



## Original research

## Surgical management of pleomorphic adenoma of parotid gland in elderly patients: Role of morphological features

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## ABSTRACT

The neoplasms of the salivary glands account for 2% of head and neck tumors and the most common form is the Pleomorphic adenoma (PA). Parotid gland is affected from 80% to 90% of cases. In elderly these tumors occur mostly in females. These benign tumors are composed of epithelial and myoepithelial cells that are arranged with various morphological patterns and subtypes. The classification of these tumors is also based on the amount and nature of the stroma. In literature there is an almost complete consensus that, in the major salivary glands, PAs are enclosed by a layer of fibrous tissue often called "capsule" but there is disagreement about the form, extension and thickness of this layer. The treatment is surgical and there are two main different surgical approaches: an enucleation (local dissection) or so-called subtotal superficial parotidectomy and lateral or superficial total parotidectomy. Histopathological characteristics of PAs especially of capsular alterations such as thin capsule areas, capsule-free regions, capsule penetration, satellite nodules and pseudopodia in the different subtypes are important for the choice of surgical treatment and the first explanation for tumor recurrence. In our study we describe a morphological features of 84 cases of pleomorphic adenoma of parotid gland from elderly patients treated by a surgical "enucleation like" method called nucleoresection.

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## 1. Introduction

Parotid gland tumors represent 2–3% of head and neck tumors and 0.6% of all tumors in the body. Parotid pleomorphic adenoma is the most common slow-growing benign neoplasm of the salivary glands. It constitute approximately 70–80% of benign parotid gland

tumors [1–5]. Sometimes these tumors could be misdiagnosed with other lateral-pharyngeal region masses [6,7]. Usually occurs in peoples of all ages, and the highest incidence is in the fourth to sixth decades [1]. In old age it occurs more frequently in males than in females patients [1,2]. These tumors are most often diagnosed and treated when the tumor is small (<4 cm), mobile, and located in superficial lobe [8–10]. The parotid gland is divided into superficial and deep lobes by virtue of the facial nerve, which passes through its substance [9,10]. The "superficial lobe" include the parenchyma's part of the gland lies lateral to the nerve. Approximately 90% of parotid tumors occur in this part of the gland. The remaining 10% occur in the "deep lobe" the lower part of the gland lying deep to the facial nerve [9]. These benign mixed tumors are characterized by cellular polymorphism and composed of an epithelial and myoepithelial cells and connective tissue component

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embedded in a stroma of mucoid, myxoid, chondroid or osteoid origin [11–15]. Macroscopically it has surrounding “capsule” from which it can be enucleated—often the treatment used in the past. Often these tumors do not have a true capsule but can press surrounding normal salivary gland, frequently having finger-like extensions into the normal tissues [16]. These and others histopathological characteristics are the main explanation for tumor recurrence after inappropriate surgical treatment. The instrumental examination used for planning the surgical treatment to be applied and for studying the relations of recurrence with glandular parenchyma were CT with contrast medium or MR of head and neck. In this context, the value of morphological findings obtained by fine needle aspiration cytology (FNAC) can be questioned [17–26]. Surgical management of pleomorphic adenoma 50 years ago consisted predominantly of local excision or “enucleation technique”, a procedure that yields recurrence rates from 20% to 45%. The hypothesis for recurrence of pleomorphic adenoma using this type of surgical approach is that microscopic portions of tumor perforate the capsule and are shared off resulting in a subtotal removal [27,28]. Nevertheless, some complications can be occurred during surgical procedure. The main complication is temporary/permanent facial nerve paralysis that compromises quality of life. Various studies showed a positive relationship between the extent of parotid surgery and postoperative facial nerve function. Another relatively frequent complication is Frey syndrome/gustatory sweating. A further unwanted complication of surgical approaches in recurrent tumors is parotid cancer development. In literature patients treated by different surgical procedures showed in different percentages these dysfunctions [29–34]. The replacement of simple enucleation of tumor with other surgical approaches like: superficial parotidectomy (SP), total parotidectomy (TP), and extracapsular dissection (ECD) as treatment of choice reduced dramatically the incidence of tumor’s recurrence and complications [35–37]. There is no hint of a remodeling of  $Ca^{2+}$  toolkit, that has been observed in other tumoral lesions, including renal cellular carcinoma [38–40], and prostate cancer [41], mielofibrosis [42], and used as target for selective molecular therapies. Actually there is a general consensus about relevance of adenoma “capsule”; in this paper we measured its thickness and try to demonstrate the precise relationship between this histopathologic feature, surgical management and tumor recurrence.

