Ultrasonography–histopathology correlation in major salivary glands lesions

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Abstract
Major salivary glands display a various and complex pathology, showing different evolution and prognosis, depending on the histopathological form. The choice of an appropriate treatment plan for the best outcome, therefore the proper surgical approach, would imply preoperative knowledge of the histopathological diagnosis. However, any core-biopsy performed prior to surgery presents the risk of a false result and increases the difficulty of latter surgery. Therefore, some complementary examinations are used, among these, ultrasonography. The retrospective study (April 2010–March 2013) conducted in the Clinic of Oral and Maxillofacial Surgery, Emergency County Hospital, Tirgu Mures, Romania, aims to evaluate the relevance of the ultrasonography by itself in leading towards a proper preoperative assessment and diagnosis, and thus, in choosing the proper treatment plan. The study included 33 lesions of the major salivary glands, undergoing first ultrasonography, then curative surgery. Different characteristics (shape, dimension, consistency, vascularization, homogeneity, delimitation) were assessed on ultrasonography as well as on histopathology; finally, the correlation between those two examinations was evaluated, by comparing diagnoses. The results of our study are similar to others, showing that ultrasonography can diagnose preoperatively the majority lesions of major salivary glands. The conclusions of the study sustain the importance of ultrasonography as a routine examination in major salivary glands lesions.

Keywords: major salivary gland lesions, ultrasonography, histopathology, salivary cancer.

Introduction
The pathology of major salivary glands comprises various and numerous lesions: tumoral, cystic, degenerative, inflammatory and traumatic [1, 2].

Preoperative assessment and diagnosis of these lesions include: anamnesis, clinical exam, imaging techniques (plain film, sialography, ultrasonography, CT – computer tomography, MRI – magnetic resonance imaging, and scintigraphy), fine-needle aspiration (FNA) biopsy, core-biopsy; among these, ultrasonography (US) is playing an important role [1–3].

Accordingly, to the preoperative diagnosis, a treatment plan will be established. However, the final diagnosis can be made strictly by histopathology, based upon the postoperative exam of the surgically removed specimen. Thus, upon histopathological (HP) diagnosis, one can verify the accuracy of the prior clinical judgment, and assess the accuracy of imaging techniques (US in this case) in providing the most appropriate preoperative diagnosis to the final, histopathological one.

Ultrasonography is a non-invasive, accessible, and low-cost imaging technique that can be easily repeated, if necessary [2, 4]. Its disadvantage is represented by the subjective interpretation, depending on operator’s “eyes”, his/her experience in a certain pathology, and device performances. US in salivary gland pathology is effective in examining the parotid gland (especially the superficial lobe), submandibular and sublingual gland. US of a salivary gland should assess glands bilaterally – volume, structure, and peri- or intra-glandular lesions (shape, dimension, consistency, homogeneity, delimitation); in addition, echo-Doppler will depict the peripheral and central vascularization, and the lesion’s relation to the intraglandular vessels; finally, it will provide an ultrasonographic diagnosis (presumed diagnosis) [3, 5]. US is a simple imaging technique that offers useful information in addition to clinical exam [6, 7]. Particular disadvantages are encountered in salivary gland US, such as difficulties in deep parotid lobe examination (due to its poor visualization) [5, 8]; similarities of ultrasonographic characteristics of some benign and malignant lesions [9]; limitation in visualization of small lesion (less than 5 mm diameter) [10]. Since 90% of parotid gland tumors are localized in the superficial lobe, US is the imaging technique of choice for a lesion displaying benign characteristics (sometimes associated with FNA biopsy) and no other preoperative methods are necessary [11]. Otherwise, if malignancy suspicion arises, CT and MRI are mandatory to be performed in order to evaluate accurately deep tumor extension, adjacent structure invasion and associated lymph nodes enlargement [5, 10]. Unfortunately, all these imaging techniques present a major disadvantage – they are not able to determine the histopathological structure of salivary gland lesions; thus, the histopathological exam becomes mandatory.
Hence, postoperative histopathological exam of removed specimen is the only one able to provide with certainty the final diagnosis. Although, even the histopathological examination of salivary glands lesions, can encounter difficulties due to presence of hybrid tumors, morphological diversity of lesions and sometimes of different tumor types in the same mass [3, 12]. The importance of the HP exam relies in establishing a final diagnosis, and thus, in appreciating the outcome and prognosis for a certain salivary gland lesion.

