

The Use of Platelet-rich Plasma Gel in Patients with Mixed Tumour Undergoing Superficial Parotidectomy: A Randomized Study

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Abstract. *Background/Aim:* Salivary gland tumors are mostly benign tumors. Whether a more conservative surgical approach at greater risk of recurrence, or a more radical intervention with an increased risk of facial paralysis is warranted is still under discussion. Our study addresses the opportunity for improving surgical outcome by employing platelet-rich plasma (PRP) gel at the surgical site. *Patients and Methods:* Twenty consecutive patients undergoing superficial parotidectomy were randomized and assigned to two groups, one with and one without PRP gel. Many parameters were evaluated after surgery and during follow-up, such as the duration of hospitalization, facial nerve deficit, onset of Frey's syndrome, relapse, cosmetic results, presence of keloid or scar depressions, behavior of several facial muscles. *Results:* Our explorative analysis suggests a positive effect of PRP on surgical outcome in patients undergoing parotidectomy, whereas no negative effects were detected. *Conclusion:* This work suggests that administration of PRP in patients undergoing parotidectomy is beneficial.

Salivary gland tumors (SGT) are mostly (80-90%) benign tumors with heterogeneous histopathology and great variety in their evolution. These tumors are uncommon, representing fewer than 3% of all head and neck tumors. The most frequent SGT is pleomorphic adenoma, representing 60-70% of all parotid gland tumors (1, 2); other SGTs are monomorphic adenomas (cysto-adenoma lymphoma or Warthin tumor, oncocytomas, monomorphic adenomas) and epithelial tumors. The parotid is by far the most affected salivary gland (3).

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Specific anatomic configuration, as well as structures involved in the tumor site and histology defines the optimal therapeutic plan; for instance, pleomorphic adenoma tends to grow locally with ramifications and neoplastic satellitosis, with a percentage of approximately 3% to 6% malignant progression. Simple enucleation or enucleoresection are considered inadequate because of a recurrence rate of more than 10% (4), tumor rupture, local dissemination of tumor cells and frequent positive resection margins. A radical approach with facial nerve preservation could be more appropriate.

However, it is still under discussion whether a more conservative approach should be followed, with greater risk of recurrence and an increased incidence of Frey's syndrome, or a more radical intervention such as total parotidectomy should be employed, with an increased risk of facial paralysis. Recent studies on superficial adenomas have shown that adopting a more conservative approach leads to fewer side-effects on the facial nerve and a comparable lower incidence of relapse (5).

Impaired postoperative facial nerve function, whether persistent or temporary, represents a substantial negative impact on the quality of life (6). It is a frequent side-effect of total parotidectomy, while in superficial parotidectomy its incidence is very low (5, 7). If the nerve itself is not interrupted (8), nervous function will recover in about three months; such reliable and more conservative approaches provide good long-term results (5).

Our study addresses the opportunity of consistently improving the surgical outcome, speeding-up the healing process and enhancing cosmetic results by employing a platelet-rich plasma (PRP) gel at the surgical site. It focuses on the possibility of reducing temporary paralysis of the facial nerve after surgery and ameliorating sensitivity of the cutaneous region innervated by the great auricular nerve. In recent literature, PRP combined with epineural or perineural suture was reported to improve re-myelination of the interrupted sciatic nerve (9). This interesting finding provides

a intriguing perspective for future studies on the effect of PRP on diseases of the nervous system.

Regenerative surgery can repair and regenerate tissues by using natural components able to recruit and proliferate progenitor cells involved in tissue regeneration. Recent advances in modern transfusion medicine have allowed regenerative surgery to adopt a new set of products and technologies, such as fibrin glue and PRP gel, that allow for improved surgical outcomes and shorter healing. In particular, PRP gel can be applied where loss of substance has occurred, filling cavities and, at the same time, stimulating tissue regeneration by the action of growth factors contained in the platelet alpha granules (10). Indeed, growth factors seem to play a crucial role in the healing process, as it was shown that the initial phase of tissue repair is triggered by platelet de-granulation when platelets aggregate. In this process, several growth factors are released which possess strong recruiting and proliferating actions: PDGF, TGF- β , IGF-I and II and VEGF (11).