## 2. Material and methods

Samples of PA affecting the major salivary gland from 84 patients were collected between 2002 and 2011. Patients with recurrent PA or history of any other parotid gland surgery were excluded from the study. According to epidemiologic data of elderly 54 patients were females (64.2%) and only 30 were males (35.7%). Mean age was 67.5 years  $\pm$  4.9 years SD. In 76 of 84 cases (90.4%) first diagnosis was performed, while in 8 cases (9.5%) recurrence occurred. All patients underwent ear, nose and throat (ENT) specialist examination, ultrasonography and preoperative fine-needle aspiration biopsy at Otolaryngology Department of Second University of Naples. A Nucleoresection surgical approach was performed in 76 patients (90.4%). In 3 patients (3.5%) and in 5 patients (5.9%) superficial parotidectomy and total parotidectomy respectively were used to remove the masses. After surgical procedures, specimens were fixed in 4% paraformaldehyde. Before dissecting the specimens for histopathological processing the surface of the parotid gland, which usually was protruded by the adenomas was inked with a specific color. We used India Ink color so it is feasible that minor capsular artifacts arose during fixation and cutting so the degree of invasion or capsular damage might have been underestimated. The inked specimens were dipped into Bouin

solution for 30 s to mordant the ink to the surface, thus minimizing smearing during dissection. The specimens were cut into a various number of slices of 4-mm thickness according to the tumor size. Slices were perpendicular to the long axis of the tumor nodule, which usually correspond to the long axis of the gland. The tip of the small tumor poles were cut perpendicular to the main slicing direction to obtain many transverse cuts of the tumor capsule of the poles. Thus, the slides were dehydrated in a graded series of alcohols and xylene and embedded in paraffin. Sections of 5- $\mu$ m thickness were stained with Emaoxilin & Eosin. A Leitz Axiophot microscope (Leitz, Germany) equipped for microphotography was used for light microscopic observations. An advanced software for the analysis of images (Quantimet 520, Leica, Germany) was used to measure some characteristics of sections. Images were directly acquired by optic microscope at 20 $\times$  enlargement using a specialized video camera (DC 200, Leica, Germany). The histologic examined areas were digitized and successively processed. The optic quality of these areas was optimized by modifying the brilliance and the contrast. The stained areas were highlighted by the program on the basis of their levels of gray and measured. This measurement was realized on 5 sections for each sample and six fields were analyzed per section. The mean value of measurement derived by the analysis of all areas in the 5 sections was reported. Sections were examined and measured by three independent observers.