In this context, the aim of the study is to search the existing correlation between preoperative US and postoperative HP, in patients having surgically removed lesions of major salivary glands (parotid, submandibular gland).

**Materials and Methods**

The retrospective study (April 2010–March 2013) was conducted in the Clinic of Oral and Maxillofacial Surgery, Emergency County Hospital, Tîrgu Mureş, Romania, and included 33 in-patients presenting major salivary glands lesions, which underwent preoperative US of salivary glands, surgical removal, and postoperative HP exam of removed specimen. Inclusion criteria: in-patients displaying parotid and submandibular gland lesion (benign and malignant tumors, cysts, chronic sialadenitis). Exclusion criteria: patients who underwent only core-biopsy; and those which were diagnosed postoperatively by HP exam with intraglandular lymphadenopathy.

Major salivary gland US used a high-frequency transducer (7.5–12 MHz). US was performed less than two months prior to surgery.

The specimens obtained by surgery were fixed in 10% buffered formalin and then embedded in paraffin blocks. Four-μm thick sections were obtained from the paraffin blocks, stained with Hematoxylin and Eosin (HE) and PAS–Alcian blue. In particular cases, immunohistochemical staining was additionally performed, using the standard Avidin–Biotin method with antibodies against cytokeratin AE1/AE3, cytokeratins 5/6, 7 and 20, vimentin, S100 protein, alpha-smooth muscle actin, p63, CD68, CD31, CD34, Factor VIII-related antigen, leukocytic common antigen and Ki67. Four-μm thick sections were obtained from the formalin-fixed and paraffin-embedded tissue specimens and were routinely dewaxed and rehydrated followed by endogenous peroxidase blocking. Antigen retrieval was performed by pressurized steam cooking (citrate, pH 10). We used the following mouse monoclonal antibodies for cytokeratin AE1/AE3 (clone Pan Ab1, Lab Vision, Fremont, CA, USA), cytokeratin 5/6 (clone D5/16B4, Dako Cytomation, Denmark), cytokeratin 7 (clone OV-TL 12/3, Dako Cytomation, Denmark), cytokeratin 20 (clone KS20.8, Dako Cytomation, Denmark), vimentin (clone V9, Dako Cytomation, Denmark), S100 protein (polyclonal, Dako Cytomation), alpha-smooth muscle actin (α-SMA) (clone 1A4, Lab Vision, Fremont, CA, USA), p63 (clone 4A4, Lab Vision, Fremont, CA, USA), CD68 (clone KP1, Dako Cytomation, Denmark), CD31 (clone JC70A, Dako Cytomation, Denmark), CD34 (clone QBend/10, Lab Vision, Fremont, CA, USA), Factor VIII-related antigen (von Willebrand factor) (clone vWF, Leica Biosystems Newcastle Ltd., UK), leukocytic common antigen (LCA) – CD45 (clone 2B11+PD7/26, Dako Cytomation, Denmark), Ki67 (clone MIB 1, Dako Cytomation, Denmark). Secondary EnVision™ Flex/HRP was used to amplify signal. 3,3’-Diaminobenzidine (DAB, Dako) development was used for detecting primary antibodies. Slides were counterstained with Mayer’s Hematoxylin, dehydrated and mounted.

Characteristics were assessed exclusively on those US and HP interpretations in which they were noted as such (occasionally, some characteristics were not specified). Regarding dimensions, lesions were evaluated in two dimensions by US, and in three dimensions by HP. Results were considered similar when two of the three HP dimensions were close to the US dimensions (variations of ±3 mm were accepted).

Statistical analysis was performed using MedCalc Software (bvba version 12.3.0, Mariakerke, Belgium). Categorical variables were summarized as percentages and compared with the Fisher exact test and chi-squared tests for two groups or more groups. We used statistical sensitivity and specificity parameters to evaluate the accuracy of US results. ROC (Receiver Operating Characteristic) curve was also used to evaluate US performance. We also applied inter-rater agreement statistic (Kappa) to evaluate the agreement between HP results and US results. A significance level of 0.05 was used for all analyses, and all p-values reported are two-tailed.

