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Diagnostic utility of immunocytochemistry by using liquid-based cytology (LBC) slides

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Abstract

Introduction. Cytological specimens, such as fine needle aspirations (FNAs) and exfoliative cytology samples, are minimally invasive options for diagnostic purposes. Liquid-based cytology (LBC), originally designed for cervical cytology, has gained prominence as an alternative technique for non-gynecological cytology. Immunocytochemistry (ICC) is an ancillary method used when diagnosis becomes challenging due to morphological overlap or the need for cellular origin clarification. This study aims to assess the diagnostic utility of ICC when applied to LBC slides and evaluate its effectiveness in relation to the waiting (lag) time of residual material.

Materials and methods. A total of 74 cases in which ICC was applied to LBC slides were studied over one year in a reference pathology laboratory (Prof. Dr. Cemil Tascioglu, Pathology Laboratory, City Hospital, Istanbul, Turkey). Cases in which immunohistochemistry (IHC) was performed on formalin-fixed paraffin-embedded cell blocks were excluded. The SurePath PAP method was used for the main LBC cytology slides. For the ICC study, 1–4 PAP-stained LBC slides were obtained from each case's residual material and stained with a primary antibody.

Results. The positive immunostaining was obtained in 81% of cases. The samples were categorized into groups based on the waiting time of residual LBC material for ICC analysis: 1–5 days, 6–10 days, 11–20 days, and 21–38 days. Comparative analysis revealed a decline in ICC efficacy as the waiting (lag) time increased. Additionally, a statistically significant difference was observed between the 11–20 days and 21–38 days groups ($P < 0.05$). An analysis of 142 LBC slides stained by ICC revealed that nuclear markers exhibited higher positivity compared to non-nuclear markers, although no significant difference was detected between the two groups.

Conclusions. High positivity rates can be obtained in ICC studies performed on additional slides obtained from residual LBC material within the first 20 days. ICC applied to LBC slides is an important and useful alternative for diagnostic and prognostic markers in cases without a cell block or with a cell block without sufficient number of cells.
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Keywords: liquid-based cytology; immunocytochemistry; immunohistochemistry; waiting (lag) time; SurePath PAP

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Introduction

Cytological specimens, such as fine needle aspirations (FNAs) and exfoliative cytology samples, are valuable resources for diagnostic purposes, offering minimally invasive procedures [1]. Liquid-based cytology (LBC), originally designed for cervical cytology, has become an alternative technique to conventional slides and cytospin preparations. It has been proven to be effective in collecting and preparing non-gynecological cytological samples, particularly thyroid FNAs [2, 3]. However, conflicting opinions and controversial data exist regarding the effectiveness of LBC, overshadowing its numerous positive attributes, including cost-effectiveness, time efficiency, and the application of ancillary techniques such as immunocytochemistry (ICC) and molecular analysis using stored LBC material for extended periods [3, 4].

Immunohistochemistry (IHC) is an adjunctive technique that uses antibodies to target specific amino acid sequences unique to proteins, aiding in the identification of proteins and other molecules within cells and tissues. When performed on formalin-fixed paraffin-embedded (FFPE) cell blocks, conventional slides, and LBC slides obtained from cytological samples, it is referred to as immunocytochemistry (ICC). ICC has proven invaluable in distinguishing overlapping entities and clarifying cellular origin when cytomorphology remains unclear. This technique, known for its simplicity and cost-effectiveness, effectively differentiates between benign and malignant cells [5–7]. In cases where a cell block can be obtained and there are sufficient cells, FFPE cell block is preferred for ICC application. However, in cases where a cell block cannot be obtained or there are not enough cells, alternative methods such as direct smears, cytospin smears, and modified LBC are used for ICC application [1, 5]. In our study, we aimed to assess the diagnostic utility of ICC when applied to LBC slides obtained from residual material and evaluate its efficacy in relation to the duration of residual LBC material storage.

Materials and methods

During a 12-month period, a total of 74 cases were included in the study from the reference pathology laboratory (Prof. Dr. Cemil Tascioglu, City Hospital, Istanbul, Turkey). These cases were selected because they had insufficient or no cell blocks and had undergone ICC using slides obtained by LBC (SurePath, BD DiagnosticsTripath, Burlington, NC, USA) technique. Cases with sections of FFPE cell blocks that underwent immunohistochemical studies were excluded from the study.

The study was approved by the Ethics Committee of the Istanbul Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Istanbul, Turkey (Approval number: 2023-391, date: 26.09.2023) and conducted following the Declaration of Helsinki.

Preparation of LBC slides, application, and evaluation of ICC. The principal LBC cytology slides were prepared using the SurePath Pap Test kit (BD Diagnostics). The samples were fixed in an ethanol-based fixative (CytoRich™ Red, BD Diagnostics) and underwent two rounds of centrifugation. The cellular samples were then homogenized using a vortex and evenly dispersed as a thin layer on microscope slides. These slides were stained using the Papanicolaou method (PAP) [8].

For the ICC study, PAP-stained LBC slides were obtained from residual material stored in CytoRich™ Red solution in the refrigerator at 4°C for each case. These slides were processed using the Ventana Autostainer BenchMark ULTRA (Ventana Medical Systems, Tucson, AZ, USA) with the same staining protocol used for surgical tissue specimens. Positive controls were FFPE samples known to be positive for specific antibodies. All procedures, including deparaffinization, were performed during the ICC process. After antigen retrieval, slides were stained with primary antibodies. Immunostained slides were evaluated using a light microscope by three pathologists with immunoreactivity in lesional cells indicative of immunostaining. The samples were categorized into groups based on the waiting (lag) time of residual LBC material for ICC analysis: 1–5 days, 6–10 days, 11–20 days, or 21–38 days.

