in a rare secondary malignancy. Ann Surg Oncol 2012; 19: 3801-8.
31. Brenn T, Fletcher CD. Radiation-associated cutaneous atypical vascular lesions and angiosarcoma: clinicopathologic analysis of 42 cases. Am J Surg Pathol 2005; 29: 983-96.
32. Hornick JL, Fletcher CD. Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. Am J Clin Pathol 2002; 117: 188-93.
33. Shet T, Malaviya A, Nadkarni M, et al. Primary angiosarcoma of the breast: observations in Asian Indian women. J Surg Oncol 2006; 94: 368-74.
34. Requena C, Rubio L, Lavernia J, et al. Immunohistochemical and fluorescence in situ hybridization analysis of MYC in a series of 17 cutaneous angiosarcomas: a single-center study. Am J Dermatopathol 2018; 40: 349-54.
35. Vorburger SA, Xing Y, Hunt KK, et al. Angiosarcoma of the breast. Cancer 2005; 104: 2682-8.
36. Sher T, Hennessy BT, Valero V, et al. Primary angiosarcomas of the breast. Cancer 2007; 110: 173-8.
37. Marchal C, Weber B, de Lafontan B, et al. Nine breast angiosarcomas after conservative treatment for breast carcinoma: a survey from French comprehensive Cancer Centers. Int J Radiat Oncol Biol Phys 1999; 44: 113-9.
38. Penel N, Bui BN, Bay JO, et al. Phase II trial of weekly paclitaxel for unresectable angiosarcoma: the ANGIOTAX study. J Clin Oncol 2008; 26: 5269-74.

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ORIGINAL ARTICLE

# Diagnostic distribution and pitfalls of glandular abnormalities in cervical cytology: a 25-year single-center study

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**Background:** Detection of glandular abnormalities in Papanicolaou (Pap) tests is challenging. This study aimed to review our institute's experience interpreting such abnormalities, assess cytohistologic concordance, and identify cytomorphologic features associated with malignancy in follow-up histology. **Methods:** Patients with cytologically-detected glandular lesions identified in our pathology records from 1995 to 2020 were included in this study. **Results:** Of the 683,197 Pap tests performed, 985 (0.144%) exhibited glandular abnormalities, 657 of which had tissue follow-up available. One hundred eighty-eight cases were cytologically interpreted as adenocarcinoma and histologically diagnosed as malignant tumors of various origins. There were 213 cases reported as atypical glandular cells (AGC) and nine cases as adenocarcinoma in cytology, yet they were found to be benign in follow-up histology. In addition, 48 cases diagnosed with AGC and six with adenocarcinoma cytology were found to have cervical squamous lesions in follow-up histology, including four squamous cell carcinomas. Among the cytomorphological features examined, nuclear membrane irregularity, three-dimensional clusters, single-cell pattern, and presence of mitoses were associated with malignant histology in follow-up. **Conclusions:** This study showed our institute's experience detecting glandular abnormalities in cervical cytology over a 25-year period, revealing the difficulty of this task. Nonetheless, the present study indicates that several cytological findings such as membrane irregularity, three-dimensional clusters, single-cell pattern, and evidence of proliferation could help distinguishing malignancy from a benign lesion.

**Key Words:** Cytopathology; Papanicolaou test; Uterine cervical neoplasms; Glandular and epithelial neoplasms; Early detection of cancer

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The introduction of cervical screening has reduced the numbers of new cases and deaths caused by cervical cancer; this success has mainly been attributed to the Papanicolaou (Pap) test, which is a main cervical screening modality used worldwide [1,2]. For instance, under the Korean cervical cancer screening program and between the years 1999 and 2017, new cervical cancer cases and deaths dropped significantly [3]. However, despite its huge success, the Pap test is by no means perfect. According to the 2020 GLOBOCAN estimates, cervical cancer still ranked as the fourth most common malignancy both in incidence (6.5%) and mortality (7.7%) in women globally [4].

Pap test is primarily considered a screening test for squamous cancer and its precursors, exhibiting high specificity for this task [5], while it is often used either as a stand-alone test or in association with human papillomavirus testing, as a part of various screening algorithms [6-9]. However, its diagnostic accuracy regarding glandular cancers and their precursors is considered suboptimal; reasons include the relative rarity of glandular abnormalities in Pap tests, compared to their squamous counterparts, the difficulty sampling them, also the pathologists' lack of experience with their cytomorphologic criteria and the reported interobserver variability [10-12]. "Atypical glandular cells" (AGC) is an uncommon cytologic interpretation used to interpret cases with cytomorphologic glandular changes exceeding reactive atypia, yet falling short to be reported as in situ or invasive carcinomas [13-15].

Squamous cell carcinoma and adenocarcinoma (ADC) are the first and second most common histologic types of cervical cancer, respectively [16,17]. Notably, whereas the incidence of squamous cell carcinoma has decreased significantly the last years mainly




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due to the successful cervical screening programs implemented, ADC has not followed a similar trend [18-22]. Adenocarcinomas often behave more aggressively, while they have been associated with worse prognosis and higher metastasis rates, also with worse response to therapy compared to squamous cervical cancers [16,17,23,24]. Consequently, the early detection of cervical ADC or its precursors would directly affect patients' quality of life and survival rates. However, this task is challenging in both conventional and liquid-based cytology (LBC) [11,25]. Due to the reported suboptimal diagnostic performance of Pap tests for the reasons mentioned before, more data are needed regarding cytologically-detected glandular abnormalities, in order to enhance our ability to detect them accurately.

In this single-center study, we evaluated the current status, diagnostic distribution, cytohistological association, and pitfalls of glandular abnormalities in cervical cytology. Additionally, we also evaluated selected cytomorphologic findings in Pap tests with glandular abnormalities associated with the presence of malignancy in subsequent follow-up biopsies.

## MATERIALS AND METHODS

In this retrospective study, the records of Seoul National University Hospital were searched within the period from January 1995 to December 2020 to identify all reported cervical cytology cases with glandular abnormalities. These cases were prepared either as LBC slides or conventional smears. Among the 683,197 Pap tests over this 25-year period, 985 cases from 923 patients were reported with a glandular abnormality, while the 657 of these with available surgical pathology follow-up were included in the study. Of the 985 Pap tests, 322 (32.7%) were prepared as conventional smears, whereas 663 (67.3%) as LBC. The cytologic interpretations regarding the glandular abnormalities were made by board-certified cytopathologists of our laboratory, based on the following categories of the Bethesda system [5,26]: atypical glandular cells not otherwise specified (AGC-NOS), atypical endocervical cells (AGC-EC), atypical endometrial cells (AGC-EM), atypical glandular cells favor neoplastic (AGC-FN), and ADC. Several Pap tests that were diagnosed as atypical glandular cells of undetermined significance, favor reactive based on the Bethesda system 1991, were considered AGC-NOS lesions in 2014 Bethesda system. Surgical pathology follow-up included any of the following samples: cervical biopsies, endocervical curettages, loop electrosurgical excision procedure or conizations, endometrial curettages, and hysterectomies.

A few cytomorphologic features of selected Pap test cases exhibiting glandular abnormalities and cytohistologic discrepant findings were also compared between the cases subsequently found to be histologically benign and malignant, to identify cytologic criteria associated with malignancy. These features included architectural characteristics (overlapping; presence of single cells; and presence of three-dimensional clusters), nuclear enlargement, irregular nuclear membranes, increased nuclear-to-cytoplasm (N/C) ratio, nuclear hyperchromasia, prominent nucleoli, and mitotic activity. The Fisher exact test was used to identify the importance of cytomorphologic features in the diagnosis of malignancies. A p-value of $< .05$ was considered statistically significant.

## RESULTS

### Distribution of glandular abnormalities in cervical cytology

Among 683,197 Pap tests interpreted from January 1995 to December 2020 in our center, the number of cases with glandular abnormalities was 985 (0.144%), of which 244 (24.8%) were diagnosed as ADC. In the cytological evaluation of the 741 cases with AGC, the distribution of AGC subcategories according to the Bethesda system was as follows: AGC-NOS 667 (67.7%), AGC-EC 32 (3.2%), AGC-EM 16 (1.6%), and AGC-FN 26 (2.6%) (Fig. 1). The mean age of the patients was 49 years (range, 14 to 86 years).

### Assessment of cytohistologic concordance in our cohort

Of the 985 Pap tests interpreted with glandular abnormalities, histologic follow-up was available for 657 cases (66.7%). Among

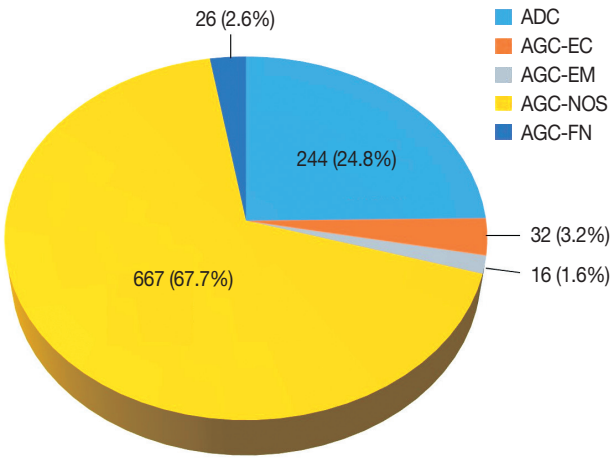


**Fig. 1.** Distribution of glandular abnormalities in the Papanicolaou tests interpreted in our institute over a 25-year period. ADC, adenocarcinoma; AGC-EC, atypical endocervical cells; AGC-EM, atypical endometrial cells; AGC-NOS, atypical glandular cells not otherwise specified; AGC-FN, atypical glandular cells favor neoplastic.

them, 409 cases (62.3%) were interpreted as AGC-NOS, 206 (31.4%) as ADC, 21 (3.2%) as AGC-FN, 12 (1.8%) as AGC-EC, and nine (1.4%) as AGC-EM. The cytohistologic concordance was summarized by referring to the Quality Improvement program recommended by the Korean Society for Cytopathology [27].

Table 1 shows the cytohistopathological correlations of the patients according to subclassification of glandular lesions. One hundred eighty-eight cases were cytologically interpreted as ADC and histologically diagnosed as malignant tumors, including the cervix, endometrium, ovary, and other organ origins. Among the cases with AGC Pap test interpretation, 213 were found to be benign in follow-up histology, while most of the others were diagnosed as malignant lesions. In addition, there were 48 cases diagnosed with an AGC and six cases with ADC cytologically, where a cervical squamous lesion was detected in their paired histology, including four squamous cell carcinomas. There was also a reported coexistence of glandular and squamous abnormalities in 50 Pap tests (AGC-NOS, 44; AGC-EM, 1; AGC-FN, 1; ADC, 4); these cases were solely categorized based on their glandular component. Notably, nine cases with an ADC cytologic interpretation were histologically diagnosed as benign.

### Cytomorphologic features of AGC associated with malignancy in follow-up histology

We also reviewed a few cytomorphologic features in some of our cytology AGC cases exhibiting cytohistologic discordance. Seventy-four AGC cases with available slides in our records were selected. Fifty-one cases with AGC cytology diagnosed as benign, 14 as cervical ADC, and nine as cervical ADC in situ (AIS) in their follow-up histology were included. Histologically, cases diagnosed

**Table 1.** Subclassification of 657 Pap test cases with glandular abnormalities and available follow-up histology

| Histopathologic result | Pap test result | | | | |
|---|---|---|---|---|---|
| | AGC-NOS | AGC-EC | AGC-EM | AGC-FN | ADC |
| Benign | 197 | 9 | 3 | 4 | 9 |
| Endometrial lesion | | | | | |
| Endometrioid adenocarcinoma | 68 | 1 | 5 | 6 | 34 |
| Serous adenocarcinoma | 7 | - | - | - | 15 |
| Clear cell carcinoma | 4 | - | - | - | 3 |
| Adenosquamous carcinoma | | | | | 2 |
| Carcinosarcoma | 5 | - | - | - | 3 |
| EM other malignant | 3 | - | - | - | - |
| EM hyperplasia | 5 | - | 1 | - | - |
| Cervical squamous lesion | | | | | |
| LSIL | 21 | - | - | - | 2 |
| HSIL | 22 | 1 | - | - | 4 |
| Squamous cell carcinoma | 4 | - | - | - | - |
| Cervical glandular lesion | | | | | |
| Adenocarcinoma in situ | 13 | - | - | - | 3 |
| Adenocarcinoma | 33 | - | - | 7 | 83 |
| Adenosquamous carcinoma | - | - | - | 1 | 6 |
| Cervical other malignant | 2 | - | - | - | 3 |
| Ovarian lesion | | | | | |
| Serous adenocarcinoma | 9 | - | - | - | 12 |
| Mucinous adenocarcinoma | - | - | - | - | 1 |
| Clear cell carcinoma | - | - | - | - | 2 |
| Endometrioid adenocarcinoma | 1 | - | - | - | - |
| Ovary other malignant | 1 | 1 | - | - | 1 |
| Vaginal lesion | | | | | |
| Poorly differentiated carcinoma | 1 | - | - | - | 1 |
| Metastatic tumor | 5 | - | - | 2 | 22 |
| Other | 2 | - | - | 1 | - |
| TIFD, unknown | 6 | - | - | - | - |
| Total | 409 | 12 | 9 | 21 | 206 |

Pap, Papanicolaou; AGC-NOS, atypical glandular cells not otherwise specified; AGC-EC, atypical endocervical cells; AGC-EM, atypical endometrial cells; AGC-FN, atypical glandular cells favor neoplastic; ADC, adenocarcinoma; EM, endometrial; LSIL, low grade squamous intraepithelial lesion; HSIL, high grade squamous intraepithelial lesion; TIFD, tissue insufficient for diagnosis.


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with diseases other than cervical ADC or AIS were excluded. We examined the abnormal glandular cells in these Pap tests to identify cytomorphologic features associated with subsequent malignant histology. Table 2 shows the comparison of selected features in the 74 eligible cases examined, according to their histologic follow-up. Whereas irregular nuclear membranes, three-dimensional clusters, single-cell pattern, and presence of mitoses were statistically significant ($p < .05$) for the presence of malignancy, increased N/C ratios, overlapping, nuclear hyperchromasia, and prominent nucleoli were not ($p > .05$). Fig. 2 shows some cytomorphologic features of the Pap smear samples from some AGC cases, histologically-confirmed as malignant.

## DISCUSSION

Although the efficacy of cervical cytology diagnosing squamous cell carcinoma and its precursors is well-established, detecting glandular abnormalities is a challenge due to their rarity, pathologists' lack of experience with their cytomorphologic criteria, and poor interobserver reproducibility [10-12]. The aim of the present single-center study was to investigate the diagnostic distribution and assess the cytohistologic concordance of glandular abnormalities in cervical cytology, also to identify selected cytomorphologic findings associated with malignancy in follow-up surgical pathology.

According to our findings, glandular abnormalities accounted for 0.144% of all cervical cytology cases in our center from 1995 to 2020, showing their rarity and the diagnostic challenge interpreting them. This finding is consistent with what is reported in the literature [15,28,29]. In respect of the distribution of glandular lesions, none was diagnosed as AIS in cytology. Of the 16 cases that were histologically-confirmed to be cervical AIS, 13 were interpreted as AGC-NOS and three as ADC in cytology. This indicates that identifying AIS in cytology is a rather challenging task. Previous studies have identified the pitfalls of the AIS cytologic diagnosis, also its suboptimal accuracy and low levels of interobserver agreement [30-32]. Some authors have also reported that many AIS lesions could be interpreted as AGC, AGC-FN, ADC, or squamous intraepithelial lesions in cervical cytology [32,33].

Assessing the cytohistologic correlation of the glandular abnormalities detected in our cervical cytology cohort, we found that most Pap test cases reported to have glandular abnormalities cytologically actually had some significant lesions in their subsequent histopathology. One hundred eighty-eight out of 657 cases (28.6%) were turned out to be completely concordant, all of which were diagnosed as malignant tumors in follow-up histology (including cervical 92, endometrial, and ovarian 73). Also, there were 121 cases reported as AGC in cervical cytology, which turned out to be malignant tumors of various origins. On the other hand, 213 AGC Pap tests were histologically benign. Zhao et al. [25] analyzed 662 patients with an AGC cytologic interpretation and available tissue biopsy material and found that AGC cytology revealed cancer in 15.3% of the cases during histologic follow-up, most likely located in the cervix (8.3%), the endometrium (6.3%), and the ovaries (0.6%), respectively. In another study by Pradhan et al. [34], the histologic diagnoses of 3,709 AGC cases consisted of: negative (70.5%), cervical intraepithelial neoplasia/low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL) (20.7%), endometrial ADC (5.5%), endocervical AIS and ADC (1.9%), and metastatic carcinomas (0.5%).

**Table 2.** Comparison of cytomorphological features between Pap test cases exhibiting atypical glandular cells

| Cytomorphological feature | Histologic diagnosis | | p-value[a] |
|---|---|---|---|
| | Benign (n=51) | ADC/AIS (n=23) | |
| Architectural features | | | |
| Overlapping | | | .058 |
| Present | 30 | 18 | |
| Absent | 21 | 5 | |
| Single-cell pattern | | | <.001 |
| Present | 1 | 8 | |
| Absent | 50 | 15 | |
| Three-dimensional clusters | | | <.001 |
| Present | 0 | 8 | |
| Absent | 51 | 15 | |
| Nuclear features | | | |
| Increased N/C ratio | | | .434 |
| Present | 50 | 22 | |
| Absent | 1 | 1 | |
| Hyperchromasia | | | .233 |
| Present | 39 | 18 | |
| Absent | 12 | 5 | |
| Membrane irregularity | | | .012 |
| Present | 18 | 15 | |
| Absent | 33 | 8 | |
| Prominent nucleoli | | | .124 |
| Present | 16 | 10 | |
| Absent | 35 | 13 | |
| Mitoses | | | .009 |
| 0/HPF | 49 | 17 | |
| >1/HPF | 2 | 6 | |

Pap, Papanicolaou; ADC, adenocarcinoma; AIS, adenocarcinoma in situ; N/C, nuclear-to-cytoplasm; HPF, high-power field.
[a]The Fisher exact test was used. p-value of <.05 was considered statistically significant.

