Original Article

Allogeneic Bone Application in Association with Platelet-Rich Plasma for Alveolar Bone Grafting of Cleft Palate Defects

Abstract

Aim: The aim of this study is to compare allogeneic bone grafts associated with platelet-rich plasma (ALBGs-PRP) to autogenous bone grafts (ATBGs) for alveolar reconstructions in patients with cleft lip and palate (CLP). **Materials and Methods:** The Maxillofacial Surgery Service of the Comprehensive Care Center for CLP (CCCLP) in Curitiba (Paraná, Brazil). **Patients:** Thirty out of 46 patients with 8–12 years of age and pre- or trans-foramen unilateral clefts were operated by the same surgeon. Groups were selected randomly after coin-toss for the first surgery to be ALBG-PRP. **Interventions:** Pre- and post-surgery cleft defect severity was registered by a score system using superimposed digitalized peri-apical radiographs. The hypothesis indicated ABG-PRP to be similar to the ABG was proved. **Results:** There was no statistically significant difference ($P < 0.05$) in bone augmentation for the ABG-PRP group (79.88%) when compared to the ABG group (79.9%). **Conclusion:** ABG-PRP is indicated as a successful treatment modality to reduce the need for additional donor sites and reduce morbidity and hospital stay.

Keywords: *Allogeneic bone graft, alveolar cleft grafting, bone reconstructive procedures, chin bone harvest, platelet-rich plasma*

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Introduction

A collaborative project on the epidemiology of craniofacial anomalies indicated in 2011 that the prevalence of cleft lip and cleft lip and palate (CLP) was 3.28/10,000, and 6.64/10,000, respectively. Multidisciplinary approach is required to treat these patients from birth to adulthood in order to rehabilitate the missing hard and soft tissues.[1] The reconstruction of the alveolar process favors permanent teeth eruption, movement of teeth through the alveolar process using orthodontic forces,[2] and reestablishment of esthetics and masticatory function with implant-supported prosthesis.[3] Bone grafts stabilize the dental arch, optimize the periodontal support of the teeth adjacent to the cleft, and close the oral-nasal clefts, reducing speech difficulties, and food regurgitation into the nasal cavity.[4]

The autogenous bone graft (ATBG) is considered the gold standard treatment in the field of alveoli reconstructions for treating the CLP patient.[5] However, the morbidity involved in the bone graft harvesting, the length of surgery, the risk of infection, and the bone graft quantity limitations have been the impetus for the use of an alternative method for bone grafting. Among these techniques, allogeneic bone grafts (ALBGs) obtained from Bone Banks and the use of platelet-rich plasma (PRP) to optimize the grafting procedure appears promising.[6]

This investigation is aimed to assess bone augmentation using X-ray analysis, after mixing the ABG-PRP and compare it to the gold standard treatment of the CLP patients, which is ATBGs.

Materials and Methods

Of 46 patients with CLP reviewed, 30 were included in the research and were offered surgery for alveolar cleft reconstruction in the Maxillofacial Surgery Service of the Comprehensive Care Center for CLP (CCCLP) in Curitiba, Paraná, Brazil. We obtained Institutional review board approval before commencing this study by the CCCLP committee. In addition, all patients included in this study had been informed of the research details and signed the consent form on this research protocol. The consent included that one of the two

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procedures would be performed and that it would be decided at the time of the surgery.

The selection criteria of the patients were according to age, sex, and type of cleft. The surgeon reviewed all patients. Patients showed unilateral, pre- and trans-foramen clefts, according to Spina's classification.[7]

The average age in the ATBG (control group) was 15.5 and varied from 11 to 23 years; 9 were male and 6 were female. In the ALGB-PRP (experimental group), the average age was 14.8 and varied from 9 to 23 years; coincidentally, 9 were male and 6 were female.

The patients were selected randomly for the surgical technique to be used. The randomization processes used was to include the first case scheduled for surgery in the experimental technique and the second in the control group and repeat this pattern until 30 cases were scheduled. The control group consisted of 15 patients receiving ATBG (from the chin and ramus of the mandible) for alveolar cleft grafting. The experimental group received ALBG-PRP.