Statistical analysis. The location of the body where the cytological samples were taken was obtained from the pathology reports. Statistical analysis was conducted using SPSS version 25 (IBM, Armonk, NY, USA). A descriptive analysis was used to present nominal variables in terms of case count and percentages. The Kruskal-Wallis test was used to evaluate the positivity of immunostaining according to ICC study day groups, while the Chi-Square test was used to assess the positive staining of ICC studies relative to staining features of markers. A p-value of less than 0.05 was considered statistically significant.

Results

Each case yielded between 1 and 4 additional LBC slides for ICC analysis, with a mean of 1.94 slides per case. Positive immunostaining was observed in 60 cases (81%), while 14 cases (19%) showed no staining. The most common specimen localizations were lymph nodes (n = 34), followed by thyroid and pleural/peritoneal effusion samples (n = 14, n = 10, respectively). Other specimens analyzed with ICC included parathyroid (n = 3), cervical smears (n = 2), salivary glands and head and neck region (n = 3), renal cyst (n = 2), pancreas (n = 1), soft tissue (arm-nerve sheath) (n = 1), lung (n = 3), and mediastinum (n = 1). Cases with no positive staining were effusion and lymph node specimens (n = 4 each), with an additional 2 thyroid cases showing negative immunoexpression. Other

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cases without positive staining included renal cyst, pancreas, soft tissue, and parotid specimens (Table 1).

The waiting (lag) time for obtaining material for ICC analysis ranged from 2 to 38 days (mean ± SD: 11.0 ± 8.4 days). The samples were categorized into groups based on the waiting time of residual LBC material for ICC analysis: 1–5 days, 6–10 days, 11–20 days, and 21–38 days. The waiting (lag) times of residual LBC material, locations and immunostaining status of each case were given in Table 2.

Comparative analysis revealed a decrease in positive staining in LBC slides as the waiting time for staining increased. A statistically significant difference was observed between the 11–20 days and 21–38 days groups ($P < 0.05$). However, no significant differences were found among groups up to 20 days (Table 3).

A total of 142 LBC slides stained immunocytochemically with 26 markers were used in the ICC analysis. The most frequently used marker was TTF1 (Thyroid transcription factor 1) (n = 28), followed by GATA3 (n = 23). Detailed information on the frequency of marker use and their corresponding positive immunostaining rates is provided in Table 4. Markers were categorized based on their staining characteristics, with 87 LBC slides exhibiting nuclear staining features and 55 showing non-nuclear (cytoplasmic/membranous) staining. Nuclear markers like TTF1, GATA3, and P40 had higher immunostaining (88.5%) frequency compared to non-nuclear markers (cytoplasmic/membranous) such as PanCK (Pan-Cytokeratin), MOC31 (Ep-CAM/Epithelial Specific Antigen), chromogranin, calcitonin, and CD68 (78.2%). However, no statistically significant difference was found between the two groups (Table 5). No staining was observed in any of the 22 slides belonging to 14 negative cases.

The immunocytochemical markers were chosen according to location. For example, Gata3 was used to detect primary breast carcionoma in lymph node metastasis, and Gata3, TTF-1, PTH were used to distinguish parathytoid-thyroid origin. In addition, BerEp4, MOC31, Calretinin, CD68 are used in effusion cytology, while P40, TTF-1 are used to differentiate adenocarcinoma from squamous cell carcinoma in liver carcinomas. TTF-1, Thyroglobulin, Calcitonin and Synaptophysin were used for the diagnosis of medullary thyroid carcinoma, while P63 and CK5/6 were used for head and neck region cancers. Prognostic markers such as ER, PR and CerbB2 were studied in cases with breast carcinoma metastasis to the lymph node. The names of the markers used clones, dilutions and name of manufactures are detailed in Table 6.

Discussion

The use of cytological specimens for diagnostic purposes, particularly through minimally invasive procedures like FNAs and exfoliative cytology, offers a valuable approach in modern pathology [1]. Our study explores the realm of LBC, an approach originally designed for cervical cytology but subsequently adopted for various anatomical sites [2, 3]. While LBC provides morphological advantages such as a clearer background, cell-rich smears, and better nuclear detail, residual LBC material is also useful for ancillary studies details like ICC [9].

Table 1. The rates of positivity of immunocytochemistry (ICC) stainings of liquid-based cytology (LBC) in terms of cases and slides according to the location of cytological samples

Location of the body	n	No of LBC slides used for ICC	No of cases with positive ICC staining according to location n (%)	No of positive immunostaining of ICC slides n (%)
Lymph nodes	34	54	30 (88.2%)	47 (87.0%)
Thyroid	14	34	12 (85.7%)	29 (85.2%)
Pleural/peritoneal effusion	10	23	6 (60.0%)	18 (78.2%)
Parathyroid	3	9	3 (100.0%)	9 (100.0%)
Salivary glands and head and neck region	3	6	2 (66.6%)	4 (66.6%)
Lung	3	6	3 (100.0%)	6 (100.0%)
Cervical smears	2	3	2 (100.0%)	3 (100.0%)
Renal cyst	2	2	1 (50.0%)	1 (50.0%)
Pancreas	1	1	0 (0.0%)	0 (0.0%)
Soft tissue (arm-nerve sheath)	1	1	0 (0.0%)	0 (0.0%)
Mediastinum	1	3	1 (100.0%)	3 (100.0%)
Total	74	142	60 (81.0%)	120 (84.5%)

