Differentiation of Malignant Salivary Gland Tumors from Pleomorphic Adenomas and Warthin’s Tumors: Combined Diagnostic Value of Tumor Blood Flow and Apparent Diffusion Coefficient by Histogram Analysis

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Abstract

We aimed to evaluate the usefulness of tumor blood flow (TBF) obtained by pseudocontinuous arterial spin labeling (pCASL) and apparent diffusion coefficient (ADC) for differentiating salivary gland malignant tumors (MTs) from pleomorphic adenomas (PAs) and Warthin's tumors (WTs). We used pCASL imaging and ADC map to evaluate 65 patients, including 16 with MT, 30 with PA, and 19 with WT. We evaluated all tumors by histogram analyses and compared various characteristics by one-way analysis of variance followed by Tukey post-hoc tests. Diagnostic performance was evaluated by receiver operating characteristic (ROC) curve analysis. There were significant differences in the mean, 50th, 75th, and 90th percentiles of TBF among the tumor types, in the mean TBFs (ml/100g/min) between MTs (57.47 ± 35.14) and PAs (29.88 ± 22.53, p = 0.039) and between MTs and WTs (119.31 ± 50.11, p < 0.001), as well as in the mean ADCs (×10⁻³ mm²/sec) between MTs (1.08 ± 0.28) and PAs (1.60 ± 0.34, p < 0.001), but not in the mean ADCs between MTs and WTs (0.87 ± 0.23, p = 0.117). In the ROC curve analysis, the highest areas under the curves (AUCs) were achieved by the 10th and 25th percentiles of ADC (AUC = 0.885) for differentiating MTs from PAs and the 50th percentile of TBF (AUC = 0.855) for differentiating MTs from WTs. The AUCs of TBF, ADC, and combination of TBF and ADC were 0.850, 0.885, and 0.950 for MT and PA differentiation and 0.855, 0.814, and 0.905 for MT and WT differentiation, respectively. The combination of TBF and ADC evaluated by histogram analysis helped differentiate salivary gland MTs from PAs and WTs.

Introduction

Parotid tumors represent nearly 70% of all salivary gland tumors, and 80% of them are benign [1]. The most frequent benign salivary gland tumors are pleomorphic adenomas (PAs), which comprise 45% of all salivary gland tumors, followed by Warthin's tumors (WTs) [2]. On the other hand, malignant tumors (MTs) represent nearly 20% of parotid tumors, approximately 40% of submandibular tumors, and 70–90% of sublingual tumors [1, 3].

Malignant salivary tumors demonstrate a range of biological behaviors. About 40% of MTs are indolent, especially in young adults [3]. The other 40% of MTs are aggressive, especially in the elderly [3]. Clinical indicators suggesting MTs are rapid growth rate, pain, facial nerve involvement, and cervical adenopathy. However, a slow growth rate of asymptomatic mass does not exclude MTs [3]. Therefore, it is important to differentiate MTs from benign salivary gland tumors, such as PAs and WTs. Fine-needle aspiration cytology is widely accepted as a reliable way to diagnose salivary gland tumors before surgical resection, but it is not appropriate for tumors located in deep areas and is an intrinsically invasive procedure [4]. Noninvasive magnetic resonance imaging (MRI) techniques may improve the diagnostic performance of salivary gland tumors regardless of tumor locations. However, conventional MRI cannot clearly distinguish between benign and malignant salivary gland tumors [5]. For instance, the apparent diffusion coefficient (ADC) obtained by diffusion-weighted imaging (DWI) reportedly provided useful information for the differentiation of WTs and PAs but remained inconclusive for differentiation of benign and malignant salivary gland tumors [6–8].
Recently, arterial spin labeling (ASL) techniques, such as pulsed ASL or pseudocontinuous ASL (pCASL), were introduced for clinical applications [9]. This method has been applied for noninvasive measurement of tumor blood flow (TBF) by using the magnetization of protons in arterial blood as an intrinsic tracer without an exogenous contrast agent [9]. There have been only a few reports on the usefulness of ASL for differentiating salivary gland tumors so far [10–12]. The use of multiparametric MRI, such as DWI and ASL, may help radiologists by increasing their efficiency in the differential diagnosis of salivary gland tumors. In addition, appropriate automated software needs to be developed so that these advanced applications can be adjusted to facilitate the workflow of radiologists and to objectively evaluate quantitative data, such as ADC and TBF. Therefore, we have developed a custom software application in MATLAB 2020a for evaluation of TBF and the ADC of salivary gland tumors by using histogram analysis. The purpose of this study was to assess the combined diagnostic value of ADC and TBF for differentiating MTs in salivary glands from PAs and WTs.

