







A Multi-Institutional Study of Salivary Gland Cytopathology: Application of the Milan System for Reporting Salivary Gland Cytopathology in Japan

Kayoko Higuchi, MD, PhD ¹; Makoto Urano, MD, PhD²; Jun Akiba, MD, PhD ³; Miwako Nogami, CT⁴; Yukiya Hirata, CT⁵; Yoko Zukeran, CT⁵; Koki Moriyoshi, MD, PhD⁶; Yuichiro Tada, MD, PhD ⁷; Mana Fukushima, MD, PhD⁸; Mariko Obayashi, DDS, PhD⁹; Shinnichi Sakamoto, DDS, PhD¹⁰; Kazuya Kuraoka, MD, PhD¹⁰; Kana Kira, CT¹¹; Akihiko Kawahara, CT, PhD ³; Taku Kato, CT¹²; Maki Tanigawa, DDS, PhD¹³; Masato Nakaguro, MD, PhD ¹⁴; Hidetaka Yamamoto, MD, PhD⁴; and Toshitaka Nagao, MD, PhD ¹³

BACKGROUND: The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) is a risk-stratification reporting system that was introduced in 2018. The objective of this multi-institutional study was to evaluate the utility of the MSRSGC in Japan. **METHODS:** In total, 1608 fine-needle aspiration samples with matching histologic diagnoses were retrieved from 12 large institutions in Japan. The diagnostic categories of the MSRSGC were assigned prospectively or retrospectively, and the results were compared with the histologic diagnoses. **RESULTS:** The cases were classified as follows: nondiagnostic, 18.1%; non-neoplastic, 4.1%; atypia of undetermined significance, 11.5%; neoplasm-benign, 43.7%; salivary gland neoplasm of uncertain malignant potential, 9.6%; suspicious for malignancy, 3.6%; and malignant, 9.4%. The risk of neoplasm and the risk of malignancy in each MSRSGC category were as follows: nondiagnostic, 72.9% and 13.4%, respectively; non-neoplastic, 15.2% and 9.1%, respectively; atypia of undetermined significance, 77.9% and 24.9%, respectively; neoplasm-benign, 99% and 1.8%, respectively; salivary gland neoplasm of uncertain malignant potential, 94.8% and 37%, respectively; suspicious for malignancy, 100% and 89.7%, respectively; and malignant, 100% and 99.3%, respectively. The accuracy of the MSRSGC for diagnosing neoplasms was 97.8%, and its accuracy for diagnosing malignancy was 97.3%. Institutions that used Romanowsky-stained preparations had lower nondiagnostic rates and lower risks of neoplasm and malignancy in the non-neoplastic category. **CONCLUSIONS:** The MSRSGC is useful for risk stratification and quality control. Widespread use of the MSRSGC would improve the accuracy of salivary gland cytology and lead to better patient care in Japan. *Cancer Cytopathol* 2021;0:1-11. © 2021 The Authors. *Cancer Cytopathology* published by Wiley Periodicals LLC on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

KEY WORDS: fine-needle aspiration; Japan; multi-institutional study; salivary gland cytopathology; the Milan system.

Corresponding Author: Kayoko Higuchi, MD, PhD, Department of Surgical Pathology, Okinawa Kyodo Hospital, 4-10-55 Kohagura, Naha, Okinawa, 900-8558, Japan (kayopathhiguchi@yahoo.co.jp).

Mana Fukushima's current address: Department of Surgical Pathology, University of Fukui Hospital, Eiheiji, Japan

¹Department of Surgical Pathology, Okinawa Kyodo Hospital, Naha, Japan; ²Department of Diagnostic Pathology, Bantane Hospital, Fujita Health University, Nagoya, Japan; ³Department of Diagnostic Pathology, Kurume University Hospital, Kurume, Japan; ⁴Division of Diagnostic Pathology, Kyushu University Hospital, Fukuoka, Japan; ⁵Department of Pathology, University of the Ryukyus Hospital, Nishihara, Japan; ⁶Department of Diagnostic Pathology, Kyoto Medical Center, Kyoto, Japan; ⁷Department of Head and Neck Oncology and Surgery, International University of Health and Welfare, Mita Hospital, Tokyo, Japan; ⁸Department of Surgical Pathology, Ehime University Hospital, Toon, Japan; ⁹Department of Diagnostic Pathology, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima, Japan; ¹⁰Department of Diagnostic Pathology, Kure Medical Center and Chugoku Cancer Center, Kure, Japan; ¹¹Department of Diagnostic Pathology, Kochi University Hospital, Nangoku, Japan; ¹²Department of Pathology, Naritatomisato Tokushukai Hospital, Tomisato, Japan; ¹³Department of Anatomic Pathology, Tokyo Medical University, Tokyo, Japan; ¹⁴Department of Pathology and Laboratory Medicine, Nagoya University Hospital, Nagoya, Japan

See related editorial on pages 1-2, this issue.