**Results**

Our study included 33 patients (20 males and 13 females); most of them were found in 61–70 years age group (Table 1). The lesions were more frequent in the parotid gland and especially in males (Table 2).

**Table 1 – Repartition of patients on age and gender**

<table>
<thead>
<tr>
<th>Age group [years]</th>
<th>Males</th>
<th>Females</th>
<th>No. of cases (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21–30</td>
<td>1</td>
<td>2</td>
<td>3 (9.1%)</td>
</tr>
<tr>
<td>31–40</td>
<td>4</td>
<td>1</td>
<td>5 (15.2%)</td>
</tr>
<tr>
<td>41–50</td>
<td>4</td>
<td>1</td>
<td>5 (15.2%)</td>
</tr>
<tr>
<td>51–60</td>
<td>3</td>
<td>3</td>
<td>6 (18.2%)</td>
</tr>
<tr>
<td>61–70</td>
<td>3</td>
<td>4</td>
<td>7 (21.2%)</td>
</tr>
<tr>
<td>71–80</td>
<td>2</td>
<td>1</td>
<td>3 (9.1%)</td>
</tr>
<tr>
<td>&lt;20</td>
<td>2</td>
<td>0</td>
<td>2 (6.1%)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>1</td>
<td>1</td>
<td>2 (6.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>13</td>
<td>33 (100%)</td>
</tr>
</tbody>
</table>

**Table 2 – Repartition of patients on gender and salivary gland**

<table>
<thead>
<tr>
<th>Salivary gland</th>
<th>Gender</th>
<th>Males</th>
<th>Females</th>
<th>No. of cases (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parotid</td>
<td></td>
<td>15</td>
<td>9</td>
<td>24 (72.7%)</td>
</tr>
<tr>
<td>Submandibular</td>
<td></td>
<td>5</td>
<td>4</td>
<td>9 (27.3%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>20</td>
<td>13</td>
<td>33 (100%)</td>
</tr>
</tbody>
</table>

Referring to histopathological type, the lesions were: 16 (48.4%) benign tumors – 11 pleomorphic adenomas, four Warthin tumors, one complex vascular malformation; four (12.1%) malignant tumors – one mucoepidermoid carcinoma, one acinic cell carcinoma, one adenoid cystic carcinoma, one myoepithelial carcinoma; four (12.1%) cysts – two retention cysts, one lymphoepithelial cyst, one dermoid cyst, and nine (27.3%) chronic sialadenitis.
Regarding parotid pleomorphic adenomas (nine cases, 27.3%), correspondence of different characteristics on US and HP was: 100% for shape, consistency, vascularization, homogeneity and delimitation; dimensions correlated in 37.5% of compared cases (the others displayed smaller dimensions on US); diagnosis in 87.5% (US diagnosis error was lymphadenopathy). For submandibular pleomorphic adenomas (two cases, 6%), all compared characteristics correlated in 100% of cases, as well as the diagnosis (Figure 1).

Warthin tumors (four cases, 12.1%) were found only in parotid gland. Concordance was: 100% for shape, consistency, homogeneity and delimitation; 50% for dimensions (on 50% US dimensions being smaller); no comparable cases for vascularization; 50% for diagnosis (US diagnosis error was lymphadenopathy).

The complex vascular malformation (one case, 3%) found in parotid gland, displayed: 100% correspondence for shape, vascularization and homogeneity; bigger dimensions on US (no concordance); unevaluated delimitation; 100% concordance for diagnosis.

Relating to malignant tumors of parotid gland (three cases, 9% – one mucoepidermoid carcinoma, one acinic cell carcinoma, one myoepithelial carcinoma), 100% correspondence was found for all characteristics, including diagnosis. In submandibular gland, one malignant tumor (adenoid cystic carcinoma – 3%) was not detected by US (US diagnosis – normal salivary gland), thus no concordance (0%) was found between the US and HP diagnoses, and the other characteristics could not be compared (Figure 2).

In chronic parotid gland sialadenitis (three cases, 9%), the study revealed: 100% US–HP correlation considering shape, consistency, vascularization and homogeneity; none (0%) considering dimensions (bigger on US); delimitation could not be compared; 66% considering diagnosis (misdiagnosis with tumor). Relating to submandibular gland sialadenitis (six cases, 18.1%), we found 83.3% correspondence for shape; 0% for dimensions; 100% for consistency, vascularization, homogeneity; no comparable cases for delimitation; 66% for diagnosis (two cases, 6%, were misdiagnosed with tumors) (Figure 3).