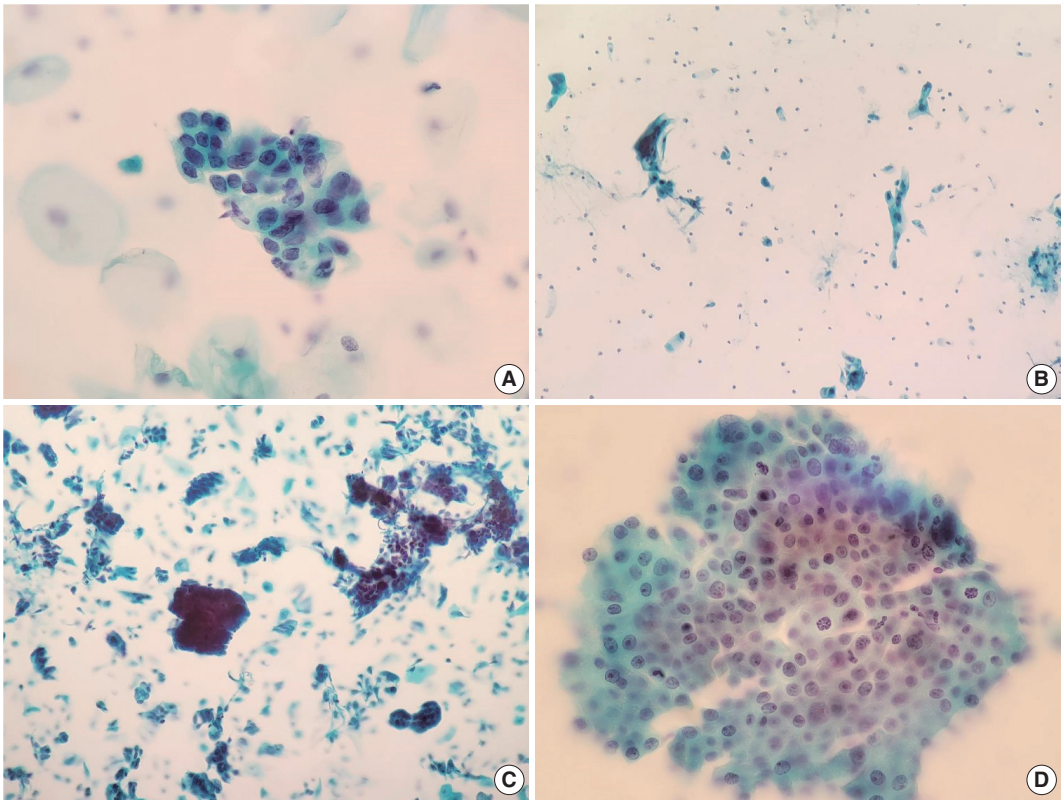


**Fig. 2.** Cytomorphologic features of histologically-confirmed malignant cases. (A) Atypical glandular cells not otherwise specified (AGC-NOS). Groups of cells showing nuclear membrane irregularity. Follow-up histology revealed adenocarcinoma in situ (Papanicolaou [Pap] stain). (B) AGC-NOS. Some scattered atypical cells showing single-cell pattern. Follow-up histology revealed adenocarcinoma (Pap stain). (C) AGC-NOS. Cell clusters showing three-dimensional architecture with sharper, smoother margins. Follow-up revealed adenocarcinoma (Pap stain). (D) Atypical glandular cells favor neoplastic. Sheet of crowded cells with mitotic activity. Histological diagnosis was adenocarcinoma (Pap stain).

We also reviewed 74 AGC Pap tests to identify any cytomorphologic feature differences between the cases confirmed as benign and malignant during follow-up surgical pathology. In this examination, irregular nuclear membranes, three-dimensional clusters, single-cell pattern, and presence of mitoses were found to be significantly associated with malignancy in subsequent histology. On the contrary, no significant association was identified regarding increased N/C ratio, nuclear hyperchromasia, prominent nucleoli, or overlapping. These particular findings are largely concordant with what has so far been reported the literature. Raab et al. [35] showed that the presence of single dysplastic cells, nuclear membrane irregularities, and reduced amount of cytoplasm indicated cancer or a cancer precursor in histology. In another study by Yucel Polat et al. [29], the presence of feathering, papillary pattern, polarity loss, 3D clusters, irregular nuclear membranes, and prominent nucleoli were found to be significant; in contrast, the formation of rosettes, overlapping, increased N/C ratio, and nuclear hyperchromasia were not significantly associated with cancer in follow-up histology. Torres et al. [36] showed that finding cells exhibiting high N/C ratio and dyskeratosis indicated intraepithelial neoplasia or cancer, whereas glandular lesions were composed of cells with reduced amount of cytoplasm, nuclear membrane irregularity, and macronuleoli. Lastly, Reynolds et al. [37] reported that the presence of single dysplastic cells, 3D clusters, intracytoplasmic neutrophils, increased N/C ratio and larger nuclei, nuclear border irregularity, reniform-shaped nuclei, polarity loss, overlapping, and macronucleoli were significantly associated with a clinically significant lesion (HSIL or cancer) in surgical pathology follow-up.

Whenever possible, it is necessary to identify the cytomorphologic differences between squamous and glandular abnormalities in Pap tests, in addition to the cytologic features observed in benign glandular lesions that could mimic malignancy, to reduce potential misinterpretations. AGC is often found to be a benign lesion or a squamous intraepithelial lesion (e.g., a HSIL with endocervical gland involvement), rather than a glandular abnormality, in follow-up histology [34,38]. HSIL with endocervical gland involvement could indeed be misinterpreted as a glandu-


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lar abnormality in cervical cytology. However, the loss of polarity within the hyperchromatic crowded groups, the flattening of the cells at the periphery of such groups, and the identification of single squamous dyskaryotic cells could help identify such lesions as squamous rather than glandular [26,38].

Some cases in our cohort included histologically benign cases interpreted as ADCs in cytology. Interestingly, among the cases interpreted as AGCs or ADCs in cytology, there were a few cases that were neither ADC nor squamous cell carcinoma in histology, including a smooth muscle tumor of uncertain malignant potential, an ovarian diffuse large B-cell lymphoma, an extramammary Paget disease of vulva, a small cell carcinoma, and a poorly differentiated carcinoma of cervix. The pathologists' lack of experience and/or the presence of ambiguous cytomorphological criteria may be responsible for their misinterpretation.

A limitation of this study is that a few of our cases cytologically containing both glandular and squamous abnormalities (e.g., ACG-NOS and atypical squamous cells-cannot exclude HSIL (ASC-H) were solely classified based on their glandular component in this study, before assessing our cases' distribution and cytology-histology correlation. According to the literature, glandular and squamous lesions in the cervix often coexist [39,40]. In our study, 50 of the 657 cases with histologic follow-up diagnosis were found to have both glandular and squamous abnormalities in their cytology reports. Forty-four of 409 AGC-NOS cases exhibited such coexistence, whereas 19 of them were finally diagnosed as cervical squamous neoplasias (LSIL, HSIL, or invasive squamous cell carcinoma) during surgical pathology follow-up, without any glandular lesions present. As previously mentioned, we evaluated only the glandular component for the cytohistologic correlation assessment of such cases. For example, a case interpreted as AGC-NOS and ASC-H in cytology followed by an LSIL diagnosis in histology, was regarded as a lesion with AGC-NOS only, ignoring its cytologic interpretation about squamous component. This way of classification, however, can be controversial, as it may not carry a major clinical impact when AGC patients end up having a squamous lesion.

In conclusion, this study showed the current status of glandular abnormalities detected by cervical cytology in a single center over a 25-year-period. They revealed a few cytomorphologic features, such as nuclear membrane irregularity, three-dimensional clusters, single-cell pattern and presence of mitoses, associated with malignancy in follow-up histology. As detecting glandular lesions is rare in Pap tests and pathologists are often unfamiliar with them, we hope this study will add some value to the relevant literature.

## Ethics Statement

All procedures performed in the current study were approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB No. H-2112-004-1277) in accordance with the 1964 Helsinki declaration and its later amendments. The committee waived the demand to obtain informed consent.

## 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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## Author Contributions

Conceptualization: CL, HSR. Data curation: JAS. Investigation: JAS. Methodology: JAS, CL, HSR. Supervision: CL. Visualization: JAS. Writing—original draft: JAS. Writing—review & editing: CL, IPN, HK. Approval of final manuscript: all authors.

## Conflicts of Interest

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

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## References

1. Peirson L, Fitzpatrick-Lewis D, Ciliska D, Warren R. Screening for cervical cancer: a systematic review and meta-analysis. Syst Rev 2013; 2: 35.
2. Arbyn M, Rebolj M, De Kok IM, et al. The challenges of organising cervical screening programmes in the 15 old member states of the European Union. Eur J Cancer 2009; 45: 2671-8.
3. Hong S, Won YJ, Park YR, et al. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2017. Cancer Res Treat 2020; 52: 335-50.
4. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209-49.
5. Nayar R, Wilbur DC. The Pap test and Bethesda 2014. "The reports of my demise have been greatly exaggerated." (after a quotation from Mark Twain). Acta Cytol 2015; 59: 121-32.
6. Kang M, Ha SY, Cho HY, et al. Comparison of papanicolaou smear and human papillomavirus (HPV) test as cervical screening tools: can we rely on HPV test alone as a screening method? An 11-year retrospective experience at a single institution. J Pathol Transl Med 2020; 54: 112-8.
7. Polman NJ, Snijders PJ, Kenter GG, Berkhof J, Meijer C. HPV-based cervical screening: rationale, expectations and future per-

spectives of the new Dutch screening programme. Prev Med 2019; 119: 108-17.
8. Naucler P, Ryd W, Tornberg S, et al. Efficacy of HPV DNA testing with cytology triage and/or repeat HPV DNA testing in primary cervical cancer screening. J Natl Cancer Inst 2009; 101: 88-99.
9. Wentzensen N, Schiffman M, Palmer T, Arbyn M. Triage of HPV positive women in cervical cancer screening. J Clin Virol 2016; 76 Suppl 1: S49-S55.
10. Moriarty AT, Wilbur D. Those gland problems in cervical cytology: faith or fact? Observations from the Bethesda 2001 terminology conference. Diagn Cytopathol 2003; 28: 171-4.
11. Bansal B, Gupta P, Gupta N, Rajwanshi A, Suri V. Detecting uterine glandular lesions: role of cervical cytology. Cytojournal 2016; 13: 3.
12. Lin M, Narkcham S, Jones A, et al. False-negative Papanicolaou tests in women with biopsy-proven invasive endocervical adenocarcinoma/adenocarcinoma in situ: a retrospective analysis with assessment of interobserver agreement. J Am Soc Cytopathol 2022; 11: 3-12.
13. Solomon D, Frable WJ, Vooijs GP, et al. ASCUS and AGUS criteria. International Academy of Cytology Task Force summary. Diagnostic cytology towards the 21st century: an international expert conference and tutorial. Acta Cytol 1998; 42: 16-24.
14. Wood MD, Horst JA, Bibbo M. Weeding atypical glandular cell look-alikes from the true atypical lesions in liquid-based Pap tests: a review. Diagn Cytopathol 2007; 35: 12-7.
15. Toyoda S, Kawaguchi R, Kobayashi H. Clinicopathological characteristics of atypical glandular cells determined by cervical cytology in Japan: survey of gynecologic oncology data from the Obstetrical Gynecological Society of Kinki District, Japan. Acta Cytol 2019; 63: 361-70.
16. Galic V, Herzog TJ, Lewin SN, et al. Prognostic significance of adenocarcinoma histology in women with cervical cancer. Gynecol Oncol 2012; 125: 287-91.
17. Gallardo-Alvarado L, Cantu-de Leon D, Ramirez-Morales R, et al. Tumor histology is an independent prognostic factor in locally advanced cervical carcinoma: a retrospective study. BMC Cancer 2022; 22: 401.
18. Smith HO, Tiffany MF, Qualls CR, Key CR. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States: a 24-year population-based study. Gynecol Oncol 2000; 78: 97-105.
19. Vizcaino AP, Moreno V, Bosch FX, et al. International trends in incidence of cervical cancer: II. Squamous-cell carcinoma. Int J Cancer 2000; 86: 429-35.
20. Wang SS, Sherman ME, Hildesheim A, Lacey JV Jr, Devesa S. Cervical adenocarcinoma and squamous cell carcinoma incidence trends among white women and black women in the United States for 1976-2000. Cancer 2004; 100: 1035-44.
21. Bray F, Loos AH, McCarron P, et al. Trends in cervical squamous cell carcinoma incidence in 13 European countries: changing risk and the effects of screening. Cancer Epidemiol Biomarkers Prev 2005; 14: 677-86.
22. Takeuchi S. Biology and treatment of cervical adenocarcinoma. Chin J Cancer Res 2016; 28: 254-62.
23. Gien LT, Beauchemin MC, Thomas G. Adenocarcinoma: a unique cervical cancer. Gynecol Oncol 2010; 116: 140-6.
24. Katanyoo K, Sanguanrungsirikul S, Manusirivithaya S. Comparison of treatment outcomes between squamous cell carcinoma and adenocarcinoma in locally advanced cervical cancer. Gynecol Oncol 2012; 125: 292-6.
25. Zhao C, Florea A, Onisko A, Austin RM. Histologic follow-up results in 662 patients with Pap test findings of atypical glandular cells: results from a large academic womens hospital laboratory employing sensitive screening methods. Gynecol Oncol 2009; 114: 383-9.
26. Nayar R, Wilbur DC. The Bethesda System for Reporting Cervical Cytology: definitions, criteria, and explanatory notes. Cham: Springer, 2015.
27. Oh EJ, Jung CK, Kim DH, et al. Current cytology practices in Korea: a nationwide survey by the Korean Society for Cytopathology. J Pathol Transl Med 2017; 51: 579-87.
28. Ajit D, Gavas S, Joseph S, Rekhi B, Deodhar K, Kane S. Identification of atypical glandular cells in pap smears: is it a hit and miss scenario? Acta Cytol 2013; 57: 45-53.
29. Yucel Polat A, Tepeoglu M, Tunca MZ, Ayva ES, Ozen O. Atypical glandular cells in Papanicolaou test: which is more important in the detection of malignancy, architectural or nuclear features? Cytopathology 2021; 32: 344-52.
30. Niu S, Molberg K, Thibodeaux J, et al. Challenges in the Pap diagnosis of endocervical adenocarcinoma in situ. J Am Soc Cytopathol 2019; 8: 141-8.
31. Chaump M, Pirog EC, Panico VJ, AB DM, Holcomb K, Hoda R. Detection of in situ and invasive endocervical adenocarcinoma on ThinPrep Pap test: morphologic analysis of false negative cases. Cytojournal 2016; 13: 28.
32. Umezawa T, Umemori M, Horiguchi A, et al. Cytological variations and typical diagnostic features of endocervical adenocarcinoma in situ: a retrospective study of 74 cases. Cytojournal 2015; 12: 8.
33. Li S, Tian D, Li Y. Cytological diagnoses of adenocarcinoma in situ of the cervix: common misdiagnoses. Acta Cytol 2015; 59: 91-6.
34. Pradhan D, Li Z, Ocque R, Patadji S, Zhao C. Clinical significance of atypical glandular cells in Pap tests: an analysis of more than 3000 cases at a large academic women's center. Cancer Cytopathol 2016; 124: 589-95.
35. Raab SS, Isacson C, Layfield LJ, Lenel JC, Slagel DD, Thomas PA. Atypical glandular cells of undetermined significance. Cytologic criteria to separate clinically significant from benign lesions. Am J Clin Pathol 1995; 104: 574-82.
36. Torres JC, Derchain SF, Gontijo RC, et al. Atypical glandular cells: criteria to discriminate benign from neoplastic lesions and squamous from glandular neoplasia. Cytopathology 2005; 16: 295-302.
37. Reynolds JP, Salih ZT, Smith AL, Dairi M, Kigen OJ, Nassar A. Cytologic parameters predicting neoplasia in Papanicolaou smears with atypical glandular cells and histologic follow-up: a single-institution experience. J Am Soc Cytopathol 2018; 7: 7-15.
38. Selvaggi SM. Cytologic features of high-grade squamous intraepithelial lesions involving endocervical glands on ThinPrep cytology. Diagn Cytopathol 2002; 26: 181-5.
39. Kumar N, Bongiovanni M, Molliet MJ, Pelte MF, Egger JF, Pache JC. Diverse glandular pathologies coexist with high-grade squamous intraepithelial lesion in cyto-histological review of atypical glandular cells on ThinPrep specimens. Cytopathology 2009; 20: 351-8.
40. Colgan TJ, Lickrish GM. The topography and invasive potential of cervical adenocarcinoma in situ, with and without associated squamous dysplasia. Gynecol Oncol 1990; 36: 246-9.

 https://doi.org/10.4132/jptm.2022.09.05

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ORIGINAL ARTICLE

# Current status of cytopathology practice in Korea: impact of the coronavirus pandemic on cytopathology practice

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**Background:** The Continuous Quality Improvement program for cytopathology in 2020 was completed during the coronavirus pandemic. In this study, we report the result of the quality improvement program. **Methods:** Data related to cytopathology practice from each institute were collected and processed at the web-based portal. The proficiency test was conducted using glass slides and whole-slide images (WSIs). Evaluation of the adequacy of gynecology (GYN) slides from each institution and submission of case glass slides and WSIs for the next quality improvement program were performed. **Results:** A total of 214 institutions participated in the annual cytopathology survey in 2020. The number of entire cytopathology specimens was 8,220,650, a reduction of 19.0% from the 10,111,755 specimens evaluated in 2019. Notably, the number of respiratory cytopathology specimens, including sputum and bronchial washing/brushing significantly decreased by 86.9% from 2019, which could be attributed to the global pandemic of coronavirus disease. The ratio of cases with atypical squamous cells to squamous intraepithelial lesions was 4.10. All participating institutions passed the proficiency test and the evaluation of adequacy of GYN slides. **Conclusions:** Through the Continuous Quality Improvement program, the effect of coronavirus disease 2019 pandemic, manifesting with a reduction in the number of cytologic examinations, especially in respiratory-related specimen has been identified. The Continuous Quality Improvement Program of the Korean Society for Cytopathology can serve as the gold standard to evaluate the current status of cytopathology practice in Korea.