In particular, TGF- β induces a chemotactic action on neutrophils and monocytes, PDGF drives the recruitment of fibroblasts and matrix remodeling, and VEGF (a permeability vascular factor) influences plasma protein extravasation in order to support epithelial and endothelial cells. Dermal fibroblasts also play an important role in tissue remodeling and wound healing (12). The literature shows improved healing as compared to standard treatments, due to autologous platelet-derived wound-healing factors, which regulate wound healing of chronic cutaneous ulcers by promoting formation of granulation tissue in the early healing phase (13).

Currently, the use of PRP is widespread in dentistry and maxillofacial surgery for implantation and bone regeneration, and is largely expanding in bone surgery (14, 15). In this series we address the role of PRP in wound healing process, in preserving facial nerve functionality and improving post-surgical cosmetic results.

Patients and Methods

Patient enrollment. From November 2005 to August 2009, 20 consecutive patients with benign parotid gland tumor referred to the National Institute for Cancer Research in Genoa were recruited in the study. Our experimental protocol was approved by the Ethical Committee of our Institute according to the precepts established by the Helsinki Declaration.

All patients with parotid gland mixed tumor were screened for the study: patients were considered eligible if suitable for surgery and if they agreed to undergo pre- and post-operative rehabilitation visits; patients were considered ineligible if they had previous neurological deficiencies of facial muscles or if they had a previous malignancy (excluding basal cell carcinoma) within five years from randomization. Eligible patients were randomized and assigned to one of two groups. In the first group, patients underwent surgery with regenerative PRP, while in the second group PRP was not

applied. All patients enrolled in the study underwent diagnostic tests including ultrasound of the parotid lodge and cytological examination, while other additional investigations such as CT scans or MRI were performed only when there was a suspicion of a malignant neoplasia revealed either by cytology or by the involvement of parapharyngeal area or deeper lobe, shown by echography. The indication for surgery was based on the diagnostic findings. During follow-up many parameters were evaluated: facial nerve deficit, onset of Frey's syndrome, time for a full recovery of facial muscles functionality, relapse (by U.S. examination), cosmetic results, presence of keloid/depressions, behavior of several facial muscles (buccinator, corrugator supercilii, frontalis, great auricular, mentalis, orbicularis oris, orbicularis oculi, platysma, zygomaticus).

In our study, all patients were affected by a mixed tumor of the parotid gland and underwent superficial parotidectomy surgery.

PRP preparation. PRP was procured by collecting a standard unit of blood (450 ml \pm 10%), usually two days before surgery. Red blood cells were re-infused to the patient, while the PRP was separated into platelet-poor plasma (PPP) and platelet concentrate (PC). PPP was immediately frozen at -80°C , while PC was stored at $+22^{\circ}\text{C}$ under continuous agitation. After freezing, PPP was placed at $+4^{\circ}\text{C}$ overnight with the satellite bag attached, and the cryoprecipitate was prepared.

Autologous thrombin was prepared starting from 20 ml of blood collected in two sterile tubes. The tube was maintained for 2 h at 37° and then was centrifuged at 3,000 rpm for 20 min. The thrombin was transferred to a second sterile tube and stored at -30°C until use.

Quality assessments performed on the final products were required to indicate the presence of the following components in at least 75% of the preparation: absolute platelet count: $60 \times 10^9/\mu\text{l}$, residual leukocytes: $0.2 \times 10^9/\mu\text{l}$, quantity: 40 ml, cryoprecipitate: factor VII: 70 IU; fibrinogen: 140 mg/unit.

The quantity of PRP was standardized (22 ml). Preparation was performed under sterile condition: 10 ml of PC and cryoprecipitate were put in one or more Petri dishes; 1 ml of autologous thrombin and 1 ml of calcium gluconate were added and mixed with a sterile pipette for a few seconds. The product was used within 8 h after delivery and was stored at room temperature until use. PRP gel was applied to the excision site and placed around nerves endings.