**Results**

A total of 65 subjects (age range, 11–86 years; mean 59 years; 34 males and 31 females) were finally included. There were 16 subjects with MTs, 30 with PAs, and 19 with WTs. The characteristics of patients are described in Table 1. The pathology of MTs was variable, including five carcinoma ex pleomorphic adenomas, two acinic-cell carcinomas, two adenocarcinomas, two adenoid cystic carcinomas, two mucoepidermoid carcinomas, one basal-cell adenocarcinoma, one epithelial myoepithelial carcinoma, and one salivary-duct carcinoma. One patient with PAs and eight patients with WTs had multiple or bilateral tumors. Among these patients, only the largest one was assessed.
## Table 1

### Patients’ characteristics

|                      | MT (n = 16) | PA (n = 30) | WT (n = 19) |
|----------------------|-------------|-------------|-------------|
| **Sex**              |             |             |             |
| Male: Female         | 9:7         | 9:21        | 16:3        |
| **Age**              |             |             |             |
| Range (year)         | 11–82       | 24–86       | 56–83       |
| Mean age (year)      | 60          | 53          | 68          |
| **Tumor diameter**   |             |             |             |
| Range (mm)           | 14–96       | 11–60       | 17–64       |
| Mean (mm)            | 34.63       | 27.37       | 35.84       |
| **Tumor sub-site**   |             |             |             |
| Parotid gland        | 11          | 22          | 19          |
| Submandibular gland  | 4           | 8           | 0           |
| Sublingual gland     | 1           | 0           | 0           |
| **Diagnostic method**|             |             |             |
| Resection            | 14          | 23          | 13          |
| Fine-needle aspiration cytology | 2       | 7           | 6           |

**Abbreviations;** MT, malignant tumor; PA, pleomorphic adenoma; WT, Warthin’s tumor

**Comparison of the parameters for TBF and ADC between MTs, PAs, and WTs.** Figures 1, 2, and 3 show representative cases of MTs, PAs, and WTs, respectively. Tables 2 and 3 show the parameter measurements of TBF and ADC, respectively, in MTs, PAs, and WTs.
Table 2
Measurements of TBF in MTs, PAs, and WTs

| TBF parameters | Mean ± standard deviation | p-value |
|----------------|---------------------------|---------|
|                | MT | PA | WT | MT vs. PA | MT vs. WT | PA vs. WT |
| Max            | 97.70 ± 54.98 | 66.11 ± 29.27 | 166.03 ± 49.85 | 0.054 | < 0.001 * | < 0.001 * |
| Min            | 22.47 ± 29.10 | 7.22 ± 14.45 | 63.74 ± 43.62 | 0.219 | < 0.001 * | < 0.001 * |
| Mean           | 57.47 ± 35.14 | 29.88 ± 22.53 | 119.31 ± 50.11 | 0.039 * | < 0.001 * | < 0.001 * |
| 10th percentile| 34.37 ± 30.95 | 14.19 ± 18.84 | 88.96 ± 48.85 | 0.127 | < 0.001 * | < 0.001 * |
| 25th percentile| 43.99 ± 32.73 | 20.84 ± 21.80 | 103.44 ± 50.52 | 0.09 | < 0.001 * | < 0.001 * |
| 50th percentile| 56.36 ± 35.35 | 28.48 ± 23.62 | 120.80 ± 51.76 | 0.044 * | < 0.001 * | < 0.001 * |
| 75th percentile| 70.71 ± 40.83 | 37.65 ± 25.32 | 135.35 ± 52.47 | 0.021 * | < 0.001 * | < 0.001 * |
| 90th percentile| 81.82 ± 46.63 | 47.29 ± 25.87 | 147.45 ± 51.63 | 0.020 * | < 0.001 * | < 0.001 * |
| Skewness       | 0.10 ± 0.52 | 0.64 ± 0.68 | −0.23 ± 0.72 | 0.029 * | 0.289 | < 0.001 * |
| Kurtosis       | −0.42 ± 0.49 | 0.62 ± 1.15 | 0.39 ± 2.82 | 0.136 | 0.364 | 0.887 |

* P-value < 0.05

minimum; MT, Abbreviations; TBF, tumor blood flow (mL/100 g/min); max, maximum; min, malignant tumor; PA, pleomorphic adenoma; WT, Warthin's tumor
Table 3
Measurements of ADC in MTs, PAs, and WTs