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INTRODUCTION

Salivary gland fine-needle aspiration cytology (FNAC) is a useful tool for preoperatively diagnosing salivary gland lesions. It can be used to differentiate neoplastic salivary gland lesions from non-neoplastic lesions and benign tumors from highly malignant tumors.¹⁻³ However, because of the inherent complexity of interpreting salivary gland FNAC findings and the lack of a uniform reporting format, salivary gland FNAC performance varies among institutions.⁴

The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) was introduced in 2018 and is an evidence-based classification scheme for addressing these issues with salivary gland cytology.⁵⁻⁷ It provides the risk of malignancy (ROM) for 7 diagnostic categories, along with recommendations for clinical management. The 7 diagnostic categories are nondiagnostic (ND), non-neoplastic (NN), atypia of undetermined significance (AUS), neoplasm-benign (NB), salivary gland neoplasm of uncertain malignant potential (SUMP), suspicious for malignancy (SFM), and malignant (M).⁵⁻⁷

Use of the MSRSGC is currently spreading throughout the world, and many studies about its application in Asian countries, including Japan, have been published.⁸⁻¹¹ Salivary gland tumors are as uncommon in Japan, where they represent 6.1% of head and neck cancers and 0.2% of all malignancies, as they are in other countries; eg, they account for 6% of head and neck cancers and 0.3% of all malignancies in the United States.^{12,13} Consequently, no large-scale studies of salivary gland FNAC have been conducted in Japan. The study reported herein was 1 of the largest studies of the MSRSGC in the world^{7-11,14-19} and the first multi-institutional study of the application of the MSRSGC in Japan. With the support of the Japanese Society of Clinical Cytology, we demonstrated the usefulness of the MSRSGC in the diagnosis of salivary gland lesions and for the quality control of salivary gland cytology results.

MATERIALS AND METHODS

This study was conducted after approval was obtained from each institutional review board. It was conducted prospectively at Kyushu University Hospital and retrospectively at the other institutions. The cytology archives of 12 institutions in Japan were electronically searched for cases of salivary gland lesions that were treated

between 2007 and 2019 for which salivary gland FNAC samples and matching histologic diagnoses were available. Each institution was numbered as follows: Tokyo Medical University, 1; Kurume University Hospital, 2; Kyushu University Hospital, 3; Fujita Health University, 4; Naritatomisato Tokushukai Hospital, 5; Kyoto Medical Center, 6; Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, 7; Kochi University Hospital, 8; Ehime University Hospital, 9; University of the Ryukyus Hospital, 10; Mita Hospital (International University of Health and Welfare), 11; and Kure Medical Center and Chugoku Cancer Center, 12. The puncture sites were grouped into the parotid gland, the submandibular gland, and others. FNAC was performed with 21-gauge to 25-gauge needles and involved 1 to 3 passes. The specimens were processed using the direct smear and/or the liquid-based cytology method and were stained with Papanicolaou stain or with both Papanicolaou and Romanowsky stains. All FNAC specimens from each institute were processed by the protocol shown in Table 1. Ancillary immunocytochemistry was performed in a total of 15 cases. The MSRSGC was applied to all cases by cytopathologists and/or cytotechnologists at each institution by reviewing glass slides. We also assessed the performance of the MSRSGC by calculating its sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) for diagnosing malignancy and neoplasms. In our analysis of the ability of the MSRSGC to diagnose malignancy, we considered the SFM and M categories to be positive and the NN and NB categories to be negative. In our analysis of the ability of the MSRSGC to diagnose neoplasms, we considered the NB, SUMP, SFM, and M categories to be positive and the NN category to be negative. We also characterized AUS, SUMP, false-positive cases, and false-negative cases, and nonmucinous cysts. The Student *t* test was used to compare the average values of continuous variables between 2 groups.

RESULTS

In total, 1608 cases from 12 institutions were analyzed, with 44 to 331 cases collected from each institution. The mean age of the patients was 56.4 years (range, 0-96 years), and the male-to-female ratio was 728/715 (1.02). The parotid gland accounted for the largest percentage of puncture sites (80.8%), followed by the submandibular gland

TABLE 1. Case Demographics and Fine-Needle Aspiration Sample Preparation

Institution	Case No.	Age (Range), y	Sex: Male/Female	Puncture Site: P/SM/O	Pap	Romanowsky	LBC
1	331	54.8 (0-96)	163/168	257/70/4	Yes	Yes	
2	121	57.1 (15-90)	68/53	104/17/0	Yes	Yes	Yes
3	44	62.0 (22-92)	19/25	32/8/4	Yes	Yes	
4	72	53.9 (11-91)	37/35	43/12/17	Yes	Yes	Yes
5	165	NA	NA	NA	Yes	Yes	
6	142	50.2 (14-85)	65/77	115/27/0	Yes	Yes	
7	107	61.7 (23-87)	62/45	87/16/4	Yes	Yes	
8	53	63.7 (25-92)	28/25	43/10/0	Yes		Yes
9	111	61.0 (16-89)	60/51	80/26/5	Yes		Yes
10	216	52.4 (11-90)	98/118	188/27/1	Yes		
11	146	57.6 (23-87)	71/75	117/27/2	Yes		
12	100	61.5 (24-88)	57/43	100/0/0	Yes	Yes	
Total	1608	56.4 (0-96)	728/715	1166/240/37			
			Ratio, 1.02:1.00	80.8%/16.6%/2.6%			

Abbreviations: LBC, liquid-based cytology; NA, not available; O, others; P, parotid gland; Pap, Papanicolaou staining; SM, submandibular gland.

