Effectiveness of fine-needle aspiration cytology in the diagnosis of lateral cervical nonthyroid tumors

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Abstract
Given that the clinical and radiological examinations of lateral cervical masses are not always sufficient for deciding on appropriate management, the cytological examination of the material obtained by fine-needle aspiration might be an efficient tool in the preoperative investigation of these lesions.

In this prospective cross-sectional study we evaluated the efficacy and diagnostic accuracy of fine-needle aspiration cytology in the assessment of lateral cervical nonthyroid tumors, by comparing its results with those of histopathology.

A total of 58 patients with lateral cervical masses were included. Preoperative cytological results were compared with the histopathologic examination of surgical specimens.

Both cytology and histology indicated that malignant tumors outnumbered benign lesions (62% vs 38%), with 88.9% of malignancies presenting in patients aged >50 years, but cytology was less effective at differentiating between benign and non-tumor lesions. Cytology had 76.5% specificity and 78.1% sensitivity for identifying malignant lateral cervical lesions, and there was a concordance between the two diagnostic tests (McNemar test, $P=0.07$, $\kappa=0.50$, $P<0.001$).

Fine-needle aspiration cytology is a simple, quick, and effective procedure that can aid in the preoperative evaluation of lateral cervical masses by differentiating benign tumors and inflammatory processes from malignancies and thus help in determining a subsequent therapeutic strategy.

Abbreviations: FN = false negative, FP = false positive, LR− = negative likelihood ratio, LR+ = positive likelihood ratio, NPV = negative predictive value, PCR = polymerase chain reaction, PPV = positive predictive value, Se = sensitivity, Sp = specificity.

Keywords: diagnostic accuracy, fine-needle aspiration cytology, histopathology, lateral cervical masses, nonthyroid tumors, sensitivity, specificity

1. Introduction
Clinical evaluation and differential diagnosis of tumor masses developed in the lateral cervical area can be difficult, because of the heterogeneity of the lesions likely involved. Most frequently these can be enlarged neck lymph nodes (inflammatory or metastatic causes), other inflammatory pathology, thyroid gland disorders, or salivary gland tumors. On rare occasions, branchial cysts, thyroglossal duct cysts, carotid glomus tumors, and cysts or tumors of the skin annexes can also be diagnosed.

The clinical diagnosis of neck masses is based on information obtained from the patient’s history and a thorough physical examination. The next steps in the workup and management of neck masses can include ultrasonography, computed tomography, fine-needle aspiration, or further follow-ups. First described by Kun in 1847, fine-needle aspiration is a simple, cheap, quick, and effective procedure, which can be used to assess neck masses. Performed either with or without imagistic assistance (ultrasound), it provides prompt information about the nature of the assessed lesion. The technique has very few contraindications and risks, and it is suitable for use in an ambulatory setting. It can offer valuable information for the diagnosis of lateral cervical masses by differentiating inflammatory or benign lesions from malignant ones and guiding therapeutic management. Although it cannot replace histologic examination, as the biological material obtained does not provide precise cellular architectural details, fine-needle aspiration has the advantage of providing samples from multiple lesion sites, thus rendering incisional biopsy unnecessary in some cases. Ideally, fine-needle aspiration should be performed preoperatively for all cases of doubtful lateral cervical swelling, as the cytological results can direct further investigations.

The procedure is widely used in the management of thyroid and breast tumors worldwide, but in our country, it is only occasionally performed for the assessment of lateral cervical masses. We aimed to comparatively evaluate the efficacy of fine-needle aspiration cytology and histopathologic examination in a series of patients treated for lateral cervical masses.
2. Methods

This prospective, cross-sectional study was conducted between May 2012 and February 2014 in the Oral and Maxillo-Facial Surgery Department of the Târgu-Mureş County Emergency Clinical Hospital, and included patients with tumor or tumor-like masses located in the lateral cervical area. The inclusion criteria were as follows: age >20 years, patients with nonulcerated tumors completely covered by intact skin, and the absence of any contraindications for performing fine-needle aspiration. Exclusion criteria were as follows: cytology slides inadequate for interpretation, patients with recurrent lesions or who have not received surgical treatment and did not have a histological examination, and patients with thyroid masses. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Târgu-Mureş (No. 30/26.06.2012). The procedure was explained to all patients and a written consent was obtained in each case.

Fine-needle aspiration was performed without radiological guidance by a maxillofacial surgeon, using a 23-G needle and a 10-mL or 20-mL syringe unattached to an aspiration syringe holder; a minimum of 2-needle passes were made in each case. The aspirated material was spread onto 2 to 4 slides and immediately fixed by immersion in 95% ethyl alcohol. Slides were stained the same day (Papanicolaou stain) and evaluated in the Pathology Department of the same hospital. Patients underwent surgical treatment at a mean of 5 days (1–12 days) after fine-needle aspiration, and the obtained histological specimens were analyzed in the same Pathology Department, by different pathologists, blinded to the cytological results. When needed special stains and immunohistochemical evaluations were also performed. To determine the efficacy of fine-needle aspiration, in each case the cytological result was compared with the final histological result, considered as “gold standard.”