| ADC parameters | Mean ± standard deviation | p-value | Mean ± standard deviation | p-value | Mean ± standard deviation | p-value | Mean ± standard deviation | p-value |
|----------------|---------------------------|---------|---------------------------|---------|---------------------------|---------|---------------------------|---------|
|                | MT | PA | WT | MT vs. PA | MT vs. WT | PA vs. WT | MT | PA | WT | MT vs. PA | MT vs. WT | PA vs. WT |
| Max            | 1.39 ± 0.31 | 1.95 ± 0.37 | 1.29 ± 0.42 | < 0.001 * | 0.684 | < 0.001 * | 0.76 ± 0.23 | 1.23 ± 0.38 | 0.51 ± 0.16 | < 0.001 * | 0.050 | < 0.001 * |
| Min            | 0.76 ± 0.23 | 1.23 ± 0.38 | 0.51 ± 0.16 | < 0.001 * | 0.050 | < 0.001 * | 1.08 ± 0.28 | 1.60 ± 0.34 | 0.87 ± 0.23 | < 0.001 * | 0.117 | < 0.001 * |
| Mean           | 1.08 ± 0.28 | 1.60 ± 0.34 | 0.87 ± 0.23 | < 0.001 * | 0.117 | < 0.001 * | 0.90 ± 0.23 | 1.40 ± 0.33 | 0.68 ± 0.15 | < 0.001 * | 0.052 | < 0.001 * |
| 10th percentile| 0.98 ± 0.27 | 1.49 ± 0.33 | 0.76 ± 0.17 | < 0.001 * | 0.057 | < 0.001 * | 1.08 ± 0.31 | 1.59 ± 0.34 | 0.85 ± 0.22 | < 0.001 * | 0.075 | < 0.001 * |
| 25th percentile| 1.16 ± 0.31 | 1.71 ± 0.36 | 0.97 ± 0.30 | < 0.001 * | 0.220 | < 0.001 * | 1.16 ± 0.31 | 1.71 ± 0.36 | 0.97 ± 0.30 | < 0.001 * | 0.220 | < 0.001 * |
| 50th percentile| 1.25 ± 0.30 | 1.80 ± 0.37 | 1.09 ± 0.39 | < 0.001 * | 0.393 | < 0.001 * | 1.25 ± 0.30 | 1.80 ± 0.37 | 1.09 ± 0.39 | < 0.001 * | 0.393 | < 0.001 * |
| Skewness       | −0.03 ± 0.64 | 0.07 ± 0.47 | 0.29 ± 0.46 | 0.805 | 0.168 | 0.32 |
| Kurtosis       | 0.29 ± 1.16 | −0.04 ± 0.95 | 0.55 ± 1.15 | 0.594 | 0.749 | 0.155 |

* P-value < 0.05

Abbreviations; ADC, apparent diffusion coefficient (×10\(^{-3}\) mm\(^2\)/sec); max, maximum; min, minimum; MT, malignant tumor; PA, pleomorphic adenoma; WT, Warthin's tumor

There were significant differences in the mean, 50th, 75th, and 90th percentiles of TBF among all three types of tumors (all p < 0.05). The mean TBF was significantly higher in MTs (57.47 ± 35.14 mL/100 g/min) than in PAs (29.88 ± 22.53 mL/100 g/min, p = 0.039) and significantly lower in MTs than in WTs (119.31 ± 50.11 mL/100 g/min, p < 0.001). The 50th percentile of TBF was significantly higher in MTs (56.36 ± 35.35 mL/100 g/min) than in PAs (28.48 ± 23.62 mL/100 g/min, p = 0.044) and significantly lower in MTs than in WTs (120.80 ± 51.76 mL/100 g/min, p < 0.001). The 75th percentile of TBF was significantly higher in MTs (70.71 ± 40.83 mL/100 g/min) than in PAs (37.65 ± 25.32 mL/100 g/min, p = 0.021) and significantly lower in MTs than in WTs (135.35 ± 52.47 mL/100 g/min, p < 0.001). The 90th percentile of TBF was significantly higher in MTs (81.82 ± 46.63 mL/100 g/min) than in PAs (47.29 ±
25.87 mL/100 g/min, \( p = 0.020 \) and significantly lower in MTs than in WTs (147.45 ± 51.63 mL/100 g/min, \( p < 0.001 \)).

There was a significant difference in the mean ADCs between MTs (1.08 ± 0.28 \( \times 10^{-3} \) mm\(^2\)/sec) and PAs (1.60 ± 0.34 \( \times 10^{-3} \) mm\(^2\)/sec, \( p < 0.001 \)) but not between MTs and WTs (0.87 ± 0.23 \( \times 10^{-3} \) mm\(^2\)/sec, \( p = 0.117 \)). There were no ADC parameters that showed significant differences for all three combinations of tumor types (MT and PA, MT and WT, and PA and WT).

**Comparison of diagnostic performance for TBF and ADC in differentiating MTs, PAs, and WTs.**

Tables 4, 5, and 6 show the diagnostic performance of each parameter determined by the receiver operating characteristic (ROC) curve analysis. When differentiating MTs from PAs, the 10th and 25th percentiles of the ADC both had the best diagnostic performance out of all TBF and ADC parameters, with areas under the curve (AUCs) of 0.885 and 0.885, respectively, which is considered medium diagnostic performance. The best detected cutoff points were 1.15 \( \times 10^{-3} \) mm\(^2\)/sec and 1.26 \( \times 10^{-3} \) mm\(^2\)/sec, respectively, yielding sensitivity and specificity for both cutoff values of 73.3% and 93.8%, respectively.
Table 4
Receiver operating characteristic curve analysis of the parameters for differentiating MT from PA