(16.6%), and other sites (2.6%). Demographic data and information about the FNAC sample preparation methods for each institution are summarized in Table 1.

The 1608 FNAC samples were classified as follows based on the MSRSGC: ND, 291 cases (18.1%); NN, 66 cases (4.1%); AUS, 185 cases (11.5%); NB, 703 cases (43.7%); SUMP, 154 cases (9.6%); SFM, 58 cases (3.6%); and M, 151 cases (9.4%) (Table 2). The matching histologic diagnoses were as follows: 1054 benign neoplasms (65.5%), 363 malignant neoplasms (22.6%), and 191 non-neoplastic lesions (11.8%). The RON and ROM for each category were as follows: ND, 72.9% and 13.4%, respectively; NN, 15.2% and 9.1%, respectively; AUS, 77.9% and 24.9%, respectively; NB, 99% and 1.8%, respectively; SUMP, 94.8% and 37%, respectively; SFM, 100% and 89.7%, respectively; and M, 100% and 99.3%, respectively (Table 2).

When the AUS and SUMP categories were excluded, the sensitivity, specificity, and accuracy of the MSRSGC for diagnosing malignancy were 91.4%, 99.1%, and 97.3%, respectively (Table 3). The PPV and NPV of the MSRSGC for diagnosing malignancy were 96.7% and 97.5%, respectively (Table 3). When the AUS category was excluded, the sensitivity, specificity, accuracy, PPV, and NPV of the MSRSGC for diagnosing neoplasms were 99.1%, 78.9%, 97.8%, 98.6%, and 84.8%, respectively (Table 4).

The characteristics of the AUS cases are summarized in Table 5. Among the 185 lesions classified as AUS, 41 (22.2%) were non-neoplastic lesions, 98 (53%) were benign neoplasms, and 46 (24.9%) were malignant neoplasms. Warthin tumors were the most common type of tumor in the AUS category (59 cases; 31.9%), followed by

pleomorphic adenoma (PA) (25 cases; 13.5%), and malignant lymphoma (23 cases; 12.4%) (Table 5). Malignant lymphoma was the most common malignant lesion in the AUS category. The malignant lymphomas included 8 mucosa-associated lymphoid tissue lymphomas, 7 diffuse large B-cell lymphomas, 6 follicular lymphomas, 1 adult T-cell leukemia/lymphoma, and 1 Burkitt lymphoma. The most common type of carcinoma among the AUS cases was mucoepidermoid carcinoma (Table 5).

The characteristics of the 154 SUMP cases are summarized in Table 6. There were 8 (5.2%) non-neoplastic lesions, 89 (57.8%) benign neoplasms, and 57 (37%) malignant neoplasms. The most common neoplasm in the SUMP category was PA (41 cases; 26.6%), followed by basal cell adenoma (31 cases; 20.1%), mucoepidermoid carcinoma (10 cases; 6.5%), adenoid cystic carcinoma (9 cases; 5.8%), and Warthin tumor (9 cases; 5.8%). The most common non-neoplastic lesion was nodular oncocytic hyperplasia (Table 6).

In the ND category, there were 48 nonmucinous cysts (16.5%). Of these, 50% were non-neoplastic lesions, and the rest were neoplasms. Lymphoepithelial cysts (22 cases; 45.8%) were the most common type of non-mucinous cyst, followed by Warthin tumors (14 cases; 29.2%), PAs (5 cases; 10.4%), and basal cell adenomas (3 cases; 6.3%). The only malignant nonmucinous cyst according to FNAC was a secretory carcinoma (Table 7).

There were 7 false-positive cases, including 4 cases of PA and 3 cases of basal cell adenoma (Table 8). The cases of PA in the SFM category involved bizarre myoepithelial cells or oncocytic metaplasia. There were 19 false-negative cases (Table 9). The most common types were carcinoma ex PA and low-grade mucoepidermoid

TABLE 2. Results of the Reclassification of Salivary Gland Lesions Using the Milan System for Reporting Salivary Gland Cytopathology

Results	Histologic Diagnosis, No.				Total No. of Cases (%) [Range]	RON, % [Range]	ROM, % [Range]	MSRSGC ROM, %
	Non-Neoplastic	Neoplasm, Benign	Malignant					
Nondiagnostic	79	173	39		291 (18.1) [4.7-34.2]	72.9 [51.3-90.9]	13.4 [0.0-41.7]	25.0
Non-neoplastic	56	4	6		66 (4.1) [0-7.7]	15.2 [0.0-62.5]	9.1 [0.0-50.0]	10.0
AUS	41	98	46		185(11.5) [4.1-25.2]	77.9 [54.5-100.0]	24.9 [3.7-100.0]	20.0
Neoplasm, benign	7	683	13		703 (43.7) [30.2-52.7]	99 [95.1-100.0]	1.8 [0.0-6.3]	<5.0
SUMP	8	89	57		154 (9.6) [3.6-15.7]	94.8 [80.0-100.0]	37.0 [18.2-57.1]	35.0
SFM	0	6	52		58 (3.6) [0-5.7]	100 [100.0]	89.7 [75.0-100.0]	60.0
Malignant	0	1	150		151 (9.4) [5.6-14.4]	100 [100.0]	99.3 [90.0-100.0]	90.0
Total no. (%)	191 (11.8)	1054 (65.5)	363 (22.6)	1608				

Abbreviations: AUS, atypia of undetermined significance; MSRSGC, Milan System for Reporting Salivary Gland Cytopathology; ROM, risk of malignancy; RON, risk of neoplasm; SFM, suspicious for malignancy; SUMP, salivary gland neoplasm of uncertain malignant potential.