Statistical analysis was performed using GraphPad InStat 3, La Jolla, USA and MedCalc software, Ostend, Belgium. For the analysis and evaluation of qualitative variables and statistically significant differences between groups, the $\chi^2$ test was used, with a level of significance set at $P<0.05$. In order to assess the efficacy of fine-needle aspiration procedure as a diagnostic test for nonthyroidal tumors of the cervical region, indicators of sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated for 95% confidence intervals.

3. Results

A total of 58 patients met the inclusion criteria, 34 (58.6%) male and 24 (41.4%) female patients, aged 23 to 81 years. Most fine-needle aspiration procedures were performed without local anesthesia, but in some cases patients opted for the comfort provided by the use of an anesthetic. There were no significant complications, except for a few cases of slight discomfort immediately after aspiration, which was controlled by oral analgesia. The lateral cervical tumors diagnosed were clinically divided into lymphadenopathies, salivary gland disorders, and tumors of other anatomical structures (branchial cysts, dermoid cysts, lipomas, and thyroglossal duct cysts).

The examined lesions involved lymph nodes in the majority of cases (36 cases, 62%), with the rest being submandibular gland pathology (13 cases, 22.4%) and other cervical tumors (9 cases, 15.6%). Both cytological and histological examinations indicated that the majority of lesions (62%) were malignant, but cytology was less effective at differentiating between benign and nontumor lesions (Table 1). The predominant lesions in our series—those involving the lymph nodes—were malignant in almost all cases (91.7%) according to the histopathological examination. We found a predominance of squamous cell carcinoma lymph node metastases; this was also shown by both cytology and histology (44.8% of cases based on histological examination, Table 1).

There was a significant association between patient age and the presence of malignant tumors, detected by both examination methods (Fischer’s exact test, $P=0.002$ for cytology and $P=0.001$ for histology, respectively). Thus, a higher frequency of malignant tumors was observed with increasing age in patients aged >50 years, compared with those <50 years (88.9% vs 11.1%), as shown in Fig. 1.

When evaluating the Sp, Se, PPV, and NPV of cytology in identifying lateral cervical nonthyroid malignancies, as compared with the histopathology examination, there was concordance between the two diagnostic tests (McNemar test, $P=0.17$); the Kappa coefficient also showed moderate concordance between

Table 1

| Histology                        | No. of cases (%) | Concordant cytology | Discordant cytology |
|----------------------------------|------------------|---------------------|---------------------|
|                                  |                  |                     |                     |
| **Primary malignancies**         |                  |                     |                     |
| Malignant                        | 4 (6.9)          | 3                   | 1                   |
| Squamous cell carcinoma metastases | 26 (44.8)      | 25                  | 1                   |
| Other tumor metastases           | 1 (1.7)          | 1                   | —                   |
| Lymphoma                         | 5 (8.6)          | 5                   | —                   |
| Pleomorphic adenoma              | Benign 25.9%     | 4                   | —                   |
| Benign lateral cervical masses    | 11 (19)          | 9                   | 2                   |
| Inflammations                     | 7 (12.1)         | 5                   | 1                   |

Figure 1. Cytological distribution of the studied lateral cervical lesions in relation to age groups.
lesions were also reported by other authors.\cite{1,18,20} Soni et al\cite{3} found salivary gland lesions in 22.03% of their cases, and Ishar et al\cite{20} reported miscellaneous lesions in 21.87% of their 160 cases of nonthyroidal head and neck swellings, with only 9.38% of lesions involving the salivary glands.

Although our cases presented significant heterogeneity in both malignant and benign lesions, the malignant pathology was dominated by squamous cell carcinoma. Metastases, alongside primary tumors located in the oral cavity (upper aerodigestive tract), lymphomas, and other metastases. Other authors have already confirmed that metastatic lymphadenopathies are the most common lateral cervical malignant lesions, and their diagnosis can be efficiently established based on cytology, using fine-needle aspiration.\cite{2,6,17,21–24}