The surgeries were performed under general anesthesia, with nasotracheal intubation contra-lateral to the fistula. Lidocaine 2% with 1:200.000 diluted epinephrine was used for the anesthesia.

A mucoperiosteal flap was used with two releasing incisions bilaterally in the premolar areas. After suturing the nasal mucosa, the cleft was filled with autogenous bone (control group) or ALBG-PRP (experimental group), according to the randomization indicated above. Next, the flap was rotated mesially and sutured with 5-0 nylon thread [Figures 1-5].

Cephalosporin was applied intravenously while the patient was hospitalized, and prescribed orally after the patient was discharged for the following 10 days. In addition, analgesia was controlled with dipyrone (450 mg/day).

Four months after surgery, the patients were reexamined clinically and radiographically by means of digital periapical radiographs (Siemens Heilodent 60B, with 60 kV and 10 mA, exposure time of 0,16s and SENS-A-RAY 2000 system sensor using SUA II648-2 of Regam Medical Systems). The magnification of this radiological apical system was of approximately 2%. In addition, this system optimizes density, sharpness, reduces radiation dosages and enhances the borders facilitating the superposition of the graphic images for analysis. All periapical X-rays were obtained using the Bisecting technique. The images were processed using Adobe Photoshop 4.5 from ADOS, and a numeric scale was created proportional to the size of the original image in a ratio of 21 pixels/mm. From this scale, parallel lines were drawn to evaluate the degree of density of the augmented bone [Figures 6-8].

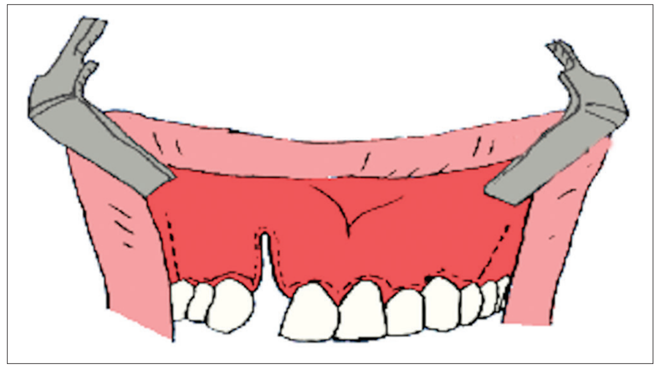


Figure 1: Schematic design delimiting the areas to be incised during the surgical procedure

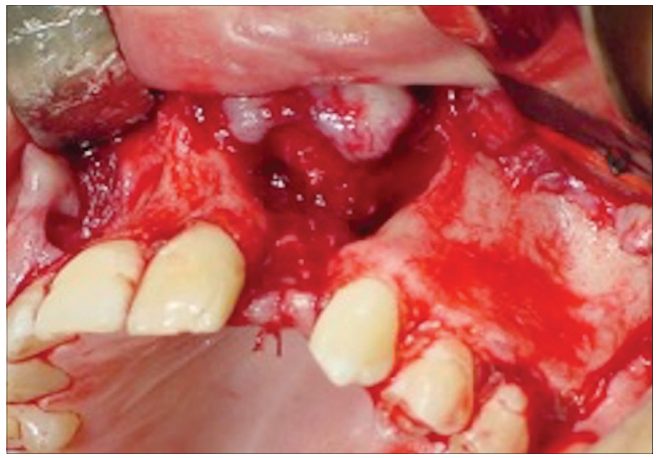


Figure 2: Reflected flap showing the buccal view of the alveolar defect. Note the extent of the alveolar defect to the nasal mucosa, which was sutured separately prior to bone grafting