Cysts (four cases, 12.1%) were located all in parotid gland. In mucous retention cysts (two cases, 6%) the correspondence was of 100% for shape, vascularization, homogeneity; bigger dimensions on US (no concordance); unevaluated delimitation; 100% concordance for diagnosis. For the lymphoepithelial cyst (one case, 3%) 100% correlation of all characteristics and diagnosis was found. For dermoid cyst (one case, 3%), dimensions were bigger on US; the other characteristics could not be analyzed; diagnosis correlated in 100% (Figure 4).
Immunohistochemical staining performed in six cases revealed no correlation between US and those markers, not even when they were found in high levels (e.g., LCA for lymphoid cells in a lymphocytic chronic parotid sialadenitis; CD68 in a granulative inflammatory process of the parotid gland; Ki67 for angiomatoid area in an infarcted submandibular pleomorphic adenoma; α-SMA, S100 protein, cytokeratins 5/6, 7 and p53 for a myo-epithelial carcinoma of the parotid gland).

Statistically, our study revealed: a 100% US–HP correlation concerning vascularization, homogeneity and delimitation; no correlation for shape and consistency – for all lesions types. Kappa test could be applied to “homogeneity” in pleomorphic adenomas, where it revealed a strong US–HP relation (result 1 – meaning 100% correlation). ROC curve was performed in two situations: for pleomorphic adenomas (sensitivity 90.9%) and for tumoral lesions vs all salivary gland lesions (sensitivity 80%).

Discussion

Benign tumors of major salivary glands are mainly epithelial tumors: pleomorphic adenoma (most frequent), Warthin tumor (cystadenolymphoma), myoepithelioma, basal cell adenoma, canalicular adenoma and oncocytoma; and rarely non-epithelial tumors: hemangioma, lymphangioma, lipoma [3, 12]. Clinically, they are usually painless, without facial nerve palsy and no associated lymph nodes enlargement, with a slow progression anamnestically. Ultrasound for all tumors (benign or malignant) must include all regional homo- and heterolateral lymph nodes (but lymph nodes are not subject of this study). US, benign tumors present as nodular lesions, rarely polylobulated, hypoechoic, well delimited, with central and peripheral blood flow, without signs of invasion of the adjacent tissues [5, 9]. Pleomorphic adenomas are also relatively homogeneous, with mostly peripheral vascularization; Warthin tumors have cystic central content (transonic), with central vascularization less abundant than in pleomorphic adenomas [5]. US can make differential diagnosis in most cases between pleomorphic adenoma and Warthin tumor [13]. HP, macroscopically, benign tumors are usually well delimited, usually with tumoral capsule, without invasion into the adjacent salivary tissue. Microscopically, there are no frequent or atypical mitoses [1, 3]. Pleomorphic adenomas display very heterogeneous aspects, probably due to an epithelial–mesenchymal transition process present in their tumorigenesis, as demonstrated by some studies [14, 15]; the most frequent patterns are the ductal and the insular type [16].

Our study included 16 (48.4%) patients with benign tumors; their US evaluation on shape, consistency, vascularization, homogeneity and delimitation were alike to HP in 100% of cases. US dimensions coincided in 46.6% of cases with those on HP, where dimensions did not correlate, US dimensions were smaller, preoperative larger dimensions on US were measured in the vascular malformation, and smaller dimensions on HP were the result of partial intraoperative evacuation of the tumoral content. US diagnosis coincided in 80% of the cases with HP diagnosis; US misdiagnosis in three (20%) cases were done with: tumoral lymph nodes (one case) – in a recurrent pleomorphic adenoma with multiple tumoral
nodules (multiple small pleomorphic adenomas, hypoechoic, round, maximum 15 mm diameter, too small for a thorough evaluation of blood flow); and with inflammatory lymph nodes (two cases) – in Warthin tumors (hypoechoic, nodular, well delimitated, vascularized). Our study result of 80% US accuracy in proper diagnosis of benign tumors, are similar to another study – 83% in determining a benign tumor of salivary glands [7]; other studies revealed even a greater sensitivity of US in diagnosing benign tumors: 87.5% [17] and 89.7% respectively [18]. Since pleomorphic adenomas were the majority of benign tumors, we determined US sensitivity in detecting that tumor, at 90.9%.