In spite of the diverseness of the pathology found, in most cases the histopathological diagnosis was suggested by the cytological examination, although sometimes only a description of the existent cellular population could be obtained. Still, in certain cases, cytology yielded false positive (FP) and false negative (FN) results. In a case of necrotizing sialometaplasia in the mouth floor associated with submandibular lymphadenopathy, based on clinical assessment and cytology, the lymph node lesion was considered a tumor metastasis, but was later identified as an inflammatory adenopathy by the histopathologic examination. Some studies also describe cases where cytology cannot distinguish between malignant squamous carcinoma and secondary changes that accompany chronic inflammatory conditions (such as squamous metaplasia).\cite{25,26} An example of a FN result is that of a clinically benign submandibular swelling, which cytologically presented inflammatory changes, but histological examination revealed an adenoid cystic carcinoma. Nevertheless, the malignant transformation was estimated to be very small (∼4–2 mm) and surrounded by an area of inflammation. It is unlikely that such a small lesion—located in an inflammatory mass of about 4 cm in diameter—can be sampled by needle during aspiration. Unfortunately, this case highlights the fact that small lesions with only partially involvement of the examined mass can be missed during the aspiration procedure.\cite{2}

Cytological examination also proved to be difficult in lymphomas, where the lesions could only be identified as lymphoproliferative masses and a conclusive diagnosis was only obtained after histopathological and immunohistochemical assessments. These cases highlight one of the major limitations of cytology, namely the fact that architectural details are poor compared with histopathology.\cite{27}

In the present classification of lymphomas, correct diagnosis is based on the combination of clinical assessment, immunophenotypic and genotypic details, and histomorphologic features,\cite{28} so biopsies and histopathological examinations are of paramount importance for an appropriate diagnosis, classification, and subclassification of these pathologic entities. A development of recent years, the application of ancillary techniques like flow cytometry on cytological material obtained by fine-needle aspiration made it possible to diagnose most types of lymphoma based on cytology alone, rendering biopsy unnecessary. Also, a significant number of lymphomas can be sub-classified solely based on cytological evaluations.\cite{24} Because material for ancillary testing may be limited, initial morphologic assessment is the most important step to determine if a particular investigative pathway will lead to the right diagnosis.\cite{27} However, some studies have shown that fine-needle aspiration cannot replace biopsy in all cases, and in these situations histopathology is a requirement for a correct diagnosis.\cite{5,29–31}

### Table 2

| Indicators                      | Estimated values | 95% Associated confidence interval |
|---------------------------------|------------------|-----------------------------------|
| Se                              | 78.1%            | 62–89                             |
| Sp                              | 76.5%            | 50–93                             |
| Accuracy                        | 77.6%            | 65–87                             |
| PPV                             | 88.9%            | 74–97                             |
| NPV                             | 59.1%            | 36–79                             |
| Rate of FP values               | 23.5%            | 7–50                              |
| Rate of FN values               | 21.6%            | 11–38                             |
| LR +                            | 3.32             | 1.39–7.94                         |
| LR –                            | 3.48             | 1.85–6.57                         |

FN = false negative, FP = false positive, LR = negative likelihood ratio, LR+ = positive likelihood ratio, NPV = negative predictive value, PPV = positive predictive value, Se = sensitivity, Sp = specificity.

**4. Discussion**

The first description of fine-needle aspiration for the evaluation of head and neck masses was given by Kun in the 19th century. Since then, the procedure was perfected by Martin and Eliss in the 1930s, whereas Scandinavian authors brought major improvements to the technique and defined its basic principles and criteria for differential diagnosis.\cite{1,11–13} Over time, fine-needle aspiration cytology became a first-line investigation in the evaluation of maxillofacial tumors, because of the advantages of the method, such as the minimally invasive nature of the procedure, its diagnostic efficacy, and cost/ efficiency ratio.\cite{14,15} Furthermore, the processing of the obtained cytological material is very quick (a few hours to 1 or 2 days, depending on the stains used and ancillary techniques applied), with readily available results (usually within a few days), far quicker than histology.\cite{16} The clinical value of aspiration cytology is not limited to tumor lesions—a series of authors have reported its use for the diagnosis of inflammatory and infectious lesions.\cite{1,14}

We found a female:male ratio of 1:1.4 for patients with lateral cervical masses, similar to the results reported by Manjula et al\cite{17} and Akhavan-Moghadam et al\cite{16} in their series of 386 and 65 patients, respectively. Still, many clinical studies reported uneven data regarding gender distribution of lateral cervical masses; in their extensive study of 850 cases, Modi et al\cite{18} found a female: male ratio of 1:0.61; whereas Soni et al\cite{3} described the involvement of 66.15% female patients and 33.89% males in their study of 59 cases. In a similar study of 50 patients, Ahmad et al\cite{19} found a female:male ratio of 2.12:1.

Regarding the nature of lateral cervical tumors, we found 62% malignancies, 25.9% benign tumors, and 12.1% inflammatory lesions. Similar studies conducted in geographic areas with a high prevalence of inflammatory lesions reported less tumor masses: the study published by Manjula et al\cite{17} found 27.36% malignant tumors, only 6.57% benign tumors, and 49.2% inflammatory lesions of the lymph nodes, whereas Akhavan-Moghadam et al\cite{16} found 40.8% malignant tumors, 19.4% benign tumors, and 39.8% nonneoplastic lesions in their series.