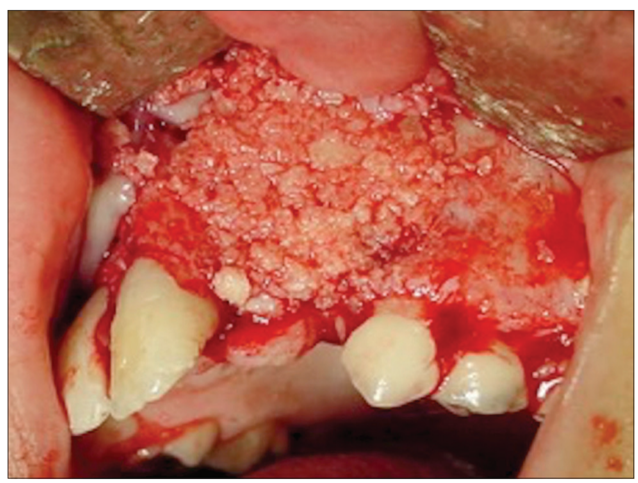


Figure 3: Buccal view of the alveolar defect filled with allogeneic bone graft material combined with PRP

The first line drawn was on the cervical region of the teeth adjacent to the cleft, representing less distortion, whereas the other lines were drawn parallel to this one with a 3 mm distance between them.

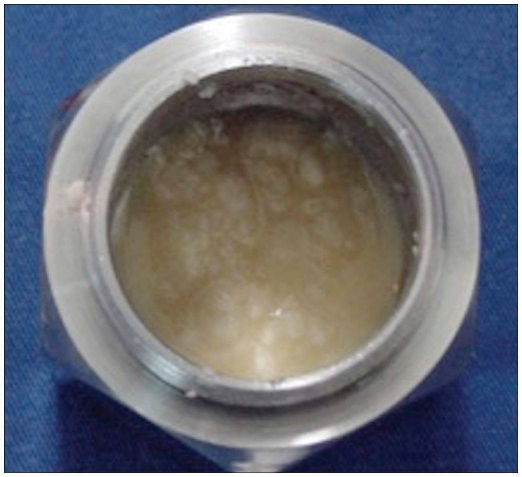


Figure 4: Allogeneic bone graft material combined with platelet-rich plasma in the clotting phase

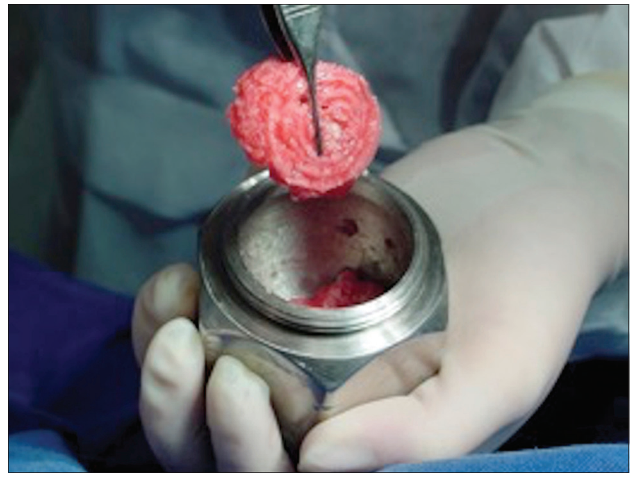


Figure 5: The same material observed in Figure 4 combined with platelet-rich plasma induced bone graft clot

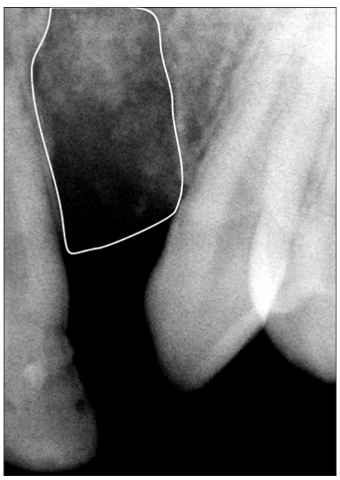


Figure 6: Area considered 100% bone augmentation (ideal bone augmentation) delimited in whiteww

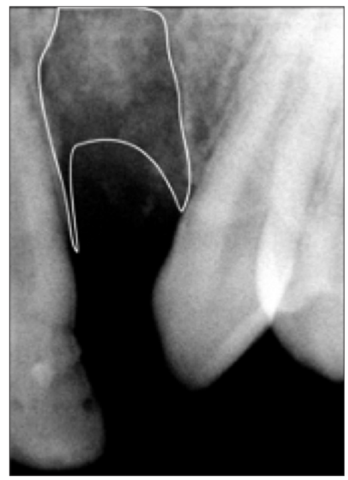


Figure 7: Area of real bone augmentation (ideal bone augmentation) delimited in white

The treatment was considered successful when the concavity format of the graft was detected between lines 1 and 2 (space A). The other graft formats, located between lines 2 and 3 (space B) or the ones located between lines 3 and 4 (space C) were considered failures. Clinically, only the grafts in space C needed an additional surgical procedure [Figure 8].