TABLE 3. Performance Characteristics of the Milan System for Reporting Salivary Gland Cytopathology for Detecting Malignancy

Performance Characteristic	Rate, %
Sensitivity	91.4
Specificity	99.1
Accuracy	97.3
PPV	96.7
NPV	97.5

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

TABLE 4. Performance Characteristics of the Milan System for Reporting Salivary Gland Cytopathology for Detecting Neoplasms

Performance Characteristic	Rate, %
Sensitivity	99.1
Specificity	78.9
Accuracy	97.8
PPV	98.6
NPV	84.8

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

carcinoma (5 cases each), followed by adenoid cystic carcinoma and malignant lymphoma (3 cases each). Four of the cases of carcinoma ex PA had small malignant components, and the malignant lymphomas included 2 cases of mucosa-associated lymphoid tissue lymphoma and 1 case of follicular lymphoma.

Next, we analyzed the effects of using Romanowsky staining in addition to Papanicolaou staining for salivary FNAC. Whereas 1082 cases from 8 institutions were diagnosed with Romanowsky staining, 526 cases from 4 institutions were diagnosed without it (Table 1). The RON for each MSRSGC diagnostic category at the institutions that did and did not use Romanowsky staining, respectively, were as follows: ND, 72.3% versus 69.7%; NN, 8.9% versus 28.6%; AUS, 73.1% versus 89.1%; NB, 99% versus 99%; SUMP, 93.3% versus 96.9%; SFM, 100% versus 100%; and M, 100% versus 100% (see Supporting Table 1). The ROM for each MSRSGC category at the institutions that did and did not use Romanowsky staining, respectively, were as follows: ND, 12.8% versus 13.8%; NN, 4.4% versus 19%; AUS, 25.4% versus 23.6%; NB, 2% versus 1.5%; SUMP, 37.8% versus 39.1%; SFM, 87.8% versus 94.2%; and M, 98.9% versus 100% (see Supporting Table 1).

DISCUSSION

Japan's universal health insurance system makes it easy for patients with neck masses to visit medical institutions.

TABLE 5. Characterization of Atypia of Undetermined Significance Cases

Histologic Category	Histologic Diagnosis	No. (%)	
Non-neoplastic	Lymphoepithelial cyst	14	
	Lymphoepithelial lesion	9	
	IgG4-related sialadenitis	6	
	chronic sialadenitis	3	
	Granulation	2	
	Lymphoid hyperplasia	2	
	Fibrosis	1	
	Necrosis	1	
	Nodular oncocytic hyperplasia	1	
	Ranula	1	
	Normal	1	
Subtotal		41 (22.2)	
Benign neoplasm	Warthin tumor	59	
	Pleomorphic adenoma	25	
	Basal cell adenoma	4	
	Lymphadenoma	4	
	Oncocytoma	1	
	Myoepithelioma	1	
	Calcifying epithelioma	1	
	Lipoma	1	
	Schwannoma	1	
	Cystadenoma	1	
	Subtotal		98 (53.0)
	Malignant	Malignant lymphoma	23
Mucoepidermoid carcinoma		7	
Acinic cell carcinoma		3	
Secretory carcinoma		3	
Carcinoma ex pleomorphic adenoma		2	
Squamous cell carcinoma		2	
Small cell carcinoma		1	
Epithelial-myoepithelial carcinoma		1	
Salivary duct carcinoma		1	
Adenoid cystic carcinoma		1	
Carcinosarcoma		1	
Metastatic squamous cell carcinoma	1		
Subtotal		46 (24.9)	
Total		185	

When a salivary gland tumor is suspected, ultrasound-guided FNAC (generally involving 1-3 passes) is usually performed followed by computed tomography and magnetic resonance imaging. The Japanese System for Reporting Salivary Gland Cytopathology (JSRSGC) has long been used for salivary FNAC diagnosis in Japan.²⁰ The JSRSGC consists of 5 categories: nondiagnostic, normal or benign, indeterminate, suspicious for malignancy, and malignant. The JSRSGC can be used to stratify salivary gland lesions according to the ROM, but it is not effective at stratifying them according to the RON (see Supporting Table 2). The MSRSGC was proposed as a tool for stratifying salivary gland lesions according to the RON and the ROM and provides recommendations for clinical management. The National Comprehensive Cancer Network guidelines recommend that all salivary

TABLE 6. Characterization of Salivary Gland Neoplasm of Uncertain Malignant Potential Cases