The majority of our cases involved lymph nodes (adenopathies of different etiologies) in 62%, with submandibular salivary gland involvement in 22.4% of cases and other pathologies in 15.6% of cases. Similarly significant numbers of lymph node
In some cases the cytological diagnosis is hard to establish. In these situations with morphologic ambiguity, ancillary testing methods—especially immunophenotyping—are considered compulsory in the diagnosis of lymphoproliferative disorders. Ancillary studies are guided by the initial cytologic evaluation of the smear that provides the baseline for the interpretation of molecular testing and immunophenotypic results. Flow cytometry has become indispensable in the diagnosis and classification of B-cell non-Hodgkin lymphomas with small-/intermediate-sized cells, whereas the selection of antibodies is guided by cytomorphology, patient history, and the amount of biological material available. In addition, flow cytometry protocols using ≥10 colors can be similarly informative when dealing with extremely paucicellular specimens. Cytogenetic testing (like fluorescence in situ hybridization) can be used to demonstrate genetic abnormalities, which can either be recurrent and diagnostic for a specific type of lymphoma, occur with a higher frequency (and thus support diagnosis), or have a prognostic value. Also, lymph node micrometastases can be detected by gene promoter hypermethylation in samples obtained by fine-needle aspiration. Molecular studies, mostly polymerase chain reaction (PCR)-based assays for clonality and translocation detection with or without sequencing, are rarely used in the diagnostic workup of lymphomas but can be used for the diagnosis of mycobacterial lymphadenitis in children.

In order to consider fine-needle aspiration cytology as a “stand-alone approach” in the diagnosis of lymphomas (especially non-Hodgkin lymphomas) interdisciplinary collaboration is needed, with multiparametric testing and on-site specimen adequacy assessment as requirements for better results. Clinical data correlated with immunophenotyping and cytogentic can offer an appropriate context for interpreting cytomorphology results and support a precise diagnosis even in difficult cases.

In regard to the diagnostic accuracy of fine-needle aspiration cytology in differentiating malignant and benign lesions, we found a Se of 78.1% and a Sp of 76.5%. Similar studies reported Se between 82.14% and 95%, and a Sp of 62.7% to 100%.[3,4,5,17,21,22,32–34] Thus, our results are in line with those found in the literature.

The major cause for a fine-needle aspiration specimen being nondiagnostic is failure to aspirate enough material for proper cytological assessment. The use of immediate on-site evaluation of the aspirated material can determine the quality of the harvested specimens and can significantly decrease nondiagnostic rates. As results regarding material adequacy are obtained immediately, it has been reported that on-site assessment of fine-needle aspiration specimens reduces nondiagnostic rates of the procedure from 20% to ~1%. Also, combining ultrasound-guided fine-needle aspiration of head and neck masses with on-site evaluation of the specimen by a cytopathologist was found to be 24% more accurate compared with that of materials obtained by clinicians without on-site assessment. Unfortunately, because of our department’s location, we did not have a possibility to perform on-site evaluation of the aspirated material.

Although our study did not include a cost analysis of the procedure, previous experience shows that fine-needle aspiration biopsy is also a cost-efficient diagnostic method. O’Donnell et al.15 aimed to determine the clinical benefits of a dedicated fine-needle aspiration service and assess if such a service is cost effective; they found that the procedure provides sufficient pathological information to avoid day case surgery in 42.2% of patients and inpatient surgery in 48.1% of cases, with significant savings.

The limitations of our study are mainly given by the relatively small number of cases and the fact that it was conducted in a single surgical department, in a short period of time. Because the patients presented a variety of lateral cervical entities—both benign and malignant—it was difficult to compare the effectiveness of cytology with histology for each individual type of lesion. More accurate results could be obtained by continuing the study over a longer period of time, thus including a larger number of patients.

Fine-needle aspiration cytology is an inexpensive, quick, and efficient diagnostic procedure that is well tolerated by patients and reliable for the preoperative assessment of tumor lesions of the lateral cervical area. In our series of patients we found that fine-needle aspiration cytology can efficiently differentiate malignancies from benign lesions, with results similar to those of histopathological examinations. A good concordance was found between the information provided by the cytological examination and histopathology for the investigated lateral cervical masses, especially for squamous cell carcinoma metastases and benign salivary gland tumors, as well as other benign lesions.

The cytological examination of the material obtained by fine-needle aspiration was efficient in differentiating inflammatory lesions from benign or malignant tumors, thus proving useful for the planning of surgical treatment. However, in some situations cytological data was insufficient for a proper assessment—for instance, in cases of lymphoma, the diagnosis is only suggested by cytology, and an accurate description can only be obtained with further histopathological and immuno-histochemical examinations.

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