**Key Words:** Cytology; Surveys; Statistics; Quality; Coronavirus; Pandemic

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The Continuous Quality Improvement program (CQI) for cytopathology laboratories was first started in 1995 by the Committee of Quality Improvement of the Korean Society for Cytopathology (CQIKSC) and has significantly contributed to the quality control and improvement of cytopathologic examination over the last two decades [1-4]. As the number of cytopathologic examinations increased from 2.8 million in 2004 to over 10 million in 2018, the number of participating institutions increased from 100 in 1996 to 214 in 2020 [2]. Despite the exponential increase in cytopathologic examinations in Korea, sample adequacy has been relatively well controlled as well as other quality parameters such as the atypical squamous cells/squamous intraepithelial lesion (ASC/SIL) ratio [2]. Meanwhile, the ratio of discordant cases with major clinical impact (category C) in the cytology-histology correlation review of gynecologic samples decreased from 1.59% in 2003 to 0.52% in 2018 [2]. In conjunction with the National Cancer Screening Program started in 1999, CQI successfully contributed to a reduction in the incidence of cervical cancers by

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improving the quality of cytopathologic examinations in Korea [2,3,5].

However, the coronavirus pandemic from late 2019 has had an unprecedented impact on all aspects of our lives and society and has fundamentally changed the whole landscape of cytopathology practice. Since the first patients with coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome-coronavirus 2 appeared in China in late 2019, 610 million confirmed cases were reported with this ongoing pandemic worldwide and over 6.5 million people were have been estimated to have died of this devastating disease until September 2022 [6]. In a recent systematic review, COVID-19 pandemic was reported to have caused large reductions in many healthcare services, major methodological changes, and the emergence of new technologies such as telemedicine and digital pathology because of measures such as lockdowns and stay-at-home orders during the pandemic period [7]. In cytopathology practice, the pandemic appears to have caused a large reduction in the number of respiratory tract samples. However, there is limited evidence and data representing the actual impact of COVID-19 on cytopathology practice. Moreover, with clinical implementation of digital cytopathology and telecytopathology gaining more attention from the cytopathology field, the availability and safety of these new technologies is also being actively questioned and tested for proper validation evidence.

During the pandemic period, CQIKSC newly introduced digital cytopathology in CQI in parallel with conventional methods and conducted an annual survey of the statistics for gynecology (GYN) cytologic exams, which included assessments of overall statistics, statistics on the diagnostic category of GYN exams, and inadequacy rates, cytology-histology correlation reviews of gynecologic samples, evaluation of the number of discordant cases, proficiency test using five glass slides and digital cytopathology with whole-slide images (WSIs), sample adequacy evaluations using both glass and digital slides, and a submission of candidate glass and digital slides for the next proficiency test. Here, we present the results of the nationwide survey of the annual statistics for cytologic exams in 2020 and the proficiency test for 2021.

## MATERIALS AND METHODS

### Outline of quality improvement program by the Committee of Quality Improvement of the Korean Society for Cytopathology

The annual CQI included the following steps: 1, collection of statistics on the cytologic examinations performed in the last year; 2, a proficiency test using five glass slides or six digital WSIs; 3, a sample adequacy evaluation of five GYN glass slides submitted by the participating institutions; and 4, submission of six candidate cases by the participating institutions to be used in the proficiency tests in the following years (Fig. 1).

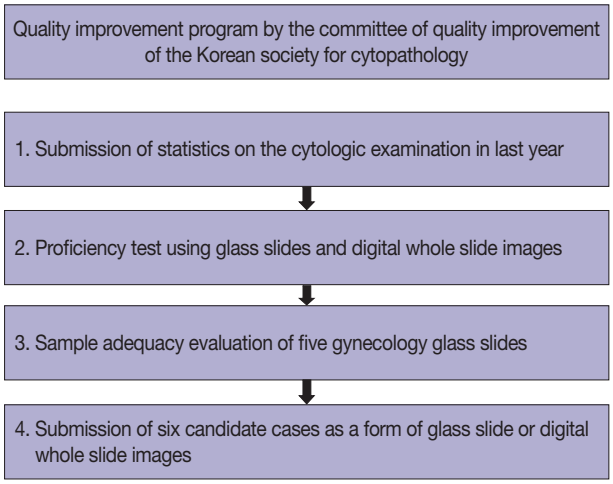


**Fig. 1.** Quality Improvement program by the Committee of Quality Improvement of the Korean Society for Cytopathology.

### Annual survey of cytopathology statistics

Internal quality control data were collected from 214 medical institutions using a portal questionnaire that included the statistical data on overall cytologic exams, the case number of GYN exams according to the diagnostic categories, the GYN sample adequacy, the cytology-histology correlation review of gynecologic sample results, and the number of discordant cases according to the discordant assessment criteria. The participating institutions were categorized into three groups: university hospitals, general hospitals, and commercial laboratories. When the cytology-histology correlation review of gynecologic sample results was evaluated, diagnostic concordance was evaluated as concordant (category O), discordant category A (minimal clinical impact), discordant category B (minor clinical impact), or discordant category C (major clinical impact). Statistical data for cytologic examinations were calculated and organized by sample categories as follows: GYN, fine-needle aspiration (FNA), and non-GYN/non-FNA sample examinations including examination using urine, body fluids, respiratory tract samples (sputum, bronchial washing, brushing, bronchioloalveolar lavage, etc.), and cerebrospinal fluid.

### Proficiency test

The second part of CQI, the proficiency test, was performed in 215 medical institutions from June 7, 2021, to June 18, 2021 using a total of 540 glass slides and 30 WSIs. Five glass slides,


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two GYN slides, two slides of body fluid and/or urine samples, and one slide of two respiratory tract or FNA samples were sent by 108 participating institutions, while six WSIs, including two GYN slides, two body fluid or urine sample while two respiratory tract samples or FNA slides were distributed randomly to 107 participating institutions and accessed by the website (Fig. 2). The cases submitted as glass slides and WSIs were different. The cases were reviewed and confirmed by members of CQIKSC. The diagnoses submitted by the participating institutions was selected using the diagnostic template (Supplementary Tables S1–S3). The diagnostic concordance was evaluated as follows: concordant (category O), discordant category A (minimal clinical impact), discordant category B (minor clinical impact), or discordant category C (major clinical impact). If an institution received one or more category C results, it was required to undergo retesting to obtain quality assurance certification.

### Sample adequacy assessment

For the sample adequacy assessment, participating institutions submitted five GYN glass slides with the consequent sample number and pathology report, including the content for sample adequacy. Sample adequacy was then reevaluated by the members of CQIKSC.

### Submission of samples for the quality assurance program

For the final part of the program, each participating institution was asked to submit six glass slides (2 GYN, 2 non-GYN, and 2 FNA) with confirmed cytologic diagnoses and the corresponding histologic diagnoses from November 26, 2018, to December 7, 2018. The eligibility of the collected samples for the proficiency test was evaluated by the members of CQIKSC, and eligible samples were archived to be used in the proficiency test in the following years.

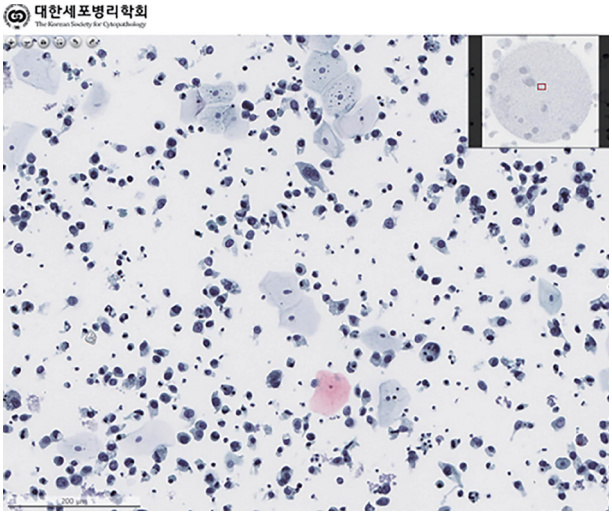


**Fig. 2.** Proficiency test using whole-slide image via web site.

## RESULTS

### Participating institutions

Responses were obtained from all 214 medical institutions, including 83 university hospitals (38.8%), 86 general hospitals (40.2%), and 45 commercial laboratories (21.0%) (Table 1). The total number of institutions that participated in the survey increased by six, in comparison with the number in 2019. The number of university hospitals, general hospitals, and commercial laboratories increased by three, two, and one, respectively.

### Overall statistics

The total number of cytopathologic examinations performed in 2020 was 8,220,650, of which 7,068,938 (86.0%) samples were GYN samples (cervical cytology, 7,063,922; gynecologic fluid samples: ovary and endometrium, 5016) and 1,151,712 (14.0%) samples were non-gynecological.

As seen in Fig. 3, the total number of cytopathologic examinations performed in Korea was increased every year, but abruptly dropped by 19% during the COVID-19 pandemic (from 10,111,755 in 2019 to 8,200,650 in 2020). In particular, the non-GYN sample number decreased significantly by 50% (from 2,294,783 in 2019 to 1,151,712 in 2020). On the other hand, the decrease in the GYN sample number was moderate, from 7,816,972 in 2019 to 7,068,938 in 2020 (about 10%).

As a result of these changes, the proportion of GYN samples in 2020 was 86%, which is an increase from before COVID-19, and the proportion of non-GYN samples was 14%, which is a reduction from that before COVID-19.

### Sample types

Among the 1,151,712 non-GYN samples in 2020, the number of FNA, urine, and non-GYN body fluid samples was 305,278, 465,219, and 381,215, respectively (Fig. 4A). The FNA samples included 211,234, 5,665 and 88,379 thyroid FNA, lung FNA,

**Table 1.** Types of institutions participating in the survey in 2021

| Types of institutions | No. of response (%) |
|---|---|
| University hospitals | 83 (38.8) |
| General hospitals | 86 (40.2) |
| Commercial laboratories | 45 (21.0) |
| Total | 214 (100) |

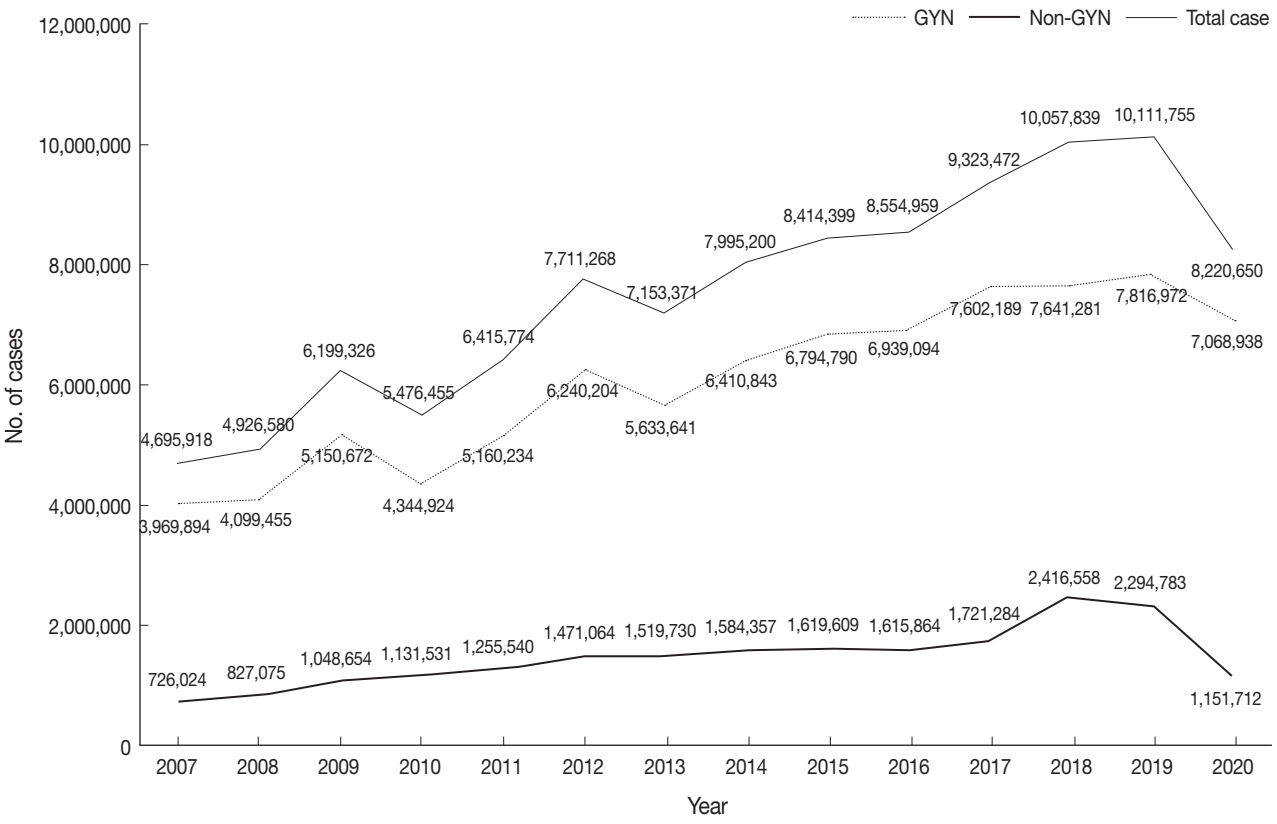


**Fig. 3.** Overall statistics of cytopathology exams from 2007 to 2020. GYN, gynecologic.

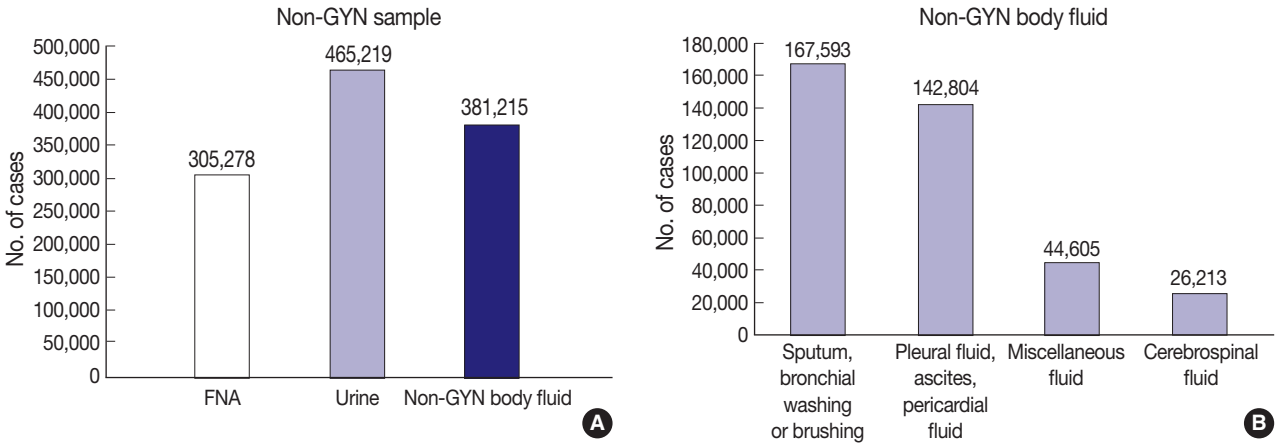


**Fig. 4.** (A) The number of non-gynecologic (non-GYN) samples, according to the type of sample. (B) The number of non-GYN body fluid, according to the type of sample.

endoscopic ultrasound/endobronchial ultrasound aspiration samples, and the body fluid samples consisted of 167,593, 142,804, 26,213, and 44,605 sputum, bronchial washing or brushing; body cavity fluid (pleural fluid, pericardial fluid or ascites); cerebrospinal fluid; other miscellaneous fluid samples (Fig. 4B).

As mentioned above, the number of non-GYN samples decreased sharply by 49.8% during the COVID-19 period, of which the number of non-GYN body fluid samples decreased by 74.5% in 2020, in comparison with 2019. In particular, the decrease in sputum, bronchial washing or brushing samples was noticeable, down 86.9% from the previous year in 2020. In contrast, the number of thyroid FNA and urine samples decreased by 6.2% and 0.6%, respectively in 2020 (Fig. 5).