| Parameters          | AUC    | Cutoff value | Sensitivity (%) | Specificity (%) | Youden index |
|---------------------|--------|--------------|-----------------|-----------------|--------------|
| TBF max             | 0.665  | 99.80        | 50.0            | 86.7            | 0.367        |
| TBF min             | 0.731  | 6.89         | 56.3            | 83.3            | 0.396        |
| TBF mean            | 0.740  | 66.82        | 50.0            | 93.3            | 0.433        |
| TBF 10th percentile | 0.738  | 6.26         | 93.8            | 46.7            | 0.404        |
| TBF 25th percentile | 0.742  | 13.48        | 93.8            | 53.3            | 0.471        |
| TBF 50th percentile | 0.744  | 20.06        | 93.8            | 50.0            | 0.438        |
| TBF 75th percentile | 0.740  | 77.91        | 50.0            | 93.3            | 0.433        |
| TBF 90th percentile | 0.721  | 83.98        | 50.0            | 93.3            | 0.433        |
| TBF skewness        | 0.710  | 0.71         | 46.7            | 100.0           | 0.467        |
| TBF kurtosis        | 0.798  | 0.00         | 66.7            | 93.8            | 0.604        |
| ADC max             | 0.877  | 1.73         | 76.7            | 93.8            | 0.704        |
| ADC min             | 0.856  | 1.01         | 70.0            | 93.8            | 0.638        |
| ADC mean            | 0.879  | 1.30         | 80.0            | 87.5            | 0.675        |
| ADC 10th percentile | 0.885  | 1.15         | 73.3            | 93.8            | 0.671        |
| ADC 25th percentile | 0.885  | 1.26         | 73.3            | 93.8            | 0.671        |
| ADC 50th percentile | 0.867  | 1.38         | 73.3            | 93.8            | 0.671        |
| ADC 75th percentile | 0.883  | 1.38         | 80.0            | 87.5            | 0.675        |
| ADC 90th percentile | 0.873  | 1.48         | 80.0            | 87.5            | 0.675        |
| ADC skewness        | 0.592  | 0.17         | 43.3            | 81.3            | 0.246        |
| ADC kurtosis        | 0.598  | -0.15        | 68.8            | 53.3            | 0.221        |

Abbreviations; TBF, tumor blood flow (mL/100 g/min); ADC, apparent diffusion coefficient (×10^-3 mm²/sec); max, maximum; min, minimum; MT, malignant tumor; PA, pleomorphic adenoma
Table 5
Receiver operating characteristic curve analysis of the parameters for differentiating MT from WT

| Parameters            | AUC   | Cutoff value | Sensitivity (%) | Specificity (%) | Youden index |
|-----------------------|-------|--------------|-----------------|-----------------|--------------|
| TBF max               | 0.829 | 151.61       | 73.7            | 87.5            | 0.612        |
| TBF min               | 0.813 | 30.21        | 73.7            | 75.0            | 0.487        |
| TBF mean              | 0.842 | 102.51       | 63.2            | 93.8            | 0.569        |
| TBF 10th percentile   | 0.845 | 20.97        | 100.0           | 56.3            | 0.563        |
| TBF 25th percentile   | 0.836 | 75.10        | 68.4            | 87.5            | 0.559        |
| TBF 50th percentile   | 0.855 | 78.02        | 84.2            | 75.0            | 0.592        |
| TBF 75th percentile   | 0.836 | 111.49       | 68.4            | 87.5            | 0.559        |
| TBF 90th percentile   | 0.832 | 124.57       | 73.7            | 87.5            | 0.612        |
| TBF skewness          | 0.651 | 0.34         | 43.8            | 89.5            | 0.332        |
| TBF kurtosis          | 0.556 | 0.11         | 31.6            | 93.8            | 0.253        |
| ADC max               | 0.671 | 1.15         | 93.8            | 57.9            | 0.516        |
| ADC min               | 0.814 | 0.62         | 81.3            | 78.9            | 0.602        |
| ADC mean              | 0.743 | 0.85         | 81.3            | 68.4            | 0.497        |
| ADC 10th percentile   | 0.806 | 0.80         | 62.5            | 89.5            | 0.520        |
| ADC 25th percentile   | 0.783 | 0.76         | 75.0            | 73.7            | 0.487        |
| ADC 50th percentile   | 0.763 | 0.80         | 87.5            | 63.2            | 0.507        |
| ADC 75th percentile   | 0.730 | 0.91         | 87.5            | 63.2            | 0.507        |
| ADC 90th percentile   | 0.727 | 1.02         | 87.5            | 68.4            | 0.559        |
| ADC skewness          | 0.701 | 0.08         | 68.4            | 75.0            | 0.434        |
| ADC kurtosis          | 0.592 | 0.13         | 63.2            | 62.5            | 0.257        |

Abbreviations; TBF, tumor blood flow (mL/100 g/min); ADC, apparent diffusion coefficient (×10⁻³ mm²/sec); max, maximum; min, minimum; MT, malignant tumor; WT, Warthin's tumor
Table 6
Receiver operating characteristic curve analysis of the parameters for differentiating PA from WT