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Table 2. The waiting (lag) times of residual LBC material, locations and immunostaining status of each cases

		Waiting (lag) time of residual LBC material				
Case no	**Location of the body**	**1–5 day**	**6–10 day**	**11–19 day**	**21–38 day**	**Immunostaining**
1.	Peritoneal effusion				X	Positive
2.	Lymph node				X	Negative
3.	Peritoneal effusion				X	Negative
4.	Pleural effusion				X	Positive
5.	Pleural effusion				X	Negative
6.	Thyroid				X	Positive
7.	Lymph node				X	Negative
8.	Cervical smear				X	Positive
9.	Thyroid				X	Positive
10.	Peritoneal effusion				X	Negative
11.	Thyroid			X		Positive
12.	Thyroid			X		Positive
13.	Renal cyst			X		Negative
14.	Parathyroid			X		Positive
15.	Lung			X		Positive
16.	Thyroid			X		Positive
17.	Parathyroid			X		Positive
18.	Pleural effusion			X		Positive
19.	Lymph node			X		Positive
20.	Thyroid			X		Positive
21.	Lung			X		Positive
22.	Thyroid			X		Positive
23.	Parathyroid			X		Positive
24.	Renal cyst			X		Positive
25.	Lymph node			X		Positive
26.	Thyroid			X		Negative
27.	Thyroid			X		Positive
28.	Thyroid			X		Positive
29.	Lymph node			X		Positive
30.	Lymph node			X		Positive
31.	Lymph node			X		Positive
32.	Lymph node			X		Positive
33.	Lymph node			X		Positive
34.	Lymph node			X		Positive
35.	Thyroid		X			Negative
36.	Parotid		X			Positive
37.	Lymph node		X			Positive
38.	Pirifom sinus (head & neck)		X			Positive
39.	Pleural effusion		X			Negative
40.	Thyroid		X			Positive
41.	Lymph node		X			Positive
42.	Thyroid		X			Positive

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Table 2 cont. The waiting (lag) times of residual LBC material, locations and immunostaining status of each cases

43.	Lymph node		X			Positive
44.	Lymph node		X			Positive
45.	Soft tissue (arm- nerve sheath)		X			Negative
46.	Pleural effusion		X			Positive
47.	Peritoneal effusion		X			Positive
48.	Lymph node		X			Positive
49.	Lymph node		X			Positive
50.	Lymph node	X				Positive
51.	Lymph node	X				Positive
52.	Lymph node	X				Positive
53.	Lymph node	X				Positive
54.	Lymph node	X				Negative
55.	Lymph node	X				Negative
56.	Thyroid	X				Positive
57.	Lymph node	X				Positive
58.	Pleural effusion	X				Positive
59.	Lymph node	X				Positive
60.	Lymph node	X				Positive
61.	Lymph node	X				Positive
62.	Pancreas	X				Negative
63.	Lymph node	X				Positive
64.	Lymph node	X				Positive
65.	Lymph node	X				Positive
66.	Parotid	X				Negative
67.	Mediastinum	X				Positive
68.	Cervical smear	X				Positive
69.	Lymph node	X				Positive
70.	Lymph node	X				Positive
71.	Lymph node	X				Positive
72.	Lymph node	X				Positive
73.	Lymph node	X				Positive
74.	Lung	X				Positive

Table 3. Distribution of immunostaining in the ICC study in relation to the waiting (lag) time of residual LBC material

Waiting time (days)	**Negative immunostaining**		**Positive immunostaining**		
	Count	**Row N**	**Count**	**Row N**	**P***
1–5	4	16.7%	20	83.3%	0.388
6–10	3	20.0%	12	80.0%	
11–20	2	8.3%	22	91.7%	0.047*
21–38	5	50.0%	5	50.0%	
Total	14	19.2%	59	80.8%	

*Kruskall-Wallis Test

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Table 4. The number of uses of the markers, positivity rates, localizations of cytological samples

ICC markers (Clones)	Number of applications	Positive IS* (n/%)	Negative IS* (n/%)	Cytological samples
TTF1 (Cell-Marque/867G3/1)	28	22/78.6	6/21.4	Thyroid, lung
GATA3 (Cell-Marque/L50-823)	23	22/95.7	1/4.3	Lymph node
PanCK (BioGenex/AE1-AE3)	13	10/76.9	3/23.1	Salivary gland, renal cyst, pancreas
PAX8 (Cell-Marque-MRQ-50)	10	7/70	3/30	Thyroid, renal cyst, cervical smear
ER (Cell-Marque-SP1)	8	8/100	0/0	Lymph node, mediastinum
PR (Invitrogen-SP2)	5	5/100	0/0	Lymph node
BerEp4 (Cell-Marque/BerEp4)	5	2/40	3/60	Pleural/peritoneal effusion
CerbB2 (Epitomics/EP3)	4	4/100	0/0	Lymph node
P40 (Bio-Care/P40M)	4	4/100	0/0	Lung
Calcitonin (SP17/Monoclonal)	4	4/100	0/0	Thyroid
Synaptophysin (Cell-Margue/MRQ-40)	4	4/100	0/0	Thyroid, lung
Chromogranin (Cell-Marque/LK2H10)	4	4/100	0/0	Parathyroid
CK7 (BioGenex/OVTL-12/30)	4	3/75	1/25	Lung, mediastinum, lymph node
MOC31 (Cell-Marque-Ep-CAM)	3	1/33.3	2/66.7	Pleural/peritoneal effusion
CD3 (Cell-Marque/polyclonal)	3	3/100	0/0	Lymph node
PAX5 (Epitomics SP34)	3	3/100	0/0	Lymph node
CD68 (Ab-1/P6-M1)	3	3/100	0/0	Pleural/peritoneal effusion
CK5/6 (Cell-Marque-D5/16B4)	3	3/100	0/0	Head and neck
P63 (Gene AB/IHC063-1)	2	2/100	0/0	Head and neck
Calretinin (Cell-Marque/SP13)	2	0/0	2/100	Pleural/peritoneal effusion
CDX2 (Cell-Marque /EPR2764Y)	2	2/100	0/0	Lymph node, pleural/peritoneal effusion
PTH (Cell-Marque/MRQ-31)	1	1/100	0/0	Parathyroid
S100 (Cell-Marque/4C4.9)	1	0/0	1/100	Soft tissue (arm-nerve sheath)
Thyroglobulin (Cell-Marque-2H11+6E1)	1	1/100	0/0	Thyroid, lymph node
P16 (Gene AB/INK4A)	1	1/100	0/0	Cervical smear
WT1 (Cell-Marque/6F/H2)	1	1/100	0/0	Cervical smear
Total	142	120/84.5	22/15.5	