The total number of GYN samples obtained in 2020 was


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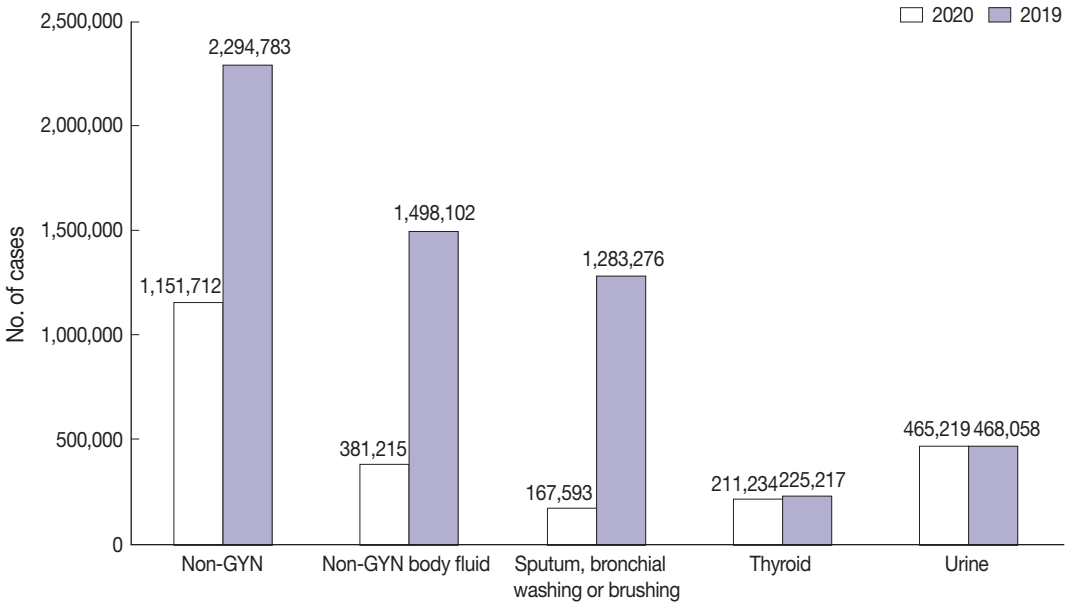


**Fig. 5.** The number of non-gynecologic samples in 2019 and 2020. Non-GYN, non-gynecologic samples.

**Table 2.** Proportion of liquid-based cytology in 2019 and 2020

| Proportion | 2019 (%) | 2020 (%) |
|---|---|---|
| GYN | 18.3 | 19.1 |
| Non-GYN | 26.3 | 52.9 |
| Urine | 49.0 | 50.1 |
| FNA | 50.0 | 54.8 |
| Body fluid | 14.1 | 54.1 |
| Sputum, bronchial washing/brushing | 6.0 | 40.2 |

GYN, gynecological sample; FNA, fine-needle aspiration.

7,068,938, and these samples were most frequently processed by commercial laboratories. In 2020, the proportion of GYN samples processed in commercial laboratories, university hospitals, and general hospitals was 83.4% (n = 5,895,184), 11.0% (n = 774,375), and 5.6% (n = 399,379), respectively. Considering the numbers of institutions (45 commercial laboratories, 83 university hospitals and 86 general hospitals), it can be inferred that commercial laboratories process a considerable amount of GYN samples and few non-gynecological samples.

## Conventional and liquid-based cytology

A total of 6,263,193 samples (76.2%) were prepared by conventional smear, and 1,957,457 samples (23.8%) were prepared by liquid-based cytology (LBC). The LBC method was used in 19.1% of the GYN (n = 1,348,699) and 52.9% of the non-GYN samples (n = 608,758), including 50.1% of the urine (n = 233,216), 54.8% of the FNA (n = 167,270), and 54.1% of the body fluid (n = 209,044) samples (Table 2). The LBC coverage of GYN samples slightly increased from 2019 to 2020 (18.3% to 19.1%). Notably, the LBC coverage of non-GYN samples dramatically increased from 2019 to 2020 (26.3% to 52.9%) due to the high LBC ratio of body fluid samples. Surprisingly, the LBC coverage markedly increased by 40.0% for sputum, bronchial washing or brushing specimens. These results can be attributed to a decreased number of sputum smears, not an increase in LBC. The LBC coverage of GYN samples slightly increased from 2019 to 2020 in all university, general hospitals, and commercial laboratories. However, in non-GYN samples, LBC coverage for all kinds of institutions increased markedly from 2019 to 2020 (university hospitals, 53.7% to 67.9%; general hospitals, 33.9% to 57.1%; commercial laboratories, 8.33% to 28.6%). Further analysis demonstrated that an increased non-GYN LBC ratio occurred from body fluid samples with an increased LBC ratio across all types of institutions.

## Distribution of GYN sample cytologic diagnoses

GYN sample cytologic diagnoses from each institution were collected and analyzed in Table 3. The percentage of the samples with "unsatisfactory adequacy" was 0.34% in 2020. The percentage of "negative" samples, including those within normal limits and those with benign cellular changes, was 95.05%. The percentages of "atypical squamous cells of undetermined significance" and "atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesions" were 3.46% and 0.18%, respectively. The percentages of "low-grade squamous intraepithelial lesion" and "high-grade squamous intraepithelial lesion" were 0.73% and 0.16%, respectively. The ASC/SIL ratio was 4.10 in 2020.

**Table 3.** Distribution of the cervicovaginal cytologic diagnosis in 2019 and 2020

| Diagnosis | 2019 | 2020 |
|---|---|---|
| Unsatisfactory | 0.13 | 0.34 |
| Negative (WNL, BCC) | 95.43 | 95.05 |
| ASC | 3.53 | 3.64 |
| ASC-US | 3.34 | 3.46 |
| ASC-H | 0.19 | 0.18 |
| AGC | 0.05 | 0.04 |
| AGC, favor neoplastic | 0.01 | 0.01 |
| LSIL | 0.65 | 0.73 |
| HSIL | 0.17 | 0.16 |
| Squamous cell carcinoma | 0.02 | 0.02 |
| Adenocarcinoma | 0.01 | 0.01 |
| ASC/SIL ratio | 4.24 | 4.02 |

WNL, within normal limit; BCC, benign cellular change; ASC, atypical squamous cells; ASC-US, atypical squamous cells of uncertain significance; ASC-H, atypical squamous cells, cannot exclude HSIL; AGC, atypical glandular cells; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ASC/SIL ratio, atypical squamous cells/squamous intraepithelial lesion ratio.

In comparison with the data for 2019, the percentage of atypical squamous cells of uncertain significance increased from 3.34% to 3.46%, and the percentage of SIL slightly increased from 0.87% to 0.89%. With regard to institutions, the ASC/SIL ratio was steadily maintained at approximately 1.81 in university hospitals and 2.71 in general hospitals. In contrast to the previously recorded continuous increasing in the ASC/SIL ratio in commercial laboratories, it slightly decreased from 5.68 in 2019 to 5.10 in 2020.

## Cytology-histology correlation review of gynecologic samples

As a part of the internal quality control program, each institution was asked to compare the diagnoses of the cytologic and histologic samples of the same individual whenever possible and to document the degree of discordance between the cytologic and histologic diagnoses according to the institutional criteria. Of the 39,456 cases, 30,451 cases showed concordant results between the cytologic and histologic diagnoses (77.2%). Discordance with minimal and minor clinical impact (categories A and B) was found in 5,948 and 2,088 cases, respectively (15.1% and 5.3%). Discordance with major clinical impact (category C) was found in 378 cases (0.96%).

## Adequacy of GYN samples

The percentages of GYN samples that were considered unsatisfactory or adequate in each institution were collected as a part of the annual survey. The unsatisfactory rate was 0.34% in 2020. It was 1.38% in university hospitals, 1.78% in general hospitals, and 0.10% in commercial laboratories in 2020.

**Table 4.** Concordant and discordant rate of proficiency test between glass slides or WSIs

| Category | Glass slide (n=540) | WSIs (n=642) | Total (n=1,182) |
|---|---|---|---|
| O | 455 (84.3) | 523 (81.5) | 978 (82.7) |
| A | 68 (12.6) | 101 (15.7) | 169 (14.3) |
| B | 14 (2.6) | 15 (2.3) | 29 (2.5) |
| C | 3 (0.6) | 3 (0.5) | 6 (0.5) |

Values are presented as number (%).
WSIs, whole-slide images; O, concordant; A, discordant with minimal clinical impact; B, discordant with minor clinical impact; C, discordant with major clinical impact.

## Proficiency test

The rates of overall concordance (category O), discordance with minimal clinical impact (category A), discordance with minor clinical impact (category B), and discordance with major clinical impact (category C) findings were 82.0%, 13.7%, 2.6%, and 0.1%, respectively. In category C, seven cases diagnosed with malignancy were interpreted as benign, while three cases with benign disease were interpreted as malignancy.

In assessments based on the source used for the proficiency test, in the 108 institutions selecting five glass slides, categories O, A, B, and C represented 84.3%, 12.6%, 2.6%, and 0.6% of the findings. On the other hand, in 107 institutions choosing six WSIs, showed categories O, A, B, and C represented 81.5%, 15.7%, 2.3%, and 0.5% of the findings (Table 4). The distribution of results in the proficiency test in relation to the source (glass slides or WSIs) was similar. Of note, the rates for categories C in both glass slides and WSIs were below 1%.

## Sample adequacy assessment

CQIKSC requested five GYN glass slides with consequent numbers. A total of 197 participating institutions submitted 985 GYN slides and their reported adequacy. The sample adequacy assessments reevaluated by the members of CQIKSC and the adequacy of the institutions were concordant in all cases. For 17 institutions that were unable to submit GYN slides, sample adequacy was examined using GYN WSIs. All participating institutions passed the tests.

## Submission of samples for the next quality assurance program

A total 176 participating institutions submitted 1,056 cases in the form of glass slides, while 11 institutions sent WSIs for 33 cases. Twenty-six institutions that were unable to submit samples, participated and passed the test for cytologic diagnosis using WSIs.


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## DISCUSSION

Four reports for the quality improvement program and its results have been published by the Korean Society for Cytopathology in the form of articles in 2008, 2017, and 2020 [1-4]. These articles showed the status of cytopathology practice in Korea, including the participating institutions, overall statistics, GYN and non-GYN samples, conventional and LBC cytology, distribution of the GYN sample cytologic diagnosis, gynecologic cytology-histology correlation review, adequacy of GYN samples, proficiency test, sample adequacy assessment, and submission of samples for the next quality assurance program. After that, the COVID-19 pandemic drastically changed the status of practice of cytopathology as well as medical practice. Therefore, a report of cytopathology practice in Korea, that reflect the impact of the COVID-19 pandemics is urgently needed.

First, the number of cytopathologic tests in 2020 was 8,200,650, a significant reduction from the number in 2019 (10,111,755 cases). According to the National Health Insurance Statistical Yearbook, the number of patients using medical institutions in 2020 dropped to 48.57 million from 49.63 million in 2019 [8]. Thus, the drop in the total number of cytologic examinations occurred due to the COVID-19 pandemics.

However, regarding the discrepancy between the cytopathologic examinations and the medical institutions, it can be reasonably speculated that cytologic tests in Korea were conducted in line with health or cancer screening programs. According to the E-national indicator issued by the Ministry of Health and Welfare, the screening rate of the National Cancer Screening Program decreased to 55.4% in 2020 from 62.2%, 2019. Furthermore, the number of cervical smear cytology specimens as a part of the National Cancer Screening Program dropped to approximately 2,756,000 from 3,141,000 [9].

The discordance rate in the cytology-histology correlation review of gynecologic samples slightly decreased in 2020 (20.54%) compared to 2019 (23.84%). The discordant cases occurred mainly with minimal clinical impact (17.84%) that was not harmful to the management of patients. The most important finding, that the rate of category C (major clinical impact) has been well controlled below 1% since 2012, indicated the usefulness of cervical cytologic examination. On the other hand, the number of submitted data points in 2020 was 39,456, only half the number of data points collected in 2018 [2]. Thus, a fine guideline on collecting, managing, and reporting data for the cytology-histology correlation review of gynecologic samples is required.

LBC offers numerous advantages over conventional smears, including advantage in interpretation, convenience, and filtering out of contaminating debris and blood [10]. Moreover, conventional smears involve a certain of workload for pathologists as well as cytotechnologists [11]. The proportion of LBC examinations in non-GYN samples has noticeably increased from 26.3% in 2019 to 52.9% in 2020. This change was not due to an increase in the absolute number of LBC examinations, but rather due to reduction in sputum smear cytology. Additional careful investigations into the health effects of the marked reduction in respiratory-related conventional smear cytology should be performed, since these results could support the usefulness of sputum cytology.

A previous study in the United States reported that the median unsatisfactory rate was 0.5% and 90% of institutions had an unsatisfactory rate below 2% [12]. The unsatisfactory rate for GYN cytology in 2020 was 0.34% across all kinds of institutions and 1.38% in university hospitals, 1.78% in general hospitals, and 0.10% in commercial laboratories. Although all institutions had unsatisfactory rate below 2%, the unsatisfactory rate for GYN cytology examinations varied substantially, according to the type of institution. In this regard, the education and the establishment of defined criteria by the Korean Society for Cytopathology would be essential.

The ASC/SIL ratio can serve as a good marker to monitor the level of certainty and specificity [13,14]. Current recommendations for the ASC/SIL ratio suggest a ratio of less than 3:1 [15]. In 2020, the ASC/SIL ratio in all institutions was 4.10. The ASC/SIL ratios of university hospitals and general hospitals were 1.81 and 2.71, respectively, i.e., < 3.0. However, the ASC/SIL ratio in commercial laboratories has remained above 5.0 from 2017 to 2020. Commercial laboratories have an important position in the diagnosis of cervical cytology because they process most of the cases in Korea. However, considering the multivariable factors affecting ASC/SIL ratio, including the workload of cytopathologists and cytotechnologists, a proper working environment and optimization of other factors is essential.

The use of WSIs in cytopathology has been more difficult than in histology because of the nature of cytological investigations, including the thickness and 3D distribution of cells [16]. To overcome this limitation, Z-stacking at multiple levels has been adopted in WSIs. However, this approach results in a long scanning time and high file size. Despite these obstacles, WSIs, obtained under quality control programs offer multiple advantages over glass slides, including standardization of the diagnostic material, same high-quality slides, rapid access via web platforms, and no risk related to transport [17]. In the 2020 proficiency test, 107 institutions conducted the test, using WSIs. The results

showed that the distribution of discordance and concordance in the institutions selecting WSIs were not different from those in the institutions using glass slides. We look forward to greater adoption and benefits from WSIs in various fields of quality control in the future.

The change of Korean cytopathology practice can be evidently associated with COVID-19 pandemic. However, the possibility of the additional factors that might be associated with those changes, cannot be rejected completely. Thus, further study would be necessary that makes an effort to analyze the complex factors which could cause the change of cytopathology practice during COVID-19 pandemic.

In summary, CQI during the COVID-19 pandemic was characterized by a reduction in the number of cytologic examinations. This change was the most prominent for respiratory-related specimens, including sputum and bronchial washing/brushing. LBC coverage has markedly increased due to the reduction of conventional sputum smears. Other indicators of quality assurance, including the distribution of GYN sample cytologic diagnoses, gynecologic cytology-histology correlation review, adequacy of GYN samples, proficiency test results, and sample adequacy assessment were shown to be well maintained, despite the social and medical crises from the COVID-19 pandemic.

## Supplementary Information

The Data Supplement is available with this article at https://doi.org/10.4132/jptm.2022.09.21.

## Ethics Statement

This study was reviewed and approved by the Institutional Review Board of The Catholic University of Korea, Uijeongbu St. Mary's Hospital (UC-21ZCSI0062) and received a waiver of the need to obtain informed patient consent.

## 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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Conceptualization: YC, SAH, GG. Data curation: YC, SAH. Investigation: YC, SAH. Methodology: YC, SAH. Project administration: YC, SAH. Resources: YC, SAH. Supervision: GG. Validation: YC, SAH. Visualization: YC, SAH, HJ, SSK, MSJ, JSP, JYJ, YC. Writing—original draft: YC, SAH, HJ, SSK, MSJ, JSP, JYJ, YC. Writing—review & editing: YC, SAH, HJ, SSK, MSJ, JSP, JYJ, YC, GG.

## Conflicts of Interest

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

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## References

1. Choi YD, Oh HK, Kim SJ, et al. Continuous quality improvement program and its results of Korean Society for Cytopathology. J Pathol Transl Med 2020; 54: 246-52.
2. Chong Y, Jung H, Pyo JS, Hong SW, Oh HK. Current status of cytopathology practices in Korea: annual report on the Continuous Quality Improvement program of the Korean Society for Cytopathology for 2018. J Pathol Transl Med 2020; 54: 318-31.
3. Lee HK, Kim SN, Khang SK, Kang CS, Yoon HK. Quality control program and its results of Korean Society for Cytopathologists. Korean J Cytopathol 2008; 19: 65-71.
4. Oh EJ, Jung CK, Kim DH, et al. Current cytology practices in Korea: a nationwide survey by the Korean Society for Cytopathology. J Pathol Transl Med 2017; 51: 579-87.
5. Kim Y, Jun JK, Choi KS, Lee HY, Park EC. Overview of the national cancer screening programme and the cancer screening status in Korea. Asian Pac J Cancer Prev 2011; 12: 725-30.
6. Johns Hopkins University of Medicine, Coronavirus Resource Center [Internet]. Baltimore: Johns Hopkins University of Medicine, 2022 [cited 2022 Aug 10]. Available from: https://coronavirus.jhu.edu/.
7. Moynihan R, Sanders S, Michaleff ZA, et al. Impact of COVID-19 pandemic on utilisation of healthcare services: a systematic review. BMJ Open 2021; 11: e045343.
8. Ministry of Health and Welfare. Yearbook of health and welfare statistics. Sejong: Ministry of Health and Welfare, 2020.
9. The rate of National Cancer Screening Program in E-national indicator [Internet]. Seoul: Ministry of Health and Welfare, 2022 [cited 2022 Aug 10]. Available from: https://www.index.go.kr/potal/main/EachDtlPageDetail.do?idx_cd=1440&param=002.
10. Cox JT. Liquid-based cytology: evaluation of effectiveness, cost-effectiveness, and application to present practice. J Natl Compr Canc Netw 2004; 2: 597-611.
11. Dowie R, Stoykova B, Crawford D, et al. Liquid-based cytology can improve efficiency of cervical smear readers: evidence from timing surveys in two NHS cytology laboratories. Cytopathology 2006; 17: 65-72.
12. Davey DD, Woodhouse S, Styer P, Stastny J, Mody D. Atypical epithelial cells and specimen adequacy: current laboratory practices of participants in the college of American pathologists interlaboratory comparison program in cervicovaginal cytology. Arch Pathol Lab Med 2000; 124: 203-11.