Histologic Category	Histologic Diagnosis	No. (%)
Non-neoplastic	Nodular oncocytic hyperplasia	5
	IgG4-related sialadenitis	2
	Normal	1
Subtotal		8 (5.2)
Benign neoplasm	Pleomorphic adenoma	41
	Basal cell adenoma	31
	Warthin tumor	9
	Myoepithelioma	8
Subtotal		89 (57.8)
Malignant	Mucoepidermoid carcinoma	10
	Adenoid cystic carcinoma	9
	Epithelial-myoepithelial carcinoma	7
	Carcinoma ex pleomorphic adenoma	7
	Myoepithelial carcinoma	4
	Basal cell adenocarcinoma	3
	Secretory carcinoma	2
	Polymorphous adenocarcinoma	2
	Adenocarcinoma, NOS	2
	Acinic cell carcinoma	2
	Intraductal carcinoma	2
	MALT lymphoma	2
	Metastatic carcinoma	1
	Salivary duct carcinoma	1
	Squamous cell carcinoma	1
	Sebaceous adenocarcinoma	1
	Carcinoma, NOS	1
Subtotal		57 (37.0)
Total		154 (100.0)

Abbreviations: MALT, mucosa-associated lymphoid tissue; NOS, not otherwise specified.

TABLE 7. Characterization of Nonmucinous Cysts

Histologic Category	Histologic Diagnosis	No. (%)
Non-neoplastic	Lymphoepithelial cyst	22 (45.8)
	Retention cyst	2 (4.2)
Subtotal Neoplasm		24 (50.0)
Benign	Warthin tumor	14 (29.2)
	Pleomorphic adenoma	5 (10.4)
	Basal cell adenoma	3 (6.3)
	Schwannoma	1 (2.1)
Malignant	Secretory carcinoma	1 (2.1)
Subtotal		24 (50.0)
Total		48 (100.0)

gland tumors, including both benign and malignant tumors, should be resected.²¹ Although the JSRSGC demonstrates a good correlation with the MSRSGC (see Supporting Table 3), the MSRSGC is a more practical cytologic reporting system and ensures that appropriate patient care is provided.

The sex and anatomic site distributions seen in the current study were similar to those reported in previous studies (Table 1).^{7,14-17,19} In contrast to many other studies, the current study included 4 institutions

TABLE 8. False-Positive Cases in the Malignant Category

Histology	Category of MSRSGC		Total No.
	Suspicious for Malignancy (No.)	Malignancy (No.)	
Pleomorphic adenoma	Acinic cell carcinoma (2) Carcinoma ex pleomorphic adenoma (2)		4
Basal cell adenoma	Acinic cell carcinoma (1)	Adenoid cystic carcinoma (2)	3
Total no.	5	2	7

Abbreviation: MSRSGC, Milan System for Reporting Salivary Gland Cytopathology.

TABLE 9. False-Negative Cases in the Benign Category

Histology	Category of MSRSGC		Total No.
	Non-Neoplastic (No.)	Neoplasm, Benign (No.)	
Carcinoma ex pleomorphic adenoma		Pleomorphic adenoma (5)	5
Mucoepidermoid carcinoma	Inflammation (1)	Pleomorphic adenoma (3) Warthin tumor (1)	5
Adenoid cystic carcinoma		Pleomorphic adenoma (2) Basal cell adenoma (1)	3
Malignant lymphoma	Lymphoid hyperplasia (3)		3
Salivary duct carcinoma	Reactive lesion (1)		1
Polymorphous adenocarcinoma		Pleomorphic adenoma (1)	1
Metastatic carcinoma, NOS	Inflammation (1)		1
Total no.	6	13	19

Abbreviations: MSRSGC, Milan System for Reporting Salivary Gland Cytopathology; NOS, not otherwise specified.

that did not perform Romanowsky staining for FNAC (Table 1).^{7-9,14,15,17}

In total, 291 cases (18.1%) were categorized as ND (Table 2), which was higher than the percentage (10%) recommended in the MSRSGC²² and in many other previous studies.^{7,9,14-16,18} In the current study, the frequency of ND cases varied among the institutions (range, 4.7%-34.2%) (Table 2; see Supporting Table 4). The percentage of ND cases is affected by various factors, including the operator's technique, the appearance of the tumor, and the specimen-processing methods used, eg, the staining method(s) used for FNAC specimens. Romanowsky staining of air-dried materials results in better specimen preservation and is useful for detecting metachromatic stromal mucin in myoepithelial salivary gland tumors.^{6,23} In this study, the mean percentage of ND cases tended to be lower at the facilities that used Romanowsky-stained preparations compared with those that only used Papanicolaou staining (14.1% vs 21.9%) (see Supporting Table 4), but the difference was not statistically significant ($P = .19$). Rapid on-site evaluation is another well known method for reducing the percentage of ND cases on FNAC.^{6,18} However, in Japan, rapid on-site evaluation is only performed at a limited number of facilities because of manpower shortages. It was only

performed at 1 facility in the current study (institution 5), where the frequency of ND lesions was as low as 7.3%. Among the ND cases, 48 lesions (16.5%) were classified as nonmucinous cysts (Table 7). The MSRSGC classifies nonmucinous cysts as ND rather than as non-neoplastic lesions²² because some of these cysts may be neoplastic cystic lesions. In our study, 50% of the nonmucinous cysts were of neoplastic origin, confirming the validity of the MSRSGC. A recent report revealed that 29.2% of cystic FNAC specimens were reclassified as ND when the MSRSGC was applied.²⁴ Pantanowitz et al²⁵ reported the utility of classifying the FNAC findings of cystic lesions based on the presence or absence of mucin and admixed lymphocytes. Our results support their proposal because approximately one-half of the nonmucinous cysts were derived from lymphoepithelial cysts.