| Parameters           | AUC   | Cutoff value | Sensitivity (%) | Specificity (%) | Youden index |
|----------------------|-------|--------------|-----------------|-----------------|--------------|
| TBF max              | 0.954 | 85.41        | 100.0           | 76.7            | 0.767        |
| TBF min              | 0.944 | 6.67         | 94.7            | 83.3            | 0.781        |
| TBF mean             | 0.954 | 68.30        | 84.2            | 93.3            | 0.775        |
| TBF 10th percentile  | 0.946 | 20.28        | 100.0           | 76.7            | 0.767        |
| TBF 25th percentile  | 0.946 | 27.39        | 100.0           | 73.3            | 0.733        |
| TBF 50th percentile  | 0.951 | 71.21        | 84.2            | 93.3            | 0.775        |
| TBF 75th percentile  | 0.963 | 70.65        | 89.5            | 90.0            | 0.795        |
| TBF 90th percentile  | 0.968 | 82.30        | 89.5            | 93.3            | 0.828        |
| TBF skewness         | 0.821 | 0.27         | 70.0            | 89.5            | 0.595        |
| TBF kurtosis         | 0.695 | -0.30        | 80.0            | 63.2            | 0.432        |
| ADC max              | 0.870 | 1.27         | 100.0           | 68.4            | 0.684        |
| ADC min              | 0.960 | 0.76         | 93.3            | 94.7            | 0.881        |
| ADC mean             | 0.960 | 1.04         | 96.7            | 84.2            | 0.809        |
| ADC 10th percentile  | 0.984 | 0.79         | 100.0           | 89.5            | 0.895        |
| ADC 25th percentile  | 0.979 | 0.95         | 96.7            | 89.5            | 0.861        |
| ADC 50th percentile  | 0.962 | 0.97         | 100.0           | 78.9            | 0.789        |
| ADC 75th percentile  | 0.933 | 1.15         | 93.3            | 84.2            | 0.775        |
| ADC 90th percentile  | 0.898 | 1.20         | 96.7            | 78.9            | 0.756        |
| ADC skewness         | 0.635 | 0.41         | 47.4            | 76.7            | 0.240        |
| ADC kurtosis         | 0.674 | 0.06         | 73.7            | 66.7            | 0.404        |

Abbreviations; AUC, area under the curve; TBF, tumor blood flow (mL/100 g/min); max, maximum; min, minimum; ADC, apparent diffusion coefficient \((\times 10^{-3} \text{ mm}^2/\text{sec})\); PA, pleomorphic adenoma; WT, Warthin's tumor

When differentiating MTs from WTs, the 50th percentile of TBF had the best diagnostic performance out of all TBF and ADC, with an AUC of 0.855, which is considered medium diagnostic performance. The best detected cutoff point was 78.02 mL/100 g/min, yielding a sensitivity and a specificity of 84.2% and 75.0%, respectively.
When differentiating PAs from WTs, the 10th percentile of ADC had the best diagnostic performance out of all TBFs and ADCs, with an AUC of 0.984, which is considered high diagnostic performance. The best detected cutoff point was $0.79 \times 10^{-3} \text{ mm}^2/\text{sec}$, yielding a sensitivity and a specificity of 100.0% and 89.5%, respectively. Figure 4 summarizes the diagnostic performance of the parameters. TBF, ADC, and the combination of TBF and ADC showed medium to high diagnostic performances, with AUCs of 0.850, 0.885, and 0.950 for differentiating MTs from PAs, 0.855, 0.814, and 0.905 for differentiating MTs from WTs, and 0.968, 1.000, and 1.000 for differentiating PAs from WTs, respectively.

**Interobserver agreement of TBF and ADC measurements.**

Table 7 shows the intraclass correlation coefficients (ICCs) of the measurements by the two observers. Excellent agreements were observed for all parameters except for the skewness of ADC, which showed good agreement.
Table 7
Interobserver agreement

| Parameters             | ICC    |
|------------------------|--------|
| TBF max                | 0.966  |
| TBF min                | 0.827  |
| TBF mean               | 0.995  |
| TBF 10th percentile    | 0.919  |
| TBF 25th percentile    | 0.962  |
| TBF 50th percentile    | 0.997  |
| TBF 75th percentile    | 0.998  |
| TBF 90th percentile    | 0.991  |
| TBF skewness           | 0.957  |
| TBF kurtosis           | 0.930  |
| ADC max                | 0.918  |
| ADC min                | 0.983  |
| ADC mean               | 0.997  |
| ADC 10th percentile    | 0.991  |
| ADC 25th percentile    | 0.999  |
| ADC 50th percentile    | 0.995  |
| ADC 75th percentile    | 0.993  |
| ADC 90th percentile    | 0.989  |
| ADC skewness           | 0.713  |
| ADC kurtosis           | 0.892  |

Abbreviations; ICC, intraclass correlation coefficient; TBF, tumor blood flow; max, maximum; min, minimum; ADC, apparent diffusion coefficient

Discussion

In this study, the diagnostic performance of the combination of TBF and ADC for differentiating MTs from PAs and WTs increased relative to the performance of each parameter alone. However, in differentiating PAs from WTs, the diagnostic performance of ADC alone showed perfect discrimination, and therefore, the value of adding the combination of ADC and TBF was low. To our best knowledge, this
is the first study to evaluate the usefulness of the combination of pCASL and the ADC map by histogram analysis for differentiating malignant salivary gland tumors from PAs and WTs.