13. Nascimento AF, Cibas ES. The ASC/SIL ratio for cytopathologists as a quality control measure: a follow-up study. Am J Clin Pathol 2007; 128: 653-6.
14. Chebib I, Rao RA, Wilbur DC, Tambouret RH. Using the ASC:SIL ratio, human papillomavirus, and interobserver variability to assess and monitor cytopathology fellow training performance. Cancer Cytopathol 2013; 121: 638-43.
15. Nayar R, Wilbur DC. The Bethesda system for reporting cervical cytology: definitions, criteria, and explanatory notes. Cham: Springer, 2015.
16. Pantanowitz L. Digital cytology: look how much has been achieved. Cytopathology 2020; 31: 370-1.
17. Eccher A, Girolami I. Current state of whole slide imaging use in cytopathology: pros and pitfalls. Cytopathology 2020; 31: 372-8.

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ORIGINAL ARTICLE

# Development of quality assurance program for digital pathology by the Korean Society of Pathologists

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**Background:** Digital pathology (DP) using whole slide imaging is a recently emerging game changer technology that can fundamentally change the way of working in pathology. The Digital Pathology Study Group (DPSG) of the Korean Society of Pathologists (KSP) published a consensus report on the recommendations for pathologic practice using DP. Accordingly, the need for the development and implementation of a quality assurance program (QAP) for DP has been raised. **Methods:** To provide a standard baseline reference for internal and external QAP for DP, the members of the Committee of Quality Assurance of the KSP developed a checklist for the Redbook and a QAP trial for DP based on the prior DPSG consensus report. Four leading institutes participated in the QAP trial in the first year, and we gathered feedback from these institutes afterwards. **Results:** The newly developed checklists of QAP for DP contain 39 items (216 score): eight items for quality control of DP systems; three for DP personnel; nine for hardware and software requirements for DP systems; 15 for validation, operation, and management of DP systems; and four for data security and personal information protection. Most participants in the QAP trial replied that continuous education on unfamiliar terminology and more practical experience is demanding. **Conclusions:** The QAP for DP is essential for the safe implementation of DP in pathologic practice. Each laboratory should prepare an institutional QAP according to this checklist, and consecutive revision of the checklist with feedback from the QAP trial for DP needs to follow.

**Key Words:** Digital pathology; Quality assurance program; Recommendations; Whole slide image; Quality control

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Digital transformation using digital pathology (DP) is currently a hot topic in pathology because it has served as a new platform for a way of making pathologic diagnosis, consultation, interdisciplinary conference, and education [1,2]. Whole slide imaging (WSI) technology is at the center of these fundamental changes, and quality assurance of WSI is essential to guarantee the safety of DP practice. In 2020, the Korean Society of Pathologists (KSP), especially members of the Digital Pathology Study Group (DPSG), formerly known as the Medical Informatics Study Group, published a consensus paper on the recommendation for pathologic practice using DP [2]. The DPSG prepared a consensus recommendation based on all previously published international guidelines and recommendations on DP, including those by the US Digital Pathology Association, College of American Pathologists, UK British Royal College of Pathologists, Canadian Association of Pathologists, Royal College of Pathologists of Australia, Federal Association of German Pathologists, Japanese Society of Pathology, and Spanish Society of Anatomic Pathology [3-10]. Consecutively, there were the Assessment and Approval Guideline on Artificial Intelligence (AI)-based Histopathologic In-vitro Diagnostic Devices (software) from the Ministry of Food and Drug Safety of Korea and the Reimbursement Assessment Guideline of Innovative Medical Technology (Pathology AI-based technology) from the Health Insurance Review and Assessment Service in 2021 [11,12].

According to the safety of DP implementation in daily routine practice, there has been accumulated evidence of comparable concordance between DP-based and conventional microscopic




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diagnosis in various sample types [13-15]. However, quality assurance program (QAP) (in-house validation) during DP implementation, continuous quality control (QC) activities including pre- and post-scan QCs are essential for guaranteeing the quality of pathologic practice in each laboratory [16]. The consensus recommendation report by the KSP includes the general principles and various consideration embracing not only basic system requirements but every QC activity [2]. However, the preparation of institutional QAP and laboratory checklists, and practical application of guidelines in each laboratory environment are not easy to implement. With this context, the Committee of Quality Assurance (CQA) of the KSP decided to develop QAP checklists for DP and plan external QAP trial to guide member institutes that implemented or are planning to implement DP.

In this report, we present a practical checklist that can be a baseline reference for each pathologic laboratory to prepare internal guidelines for DP QAP, and the results of DP QAP trial that was performed in four leading hospitals with DP systems in Korea along with their feedback.

## MATERIALS AND METHODS

Since the draft of the consensus recommendation paper by the KSP DPSG was prepared in May 2020, the CQA of the KSP initiated the task force team to develop a checklist for DP QAP (YC, JMB, DWK, and HSH) (Table 1). The draft checklist consisted of 39 items (216 score) to check: eight items for QC of DP systems; three items for DP personnel; nine items for hardware and software requirements for DP systems; 15 items for validation, operation, and management of DP systems; and four items for data security and personal information protection (Tables 2, 3). After the preparation of a draft checklist, it was serially reviewed by the DPSG and the members of the CQA. As the consensus recommendation paper was published in October 2020, the final version of the DP QAP checklist was prepared after final revision by the CQA. After the introduction of the checklist to the members of the KSP, the first QAP trial for DP was performed the following year, 2021. Unfortunately, because of the coronavirus disease 2019 (COVID-19) pandemic, the QAP trial could be conducted online in four leading hospitals with DP systems without on-site inspection. After the QAP trial, feedback from technicians, DP users, and DP managers of the hospitals preparing DP implementation was collected (Table 4).

## RESULTS

### Checklist for DP quality assurance program

*Preface*

- This checklist was developed based on the 'Recommendations for Pathologic Practice Using Digital Pathology: Consensus Report of the Korean Society of Pathologists' (2020.10) [2]. It is highly recommended to refer to the recommendations for understanding the background information related to DP (Supplementary Table S1).

**Table 2.** QAP checklist items and assigned scores according to the subjects of DP

| Subjects | Checklist items | Score |
|---|---|---|
| Quality control of DP systems | 8 | 62 |
| Personnel in DP systems | 3 | 16 |
| The hardware and software used in DP systems | 9 | 28 |
| Operation, management, and validation of DP systems | 15 | 82 |
| Personal information protection and information security of DP image data | 4 | 28 |
| Total | 39 | 216 |

QAP, quality assurance program; DP, digital pathology.

**Table 1.** Timeline of the DP QAP checklist preparation process

| | Timeline | Checklist preparation process |
|---|---|---|
| 2020 | May | First draft of DP recommendation consensus paper of KSP DPSG |
| | June | Initiation of the task force team for developing the DP QAP checklist |
| | July | Draft checklist review by DPSG |
| | August | Revised checklist review by CQA of KSP |
| | September | Peer review of DP recommendation consensus paper by Journal of Pathology and Translational Medicine |
| | October | DP recommendation consensus paper published<br>Final review of the DP QAP checklist by CQA of KSP |
| | November | Introduction of DP QAP checklist to KSP members during KSP QAP education |
| 2021 | June–July | DP QAP trial of 4 hospitals with DP systems |
| | August–October | Collection of the feedback of DP QAP trial from the technicians, DP users, and managers of 7 hospitals preparing DP systems |

DP, digital pathology; QAP, quality assurance program; KSP, Korean Society of Pathologists; DPSG, Digital Pathology Study Group; CQA, Committee of Quality Assurance.

**Table 3.** The detailed QAP checklist items and assigned scores

| No. | Checklist item | Score |
|---|---|---|
| Quality control of DP systems | | 62 |
| 01.001 | Do you have laboratory guidelines for DP covering overall DP workflow and is it easily accessible? | 4 |
| 01.002 | Does the DP director regularly review and check all guidelines for DP system? | 2 |
| 01.003 | If the guidelines are changed, or added, or revised, or partly discarded, does the person in charge of the DP system review and confirm the content? | 2 |
| 01.004 | When the DP system director changes, does the new director review and confirm all guidelines? | 4 |
| 01.005 | Is there a system to ensure that all employees using the DP system are all aware of the guidelines? | 2 |
| 01.006 | Do you have an internal quality control system for the DP system in place and implemented? | 32 |
| 01.007 | Do you regularly conduct internal quality control of DP systems? | 8 |
| 01.008 | Does the person in charge of the DP systems review and resolve the internal QC results in documentation? | 8 |
| Personnel in DP systems | | 16 |
| 02.001 | Are the personnel managing the DP system adequate in numbers? | 4 |
| 02.002 | Is the education/training for personnel using/managing DP systems being documented? | 4 |
| 02.003 | Are there personnel primarily assigned to whole slide scanning? | 8 |
| The hardware and software used in DP systems | | 28 |
| 03.001 | Is the WSS in the DP systems approved by the Ministry of Food and Drug Safety? | 4 |
| 03.002 | Is the performance of the WSS regularly checked and maintained by the manufacturer? | 4 |
| 03.003 | Is the performance of the WSS and the quality of scanned WSIs regularly evaluated by DP personnel? | 4 |
| 03.004 | Does the image database system guarantee that the identification information of the glass slide matches that of the digital image? | 2 |
| 03.005 | Even though the version of the image archiving software changes, is it still available to view and use the archived data with a new software without any technical difficulty? | 2 |
| 03.006 | Does the image storage method take a form of backup or mirroring? | 4 |
| 03.007 | Do the image display devices (e.g., monitor) have the appropriate image quality for primary diagnosis? | 2 |
| 03.008 | Does the image viewing software properly implement overview image functions, annotation functions, and image comparison functions to suit the pathological workflow? | 2 |
| 03.009 | Are DP systems properly linked to the LIS, EMR, or HIS in an appropriate manner? | 4 |
| Operation, management, and validation of DP systems | | 82 |
| 04.001 | Is the laboratory SOP well prepared stating the following? | 10 |
| 04.002 | Is an in-house validation on newly introduced devices of DP system being conducted and documented? | 12 |
| 04.003 | Is the validation study conducted under conditions that are consistent with the clinical use intended by the DP system manufacturer? | 4 |
| 04.004 | Is the validation study designed to be as similar as possible to the actual clinical settings in which the technology will be used? | 4 |
| 04.005 | Does the validation study cover the entire DP system? | 4 |
| 04.006 | When there are significant changes in the composition of the DP system, is the revalidation on the whole DP system being conducted? Or do you have any guidelines for this? | 8 |
| 04.007 | Is the validation intended to be conducted by at least one pathologist who has been acclimated to the DP system? | 4 |
| 04.008 | Is the validation carried out using a comparative analysis of concordance between microscopic and WSI-based diagnoses made by a single observer (intra-observer variability assessment)? | 4 |
| 04.009 | Did you have a washout period of at least 2 weeks to minimize the influence of recall bias during the validation? | 4 |
| 04.010 | During validation, do you assess data integrity of image acquisition by verifying whether all tissues on the glass slide have been properly scanned to form the digital image? | 8 |
| 04.011 | Is the additional validation being conducted on the samples for the detection of microorganisms (e.g., *Helicobacter pylori*), the cytology slides (cell smears, liquid-based cytology, or blood smears), and the cases suspicious for lymphoreticular neoplasms? | 4 |
| 04.012 | Are all the errors, its statistics and cause analysis being recorded during the whole slide scanning? | 4 |
| 04.013 | Are WSIs being scanned at a minimum magnification of 20x (In case of H&E slides)? | 4 |
| 04.014 | Do you manage regular inspection results for the image display device (e.g., monitor)? | 4 |
| 04.015 | Is the data being stored according to the data preservation period determined by the individual laboratory? | 4 |
| Personal information protection and information security of DP images | | 28 |
| 05.001 | Do the guidelines include the instruction and regulation on the collection and management of personal information and information safety? | 16 |
| 05.002 | Does the DP guideline specify external personnel or institutions that can access medical information and access data using DP systems? | 4 |
| 05.003 | Is there a person in charge of information security for DP system? | 4 |
| 05.004 | Is there a suitable system to prevent the loss of personal medical information in case of hardware or software failure in DP system and other emergencies or disasters (e.g., power outages due to natural disasters)? | 4 |

QAP, quality assurance program; DP, digital pathology; QC, quality control; WSS, whole slide scanner; WSI, whole slide imaging; LIS, laboratory information systems; EMR, electronic medical records; HIS, hospital information systems; SOP, Standard Operating Procedures; H&E, hematoxylin and eosin.


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**Table 4.** Survey questionnaire for DP QAP trial

| No. | Questionnaire | Answer |
|---|---|---|
| 1 | Please fill in your institute name. | |
| 2 | What is your current position? | 1) DP director (pathologists)<br>2) WSS manager (technician)<br>3) DP user |
| 3 | In which part DP is being applied? (multiple) | 1) Referred slides<br>2) Some H&E slides<br>3) All H&E slides<br>4) Special stains<br>5) IHC stains<br>6) SISH, IF, FISH |
| 4 | In which part DP will be extended in the future? (multiple) | 1) Referred slides<br>2) Some H&E slides<br>3) All H&E slides<br>4) Special stains<br>5) IHC stains<br>6) SISH, IF, FISH |
| 5 | How many cases are being handled with DP annually? | 1) Less than 5000 cases<br>2) 5,000–10,000 cases<br>3) 10,000–15,000 cases<br>4) 15,000–25,000 cases<br>5) 25,000–50,000 cases |
| 6 | How many percentages of cases are being handled with DP annually? | |
| 7 | Is there any errors or something that needs to be improved in the DP checklist? | |
| 8 | Is there anything that are unrealistic or | |
| 9 | Is there anything that needs to be added to the checklist? | |
| 10 | Do you think the checklist was helpful for preparing in-house QAP or DP guidelines? | 1) Very likely<br>2) A little bit<br>3) I don't know<br>4) Not really<br>5) Not at all |
| 11 | Please share any suggestions to improve DP checklist. | |

DP, digital pathology; QAP, quality assurance program; WSS, whole slide scanner; H&E, hematoxylin and eosin; IHC, immunohistochemistry; SISH, silver in situ hybridization; IF, immunofluorescence; FISH, fluorescence in situ hybridization.

- "DP" used in this checklist for DP QAP refers to a relatively narrow perspective of DP, that is primary diagnosis or consultation using WSIs via displays such as monitors instead of a microscope, by digitalizing all or a part of pathological samples. The DP QAP of the KSP is intended only for institutions conducting primary diagnoses using DP systems.
- "DP" is a dynamic imaging environment (or academic field related to this environment) that involves the acquisition and management of pathologic information, by converting microscopic glass slides into digital files, and the pathologic diagnosis and interpretation of those images using display devices (e.g., monitors). The scope of the application includes education, diagnosis, research, image analysis, archiving, retrieval, expert consultations, and data sharing. In this checklist, its meaning is limited to the primary diagnosis or consultation as part of the pathologic diagnosis process.
- "The digital pathology system" is a computer system that enables the collection, management, and interpretation of pathologic image data by digitalizing glass slides. It includes a whole slide scanner (WSS), computer workstation, operating and managing software (scanner operation software, image viewer, and image analysis software), and network system (server and network environment).
- "Telepathology" is a digital or real-time pathologic image communication environment using wired or wireless networks or a related academic field. Telepathology could be used either for consultation with specialists in a distant location or for the diagnosis of samples in a remote facility.
- "Whole slide image/imaging (WSI)" is a single high-resolution image file or associated technology scanned from a single glass slide using a WSS. WSI can be considered a high-resolution copy or mirrored image of a glass slide.
- "Focus stacking (Z-stacking)" is an image-processing technique that displays multilayer digital images acquired at varying focus levels to obtain a much greater depth of field. In samples with many 3-dimensional microstructures and cell clusters, such as cytology slides, it is difficult to obtain the appropriate depth of field with a single focus. Multiple images at slightly different levels of the z-axis should be combined using various image processing methods to generate a single image file.
- "Pathology picture archiving and communication system (Pathology PACS)" is a system that archives, processes, and transmits DP images in accordance with international standards such as the Digital Imaging and Communications in Medicine (DICOM) format. Pathology PACS consists of an image-viewing and archiving software, a mass storage device, and a computer hardware system.
- "A laboratory information management system, or laboratory information system (LIS)", is a software system designed to manage information related to the overall operation and management of a laboratory.
- "Validation" describes the process of confirming whether equipment, reagents, and test methods that have already been verified can be appropriately applied to an individual laboratory according to certain standards before implementation. Validation should be conducted using documents that provide a high level of assurance.

*QC of DP systems*

W.01.001 Do you have laboratory guidelines for DP cover-

ing overall DP workflow and is it easily accessible? (4)

Yes ( ) No ( )

* Laboratory DP guidelines can include the following content according to the scope of application and individual needs.

- Principles/guidelines for the operation of DP systems (Standard Operating Procedures, SOP)
- Information on personnel managing and using DP systems (organizational charts and roles)
- Principles/guidelines for the maintenance and repair of all facilities and equipment in a DP system
- Principles/guidelines for the collection, storage, use, and disposal of digital image data
- Principles/guidelines for personal information protection, management, and security
- Principles/guidelines related to telepathology and the use of portable devices
- Principles/guidelines for coping with emergencies and disasters
- Principles/guidelines for education of all DP system users
- Principles/guidelines for QC in DP systems
- Principles/guidelines for validation of DP systems
- Principles/guidelines for the other operation in DP systems

W.01.002 Does the DP director regularly review and check all the guidelines for the DP system? (2)

Yes ( ) No ( )

* The director of the DP system should review and confirm all the guidelines at least once a year, leaving a date and signature.

W.01.003 If the guidelines are changed, added, revised, or partly discarded, does the person in charge of the DP system review and confirm their content? (2)

Yes ( ) No ( )

* When major equipment constituting the DP system is newly introduced/changed, the content of the corresponding guidelines should be changed or added accordingly.

* The director of DP systems should review and leave a date and signature whenever there is a change in the guideline content.

* The contents of the guidelines that are changed or discarded should be retained for at least 2 years.

* Any changes, abolitions, or additions to the guidelines should be dated.

W.01.004 When the DP system director changes, does the new director review and confirm all the guidelines? (4)

Yes ( ) No ( )

* The new director should review and confirm the guidelines, and leave the date and signature.