In the NN category, the RON and ROM for NN lesions tended to be lower at the facilities that used Romanowsky-stained preparations compared with those that only used Papanicolaou staining (8.9% vs 28.6% and 4.4% vs 19%, respectively) (see Supporting Table 1). However, the differences were not statistically significant ($P = .31$ and $P = .57$ for RON and ROM, respectively). Romanowsky staining might be beneficial to facilitate the correct classification of neoplasms partly because it

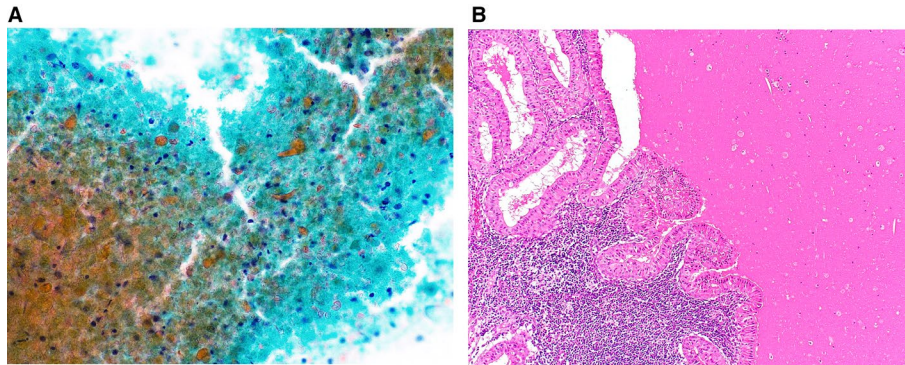


Figure 1. (A) Cytological features of a Warthin tumor that was categorized as atypia of undetermined significance. Only a few degenerated columnar cells and degenerated lymphocytes can be seen on a dirty background (Papanicolaou stain, original magnification $\times 400$). (B) The histologic specimen shows a Warthin tumor (H&E stain, original magnification $\times 100$).

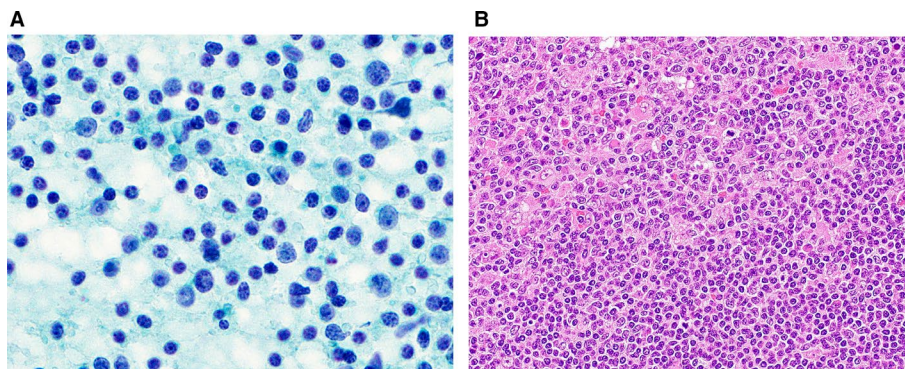


Figure 2. (A) Cytological features of a case of T-cell lymphoma that was categorized as atypia of undetermined significance. The aspirate shows a mixed lymphoid pattern with small, intermediate-sized, and large lymphocytes without distinct atypia (Papanicolaou stain, original magnification $\times 1000$). (B) The histologic specimen shows a transition from an area of non-neoplastic, small lymphocytes (lower right) to an area of atypical neoplastic lymphocytes (upper left; H&E stain, original magnification $\times 400$).

prevents the underdiagnosis of neoplastic lesions, including malignant tumors. It is important to highlight the advantages of using Romanowsky staining in combination with Papanicolaou staining for salivary FNAC in Japan.

In total, 185 cases were categorized as AUS (Table 5). Warthin tumors were the most common type of tumor (59 cases) in the AUS category, accounting for 31.9% of all AUS cases. Japanese studies tend to include more Warthin tumors in the AUS category^{11,26} (range, 41.9%-50%) than those from other countries (range, 5.6%-16.7%).¹⁵⁻¹⁷ Cytologic specimens of typical Warthin tumors show oncocytic cell clusters with lymphocytes on a necrotic background, and such cases can be diagnosed by cytology with a high degree of certainty.²⁷ However, aspiration cytology often yields necrotic material containing only degenerated columnar cells (Fig. 1) or a few oncocytic cells.²⁸ Such FNAC specimens tend to

be classified as AUS in Japan, although these features are characteristic of Warthin tumors.²⁸ For these reasons, the RON in the AUS category tended to be higher than that reported in previous studies (Table 2).¹⁵⁻¹⁷

Warthin tumors have characteristic clinical features, eg, they are soft, cystic tumors arising in the lower pole of the parotid gland in middle-aged to older men. On the basis of these characteristic features, a clinical diagnosis can be made in many cases.²⁹ It might be necessary to reconsider the clinicocytologic diagnostic criteria for Warthin tumors used in Japan to reduce the RON value of the AUS category.