Kato et al. reported that qualitative analysis showed that TBF was significantly higher in WTs than PAs and MTs, but did not show a significant difference between PAs and MTs [10]. However, we demonstrated that the mean, 50th, 75th, and 90th percentiles of TBF could differentiate MTs, PAs, and WTs. We speculate that the differences in ASL methods may explain why their results differed from ours. They placed the regions of interest (ROIs) on both a tumor and the contralateral normal parotid gland parenchyma at the same level and then evaluated tumor-to-parotid signal intensity ratios from ASL images supposing that those ratios are surrogates of TBF [10]. They measured the relative ratio of salivary gland tumors to normal parotid glands, whereas we measured the TBF values of tumors quantitatively. Consequently, histogram analysis may overcome the limitations of qualitative analysis. Moreover, they used an alternating radio-frequency ASL sequence with gradient echo-type single-shot echo-planar imaging (MP-EPISTAR), which suffers from susceptibility artifacts more seriously than pCASL sequences that use 3D turbo spin echo (TSE) acquisition [10]. In addition, MP-EPISTAR used in the study of Kato et al. has a lower signal-to-noise ratio than that of pCASL [11]. Thus, the pCASL technique may be more suitable for imaging compared to the ASL sequence that Kato et al. used for differentiation among MTs, PAs, and WTs.

A recent report stated that metrics, such as percentiles, kurtosis, and skewness, calculated by histogram analysis are strong and reliable quantitative surrogate markers of tumor heterogeneity [13]. Thus, we consider that microenvironments of tumors could be masked by evaluating only a single parameter, such as the mean value. Yamamoto et al. demonstrated that the mean TBF value was significantly higher in WTs than in PAs by using the pCASL sequence with conventional ROI analysis [11]. They also showed that the higher mean TBF of WTs than of PAs was attributable to higher micro-vessel density in WTs than in PAs [11]. Furthermore, our results revealed that the 75th and 90th percentiles of TBF exhibited higher AUC values than the mean TBF. Consequently, histogram analysis appears to provide more detailed information about TBF.

Kato et al. reported that the mean ADC values were significantly higher in PAs than in WTs and MTs but were not significantly different between WTs and MTs [10]. Their results were consistent with our results showing that all ADC parameters except for skewness and kurtosis were significantly different between PAs and WTs and between PAs and MTs, but not between WTs and MTs. Razek et al. studied ADC values by histogram analysis for diagnosis of PAs, WTs, and MTs and reported significant differences in the means and skewness of ADC among all three tumors, although these differences between WTs and MTs were weaker than those between PAs and WTs and PAs and MTs [14]. Histopathologically, PAs comprise an abundant myxoma-like stroma [6, 11], which probably contributes to the highest ADC value among the three types of tumors in all parameters except for skewness and kurtosis in our study. In contrast, WTs showed the lowest ADC among all parameters except for skewness and kurtosis, which might reflect epithelial and lymphoid stromata with microscopic slit-like cysts filled with proteinous fluid [2, 6].
There were several limitations in this study. First, the study was conducted at a single institution with a relatively small number of subjects. Studies with a larger number of subjects would be required to confirm the efficacy of pCASL imaging and ADC mapping for evaluating salivary gland tumors. Second, we could not evaluate the whole pCASL image slices and ADC maps for each tumor. Particularly, MTs tend to have heterogeneous characteristics. Thus, whole-tumor evaluation would be desirable in future studies.

In conclusion, the combination of TBF and ADC evaluated by histogram analysis was found to be helpful for differentiating MTs from PAs and WTs in salivary glands.

**Methods**

**Subjects.**

This study was approved by the ethics committee of our university, and the requirement for written informed consent was waived because of the retrospective study design. All study procedures were conducted according to the principles of World Medical Association Declaration of Helsinki. We retrospectively collected data from the patients who fulfilled the following inclusion criteria: (a) underwent an MRI scan to evaluate clinically suspected major salivary gland tumors between December 2015 and September 2020; (b) available preoperative 3T MRI, including pCASL images, DWI, T1-weighted images, contrast-enhanced T1-weighted images, and T2-weighted images; (c) tumor size > 10 mm; (d) pathologically proven tumors by fine-needle aspiration biopsy or surgical resection; and (e) diagnosed as an MT, PA, or WT of the salivary gland.

**Conventional MRI protocol.**

All patients underwent MRI on a 3T MRI system (Ingenia; Philips Medical Systems, Best, the Netherlands) with a Head/Neck coil. The pulse sequence parameters were as follows. T2-weighted imaging: repetition time (TR)/echo time (TE), 6528/90 ms; number of signals averaged (NSA), 1; field of view (FOV), 240 x 240 mm; matrix, 384 x 271; slice thickness, 4 mm; number of slices, 22; acceleration factor, 1.5; and scanning time, 1 min 57 s. T1-weighted imaging: TR/TE, 614/14 ms; NSA, 1; FOV, 240 x 240 mm; matrix, 352 x 246; slice thickness, 4 mm; number of slices, 22; acceleration factor, 2; and acquisition time, 2 min 34 s. DWI: TR/TE, 5000/88 ms; fat suppression, short-tau inversion recovery; inversion time, 250 ms; NSA, 2; b value, 0 and 1,000 s/mm²; FOV, 240 x 240 mm; matrix, 96 x 125; slice thickness, 4 mm; number of slices, 22; acceleration factor, 2; and acquisition time, 3 min 30 s. Contrast-enhanced 3D-T1-weighted imaging: slice orientation, sagittal; TR/TE, 5.3/2.4 ms; flip angle (FA), 10; fat suppression, spectral-attenuated inversion recovery; FOV, 250 x 225 mm; matrix, 256 x 256; slice thickness, 1 mm; number of slices, 180; acceleration factor, 1.8; and acquisition time, 3 min 24 s. The contrast-enhanced 2D-T1-weighted imaging parameters were the same as the non-contrast parameters.
pCASL MRI protocol.