W.01.005 Is there a system to ensure that all employees using the DP system are aware of the guidelines? (2)

Yes ( ) No ( )

* In-house education for the guidelines should be implemented, and trainees should leave training dates and signatures.

W.01.006 Do you have an internal QC system for the DP system in place and implemented? (32)

Yes ( ) No ( )

* Internal QC of DP systems should include:

- Regular validation of DP systems
- Documentation, causal analysis, and statistics on errors that occur during whole slide scanning
- Maintenance check report for overall equipment of DP system
- Documentation and troubleshooting of errors between DP systems and LIS (or hospital information systems, HIS)
- Inspections related to privacy and personal information security
- Guidelines for follow-up measures of errors in internal QC

W.01.007 Do you regularly conduct internal QC of DP systems? (8)

Scoring: _____

* Internal QC should be implemented periodically, daily, weekly, monthly, and quarterly, in accordance with internal regulations as well as validation after major changes in the components of the entire DP system due to the introduction of new equipment and others. If it is conducted irregularly (when 20%–80% is satisfied), half of the score (4 points) is given, and if it is conducted with less than 20%, 0 points are given.

W.01.008 Does the person in charge of the DP system review and resolve the internal QC results in documentation? (8)

Yes ( ) No ( )

*Personnel in DP systems*

W.02.001 Are the personnel managing the DP system adequate in number? (4)

Yes ( ) No ( )

* The management team of DP systems consists of a variety of people, including pathologists, technicians, personnel who perform whole slide scanning, IT managers, and others.


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* Information on personnel managing DP systems (such as organization charts and roles) should be thoroughly prepared and updated.

* Personnel managing DP systems should understand the subjects related to implementation, management, and maintenance of DP systems. DP personnel should continuously educate themselves for SOP and understand the difference in the diagnosis process between DP systems and conventional microscopy.

* The appropriate number of personnel managing the DP system may be determined according to the scale and workflow of the DP system in individual institutions.

W.02.002 Is the education/training for personnel using/managing DP systems being documented? (4)

Yes ( ) No ( )

* Training programs for new personnel using/managing DP systems should be prepared, and regular training should be provided for existing members.

* New training should be provided to employees using/managing digital pathology systems in the following circumstances: (1) introduction/replacement of key equipment in the DP system and corresponding changes in the laboratory guidelines, (2) change in the operational scope and method in the DP system, and 3) validation is newly performed.

W.02.003 Are there personnel primarily assigned to whole slide scanning? (8)

Yes ( ) No ( )

* Scanning personnel are required to undergo a certain training period according to the laboratory DP guidelines.

*The hardware and software used in DP systems*

W.03.001 Is the WSS in the DP system approved by the Ministry of Food and Drug Safety? (4)

Yes ( ) No ( )

W.03.002 Is the performance of the WSS regularly checked and maintained by the manufacturer? (4)

Yes ( ) No ( )

W.03.003 Is the performance of the WSS and the quality of scanned WSIs regularly evaluated by DP personnel? (4)

Yes ( ) No ( )

* Considerations and recommended functional requirements for a WSS (Supplementary Material S1) [2].

W.03.004 Does the image database system guarantee that the identification information of the glass slide matches that of the digital image? (2)

Yes ( ) No ( )

* Recommended functional requirements for image database systems (Supplementary Material S2) [2].

W.03.005 Even if the version of the image-archiving software changes, is it still available to view and use the archived data with updated software without any technical difficulty? (2)

Yes ( ) No ( )

* Recommended functional requirements for image database systems (Supplementary Material S3) [2].

W.03.006 Does the image storage method take a form of backup or mirroring? (4)

Yes ( ) No ( )

* Recommended functional requirements for image database systems (Supplementary Material S4) [2].

W.03.007 Do the image display devices (e.g., monitor) have the appropriate image quality for primary diagnosis? (2)

Yes ( ) No ( )

* Considerations and recommended functional requirements for image display devices and image viewing software (Supplementary Material S5, Supplementary Table S2) [2].

W.03.008 Does the image viewing software properly implement overview image, annotation, and image comparison functions to suit the pathological workflow? (2)

Yes ( ) No ( )

* Considerations and recommended functional requirements for image display devices and image viewing software (Supplementary Material S6) [2].

W.03.009 Are DP systems appropriately linked to the LIS, electronic medical records (EMR), or HIS? (4)

Yes ( ) No ( )

* Issues related to integration/links with LIS and EMR systems (Supplementary Material S7) [2].

*Operation, management, and validation of DP systems*

W.04.001 Is the laboratory SOP well prepared stating the following? (10)

1) Types of samples and staining methods to be used for primary diagnosis using a DP system (scope) Yes ( ) No ( )

2) Components of the DP system and SOP for each device Yes ( ) No ( )

3) Role and training records of the personnel using and managing DP system Yes ( ) No ( )

4) Guidelines on consultation and management of the referred patient samples using the DP system Yes ( ) No ( )

5) Guidelines for the storage, management, and disposal of DP image data Yes ( ) No ( )

6) Guidelines for privacy and information security Yes ( ) No ( )

7) Principles/guidelines related to telepathology and portable device use Yes ( ) No ( )

8) Guidelines for validation Yes ( ) No ( )

9) Plans/programs for QC Yes ( ) No ( )

10) Principles/guidelines for emergencies and disasters Yes ( ) No ( )

* 1 point to each item.

W.04.002 Is in-house validation of newly introduced devices of the DP system being conducted and documented? (12)

Yes ( ) No ( )

* Guidelines and considerations for validation needed for the implementation of DP systems and internal QC needed during operation (Supplementary Material S8) [2].

W.04.003 Is the validation study conducted under conditions that are consistent with the clinical use intended by the DP system manufacturer? (4)

Yes ( ) No ( )

* Guidelines and considerations for validation needed for the implementation of DP systems and internal QC needed during operation (Supplementary Material S9) [2].

W.04.004 Is the validation study designed to be as similar as possible to the actual clinical settings in which the technology will be used? (4)

Yes ( ) No ( )

* Guidelines and considerations for validation needed for the implementation of DP systems and internal QC needed during operation (Supplementary Material S10) [2].

W.04.005 Does the validation study cover the entire DP system? (4)

Yes ( ) No ( )

* Guidelines and considerations for validation needed for the implementation of DP systems and internal QC needed during operation (Supplementary Material S11) [2].

W.04.006 When there are significant changes in the composition of the DP system, is the revalidation on the whole DP system being conducted? Do you have guidelines for this? (8)

Yes ( ) No ( )

* Guidelines and considerations for validation needed for the implementation of DP systems and internal QC needed during operation (Supplementary Material S12) [2].

W.04.007 Is the validation intended to be conducted by at least one pathologist who has acclimated to the DP system? (4)

Yes ( ) No ( )

* For general hematoxylin and eosin (H&E) slides, frozen sections, cytology slides, and blood smears, validation must be performed on at least 60 samples for a single applicable field. For additional applicable fields such as immunohistochemical stains and special stains, validation could be performed by adding an additional 20 samples.

* Guidelines and considerations for validation needed for the implementation of DP systems and internal QC needed during operation (Supplementary Material S13) [2].

W.04.008 Is the validation carried out using a comparative analysis of concordance between microscopic and WSI-based diagnoses made by a single observer (intra-observer variability assessment)? (4)

Yes ( ) No ( )

* Guidelines and considerations for validation needed for the implementation of DP systems and internal QC needed during operation (Supplementary Material S14) [2].

W.04.009 Did you have a washout period of at least 2 weeks to minimize the influence of recall bias during the validation? (4)

Yes ( ) No ( )

* Guidelines and considerations for validation needed for the implementation of DP systems and internal QC needed during operation (Supplementary Material S15) [2].

W.04.010 During validation, do you assess the data integrity of the image acquisition by verifying whether all tissues on the glass slide have been properly scanned to form the digital image? (8)

Yes ( ) No ( )

* Guidelines and considerations for validation needed for the


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implementation of DP systems and internal QC needed during operation (Supplementary Material S16) [2].

W.04.011 Is additional validation being conducted on the samples for the detection of microorganisms (e.g., *Helicobacter pylori*), the cytology slides (cell smears, liquid-based cytology, or blood smears), and the cases suspicious for lymphoreticular neoplasms? (4)

Yes ( ) No ( )

* Scope of application (Supplementary Material S17) [2].

W.04.012 Are all the errors, its statistics, and its cause analysis being recorded during the whole slide scanning? (4)

Yes ( ) No ( )

W.04.013 Are WSIs being scanned at a minimum magnification of 20× (in case of H&E slides)? (4)

Yes ( ) No ( )

W.04.014 Do you manage regular inspection results for the image display device (e.g., monitor)? (4)

Yes ( ) No ( )

* Considerations and recommended functional requirements for image display devices and image viewing software (Supplementary Material S18) [2].

W.04.015 Is the data being stored according to the data-preservation period determined by the individual laboratory? (4)

Yes ( ) No ( )

* Considerations and recommended functional requirements for image database systems (Supplementary Material S19) [2].

### *Personal information protection and information security of DP image data*

W.05.001 Do the guidelines include the instruction and regulation on the collection and management of personal information and information safety? (16)

Yes ( ) No ( )

* Digital pathological imaging data should be stored systematically with the help of information processing experts and easily found as needed, and methods for regulating access to appropriate security and information should be established to protect privacy.

* There should be appropriate guidelines for data sharing and wired/wireless transmission that include the extent to which personal information is shared and how it is protected.

* Issues related to telepathology, firewalls, protection of personal information, and mobile device use (Supplementary Material S20) [2].

W.05.002 Does the DP guideline specify the external personnel or institutions that can access medical information and data using DP systems? (4)

Yes ( ) No ( )

W.05.003 Is there a person in charge of information security for the DP system? (4)

Yes ( ) No ( )

W.05.004 Is there a suitable system to prevent the loss of personal medical information in the case of hardware or software failure in the DP system, other emergencies, or disasters (e.g., power outages due to natural disasters)? (4)

Yes ( ) No ( )

* Measures and guidelines should be implemented to prevent the spread of failures or disasters that cause problems, plan periodic data/information backups, and recover corrupted data/information.

### *Quality assurance program trial for DP*

Four leading hospitals, Seoul St. Mary's Hospital, Seoul National University Hospital, Yongin Severance Hospital, and National Cancer Center participated in the QAP trial for DP. At the 46th Annual Spring Meeting of the KSP and the 73rd Annual Autumn Meeting of the KSP, eight hospitals, including the Samsung Medical Center, Yonsei Severance Hospital, Kangbuk Samsung Hospitals, and Seoul Asan Medical Center, in addition to the aforementioned hospitals, shared institutional experience on DP implementation and feedback after participating in the trial [17-24]. In addition, 11 DP directors, WSS technicians, and DP users from these hospitals submitted a feedback survey.

### *Current status of DP implementation in Korea*

As of 2022, 40 out of 214 pathology laboratories (18.7%) implemented DP for primary diagnosis, archiving, consultation, and research and 2–10 more laboratories are expected to implement DP within next 1–2 years. Majority of the laboratories with DP is using DP for research, followed by archive, and consultation. Only about 15% of these laboratories are being estimated to use DP for primary diagnosis. The feedback survey showed that these pathology laboratories are currently processing 40%–100% of the total H&E examinations digitally (5,000 to 50,000 cases

annually per lab). These labs are scanning mostly a part of H&E, some special and immunohistochemical stains, rarely immunofluorescence, silver in situ hybridization, or fluorescence in situ hybridization and are planning to expand the coverage.

*QAP trial results*

Four leading hospitals, Seoul St. Mary's Hospital, Seoul National University Hospital, National Cancer Center, and Yongin Severance Hospital, participated in the QAP trial for DP. Table 5 summarizes the results. Average score was 209.75 out of 216 ranging from 204 to 216 with 1 to 2 missed items in each lab. All checklist items according to the hardware and software used in DP systems and personal information protection and information security of DP images were well prepared by all laboratories. Missed items were W.01.007, W.02.003, W04.001.004, W.04.011, and W.04.014, which do not affect the integrity of DP system seriously and can be improved in the future.

*Feedback survey after DP QAP trial*

Jang K-T from Samsung Medical Center highlighted focusing error, robotic motor dysfunction, localization error of the region of interest, software upgrades issue, compatibility between server and storage, and security software troubles as major issues during DP implementation [20]. He mentioned fast and convenient accessibility and reduced time of tumor annotation for additional ancillary tests, convenient application in multidisciplinary conferences, and education as the major benefits of the DP system. He also pointed out that the current DP systems fall behind the users' expectations because there are still many problems in incorporating the system into the conventional pathology workflow, in which thorough QAP is required.

Lee KB from Seoul National University Hospital mentioned delayed turn-around time (TAT) due to scanning, unfamiliarity with WSIs, and increased workload of pathology lab personnel as the most worried aspects during DP implementation [24]. However, it turned out that the TAT could be reduced as the DP systems were expanded to the whole sample, including immunohistochemistry. The number of active user pathologists could increase over time, and gradual expansion of the DP system could increase the flexibility of the rapid workload increase. She highlighted the increased patient safety of the DP system, which is based on easy access to the archive, fast comparison of previous examinations, and instant intra- or interdepartmental consultation. She also mentioned that this could be very important for the general quality of pathologic diagnosis services.

Hong SW from Yongin Severance Hospital, Yonsei University introduced the advanced facilities of the digital hospital of Yongin Severance Hospital, including the Integration and Response Space, Real Time Location System, artificial intelligence-based chest X-ray interpretation system, mobile PACS, 5G mobile network-based server and storage, and DP solution, including voice recognition system, digital gross lab recording and photography system, and barcode/QR code sample tracking system [19]. She also shared that most errors were found during the pre- and post-scanning quality checks, such as localization error of the region of interest, foreign body/air bubble during the mounting, and handling errors by lab personnel. Over a year and a half after DP implementation, the error rate of her laboratory decreased from 0.61 to 0.02 as the DP coverage increased from 75% to 100%. She also mentioned witnessing a significantly reduced TAT, especially for reviewing prior exams and intra-/interdepartmental consultation, as well as a possible reduction of workload and slide storage space.

Some surveyees did not seem to understand the DP checklist items properly and they raised questions, the need for revision of some items for better clarification, or the need for more educational opportunity on DP from the KSP. Overall, the surveyees replied that the DP checklist was helpful in preparing internal QC guidelines on DP and laboratory QAP on DP.

## DISCUSSION

DP will be a core of pathology in future medicine, playing a central role both in clinical practice and in various fields of translational research by providing faster and more accurate diagnosis and enhanced patient safety, and by becoming valuable data with enormous potential [1]. QAP holds the key to the successful utilization of DP systems considering the many practical difficulties that arise during the incorporation of DP systems into the conventional pathology workflow. As each laboratory should prepare institutional guidelines on DP and QAP for DP, it is highly recommended to refer to this checklist along with consensus recommendations for pathologic practice using DP. In addition to other QAP activities in the pathology field, initial validation when implementing new DP systems, internal pre- and post-scanning quality checks with laboratory QAP for DP during daily routine practice, and continuous education for lab personnel should be prepared and conducted properly.

As the survey after the DP QAP showed, most participants agreed that there should be more educational opportunities on DP from the KSP. Currently, DPSG provides workshops on DP and other newly developed technology to KSP members every

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**Table 5.** Results of QAP trial for DP