Among the malignant tumors classified as AUS, malignant lymphoma was the most common type (23 cases) (Fig. 2), accounting for 12.4% of all cases in the AUS category (Table 5). Lymphoid-rich entities, including IgG4-related sialadenitis, chronic sialadenitis, lymphoid

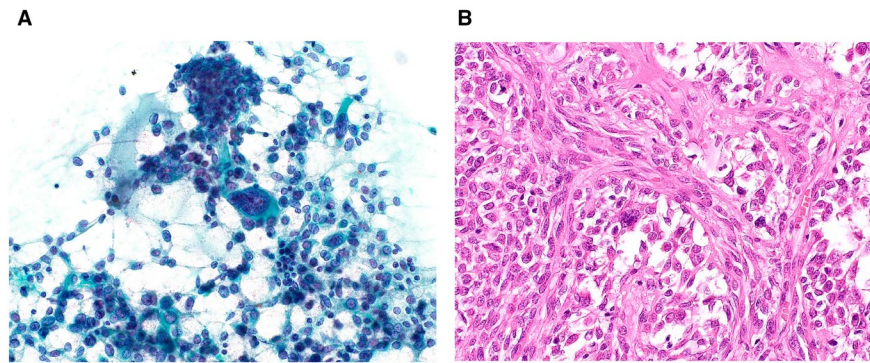


Figure 3. (A) Cytological features of a pleomorphic adenoma that was categorized as salivary gland neoplasm of uncertain malignant potential. Many myoepithelial cells and mucin can be seen. Among these, a few large, atypical cells are present (Papanicolaou stain, original magnification $\times 400$). (B) Histology of the resected specimen reveals cellular pleomorphic adenoma in which interspersed, bizarre myoepithelial cells can be seen (H&E stain, original magnification $\times 400$).

hyperplasia, and malignant lymphoma, accounted for 23.2% of the AUS lesions in this study. Alruwaili et al³⁰ reported a higher percentage (50%) of lymphoid-rich lesions in the AUS category, which pushed up the ROM in the AUS category to 37.5%. In general, making a definitive diagnosis of malignant lymphoma based solely on cytology is difficult.³¹ Ancillary immunophenotyping examinations, especially flow cytometry, are useful for differentiating malignant lymphoma from other lymphoid lesions.^{32,33} In the current study, the ROM for AUS lesions was 24.9%, which was slightly higher than the percentage recommended in the MSRS GC (20%). Ancillary examinations were only performed in 15 cases in this study, and flow cytometry was not applied to any lymphoid lesions. It will be necessary to further promote the use of flow cytometry for cases in which lymphoid lesions are suspected to reduce the ROM value for the AUS category in Japan.

In total, 154 cases were classified as SUMP (Table 6). Of the 57 malignant tumors, so-called basaloid neoplasms, including adenoid cystic carcinoma, myoepithelial carcinoma, and basal cell adenocarcinoma, accounted for 16 of the malignant SUMP cases (28.1%). Other low-grade carcinomas that exhibited slight atypia, including mucoepidermoid carcinoma, epithelial-myoepithelial carcinoma, secretory carcinoma, polymorphous adenocarcinoma, acinic cell carcinoma, and intraductal carcinoma, were also included in the SUMP category. When combined with basaloid cell tumors, they accounted for 41 cases (71.9%). To differentiate among them, we should use ancillary techniques, for example, immunostaining of PLAG1 or MYB or *ETV6* fluorescence in situ

hybridization using cell blocks or liquid-based cytology samples.³⁴ Of the benign tumors, 41 (46.1%) were PAs, and 31 (34.8%) were basal cell adenomas. The PAs categorized under SUMP included cases of the spindle cell type; the basaloid cell type; PA with bizarre giant myoepithelial cells (Fig. 3); and PA with many hyaline globules, which is sometimes difficult to differentiate from adenoid cystic carcinoma.²⁸ The most common non-neoplastic lesion was nodular oncocytic hyperplasia (5 cases) (Fig. 4). Liu et al also reported 59 cases of SUMP, including 7 cases of nodular oncocytosis,³⁵ and Rooper et al reported that nodular oncocytosis was difficult to differentiate from oncocytoma.³⁶

There were significant differences in the RON and ROM values for each MSRS GC category among the institutions (Table 2). The adoption of the MSRS GC will require education and quality control to ensure appropriate performance.³⁷

Although overall ROM values for each category were not calculated in this study, the mean percentage of malignant lesions among all of the aspiration samples from 7 institutions (institutions 1, 2, 4, 5, 8, 10, and 11) was 11.8% (range, 5.9%-20%), which is similar to that reported in another study.¹⁷

The sensitivity, specificity, accuracy, PPV, and NPV of the MSRS GC for diagnosing malignant lesions were as good as those described in previous studies.^{11,14,16-19} In the current study, there were 7 false-positive cases (see Supporting Table 2). One PA that was misdiagnosed as an acinic cell carcinoma showed cellular atypia with granular cytoplasm, enlarged nuclei, and conspicuous nucleoli. A retrospective examination indicated that these features