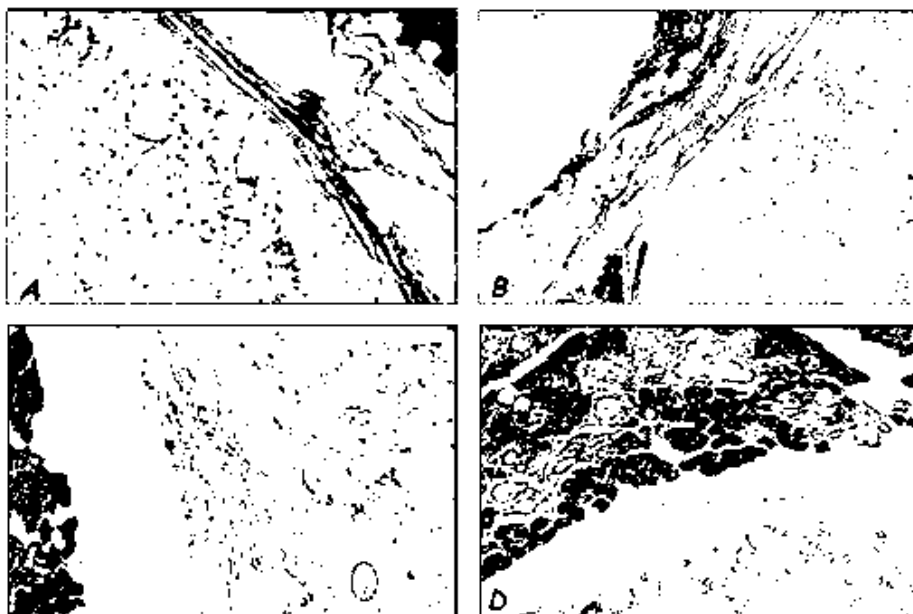
## 3. Results

In our series, cytological study had an excellent diagnostic value with a higher sensitivity in comparison with MRI scan. According to Seifert et al. original classification partially revised by Stennert et al., in 2001 our samples were graded in four types [11,12]. Type I comprised 13 patients (15.4%), type II comprised 44 patients (52.4%), type III included 18 subjects (21.4%) and finally 9 patients (10.7%) were assigned to type IV. Results of capsule measurement performed using analysis of images software are summarized in Table 1. Parenchyma-rich tumors showed a thicker capsule than in stroma-rich tumors. In our samples hypercellular PAs have a thick capsule (Fig. 1(A) and (B)). Hypocellular tumors have a thin capsule and constitute the most frequently encountered histological type in recurrence (Fig. 1(C) and (D)). In stroma rich tumors the largest amount of stromal differentiation were represented by myxomatous tissue followed by chondroid and mixed mucochondroid stroma. According to literature tumors of deep lobe have a thicker capsule than those located in the superficial lobe. Tumors pseudopodias or grow through capsular breaches to extend to adjacent parotid or adipose or other soft tissues. Pseudopodias or *capsule bulges* are considered as an additional factor in recurrence. Capsule dimensions and integrity represented a good tool to establish the better surgical technique. We used in 76 patients (90.4%) a surgical approach called nucleoresection (Fig. 2). In 3 patients (3.5%) and in 5 patients (5.9%) superficial parotidectomy and total parotidectomy

**Table 1**

In this table are reported measures of capsule thickness and their relationship with tumor grading.

Grading	Capsule thickness
Tipo I (>8 mm < 25 mm)	215.6 $\mu$ m
Tipo I (>25 mm)	211.8 $\mu$ m
Tipo II (>8 mm < 25 mm)	221.3 $\mu$ m
Tipo II (>25 mm)	219.7 $\mu$ m
Tipo III (>8 mm < 25 mm)	255.5 $\mu$ m
Tipo III (>25 mm)	223.6 $\mu$ m
Tipo IV (>8 mm < 25 mm)	229.6 $\mu$ m



**Fig. 1.** Morphological features of capsule: hypercellular PA showing a thick capsule (A); hypercellular PA with a thick capsule marked using India Ink (B); hypercellular PA showing a thick capsule and a mixomatous stroma (C); hypercellular PA with a thick capsule and mixomatous and chondroid stromal tissue (Hematoxylin & Eosin stain. Original magnification  $\times 150$ ).



**Fig. 2.** Nucleoresection surgical technique (A and B: two different times of surgical procedure).

respectively were used to remove the tumors. At a mean follow-up of 24 months no local recurrences have been described and no patients presented local complications in 76 patients undergone to surgery at first time. In 8 patients surgically treated for tumor recurrence using superficial (3 patients) or total parotidectomy (5 patients) complications occurred. After superficial parotidectomy one patient showed a Frey Syndrome while one patient treated with total parotidectomy showed a facial nerve branches little dysfunction.