Among the malignant salivary tumors, carcinomas are frequent, followed by lymphomas and rarely by sarcomas [11]. Clinically, in early stage, they look like benign lesions; later, the tumor becomes adherent to the surrounding tissues, painful, invades facial nerve causing facial palsy, could exulcerate, and tumoral lymph nodes are associated. US, malignant lesions have: irregular shape, numerous blood vessels with anarchic distribution, inhomogeneous structure, imprecise delimitation, sometimes visible invasion of adjacent structures [3, 5]. Malignant lymph nodes are visualized typically having round shape and increased central blood flow. In US, similar to the clinical exam, malignant tumors may display benign characteristics, and so malignant-benign differentiation cannot be made based upon US alone [9, 19–21]. HP, macroscopically, malignant tumors display: irregular shape (but sometimes may be nodular), rich vascularization, inhomogeneous feature, tumoral capsule (but mostly capsule is missing and surrounding salivary tissue is inhomogeneous feature, tumoral capsule (but mostly may be nodular), rich vascularization, inhomogeneous structure, imprecise delimitation, sometimes visible invasion of adjacent structures [3, 5]). Malignant lymph nodes are visualized typically having round shape and increased central blood flow. In US, similar to the clinical exam, malignant tumors may display benign characteristics, and so malignant-benign differentiation cannot be made based upon US alone [9, 19–21]. HP, macroscopically, malignant tumors display: irregular shape (but sometimes may be nodular), rich vascularization, inhomogeneous feature, tumoral capsule (but mostly capsule is missing and surrounding salivary tissue is invaded); in acinic cell carcinomas, there is also active intratumoral angiogenesis [22]. Microscopically, they present infiltrative margins, necrosis, cellular and nuclear pleomorphism [2, 12].

Our study included four (12.1%) patients with malignant tumors, all carcinomas. For those, US described shape, dimensions, consistency, vascularization, homogeneity and delimitation similar to histopathology in 100% of compared cases. These characteristics could not be compared in one case, where US described a normal gland and HP revealed an adenoid cystic carcinoma, visible only macroscopically (dimensions measured on stained section being 2×4 mm). As a result, US diagnosis correlated in 75% of cases with HP; incorrect diagnosis was made with the submandibular carcinoma mentioned above. We think that US is useful in diagnosing almost any malignant lesion, larger than 5 mm diameter; the misdiagnosed tumor in our study being difficult to detect with most existing imaging methods. An article published in 2011 shows even that US has greater sensitivity in detecting malignant tumors than CT or MRI [4]. Ultrasound sensitivity in diagnosing malignant lesions revealed by our study was: similar to another study made in 2000 (75.5%) [17]; smaller than values found by other studies – 81.8% in a 2012 [23], 81.8% in a 1997 [6]; but higher than 57% found in a 1989 study [7].

Chronic sialadenitis is a group of inflammatory diseases where affected salivary gland presents chronic inflammation due to a prolonged obstruction, usually caused by a calculus, or canalicul fibrosis or canalicul wall thickening due to repeated infections [1–3]. Clinically, affected glands display: volume enlargement, firm consistency, reduced spontaneous pain, tenderness to palpation, decreased salivary flow; and acute episodes are associated [1, 2]. US exhibits: pseudonodular shape, inhomogeneous structure, more echoic, increased blood flow, association of inflammatory lymph nodes. When lithiasis is the cause of chronic inflammation, sialoliths can be visualized if they are at least of 5 mm diameter [5, 10]. HP, macroscopically, gland presents: increased consistency, but lobular architecture is conserved. Microscopically, increased periductal and periacinar fibrosis, association of chronic inflammatory lymphoplasmocytic infiltrate with variable density, acinar atrophy and squamous metaplasia of ductal epithelium and irregular canalar dilatation, can be seen [3].