Statistical analysis. In order to better-evaluate the potential effects of PRP gel use, we performed a *t*-test (16) for each measured clinical parameter. The main aim was to identify the parameters that are significantly altered in patients to whom PRP was applied, with respect to patients that did not undergo gel application. We considered as relevant those parameters associated with a *p*-value less than 0.1.

Results

The two groups were homogeneous for male/female ratio ($p=0.84$) and age ($p=0.60$). The median age for the PRP group was 46 years (range=24-55), while that for the other group was 44 years (range=28-80).

The median duration of drainage maintenance was almost equivalent: 2.1 days (range=2-3 days) in the PRP group and 2.4 days (range=1-3 days) in not PRP group (*p*-value not statistically significant).

Table I. Parameters evaluated during follow-up.

	PRP (n=8)	No PRP (n=12)
Median drainage (cc)	15	22.5
Mean recovery (days)	2.38	2.42
Frey's syndrome	1	6
Average time for total muscle functionality (days)	57	73
Facial nerve deficit	0	2

As reported in Table I, median serum drainage was 15 cc (range: 5-50 cc) in the PRP-group, and 22.5 cc (range: 10-340 cc) in the not-PRP-group (the difference was not statistically significant). Patients from both groups were discharged after a median of two days of hospitalization (range=2-4 days).

None of PRP-treated patients reported any transient facial nerve deficit, whereas two cases not treated with PRP presented compromised facial nerve function.

Frey's syndrome was diagnosed in four out of eight PRP-treated patients and in nine out of twelve patients not treated with PRP.

No patient developed recurrence in the postoperative period. Only one patient developed a second tumor.

A thorough evaluation of the facial muscles was performed during the postoperative period and follow-up, recording for each one of the days needed to recover full functionality. Table II reports the average amount of time for muscle recovering in the PRP and no PRP groups.

For each measured clinical parameter, we performed a *t*-test (16). The *p*-value for each parameter is reported in Table III. Parameters that were significantly different (*p*-value <0.1) across the two sets of patients were keloids/depression, cosmetic result, great auricular nerve conservation and Frey's syndrome. For all the parameters above, PRP gel application had a positive effect when applied.

Discussion

Our explorative analysis on PRP gel application in patients undergoing parotidectomy suggests a positive effect of PRP on surgical outcome, with no negative effects detected. In fact, some parameters measured during follow-up were significantly improved in patients to whom gel was applied as compared patients to whom it was not (Table III).

PRP is a valid choice for the treatment of Frey's syndrome and an alternative to treatment with Botulin toxin type A, as reported in literature (17). Furthermore, facial nerve function was conserved in all patients treated with PRP gel, whereas two patients not treated with PRP presented a significant deficit.

Table II. Average days required for recovery of each muscle.

	PRP (n=8)	No PRP (n=12)
<i>Frontalis</i>	11	27
<i>Corrugator supercilii</i>	15	13
<i>Orbicularis oculi</i>	17	21
<i>Quadratus labii superioris</i>	35	34
<i>Zygomaticus</i>	10	17
<i>Risorius</i>	12	13
<i>Orbicularis oris</i>	34	50
<i>Buccinator</i>	33	17
<i>Triangularis</i>	55	51
<i>Mentalis</i>	46	45
<i>Platysma</i>	44	36

Table III. Significantly improved parameters in the group treated with PRP gel.

	<i>p</i> -Value
Keloids/depression	0.07
Cosmetic result	0.01
Great auricular conservation	<0.001
Frey's syndrome	0.09

We must note our small sample size, as well as the categorical nature of the collected data that could have affected statistical analysis. Nonetheless, our study is meant to be an explorative analysis, in order to evaluate the possibility of carrying out a large-size clinical trial with a better experimental design.

Indeed this work highlights the positive effect of PRP on patients undergoing parotidectomy, and indicates that more effort has to be put on the study of the effect of PRP gel.

Disclosure

Marco Scala, Paola Mereu, Francesco Spagnolo, Michela Massa, Annalisa Barla, Sofia Mosci, Gilberto Forno, Andra Ingenito and Paolo Strada have no financial interest in any of the products, devices, or drugs mentioned in this article.

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