IS — immunostaining.

Immunocytochemistry has become an indispensable method for diagnosis and evaluation of prognostic and predictive markers when only cytology samples are available for diagnostic studies [10]. Several studies have confirmed the use of ICC on variously prepared and fixed slides from cytology samples [3–5, 11]. However, ICC on cytology samples other than immunohistochemistry on FFPE cell block sections has been neglected in terms of quality assurance, control, and validation [10]. In cases where the cell amount is low and a cell block is not obtained, ICC performed on other types of cytological samples becomes more important [5, 12].

Our study aimed to demonstrate the diagnostic utility of ICC when applied to LBC slides and evaluate its efficacy in relation to the waiting (lag) time of residual material. This investigation, involving 74 patients over a 12-month period, revealed intriguing findings.

The positive immunostaining in ICC was achieved in 81% of cases, highlighting its potency as a diagnostic tool. However, in 19% of cases, positive immunostaining could not be achieved. The most common specimen localization was lymph nodes, followed by thyroid and effusion samples, demonstrating the diverse range of applications for ICC. Other specimen types, such as parathyroid, cervical smear, parotid, head and neck region, renal cyst, pancreas,

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Table 5. Positive immunostaining rates of immune markers according to staining features in the immunocytochemistry (ICC) study

	Number of applications	Positive immunostaining		P*
		n	%	
Nuclear staining ICC markers (TTF1, GATA3, ER, P40, P16, P63, WT1, CDX2)	87	77	88.5	0.99
Non-nuclear (cytoplasmic & membranous) staining ICC markers (PanCK, MOC31, Chromogranin, Calcitonin, CD68, PAX8, PAX5, CerbB2, BerEp4, Synaptophysin, CK7, CD3, CK5/6, Calretinin, PTH, S100, Thyroglobulin)	55	43	78.2	
Total	142	120	84.5	

*Chi Square test; ICC — immunocytochemistry

Table 6. The technical features of immune markers

Name	Clone	Dilution	Manufacture
TTF1	867G3/1	1:100	Cell-Marque
GATA3	L50-823	1:200	Cell-Marque
PanCK	AE1-AE3	1:200	BioGenex
PAX8	MRQ-50	1:100	Cell-Marque
ER	SP1	1:200	Cell-Marque
PR	SP2	1:100	Invitrogen
BerEp4	BerEp4	1:100	Cell-Marque
CerbB2	EP3	1:300	Epitomıcs
P40	P40M	1:1000	Bio-Care
Calcitonin	SP17	1:200	Abcam
Synaptophsin	MRQ-40	1:150	Cell-Marque
Chromogranin	LK2H10	1:300	Cell-Marque
CK7	OVTL-12/30	1:500	BioGenex
MOC31	Ep-CAM	1:150	Cell-Marque
CD3	CD3	1:200	Cell-Marque
PAX5	SP34	1:50	Roche
CD68	Ab-1/PG-M1	1:100	Abcam
CK5/6	D5/16B4	1:100	Cell-Marque
P63	IHC063-1	1:150	Gene AB
Calretinin	SP13	1:300	Cell-Marque
CDX2	EPR2764Y	1:100	Cell-Marque
PTH	MRQ-31	1:100	Cell-Marque
S100	4C4.9	1:200	Cell-Marque
Thyroglobulin	2H11+6E1	1:300	Cell-Marque
P16	INK4A	1:100	Gene AB
WT1	6F/H2	1:200	Cell-Marque

soft tissue, lung, and mediastinum, also showed the versatility of this technique. Interestingly, cases with ineffective staining were mostly effusion and lymph node specimens with low cell numbers. It was thought that the waiting time without the addition of fixative in the preanalytical process affected the ICC study, as 4 out of the 14 cases in which immunostaining was not observed were effusions.