US and HP were compared for nine (27.3%) patients with chronic sialadenitis. Shape corresponded in 88.8% cases; consistency, vascularization, homogeneity and delimitation correlated in 100% cases. Dimensions coincided in 14.2% of compared cases. US diagnosis corresponded in 66.6% of cases with HP; the confusion was done with tumors (polynodular shape, hypoechoic, firm, increased blood flow, inhomogeneous). Sialolithiasis present in one (3%) case in our study was revealed by US (100%). For chronic sialadenitis, ultrasound sensitivity was 66.6%, higher than results of other studies: a 1993 study – 58% [24], and a 1989 study – 54% [25].

Cysts are non-neoplastic lesions (pseudotumors) and they include mucous retention cysts, lymphoepithelial cysts, dermoid cysts, epidermoid cysts, branchial cleft cysts. Retention cysts (salivary duct cysts) in major salivary glands are produced by chronic but incomplete duct obstruction. Lymphoepithelial cysts are frequently encountered in patients with HIV infection. Dermoid cysts are congenital lesions rarely encountered in parotid gland, caused by epidermal inclusions within the line of embryonic closure [27]. Clinically, cysts have a history of slow, painless evolution, but pain could be caused by infection or trauma, fluctuency [3]. US reveals: round shape, hypoechoic with central transonic image, well delimited, without central vascularization [5, 9]. HP, macroscopically, cysts are elastic lesions, with liquid content, round or oval shape, blue colored if they are superficial or similar color to the surrounding tissues [3]. Microscopically, retention cysts are delineated by epithelium with frequent squamous or mucinous metaplasia, easily to mistake for a low-grade mucoepidermoid carcinoma [26]; lymphoepithelial cysts have typical lymphoepithelial islands; dermoid cysts are delineated by squamous epithelium, which contains all type of skin adnexa (sebaceous glands, sweat glands, hair follicles).

Our study included four (12%) patients with cysts. Correlation between US and HP was found to be 100% in all compared cases for shape, vascularization, homogeneity and delimitation. Consistency correlated in 66.6%. US dimensions were the same as in histopathology in 25% cases; they were smaller than HP dimensions in 75% cases, possibly due to intraoperative evacuation of cysts content. US diagnosis correlated in 100% with HP, similar to a 2012 study, where ultrasound sensitivity in detecting cystic lesions was 92.3% [18].
The results of our study, similar to others, sustain the accuracy of US in determining if a salivary gland lesion is of neoplastic, inflammatory or cystic nature. By using a ROC curve, our study established a sensitivity of 80% and a specificity of 76.9% in detecting tumoral lesions (Figure 5), with a positive predictive value of 84.2% and a negative predictive value of 71.4%. Sensitivity was smaller than in other studies: 98.5% in a 2011 study [28], 89.3% in a 1999 study [30], and 97.5% in a 1987 study [29], 89.3% in a 1999 study [30], 98.5% in a 2011 study [28], with a positive predictive value of 84.2% and a specificity of 76.9% in detecting tumoral lesions. By using a ROC curve, our study established a sensitivity of 80% and a specificity is obtained.

However, limitations of this study should be mentioned. One is represented by the small sample of patients, which did not permit realization of certain statistical tests (e.g., inter-rater agreement – kappa, ROC curve for more parameters) for every characteristic and every type of salivary gland lesion. Therefore, we could not identify one single US characteristic or an association of those, which could have display a strong predictive value for the final HP diagnosis, although strong correlations were found, but without statistical significance. Another limitation is represented by the retrospective type of this study; therefore, we could not find all characteristics we wanted to compare in both US and HP interpretations, and so, the sample decreased even more, making a coherent statistical analysis difficult.

A more extensive study and of prospective type would be useful in the future, in order to evaluate the same characteristics in ultrasound and histopathology for all major salivary glands lesions.

Conclusions

This study enhances the importance of US in diagnosing a major salivary gland lesion, by studying the correlation ultrasonography–histopathology exam for some of the most frequent types of diseases. For all types of lesions, US diagnosis correlates in 78.1% of cases with HP diagnosis. Even if this study did not produce entirely the expected results, we find it promising, and we think that it could become more relevant if a larger number of cases would be enlisted. In our opinion, surgeons should use US as a first line diagnosis tool for any major salivary gland lesion (preferably associated with FNA, in order to increase its accuracy), but always keeping in mind its limitations and the possibility of error.

Conflict of interests

The authors declare that they have no conflict of interests.

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References

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