| No. | Checklist items | Score | Lab A | Lab B | Lab C | Lab D |
|---|---|---|---|---|---|---|
| Quality control of DP systems | | 62 | 62 | 62 | 54 | 62 |
| 01.001 | Do you have laboratory guidelines for DP covering overall DP workflow and is it easily accessible? | 4 | 4 | 4 | 4 | 4 |
| 01.002 | Does the DP director regularly review and check all guidelines for DP system? | 2 | 2 | 2 | 2 | 2 |
| 01.003 | If the guidelines are changed, or added, or revised, or partly discarded, does the person in charge of the DP system review and confirm the content? | 2 | 2 | 2 | 2 | 2 |
| 01.004 | When the DP system director changes, does the new director review and confirm all guidelines? | 4 | 4 | 4 | 4 | 4 |
| 01.005 | Is there a system to ensure that all employees using the DP system are all aware of the guidelines? | 2 | 2 | 2 | 2 | 2 |
| 01.006 | Do you have an internal quality control system for the DP system in place and implemented? | 32 | 32 | 32 | 32 | 32 |
| 01.007 | Do you regularly conduct internal quality control of DP systems? | 8 | 8 | 8 | 0 | 8 |
| 01.008 | Does the person in charge of the DP systems review and resolve the internal QC results in documentation? | 8 | 8 | 8 | 8 | 8 |
| Personnel in DP systems | | 16 | 16 | 8 | 16 | 16 |
| 02.001 | Are the personnel managing the DP system adequate in numbers? | 4 | 4 | 4 | 4 | 4 |
| 02.002 | Is the education/training for personnel using/managing DP systems being documented? | 4 | 4 | 4 | 4 | 4 |
| 02.003 | Are there personnel primarily assigned to whole slide scanning? | 8 | 8 | 0 | 8 | 8 |
| The hardware and software used in DP systems | | 28 | 28 | 28 | 28 | 28 |
| 03.001 | Is the WSS in the DP systems approved by the Ministry of Food and Drug Safety? | 4 | 4 | 4 | 4 | 4 |
| 03.002 | Is the performance of the WSS regularly checked and maintained by the manufacturer? | 4 | 4 | 4 | 4 | 4 |
| 03.003 | Is the performance of the WSS and the quality of scanned WSIs regularly evaluated by DP personnel? | 4 | 4 | 4 | 4 | 4 |
| 03.004 | Does the image database system guarantee that the identification information of the glass slide matches that of the digital image? | 2 | 2 | 2 | 2 | 2 |
| 03.005 | Even though the version of the image archiving software changes, is it still available to view and use the archived data with a new software without any technical difficulty? | 2 | 2 | 2 | 2 | 2 |
| 03.006 | Does the image storage method take a form of backup or mirroring? | 4 | 4 | 4 | 4 | 4 |
| 03.007 | Do the image display devices (e.g., monitor) have the appropriate image quality for primary diagnosis? | 2 | 2 | 2 | 2 | 2 |
| 03.008 | Does the image viewing software properly implement overview image functions, annotation functions, and image comparison functions to suit the pathological workflow? | 2 | 2 | 2 | 2 | 2 |
| 03.009 | Are DP systems properly linked to the LIS, EMR, or HIS in an appropriate manner? | 4 | 4 | 4 | 4 | 4 |
| Operation, management, and validation of DP systems | | 82 | 82 | 78 | 82 | 75 |
| 04.001 | Is the laboratory SOP well prepared stating the following? | 10 | 10 | 10 | 10 | 9 |
| 04.002 | Is an in-house validation on newly introduced devices of DP system being conducted and documented? | 12 | 12 | 12 | 12 | 12 |
| 04.003 | Is the validation study conducted under conditions that are consistent with the clinical use intended by the DP system manufacturer? | 4 | 4 | 4 | 4 | 4 |
| 04.004 | Is the validation study designed to be as similar as possible to the actual clinical settings in which the technology will be used? | 4 | 4 | 4 | 4 | 4 |
| 04.005 | Does the validation study cover the entire DP system? | 4 | 4 | 4 | 4 | 4 |
| 04.006 | When there are significant changes in the composition of the DP system, is the revalidation on the whole DP system being conducted? Or do you have any guidelines for this? | 8 | 8 | 8 | 8 | 8 |
| 04.007 | Is the validation intended to be conducted by at least one pathologist who has been acclimated to the DP system? | 4 | 4 | 4 | 4 | 4 |
| 04.008 | Is the validation carried out using a comparative analysis of concordance between microscopic and WSI-based diagnoses made by a single observer (intra-observer variability assessment)? | 4 | 4 | 4 | 4 | 4 |
| 04.009 | Did you have a washout period of at least 2 weeks to minimize the influence of recall bias during the validation? | 4 | 4 | 4 | 4 | 4 |
| 04.010 | During validation, do you assess data integrity of image acquisition by verifying whether all tissues on the glass slide have been properly scanned to form the digital image? | 8 | 8 | 8 | 8 | 8 |
| 04.011 | Is the additional validation being conducted on the samples for the detection of microorganisms (e.g., Helicobacter pylori), the cytology slides (cell smears, liquid-based cytology, or blood smears), and the cases suspicious for lymphoreticular neoplasms? | 4 | 4 | 0 | 4 | 4 |
| 04.012 | Are all the errors, its statistics and cause analysis being recorded during the whole slide scanning? | 4 | 4 | 4 | 4 | 4 |
| 04.013 | Are WSIs being scanned at a minimum magnification of 20× (In case of H&E slides)? | 4 | 4 | 4 | 4 | 4 |
| 04.014 | Do you manage regular inspection results for the image display device (e.g., monitor)? | 4 | 4 | 4 | 4 | 0 |
| 04.015 | Is the data being stored according to the data preservation period determined by the individual laboratory? | 4 | 4 | 4 | 4 | 4 |
| Personal information protection and information security of DP images | | 28 | 28 | 28 | 28 | 28 |
| 05.001 | Do the guidelines include the instruction and regulation on the collection and management of personal information and information safety? | 16 | 16 | 16 | 16 | 16 |
| 05.002 | Does the DP guideline specify external personnel or institutions that can access medical information and access data using DP systems? | 4 | 4 | 4 | 4 | 4 |
| 05.003 | Is there a person in charge of information security for DP system? | 4 | 4 | 4 | 4 | 4 |
| 05.004 | Is there a suitable system to prevent the loss of personal medical information in case of hardware or software failure in DP system and other emergencies or disasters (e.g., power outages due to natural disasters)? | 4 | 4 | 4 | 4 | 4 |
| | Total | 216 | 216 | 204 | 208 | 211 |

QAP, quality assurance program; DP, digital pathology; QC, quality control; WSS, whole slide scanner; WSI, whole slide imaging; LIS, laboratory information systems; EMR, electronic medical records; HIS, hospital information systems; SOP, Standard Operating Procedures; H&E, hematoxylin and eosin.

4–5 years and last May, the latest workshop includes sessions for DP terminology, practical recommendations for DP implementation and its integration into HIS, standardization of DP data, introduction of WSSs and displays for DP, morphometric analysis software, AI-based computer-assisted diagnosis software, and other new technologies such as block chain and non-fungible token-based medical data sharing and common data model-based distributed research network. The DPSG also plans a regular educational program for pathology residents and fellows. Continuous efforts to educate new and existing lab personnel and pathologists should be made by the KSP DPSG, and each lab should also encourage personnel to participate in these educational opportunities in addition to the internal education plan. In addition, the CQA of the KSP needs to continue to educate examiners and examinants with the DP checklist items until they become familiar.

Although the long-term benefits of DP are profound and obvious, the initial cost of DP implementation is a huge obstacle for a lab to accept this new technology. In a study on the preparation of the Reimbursement Assessment Guideline of Innovative Medical Technology (Pathology AI-based technology), Lee KB at Seoul National University suggested that government authorities make new reimbursement codes for DP or make an indirect method to support DP implementation [25]. She highlighted that DP can reduce the total amount of medical costs over time and enhance the overall quality of pathologic diagnosis service, as well as the quality of healthcare service, although DP might take more time, costs, and manpower during the initial time for implementation. She also mentioned that it can save a lot of medical costs by increasing specialist consultation and reducing overtreatment or missing treatment time, resulting in increased diagnostic accuracy of pathologic diagnosis and enhanced treatment. It also might reduce redundant tests during hospital transfer, such as immunohistochemistry, and increase patient safety by facilitating the use of sample tracking systems such as barcodes or QR codes. Although DP technology has been implemented since mid-2000s, it took almost 20 years for some leading institutes to become fully digital. The national healthcare service and insurance system should support DP technology to bring about this fundamental change.

The last 2 years were a monumental period for every aspect of society and way of living and working because the COVID-19 pandemic forced us to change. The need for remote communication, working from home, and distant, 'untact' medical services has increased. DP has also received attention as the best solution to this unexpected crisis in the field of pathology. Many institutes in Korea have also begun to accelerate the implementation of DP after the pandemic. However, there are still many practical issues during DP implementation that affect the process. Chae SW from Kangbuk Samsung Hospital highlighted the need for standardization of pathologic data. Since the file formats differ by WSS domain, a standardized format such as DICOM in radiologic imaging is essential for smooth incorporation into the electronic health record system or LIS and better utilization for research and collaboration between laboratories [17,26-28]. Jung CK from Seoul St. Mary's Hospital, Catholic University of Korea mentioned that developing artificial intelligence and other 4th industrial revolution technology was impossible without data standardization [22,29].

In summary, digital transformation in pathology is an inevitable change of timely importance and is the center of next-generation pathologic practice, healthcare service, education, and research. For the successful implementation of DP systems in the pathologic diagnosis process, continuous QAP from each laboratory should be accompanied by institutional, governmental, and policy support. Further efforts on standardization of pathologic data from the market are also needed to inspire innovative applications to new technologies, such as artificial intelligence prediction for cancer classification and mutation [29-31]. The CQA of the KSP will continue to revise this checklist according to feedback from the following QAP trials for DP.

## Supplementary Information

The Data Supplement is available with this article at https://doi.org/10.4132/jptm.2022.09.30.

## Ethics Statement

All procedures performed in the current study were approved by the Institutional Review Board of the Catholic University of Korea (UC21ZCSI0057) in accordance with the 2018 Helsinki declaration. Formal written informed consent was not required with a waiver by the appropriate the Institutional Review Board of the Catholic University of Korea (UC21ZCSI0057).

## 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

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

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## References

1. Nam S, Chong Y, Jung CK, et al. Introduction to digital pathology and computer-aided pathology. J Pathol Transl Med 2020; 54: 125-34.
2. Chong Y, Kim DC, Jung CK, et al. Recommendations for pathologic practice using digital pathology: consensus report of the Korean Society of Pathologists. J Pathol Transl Med 2020; 54: 437-52.
3. Canadian Association of Pathologists Telepathology Guidelines Committee; Bernard C, Chandrakanth SA, et al. Guidelines from the Canadian Association of Pathologists for establishing a telepathology service for anatomic pathology using whole-slide imaging. J Pathol Inform 2014; 5: 15.
4. College of American Pathologists. Validating whole slide imaging for diagnostic purposes in pathology. Northfield: College of American Pathologists, 2013.
5. College of American Pathologists. Anatomic pathology checklist: CAP accreditation program. Northfield: College of American Pathologists, 2011.
6. Cross S, Furness P, Igali L, Snead D, Treanor D. Best practice recommendations for implementing digital pathology. London: The Royal College of Pathologists, 2018; 1-43.
7. Federal Association of German Pathologists Bundesverband deutscher Pathologen (FAGP-BDP). Guidelines digital pathology for diagnosis on (and reports of) digital images, 2018. Berlin: Federal Association of German Pathologists Bundesverband deutscher Pathologen (FAGP-BDP), 2018.
8. Garcia-Rojo M, Conde A, Ordi J, et al. Guia practica para la implantacion de la patologia digital [Practical guide for the implementation of digital pathology]. In: Guerra Merino I, ed. Libro Blanco de la Anatomia Patologica en Espana 2015 [White book of pathological anatomy in Spain 2015]. Madrid: Sociedad Espanola de Anatomia Patologica, 2015; 247-78.
9. Japanese Society of Pathology. Digital Pathology Assessment Committee: technical standards for digital pathology system for pathologic diagnosis. Tokyo: Japanese Society of Pathology, 2018.
10. Japanese Society of Pathology. Digital Pathology Assessment Committee: guidelines for pathologic diagnosis using digital pathology images (clinical questions and answers). Tokyo: Japanese Society of Pathology, 2019.
11. Department of In-Vitro Diagnostic Device, Ministry of Food and Drug Safety. Assessment and approval guideline on the artificial intelligence (AI)-based histopathologic in-vitro diagnostic devices (software). Cheongju: Ministry of Food and Drug Safety, 2021.
12. Health Insurance Review and Assessment Service. Reimbursement assessment guideline of innovative medical technology (pathology AI-based technology). Wonju: Health Insurance Review and Assessment Service, 2021.
13. Mukhopadhyay S, Feldman MD, Abels E, et al. Whole slide imaging versus microscopy for primary diagnosis in surgical pathology: a multicenter blinded randomized noninferiority study of 1992 cases (pivotal study). Am J Surg Pathol 2018; 42: 39-52.
14. Pantanowitz L, Sinard JH, Henricks WH, et al. Validating whole slide imaging for diagnostic purposes in pathology: guideline from the College of American Pathologists Pathology and Laboratory Quality Center. Arch Pathol Lab Med 2013; 137: 1710-22.
15. Snead DR, Tsang YW, Meskiri A, et al. Validation of digital pathology imaging for primary histopathological diagnosis. Histopathology 2016; 68: 1063-72.
16. Fraggetta F, Yagi Y, Garcia-Rojo M, et al. The importance of eSlide macro images for primary diagnosis with whole slide imaging. J Pathol Inform 2018; 9: 46.
17. Chae SW. Present and future of digital pathology: experience of Kangbuk Samsung Hospital. In: Proceedings of the 73rd Annual Fall Meeting of The Korean Society of Pathologists; 2021 Oct 27-29; Seoul, Korea. Seoul: The Korean Society of Pathologists, 2021; 21.
18. Han N. Present and future of digital pathology: experience of National Cancer Center. In: Proceedings of the 73rd Annual Fall Meeting of The Korean Society of Pathologists; 2021 Oct 27-29; Seoul, Korea. Seoul: The Korean Society of Pathologists, 2021; 21.
19. Hong SW. Present and future of digital pathology: experience of Yongin Severance Hospital. In: Proceedings of the 73rd Annual Fall Meeting of The Korean Society of Pathologists; 2021 Oct 27-29; Seoul, Korea. Seoul: The Korean Society of Pathologists, 2021; 21.
20. Jang KT. Present and future of digital pathology: experience of Samsung Medical Center. In: Proceedings of the 46th Annual Spring Meeting of The Korean Society of Pathologists; 2021 May 13-14; Virtual. Seoul: The Korean Society of Pathologists, 2021; 17.
21. Jang S. Present and future of digital pathology: experience of Seoul Asan Medical Center. In: Proceedings of The 46th Annual Spring Meeting of The Korean Society of Pathologists; 2021 May 13-14; Virtual. Seoul: The Korean Society of Pathologists, 2021; 21.
22. Jung CK. Present and future of digital pathology: experience of the Catholic University of Korea St. Mary's Hospital. In: Proceedings of The 46th Annual Spring Meeting of The Korean Society of Pathologists; 2021 May 13-14; Virtual. Seoul: The Korean Society of Pathologists, 2021; 19.
23. Kim S. Present and future of digital pathology: experience of Yonsei University Severance Hospital. In: Proceedings of The 46th Annual Spring Meeting of The Korean Society of Pathologists; 2021 May 13-14; Virtual. Seoul: The Korean Society of Pathologists, 2021; 20.
24. Lee KB. Present and future of digital pathology: laboratory information system leads whole slide image diagnosis: integration digital pathology with paperless pathology workflow in SNUH. In: Proceedings of The 46th Annual Spring Meeting of The Korean Society of Pathologists; 2021 May 13-14; Virtual. Seoul: The Korean Society of Pathologists, 2021; 18.

25. Lee KB. Preparation of Reimbursement Assessment Guideline of Innovative Medical Technology (pathology AI-based technology). Seoul: Health Insurance Review and Assessment Service, 2019.
26. DICOM Standards Committee Working Groups 26, Pathology. Digital imaging and communications in medicine (DICOM). Supplement 145: Whole slide microscopic image IOD and SOP classes. Rosslyn: DICOM, 2010.
27. Singh R, Chubb L, Pantanowitz L, Parwani A. Standardization in digital pathology: Supplement 145 of the DICOM standards. J Pathol Inform 2011; 2: 23.
28. Zwonitzer R, Kalinski T, Hofmann H, Roessner A, Bernarding J. Digital pathology: DICOM-conform draft, testbed, and first results. Comput Methods Programs Biomed 2007; 87: 181-8.
29. Ailia MJ, Thakur N, Abdul-Ghafar J, Jung CK, Yim K, Chong Y. Current trend of artificial intelligence patents in digital pathology: a systematic evaluation of the patent landscape. Cancers (Basel) 2022; 14: 2400.
30. Alam MR, Abdul-Ghafar J, Yim K, et al. Recent applications of artificial intelligence from histopathologic image-based prediction of microsatellite instability in solid cancers: a systematic review. Cancers (Basel) 2022; 14: 2590.
31. Kim H, Yoon H, Thakur N, et al. Deep learning-based histopathological segmentation for whole slide images of colorectal cancer in a compressed domain. Sci Rep 2021; 11: 22520.


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NEWSLETTER



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# What's new in kidney tumor pathology 2022: WHO 5th edition updates

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## Abstract

The 5th edition WHO Classification of Urinary and Male Genital Tumours (2022) introduces significant changes relevant to daily practice, especially in the completely restructured renal cell tumor chapters. Herein we highlight the most important diagnostic updates of known kidney tumor types, new and molecularly defined entities and emerging/provisional entities.

## UPDATES IN ESTABLISHED RENAL TUMORS

### Papillary renal cell carcinoma (PRCC)

- Subclassification into type 1 and type 2 is no longer recommended.
- PRCC has classic morphology (papillae with vascular cores, foamy histiocytes and psammoma bodies) but can exhibit other appearances, including predominant solid phenotype, biphasic pattern with squamoid alveolar cells, eosinophilic cells with brisk inflammation mimicking Warthin tumor or predominant vacuolated cells mimicking clear cell RCC.
- Many tumors previously diagnosed as type 2 PRCC now constitute independent entities.

### Clear cell papillary renal cell tumor (CCPRCT)

- **Renamed** from carcinoma to tumor due to uniformly indolent behavior.
- Low-stage, low-grade tumor with tubulopapillary and cystic architecture composed of clear cells with linearly aligned luminally oriented nuclei.
- Co-express CK7 and CAIX (cup-like), often positive for HMWCK, but negative for CD10, and lack recurrent cytogenetic abnormalities or *VHL* gene alterations.

### Chromophobe RCC (ChRCC)

- ChRCC can have non-conventional morphology with trabecular, alveolar, papillary, microcystic or cystic architecture, but all these phenotypes typically maintain CK7/CKIT co-expression, characteristic chromosomal monosomies and favorable prognosis.

### Diagnostic recommendations

- An unequivocal diagnosis of multilocular cystic neoplasm of low malignant potential (MCN-LMP), CCPRCT and oncocytoma should not be made on needle biopsy alone because of limited sampling and overlapping features with malignant counterparts.

## NEW CATEGORY OF MOLECULARLY DEFINED RENAL TUMORS

This heterogeneous group of tumors often shows significant morphologic overlap with other renal tumors. Definitive diagnosis requires molecular studies like NGS, RNAseq, FISH or RT-PCR [1].

### *TFE3*-rearranged RCC (formerly named MiTF family Xp11 translocation RCC)

- Heterogeneous tumors in younger patients with mixed papillary and solid architecture, psammoma bodies and clear to eosinophilic cytoplasm.
- Express nuclear TFE3 and variably melanocytic markers and cathepsin K.
- *TFE3* rearrangement with > 20 different gene partners creates fusion subtypes with variable tumor morphology, immunoprofile and clinical behavior.

### *TFEB*-rearranged RCC

- Tumor has either translocation or amplification of *TFEB* on t(6;11).
- *TFEB*-**translocation** RCC is a low-stage indolent biphasic neoplasm with nests of large clear cells and smaller cells clustered around basement membrane material.
- *TFEB*-**amplified** RCC is an often high-grade and high-stage tumor with frequent oncocytic and papillary morphology affecting older patients (Fig. 1).