In a study by Sharma *et al.*, the diagnostic performance of dual immunostaining for p16 and Ki-67 in SurePath-stained LBC cervico-vaginal samples was evaluated. They found dual positivity in most high-grade squamous intraepithelial lesion (HSIL) and squamous cell carcinoma (SCC) cases when comparing ICC positivity (78.8%) with Human papillomavirus (HPV) positivity [13]. Similarly, Song *et al*. applied

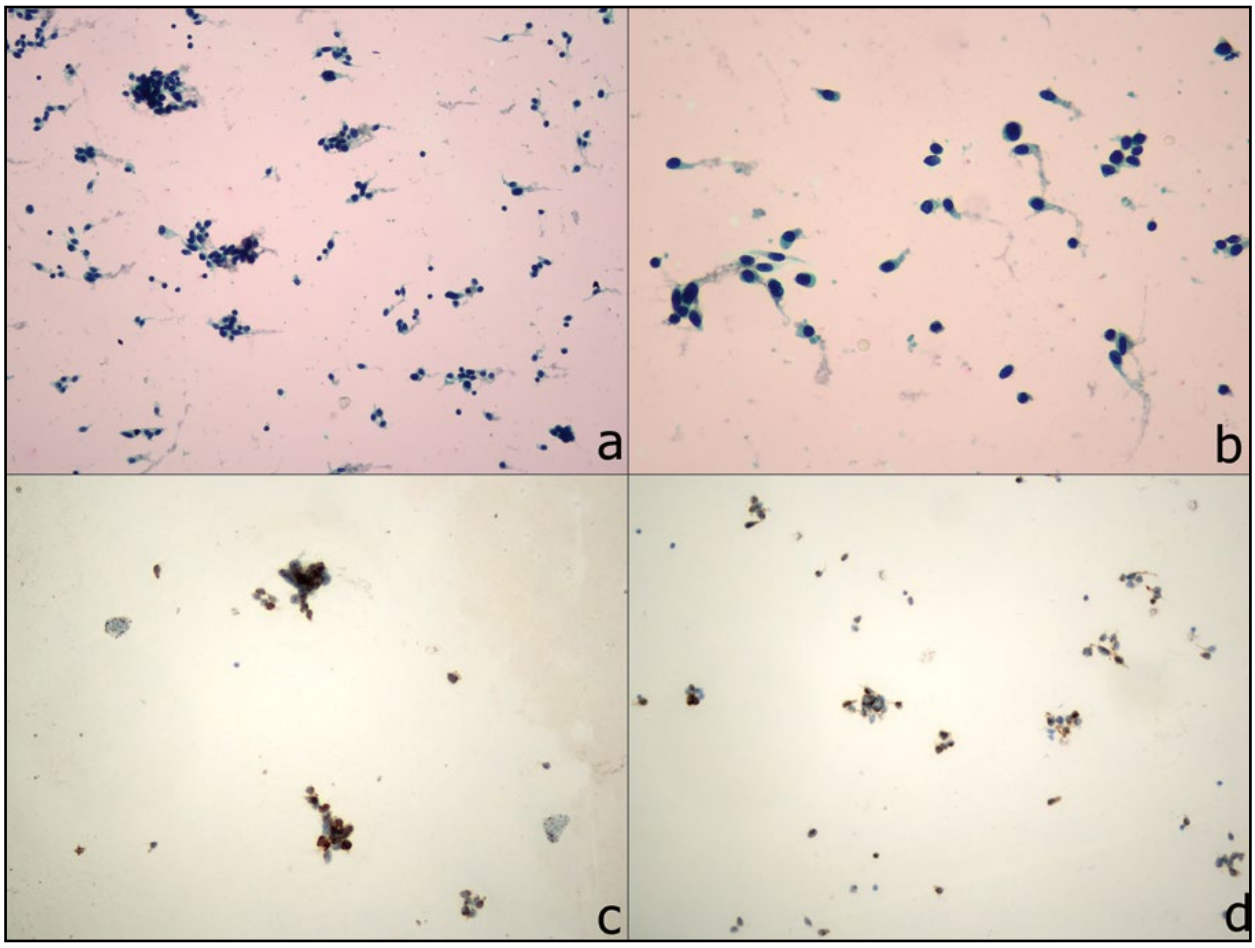

Figure 1. Case in which fine-needle aspiration (FNA) was performed from a 2 cm-wide thyroid nodule. There was no FFPE cell block. ICC staining was applied to slides obtained from residual liquid-based cytology (LBC) material. A diagnosis of medullary thyroid carcinoma was established. **A.** LBC, PAP staining, 200×. **B.** LBC, PAP, 400×. **C.** Calcitonin, 200×. **D.** Synaptophysin, 200×.

p16 to cervical smears with ICC study and suggested that p16INK4a immunocytology may be a favorable technique for cervical cancer screening [14].

Since cell blocks cannot generally be obtained in urine cytology, ICC studies are getting importance [15, 16]. Moon *et al.* [16], applied anti-human telomerase reverse transcriptase (hTERT) antibody (SCD-A7) to LBC slides obtained from urine samples and found very high sensitivity and specificity rates. The authors suggested that this antibody, when applied with ICC, was a valuable auxiliary test in detecting malignant urothelial cell.

Immunocytochemistry has also been used to distinguish normal and cancer cells in effusion cytology. In a study, LBC slides prepared from 110 serous effusion samples and a panel marker, comprising EMA (Epithelial membrane antigen), Ber-EP4 (Anti-EpCam antibody), and calretinin, were used to differentiate between cases of metastatic adenocarcinoma and benign mesothelium. The authors suggested that the serous effusion specimen collected by the modified LBC technique is an effective preparation method for ICC [5].