#### 4. Discussion

Aging is accompanied by a decline in the healthy function of several organs in response to different mechanisms like oxidative stress [43–45]. PA is a slowly growing, usually demarcated, and mobile tumor occurring frequently in females elderly patients. It constitute approximately 40–60% of benign salivary gland tumors located in the superficial parotid gland lobe in about 80% of cases [1–5,46]. This benign tumor is characterized by 3 main morphological features. The capsule is the most relevant structural components of PA. The other two morphological features are

parenchyma (tumor epithelial cells) and stroma [11–15,47]. The parenchymal/stromal ratio varies, and was used to classify these tumors in four types. Many Authors have been recognized parenchyma-rich and stroma-rich variants [11–15]. There is no consensus whether the capsule is newly formed structure or reflects pre-existing connective tissue compressed by the growing tumor. Morphologically, the capsule could be consist as thick, dense, fibrous tissue that may be discontinuous or absent or become invaded and even penetrated by a tumor. Correlation between capsular features and parenchymal/stromal ratio or the location of the tumor has been made. The capsule is usually 0.015–1.75-mm thick and it is thicker in parenchyma-rich tumors than in stroma-rich tumors. In our series of cases hypercellular PAs have a thick capsule while hypocellular tumors have a thin capsule and constitute the most frequently encountered histological type in recurrence [48–50]. Half to two thirds of stroma rich tumors show a variable/focal absence of the capsule. Tumors pseudopodias or bulge or grow through capsular breaches to extend to adjacent parotid or soft tissues. Where the capsule is absent, tumor invades adjacent parotid or adipose tissue either as a broad advancing front or as small mammillations bulging out from the main mass

[48–50]. Observation of capsular features in PA naturally leads to consideration of the so-called tumor satellites [51]. In particular satellites could correspond with section profiles of extracapsular tumor extension; continuity with the main mass is outside of the plane of that section. These may be alternatively attributed to the multifocal/multicentric origin of PAs [51]. The instrumental examination used for diagnosis and to plan the best were CT with contrast medium or MR of head and neck. There is no general consensus about fine needle aspiration cytology (FNAC) which morphological findings could be useful to surgical treatment [17–26]. Almost all PAs can be effectively treated by surgical procedures. PAs, small, mobile, with a clearly observed capsule located in the superficial lobe/tail of the parotid gland can be removed by limited surgery with few complications. Some complications can be occurred during surgical procedure. The main complications are: temporary/permanent facial nerve, Frey syndrome/gustatory sweating and parotid cancer development. Patients treated by different surgical procedures can be affected by these dysfunctions. Superficial parotidectomy (SP), total parotidectomy (TP), and extracapsular dissection (ECD) replaced enucleation surgical technique [52–54]. Alternative really novel therapeutic approach might consist in injecting the patients with autologous endothelial progenitor cells, to accelerate tissue revascularization and prevent a surgical approach [55–59]. We used in our series of cases nucleoresection technique as surgical treatment of choice to reduce dramatically the incidence of tumor's recurrence and complications. Nucleoresection is an “enucleation like” minimal margin surgery utilizing a capsule margins as plane of dissection. In this technique, the removal of healthy parotid tissue compared with formal parotidectomy is limited, thus minimizing recurrence rate and complications such as facial nerve dysfunction and Frey syndrome. Our results suggest that nucleoresection surgical approach using capsule characteristics and other morphological features could be considered a suitable option for first time diagnosed patients, reducing significantly tumor recurrence and complications development. Nevertheless we need longer follow-up to draw conclusive results.

### Ethical approval

Ethical approval was requested and obtained from the “Second University of Naples” ethical committee.

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### Author contribution

**Germano Guerra:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Domenico Testa:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

**Stefania Montagnani:** Participated substantially in the analysis and interpretation of data and revised the manuscript.

**Domenico Tafuri:** Revised the manuscript.

**Francesco Salzano:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data.

**Aldo Rocca:** Participated substantially in the analysis and interpretation of data.

**Bruno Amato:** Revised the manuscript.

**Giovanni Salzano:** Participated substantially in the analysis and interpretation of data.

**Giovanni Dell'Aversana Orabona:** Revised the manuscript.

**Pasquale Piombino:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data.

**Gaetano Motta:** Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the editing of the manuscript.

### Conflicts of interest

All Authors have no conflict of interests.

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