The pulse sequence parameters for 3D TSE pCASL were as follows: TR/TE, 6000/40 ms; FA, 90°; labeling duration, 1650 ms; post-label delay, 1800 ms; number of shots, 3; FOV, 240 × 240 mm; matrix, 80 × 80; slice thickness, 4 mm; number of slices, 22; acceleration factor, 2.5; and acquisition time, 5 min 36 s. The labeling plane was set parallel to the imaging volume and perpendicular to the common carotid artery.

TBF was calculated according to the following equation [8]:

$$\text{TBF} = \frac{6000 \cdot \lambda \cdot (\text{SI}_{\text{control}} - \text{SI}_{\text{label}}) \cdot e^{\frac{\text{PLD}}{T_{1,\text{blood}}}}}{2 \cdot \alpha \cdot T_{1,\text{blood}} \cdot \text{SI}_{\text{PD}} \cdot (1 - e^{-\frac{\tau}{T_{1,\text{blood}}}})} \quad [\text{mL/100 g/min}]$$

where $\lambda$ is the blood/tumor-tissue water partition coefficient (1.0 g/mL), and $\text{SI}_{\text{control}}$ and $\text{SI}_{\text{label}}$ are the time-averaged signal intensities in the control and label images, respectively. $T_{1,\text{blood}}$ is the longitudinal relaxation time of blood (1650 ms), $\alpha$ is the labeling efficiency (0.85), $\text{SI}_{\text{PD}}$ is the signal intensity of a proton density-weighted image, and $\tau$ is the label duration (1650 ms). The value of $\lambda$ was 1.0 mL/g. To calculate TBF, we used the same model and conditions as those used for calculating blood flow in the brain.

Image analysis.

Image analysis was performed by using a custom software application developed in MATLAB 2020a. The custom software displays the ADC map and the pCASL map for the same patient side by side on the monitor. A slice image of each map for display can be moved. Two board-certified neuroradiologists (F.T and R.K) reviewed all MRI sequences. First, we identified the tumors on T1-weighted images, T2-weighted images, and contrast-enhanced T1-weighted images. The ROIs were manually drawn around the tumor margin in the maximum diameters on the ADC map by using the software. The ROIs were within an entire solid part of a tumor as much as visually traced, avoiding areas of necrosis, cyst, or hemorrhage. Then, the segmented ROI was copied from the ADC map and pasted to the pCASL image by using the software. The histogram features for each image were determined using those histograms. The following 10 objective features were determined as histogram features in the custom software: (1) minimum (min), (2) mean, (3) maximum (max), (4) 10th percentile, (5) 25th percentile, (6) 50th percentile, (7) 75th percentile, (8) 90th percentile, (9) skewness, and (10) kurtosis. The histogram features of TBF and ADC were measured twice in each ROI, and these measurements were averaged.

Statistical analysis.

Statistical analysis was performed by using SPSS v. 25.0 software (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY). All 10 parameters of the TBF and ADC values were assessed. Significant
differences among the groups were analyzed by one-way analysis of variance followed by Tukey post-hoc tests. A \( p \)-value of < 0.05 was considered to be indicative of statistical significance.

ROC curve analyses were performed to investigate the diagnostic performance of the parameters in differentiating among PAs, WTs, and MTs. We considered AUC values < 0.7, 0.7–0.9, and > 0.9 to indicate low, medium, and high diagnostic performance, respectively. Cutoff values were calculated with the maximum of the Youden index (Youden index = sensitivity + specificity – 1). A \( p \)-value of < 0.05 was considered significant to be indicative of statistical significance.

Interobserver agreement on TBF and ADC values between two readers was evaluated by ICC. ICCs are considered excellent if > 0.74 [15].

**Declarations**

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**Author contributions**

Study concept and design: T.T., M.U., and M.M. Acquisition of data: K.I. and M.O., development of the software for image analysis: R.N. Analysis and interpretation of data: F.T. and M.M. Drafting of the manuscript: F.T. and M.M. Statistical analysis: F.T. Study supervision: M.M. and H.S. All authors reviewed the manuscript.

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**Competing interests**

Makoto Obara is an employee of Philips Japan. The other authors declare no competing interests.

**Data availability**

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

**Ethics Statement**

This study was approved by the ethics committee of Mie University School of Medicine, and the requirement for written informed consent was waived because of the retrospective study design. All study
procedures were conducted according to the principles of World Medical Association Declaration of Helsinki.