It is still uncertain whether prognostic and therapeutic markers such as Estrogen receptor (ER), Progesterone receptor (PR), and CerbB2 (HER2/neu, human epidermal growth factor receptor 2) can be studied in cytological material without a cell block. In a study where ER, PR, and CerbB2 were applied to LBC (Thinprep) slides and FFPE tissues of biopsy specimens obtained from breast cancer patients, LBC allowed easy preparation of slides, and ER/PR status showed good concordance with IHC. ICC for HER2 results also showed good agreement [17].

In our study, ICC was used to diagnose the primary tumor or determine the origin of metastases/cells and study prognostic factors such as ER, PR, and CerbB2 to LBC slides prepared with the SurePath system. The BD SurePath liquid contains ethanol (15–20%), < 1% isopropyl alcohol, < 1% methanol, and approximately 0.1% formaldehyde (Becton Dickinson Ltd., Wokingham, UK). In a study that investigated the

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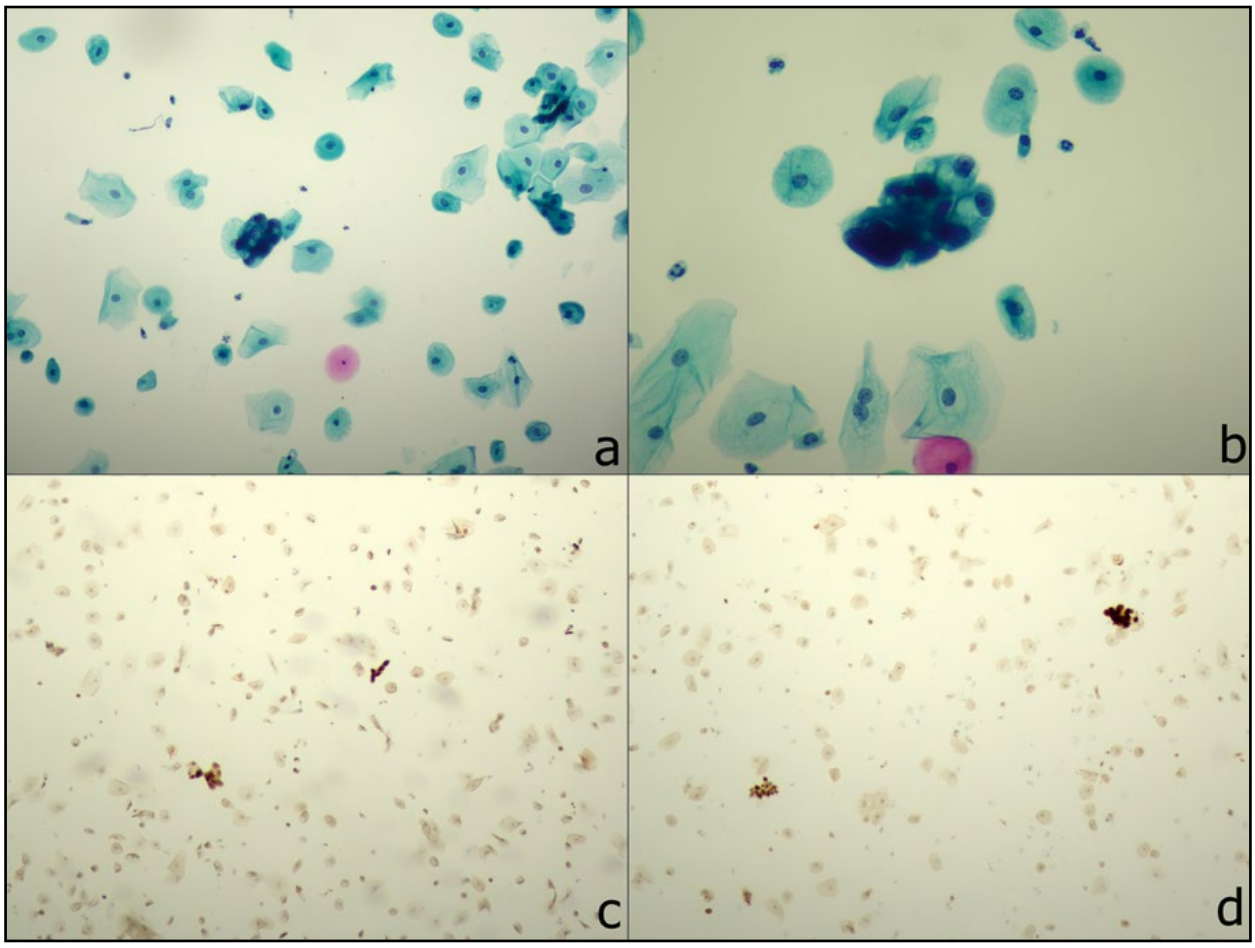

Figure 2. In the cervical smear sample, while the squamous epithelium was normal, tumor cells with high nucleocytoplasmic ratio and irregular nuclear contour as a glandular structure were observed. In the ICC study, these tumor cells were positive for WT1 and PAX8 staining. The LBC was diagnosed as metastasis in a patient with known ovarian carcinoma. **A.** LBC, PAP staining, 200×. **B.** LBC, PAP, 400×. **C.** WT1, 200×; **D.** PAX8, 200×.

immunoreactivity of cells stored for different lengths of time in the SurePath liquid, the immunostaining intensity was observed to decrease within 5 days of storage [18]. The authors reported that small amounts of formaldehyde in SurePath fluid affect immunoreactivity, and cytology laboratories should consider this when optimizing their procedures [18]. In our study, there was no significant difference between the lag time groups in obtaining positive immunostaining up to 20 days.