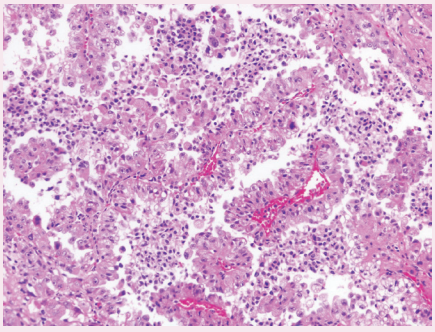

**Fig. 1.** *TFEB*-amplified RCC.

- Both subtypes consistently express nuclear TFEB, cathepsin K and melanocytic markers.

### ELOC (formerly *TCEB1*)-mutated RCC (novel entity)

- Uncommon indolent clear cell tumor with solid and papillary growth patterns and nodular appearance due to traversing fibromuscular bands and septa.

- Morphologically mimic conventional clear cell and tuberous sclerosis-associated RCCs.
- Consistently immunoreactive for CK7 and can be focally positive for HMWCK.
- Develops due to bi-allelic inactivation of TCEB1 (*ELOC*) on chromosome 8 encoding for elongin C of the VHL complex with intact *VHL* and mTOR pathway genes.

### Fumarate hydratase (*FH*)-deficient RCC

- **Renamed** from hereditary leiomyomatosis-associated RCC.
- Aggressive tumor with mixed papillary, solid, tubulocystic and cribriform architecture, composed of high-grade cells with cherry-red macronucleoli.
- Germline (majority of cases) or somatic *FH* gene mutations should be suspected with immunostaining demonstrating FH protein loss and/or 2-succinocysteine (2SC) gain.

### Succinate dehydrogenase (SDH)-deficient RCC

- Rare tumor with distinct solid morphology of bland eosinophilic cells with bubbly inclusions.
- Loss of SDHB protein expression and germline mutation in *SDH* gene complex.

### ALK-rearranged RCC (novel entity)

- Very rare group of extremely heterogeneous eosinophilic tumors which develop due to fusions of anaplastic lymphoma kinase gene (*ALK*) at 2p23 resulting in ALK protein overexpression.
- May show cytoplasmic vacuolization; solid, papillary or cribriform architecture with mucin production; psammoma bodies; metanephric-like, rhabdoid or spindle cell morphology (Fig. 2).

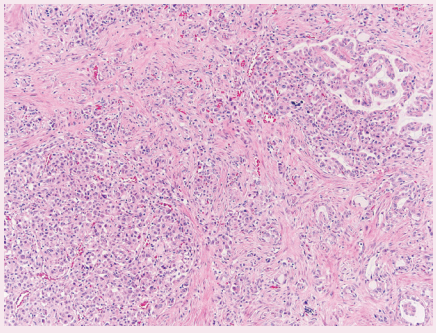

**Fig. 2.** *ALK*-rearranged RCC.

### SMARCB1-deficient renal medullary carcinoma

- **Renamed** from renal medullary carcinoma.
- Highly aggressive medulla-centered adenocarcinoma predominantly affecting patients with sickle cell trait (hemoglobinopathy) and of African ancestry.
- Presents as locally advanced or metastatic disease with fast-growing infiltrating tumor composed of cords, nests, tubules and cribriform structures in desmoplastic background with brisk mitoses.
- Loss of SMARCB1 (INI1, SNF5, BAF47) protein expression on immunostaining reflects inactivation of *SMARCB1* at 22q11.23 by chromosome translocations or deletions.

### Eosinophilic solid and cystic RCC

- Novel distinct entity in "OTHER RENAL TUMORS" category
- Originally described in patients with tuberous sclerosis complex, but can occur sporadically due to *TSC1* or *TSC2* mutations.
- Indolent tumor disproportionately affecting women, with only rare reported metastases.
- Solid and cystic architecture, voluminous eosinophilic cytoplasm and coarse basophilic granularity.
- CK20 and cathepsin K are positive and there is a lack of CK7/CKIT expression (Fig. 3).

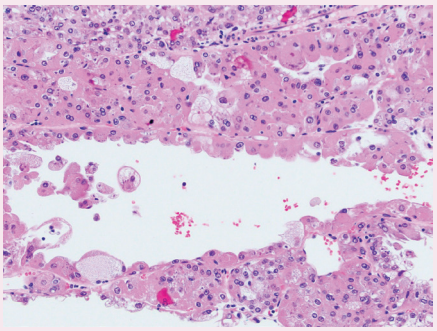

**Fig. 3.** Eosinophilic solid and cystic RCC.

## EMERGING/PROVISIONAL ENTITIES

- These tumor types are still not part of classification, but discussed in WHO [2].

### Thyroid-like follicular carcinoma

- Rare kidney tumor composed of tightly packed follicle-like cysts filled with eosinophilic colloid-like material and lined by cuboidal cells with scant cytoplasm and oval to round nuclei.
- Positive for PAX8, CK19 and CK7; negative for TTF1 and thyroglobulin.
- Recurrent *EWSR1::PATZ1* fusion.

### Other oncocytic tumors (oncocytic tumor, NOS)

- These are heterogeneous groups of tumors that do not fulfill criteria for oncocytoma or eosinophilic variant of chromophobe RCC (or other specific entities).
- **Hybrid oncocytic chromophobe tumor (HOCT)** is an indolent oncocytic neoplasm with borderline (intermediate) features between oncocytoma and ChRCC. It can be solitary and sporadic, but in Birt-Hogg-Dubé syndrome often is multifocal and bilateral, exhibiting a checkerboard mosaic pattern and harboring mutations in *FLCN*.
- **Eosinophilic vacuolated tumor (EVT)** is characterized by solid growth, cytoplasmic vacuolization, entrapped tubules and large vessels, prominent nucleoli, CK7-/CKIT+ immunoprofile and mutations in mTOR pathway genes.
- **Low-grade oncocytic tumor (LOT)** is a solid neoplasm with bland low-grade nuclei, CK7+/CKIT- immunoprofile and mutations in mTOR pathway genes.

### Biphasic hyalinizing psammomatous RCC

- Rare biphasic tumor with larger cells forming tubules, papillae and acini and smaller cells clustered around hyalinized basement membrane material in glomeruloid or nested pattern.
- It has sclerotic stroma with abundant psammoma bodies and bi-allelic loss of *NF2*.

### Papillary renal neoplasm with reverse polarity

- Formerly considered as a subtype of PRCC.
- Eosinophilic tumor with branching papillary architecture and reverse polarity of low-grade nuclei (Fig. 4).

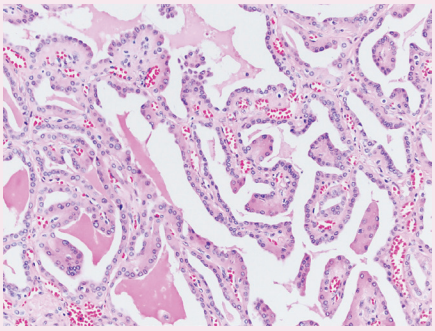

**Fig. 4.** Papillary renal neoplasm with reverse polarity.

- Positive for GATA3, negative for vimentin and variable for AMACR.
- Has recurrent mutations of *KRAS* and lacks trisomy 7/17.


### Reference

1. Trpkov K, Hes O, Williamson SR, et al. New developments in existing WHO entities and evolving molecular concepts: The Genitourinary Pathology Society (GUPS) update on renal neoplasia. Mod Pathol 2021; 34: 1392-424.
2. Trpkov K, Williamson SR, Gill AJ, et al. Novel, emerging and provisional renal entities: The Genitourinary Pathology Society (GUPS) update on renal neoplasia. Mod Pathol 2021; 34: 1167-84.


### Meet the Author

Dr. Tretiakova has been an author for PathologyOutlines since 2015, part of the PathologyOutlines editorial board since 2019, and Deputy Editor in Chief for GU Pathology since 2021. She is currently a Professor at the University of Washington where she primarily practices GU Pathology. Dr. Tretiakova is an international expert in GU pathology and has co-authored several topics in the WHO 2022 Blue Book.

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# What's new in soft tissue and bone pathology 2022—updates from the WHO classification 5th edition

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## Abstract

The 2020 release of the WHO Classification of Soft Tissue and Bone Tumors, 5th edition, contains several changes driven by new knowledge in the field. These include reclassification of some entities, refinement of risk classification systems, and the inclusion of novel disease processes, many of which are driven by recurrent gene fusions. The most notable changes are described here.

## SELECT NEW ENTITIES

### Lipomatous tumors

- Atypical spindle cell/pleomoprhic lipomatous tumor
  - Composed of variable proportions of atypical spindle cells, adipocytes, univacuolated or multivacuolated lipoblasts, pleomorphic to multinucleated cells, and myxoid to collagenous stroma.
  - Lack of *MDM2* or *CDK4* amplification.
  - Rb expression is generally lost.
  - Low rate of local recurrence (10%–15%); no known risk of dedifferentiation.
- Myxoid pleomorphic liposarcoma
  - Occurs predominantly in children and young adults with a predilection for the mediastinum.
  - Admixture of areas resembling myxoid liposarcoma with more cellular areas containing overt nuclear pleomorphism, which resembles pleomorphic liposarcoma.
  - Lacks recurrent chromosomal changes, namely *MDM2* amplification and *DDIT3* gene fusion.
  - Clinical behavior akin to pleomorphic liposarcoma.

### Fibroblastic/myofibroblastic tumors

- *EWSR1::SMAD4* positive fibroblastic tumor
  - Small dermal and subcutaneous acral nodule, with indolent biological behavior.
  - Histologic zonation with acellular hyalinized center and peripheral fascicular monomorphic spindle cell growth.
  - Diffuse ERG nuclear expression in the absence of CD34 and SMA expression.
  - *EWSR1::SMAD4* fusion.
- Angiofibroma of soft tissue
  - Benign neoplasm with rare local recurrence.
  - Uniformly bland short spindle cells in variably myxoid to collagenous stroma, with prominent vascular network of small thin-walled branching blood vessels (Fig. 1).
  - *NCOA2* gene rearrangements in up to 80%.
- Superficial CD34-positive fibroblastic tumor
  - Rare, slow-growing, indolent neoplasm.
  - Superficial location, typically in the lower extremities.
  - Large eosinophilic cells with granular to glassy cytoplasm; marked pleomorphism with low mitotic count (Fig. 2).
  - CD34 and frequent keratin expression.

### Smooth muscle tumors

- Inflammatory leiomyosarcoma
  - Rare, and thought to be relatively indolent compared to conventional leiomyosarcoma.
  - Typically arise in the deep extremities.
  - Variably atypical eosinophilic spindle cells in fascicles, with mitotic activity and prominent, usually diffuse, mixed (predominantly mononuclear) inflammatory infiltrate.
  - Near-haploid karyotype.
- EBV-associated smooth muscle tumor
  - Associated with EBV infection, usually in the setting of immunosuppression.
  - Can arise in any anatomic location, most often in visceral sites and CNS.
  - Prognosis depends on the patient's immune condition. Most tumors do not metastasize.
  - Cytologic atypia is highly variable. In half of cases, a second population of more primitive

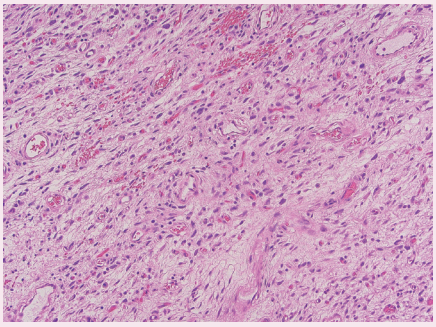

Fig. 1. Angiofibroma of soft tissue.

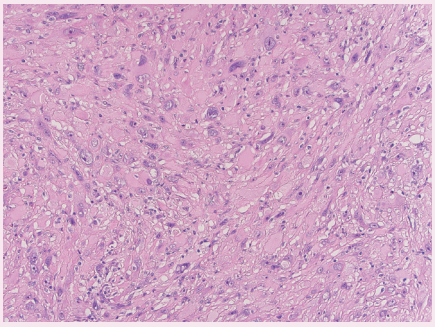

Fig. 2. Superficial CD34-positive fibroblastic tumor.

appearing round cells are seen. T-cell inflammatory infiltrates are common.
- Invariably positive expression of EBER.

### Vascular tumors

- Anastomosing hemangioma
  - Benign vascular neoplasm. Often arises in viscera and can be multifocal.
  - Thin-walled anastomosing vessels lined by hobnail endothelial cells. Vascular thrombi are typical. Loosely lobulated architecture with focal infiltration into adjoining tissue. May be associated with a medium-caliber vessel.
  - Activating mutations in *GNAQ* or *GNA14*.
- Epithelioid hemangioendotehlioma with *YAP1::TFE3* gene fusion
  - Considered to have a generally more aggressive behavior.
  - Tends to have more solid growth and be vasoformative, compared to cases harboring *WWTR::CAMTA1* fusion.

### Tumors of uncertain differentiation

- Kinase gene-rearranged spindle cell neoplasms
  - Outside of infantile fibrosarcoma, this represents an emerging group of tumors with a wide morphologic spectrum.
  - Most tumors have co-expression of S100 and CD34 in the absence of SOX10.
  - They can resemble lipofibromatosis or can be composed of monomorphic spindle cells with prominent collagen deposition and hyalinization; amianthoid-like fibers and infiltrative growth may also be present.
  - Harbor gene fusions involving kinase genes, such as *NTRK, ALK, RAF1* and *BRAF*.

### Undifferentiated round cell sarcomas

Due to the recent expansion in molecular studies, multiple recurrent gene fusions have been described in previously unclassified round cell sarcomas. These lesions have been shown to have particular clinical and morphologic features. These include:

- Round cell sarcoma with *EWSR1*::non-*ETS* fusions
  - Round and spindle cell sarcomas with fusions in *EWSR1* or *FUS* with partners unrelated to the *ETS* gene family, most commonly *NFATC2* or *PATZ1* genes.
  - Tumors with *EWSR1::NFATC2* consist of round or spindled cells arranged in cords, nests and trabeculae in a myxohyaline background (Fig. 3). They may have focal dot-like keratin and CD138 expression. They may morphologically mimic myoepithelial neoplasms or ossifying fibromyxoid tumor, among others.
  - Sarcomas with *EWSR1::PATZ1* fusion have a broad morphologic spectrum. They may have round to spindle cells within a fibrous stroma. Co-expression of myogenic and nerve sheath markers has been described.
- *CIC*-rearranged sarcoma
  - Round cell sarcoma characterized by *CIC* gene fusions, most commonly *CIC::DUX4*. Other fusion partners include *FOXO4, LEUTX, NUTM1* and *NUTM2A*.
  - Composed of sheets of large round cells with mild nuclear pleomorphism, lightly eosinophilic cytoplasm, geographic necrosis and brisk mitotic activity (Fig. 4).
  - Immunohistochemically, they show variable CD99 expression, nuclear WT1 and DUX4 reactivity.
  - Response to chemotherapy is poor compared to Ewing sarcoma and *BCOR* fusion sarcomas.
- Sarcoma with *BCOR* alterations
  - Primitive round cell sarcoma with *BCOR* gene fusions, most commonly *BCOR::CCNB3*, followed by *BCOR* internal tandem duplication.
  - Composed of primitive round to spindled cells in nests, sheets or fascicles in variably myxoid stroma, which may morphologically resemble synovial sarcoma. Their clinical response to chemotherapy is favorable when compared to Ewing sarcoma and *CIC*-fusion sarcomas.
  - Sarcomas with *BCOR* fusion are slightly more common in bone and tend to arise in patients younger than 20 years. On the other hand, sarcomas with *BCOR* internal tandem duplication tend to arise in the soft tissues of the trunk, retroperitoneum, and head and neck. These usually occur within the first year of life or may present at birth.

## CHANGES IN NOMENCLATURE

- Mammary-type myofibroblastoma has been renamed as myofibroblastoma.
- Melanotic schwannoma has been renamed to malignant melanotic nerve sheath tumor. This change better reflects its aggressive clinical behavior.

## OTHER CHANGES

- Solitary fibrous tumor
  - Risk stratification based on age, tumor size, mitotic activity, and necrosis is recommended [1].
- Spindle cell/sclerosing rhabdomyosarcoma
  - Subclassification depends on the presence of genetic alterations associated with prognosis.
  - Tumors with *VGL2L, NCOA2* and *CITED2* gene rearrangements typically arise in infants and have favorable prognosis.
  - Tumors with *MYOD1* mutations usually arise in adolescents and adults, and have unfavorable prognosis.
  - Tumors with *TFCP2/NCOA2* gene rearrangements can be intraosseous.

### Reference

1. Demicco EG, Wagner MJ, Maki RG, et al. Risk assessment in solitary fibrous tumors: validation and refinement of a risk stratification model. Mod Pathol 2017; 30: 1433-1442.

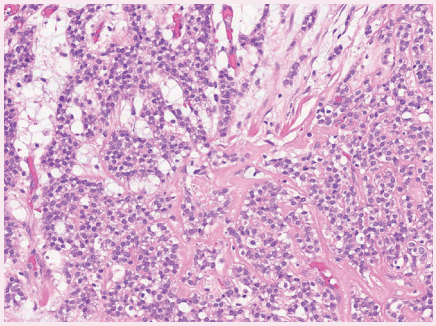

**Fig. 3.** Round cell sarcoma with *EWSR1*::non-*ETS* fusions.

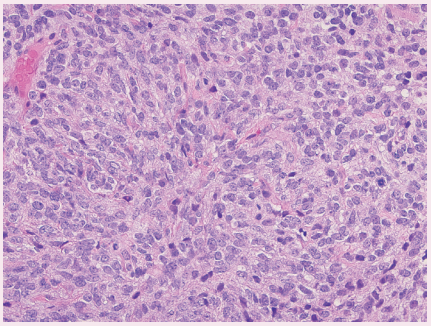

**Fig. 4.** *CIC*-rearranged sarcoma.

### Meet the Authors

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