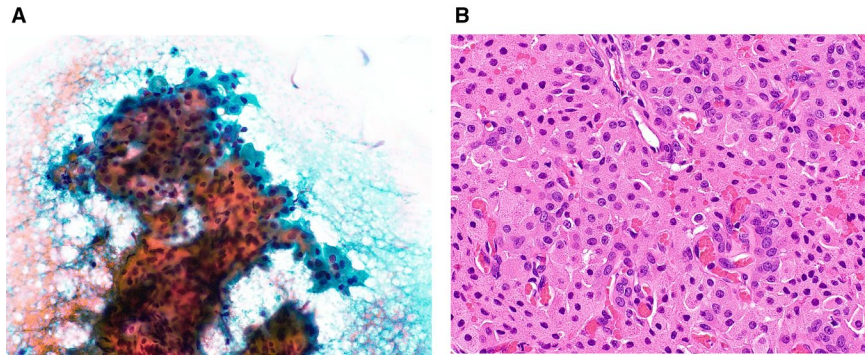


Figure 4. (A) Cytological features of a case of nodular oncocytic hyperplasia that was categorized as salivary gland neoplasm of uncertain malignant potential. Large oncocytic cell clusters with polygonal eosinophilic cytoplasm can be seen (Papanicolaou stain, original magnification $\times 400$). (B) The histologic specimen shows hyperplasia with ductal components (H&E stain, original magnification $\times 200$).

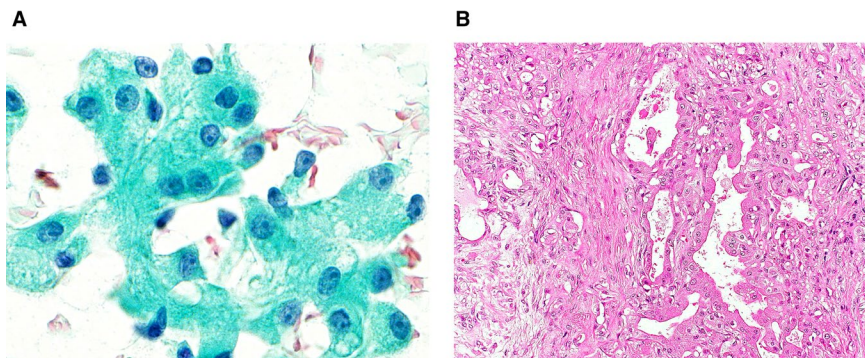


Figure 5. (A) Cytological features of a pleomorphic adenoma that was misdiagnosed as acinar cell carcinoma. Acinar-like clusters of epithelial cells with large amounts of lacy and granular cytoplasm and distinct nucleoli can be seen (Papanicolaou stain, original magnification $\times 1000$). (B) On histology of the resected specimen, oncocytic changes in the ductal epithelium (right) can be seen in this typical pleomorphic adenoma (left) (H&E, original magnification $\times 200$).

were caused by oncocytic metaplasia (Fig. 5).³⁸ There were 19 false-negative cases (see Supporting Table 3). The most common misdiagnosis was carcinoma ex PA being misdiagnosed as PA (5 cases; 26.3%). The cytologic diagnosis of carcinoma ex PA might be difficult when a carcinoma component is poorly sampled.³⁹ When relevant clinical findings, such as a sudden increase in the size of a long-standing mass in an elderly patient, are present, the possibility of carcinoma ex PA should be considered, even if PA is suspected.

In conclusion, the MSRSGC can be implemented as an alternative reporting format for salivary gland cytology to the JSRSGC. The MSRSGC is an excellent tool for ensuring quality control of salivary gland cytology, and the widespread use of the MSRSGC would improve the accuracy of salivary gland cytology and lead to better patient care in Japan.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Kayoko Higuchi: Conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, writing—original draft, and writing—review and editing. **Makoto Urano:** Conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, and writing—review and editing. **Jun Akiba:** Conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, and writing—review and editing. **Miwako Nogami:** Data curation, formal analysis, investigation, resources, validation, and writing—review and editing. **Yukiya Hirata:** Data curation, formal analysis, investigation, resources, validation, and writing—review and

editing. **Yoko Zukeran:** Data curation, formal analysis, investigation, resources, and validation. **Koki Moriyoshi:** Data curation, formal analysis, investigation, methodology, resources, validation, and writing–review and editing. **Yuichiro Tada:** Data curation, resources, validation, and writing–review and editing. **Mana Fukushima:** Data curation, formal analysis, investigation, resources, validation, and writing–review and editing. **Mariko Obayashi:** Data curation, formal analysis, investigation, resources, validation, and writing–review and editing. **Shinnichi Sakamoto:** Data curation, formal analysis, investigation, resources, validation, and writing–review and editing. **Kazuya Kuraoka:** Data curation, formal analysis, investigation, resources, validation, and writing–review and editing. **Kana Kira:** Data curation, formal analysis, investigation, resources, validation, and writing–review and editing. **Akihiko Kawahara:** Data curation, formal analysis, investigation, resources, validation, and writing–review and editing. **Taku Kato:** Data curation, formal analysis, investigation, methodology, resources, validation, and writing–review and editing. **Maki Tanigawa:** Data curation, formal analysis, investigation, methodology, resources, validation, and writing–review and editing. **Masato Nakaguro:** Conceptualization, validation, and writing–review and editing. **Hidetaka Yamamoto:** Data curation, formal analysis, investigation, methodology, resources, validation, and writing–review and editing. **Toshitaka Nagao:** Conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, and writing–review and editing.

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