In a recent study the relationship between the LBC (SurePath) fixing solution and ICC and the usefulness of antigen retrieval in LBC specimens of cell lines has been investigated [19]. Insufficient reactivity was observed without heat-induced antigen retrieval in the staining of nuclear antigens. The percentage of positive cells was lower in certain LBC samples for Ki-67, estrogen receptor, and p63. For cytoplasmic antigens, the percentage of positive cells without antigen retrieval treatment was low. However, the number of positive cells increased in all LBC specimens with antigen retrieval for cytokeratin 5/6 (CK 5/6). For cell membrane antigens, certain LBC samples had a lower percentage of positive cells. The authors conclude that ICC using LBC specimens is a useful technique, but the staining conditions should be examined before performing ICC [19]. In our study CytoRich Red was used as the fixative for all samples. Our analysis of 142 LBC slides stained by ICC with 26 markers revealed that nuclear markers exhibited higher positive immunostaining compared to non-nuclear markers (cytoplasmic/membranous), although there was no statistically significant difference between the two groups. This suggests the need for continued scrutiny of ICC marker selection and its implications for immunostaining.

There were some limitations in our study. One of these is that although the number of antibodies used is high, the number of cases is low. Additionally, very few of some antibodies could be used. These limits making comments specifically on these antibodies.

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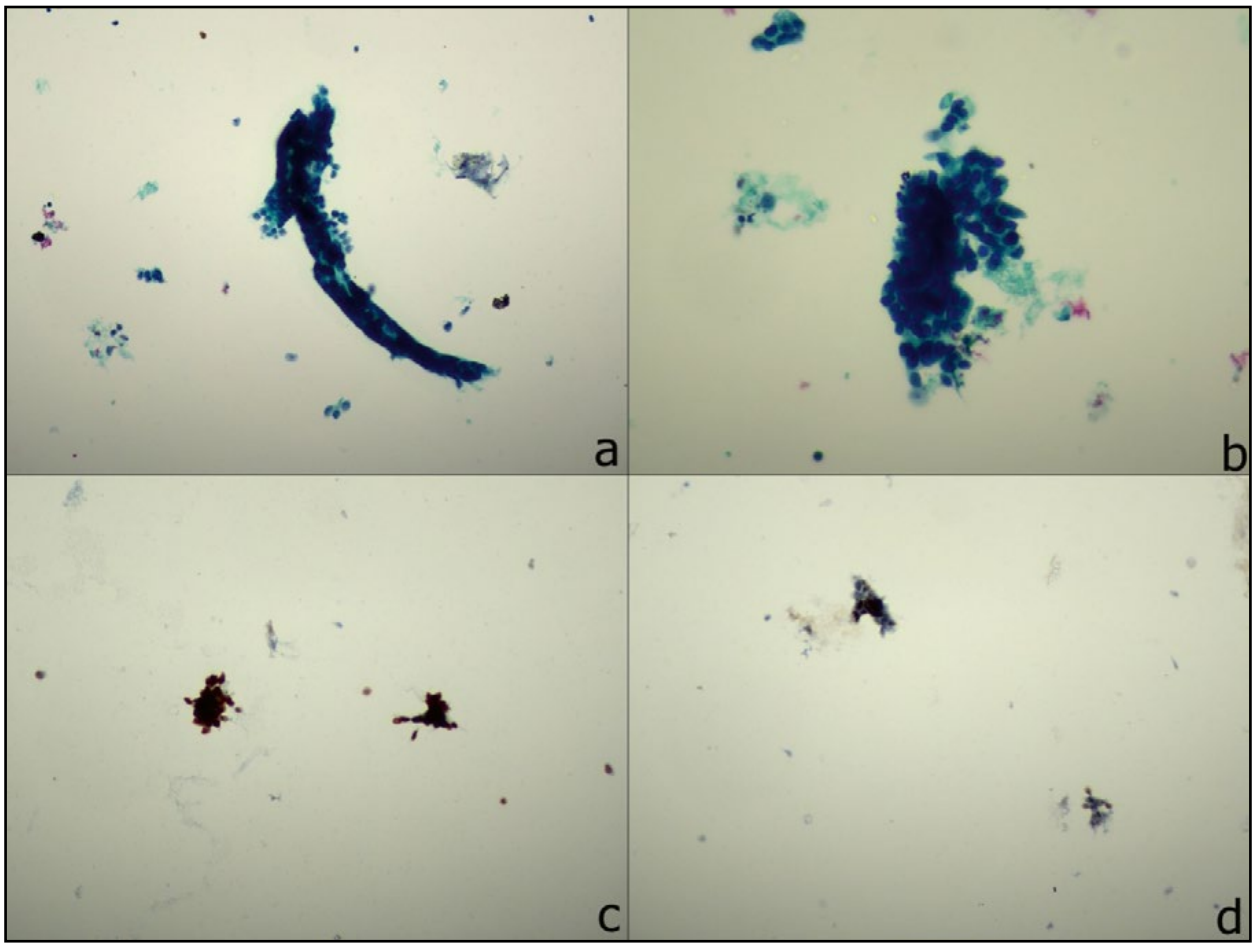

Figure 3. In the axillary lymph node FNA sample metastatic tumor cells as cohesive groups were seen. In the ICC study, these tumor cells were positive for GATA3 and estrogen receptors (ER). The patient with known breast carcinoma was diagnosed as metastasis of this tumor. **A.** LBC, PAP staining, 200×. **B.** LBC, PAP, 200×. **C.** GATA3, 200×. **D.** ER, 200×.