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Figures

Figure 1

An 80-year-old male with a carcinoma ex PA in the left parotid gland. T2-weighted image (a) showing an iso signal intensity lesion (arrow). Contrast-enhanced 3D-T1-weighted image (b) showing homogeneous contrast enhancement (arrow). TBF color map (c) showing medium TBF (arrow). The ROI was manually drawn on the ADC map of the software (d, yellow) and the ROI was copied from the ADC map to the TBF map of the software (e, yellow). The TBF histogram (f) and ADC histogram (g) are presented. The TBF histogram values are as follows: max, 67.98 mL/100 g/min; min, 22.46 mL/100 g/min; mean, 49.34 mL/100 g/min; 10th percentile, 32.84 mL/100 g/min; 25th percentile, 43.08 mL/100 g/min; 50th
percentile, 50.92 mL/100g/min; 75th percentile, 57.46 mL/100g/min; 90th percentile, 61.05 mL/100 g/min; skewness, −0.62; kurtosis, −0.42. The ADC histogram values are as follows: max, $1.60 \times 10^{-3}$ mm$^2$/sec; min, $0.70 \times 10^{-3}$ mm$^2$/sec; mean, $0.95 \times 10^{-3}$ mm$^2$/sec; 10th percentile, $0.82 \times 10^{-3}$ mm$^2$/sec; 25th percentile, $0.85 \times 10^{-3}$ mm$^2$/sec; 50th percentile, $0.91 \times 10^{-3}$ mm$^2$/sec; 75th percentile, $0.99 \times 10^{-3}$ mm$^2$/sec; 90th percentile, $1.16 \times 10^{-3}$ mm$^2$/sec; skewness, 1.90; kurtosis, 4.08.

Figure 2

A 77-year-old female with a PA in the left parotid gland. T2-weighted image (a) showing a high signal intensity lesion (arrow). Contrast-enhanced 3D-T1-weighted image (b) showing a little heterogeneous contrast enhancement (arrow). TBF color map (c) showing low TBF (arrow). The ROI was manually drawn on the ADC map of the software (d, yellow), and the ROI was copied from the ADC map to the TBF map of the software (e, yellow). The TBF histogram (f) and ADC histogram (g) are presented. The TBF histogram values are as follows: max, 40.61 mL/100 g/min; min, 0.07 mL/100 g/min; mean, 13.07 mL/100 g/min; 10th percentile, 2.21 mL/100 g/min; 25th percentile, 6.13 mL/100 g/min; 50th percentile, 11.17 mL/100 g/min; 75th percentile, 18.32 mL/100 g/min; 90th percentile, 25.58 mL/100 g/min; skewness, 0.74; kurtosis, 0.07. The ADC histogram values are as follows: max, $2.39 \times 10^{-3}$ mm$^2$/sec; min, $1.52 \times 10^{-3}$ mm$^2$/sec; mean, $1.91 \times 10^{-3}$ mm$^2$/sec; 10th percentile, $1.71 \times 10^{-3}$ mm$^2$/sec; 25th
percentile, $1.78 \times 10^{-3}$ mm$^2$/sec; 50th percentile, $1.89 \times 10^{-3}$ mm$^2$/sec; 75th percentile, $2.00 \times 10^{-3}$ mm$^2$/sec; 90th percentile, $2.17 \times 10^{-3}$ mm$^2$/sec; skewness, 0.59; kurtosis, 0.11.

**Figure 3**

An 83-year-old male with a WT in the left parotid gland. T2-weighted image (a) showing iso signal intensity lesion (arrow). Contrast-enhanced 3D-T1-weighted image (b) showing homogeneous contrast enhancement (arrow). TBF color map (c) showing high TBF (arrow). The ROI was manually drawn on the ADC map of the software (d, yellow) and the ROI was copied from the ADC map to the TBF map of the software (e, yellow). TBF histogram (f) and ADC histogram (g) are presented. The TBF histogram values are as follows: max, 172.47 mL/100 g/min; min, 29.32 mL/100 g/min; mean, 113.60 mL/100 g/min; 10th percentile, 76.90 mL/100 g/min; 25th percentile, 96.35 mL/100 g/min; 50th percentile, 117.90 mL/100 g/min; 75th percentile, 131.39 mL/100 g/min; 90th percentile, 143.38 mL/100 g/min; skewness, −0.44; kurtosis, 0.09. The ADC histogram values are as follows: max, $0.91 \times 10^{-3}$ mm$^2$/sec; min, $0.45 \times 10^{-3}$ mm$^2$/sec; mean, $0.62 \times 10^{-3}$ mm$^2$/sec; 10th percentile, $0.53 \times 10^{-3}$ mm$^2$/sec; 25th percentile, $0.56 \times 10^{-3}$ mm$^2$/sec; 50th percentile, $0.62 \times 10^{-3}$ mm$^2$/sec; 75th percentile, $0.67 \times 10^{-3}$ mm$^2$/sec; 90th percentile, $0.72 \times 10^{-3}$ mm$^2$/sec; skewness, 0.59; kurtosis, 0.38.
Figure 4

ROC curve analyses for differentiating MT from PA (a), for differentiating WT from MT (b), and for differentiating PA from WT (c). (a) The AUCs for the TBF and ADC show medium diagnostic performances (AUC = 0.850 and 0.885, respectively). The AUC for combination of the TBF and ADC show high diagnostic performance (AUC = 0.950). (b) The AUCs for the TBF and ADC show medium diagnostic performances (AUC = 0.855 and 0.814, respectively). The AUC for the combination of TBF and ADC
shows high diagnostic performance (AUC = 0.905). (c) The AUCs of the TBF, ADC, and combination of the TBF and ADC show high diagnostic performances (AUC = 0.968, 1.000, and 1.000, respectively).