If the diagnostic utility is evaluated in more cases with a single specific marker in further studies, a more specific interpretation can be made on the waiting time of LBC residual material.

In conclusion, our study showed that positive immunostaining was obtained in 81% of cases when ICC was applied to additional LBC slides from residual LBC material. Our study has supported that ICC application to additional LBC slides from residual LBC material in cytology samples where a cell block is not formed or there are not enough cells in the block can be safely applied for up to 20 days. This finding may contribute to the primary diagnosis of tumors, metastasis, distinguish of cell origin or could be used for prognostic factors such as ER, PR, and CerbB2.

Article information and declarations

Data availability statement

The authors elect not to share data.

Ethics statement

The study was approved by the Ethics Committee of the Istanbul Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Istanbul, Turkey. (Approval number:2023-391, date: 26.09.2023) and conducted following the Declaration of Helsinki.

Author contributions

Conceptualization — OY, SED; methodology — SED, EY; formal analysis — SED, EY; investigation — OY, SED; data curation — SED, OY; writing — original draft preparation — SED, EY; writing — review and editing — SED, OY, EY.

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Conflict of interest

The authors declare that there is no conflict of interest.

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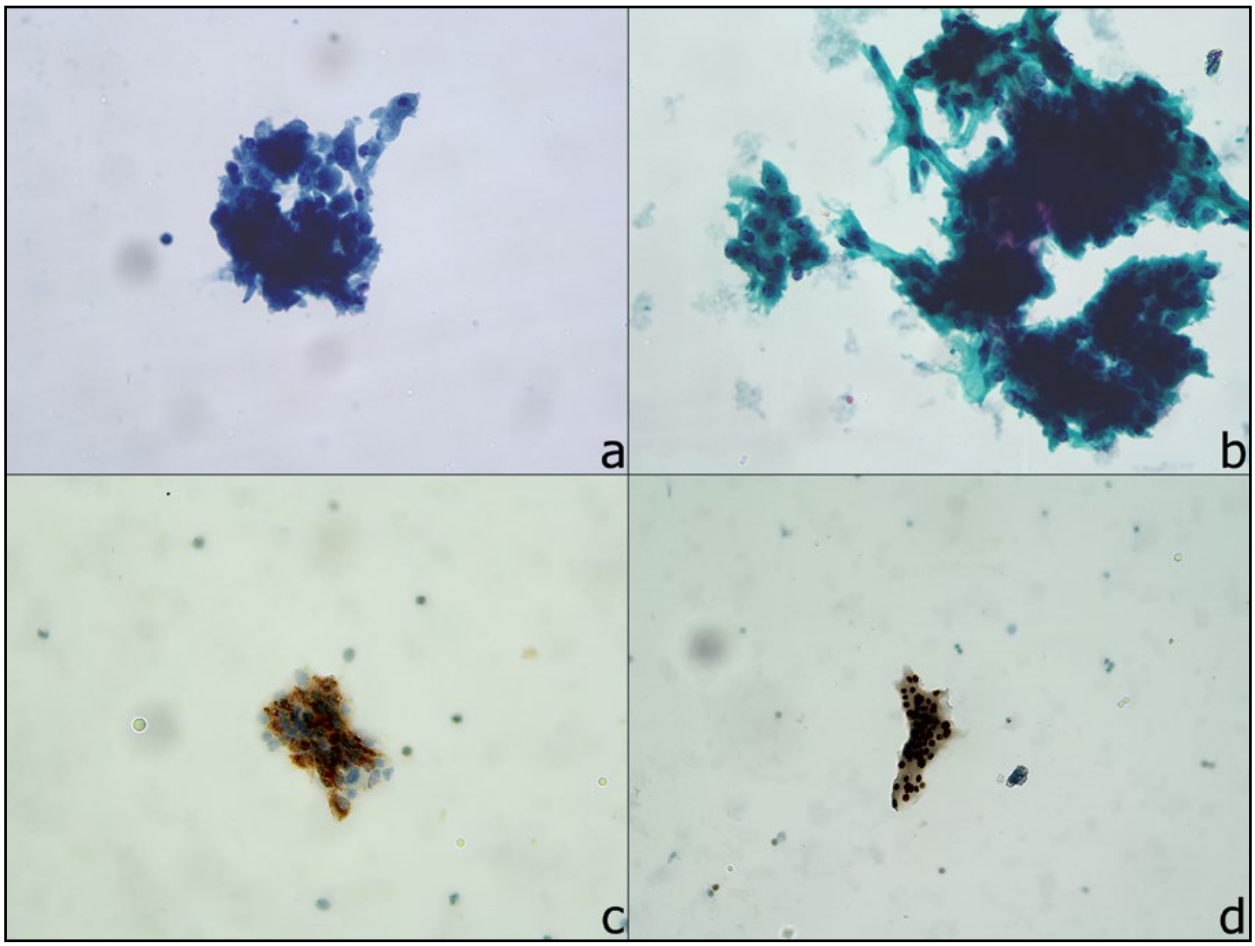

Figure 4. Fine-needle aspiration sample of the parathyroid gland. The parathyroidal cells with large cytoplasm and salt pepper chromatin as cohesive structures were seen. In the ICC study, these cells were positive for PTH (parathyroid hormone) and GATA3. The origin of the cells was determined by ICC. **A.** LBC, PAP staining, 200×. **B.** LBC, PAP, 400×. **C.** PTH, 200×. **D.** GATA3, 200×.

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