The ALBG was obtained from the Bone Bank of the Clinical Hospital of the Federal University of Parana, in Brazil. We chose to use the cortical-cancellous bone, with particle sizes ranging from 0.5 to 1.0 mm, which came in packages containing 5 g.

The PRP was obtained in the laboratory where the blood was drawn, through brachial vein, to 5 ml test tubes containing 0.5 ml of sodium citrate (anticoagulant). Twenty-two milliliters of blood were drawn from each patient to obtain 5 ml of PRP.

The blood was centrifuged with 1000 rpm (Biofixette) for 7 min. After centrifugation, the plasma was separated from the red blood cells by means of pipetting 0.5 ml. Platelet count was performed using a platelet counter before and after the centrifugation.

The success and failure of the control and test groups were assessed using the Multifactor ANOVA test.

Results

Data from the experimental group (number of charts, sex and age) and the measurements of the ideal bone augmentations are presented in Table 1.

The bone augmentation for each patient of the control group ranged from 34.12% to 100% of the total area planned for the bone fill of the defect. The mean bone augmentation for this group was 79.9%.

The individual variation is in Table 2. The gender distribution among the patients showed 60% (n = 9) of male and 40% (n = 6) female. The age distribution was, only 26.5% (n = 4) of the patients were between 9 and

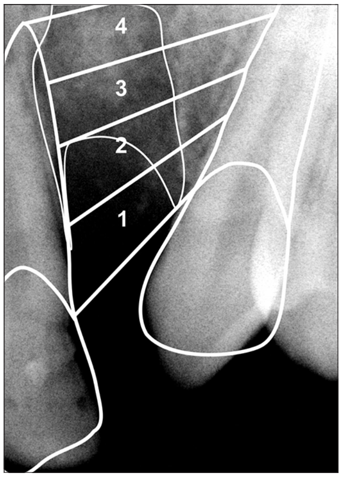


Figure 8: Scale showing the first line drawn on the cervical aspect of the teeth adjacent to the cleft, representing less distortion, and the other lines drawn parallel to this one with a 3 mm distance between them

12 years old, which is the recommended age for surgery,[8] whereas the great majority, 73.3% (n = 11) were operated in a nonideal age range.

Whereas In the test group, the worst performance was of 14.16% and the best was of 100% of the total area of bone augmentation, whereas the general mean value of bone augmentation for the group was 79.88%. The result of each patient was expressed in Table 1.

The gender distribution, shows 60% (n = 9) of the patients were male and 40% (n = 6) were female. In relation to the age range, 40% (n = 6) were in the recommended age for surgery (9 and 12 years old), and 60% (n = 9) were operated on after that age. The results are registered in Tables 2 and 3. The results of the total bone gain for each patient in the control group varied between 34.12% up to 100% of the total area that was planned for the graft to fill the defect. The mean bone gain for this group was 79.9%. The individual variation can be visualized in Table 2.

The statistical method applied to verify statistical significance for the independent groups was the Student's t-test [Table 3]. ANOVA statistical test was used to verify differences within the groups. This analysis decomposed the variability of the digital measurement into contributions per factor: group, sex, and age. Once the sum of the Type III squares was chosen, the contribution of each factor was measured with the effect of the other factors being removed. The probability values tested the statistical significance of each factor [Table 4].

Through direct comparison of the confidence intervals of the digital measurement variable under the influence of the group factors [Graph 1], due to the coincidence of the numeric intervals, the authors concluded that these values cannot be considered statistically different. Comparing confidence intervals in 95% of the digital measure variable under the influence of the age factor [Graph 2], statistically,

Table 1: Data from the experimental group (number of charts, sex and age) and the measurements of the ideal bone augmentations

	Chart	Sex	Age (years)	Digital measure
1	33876	M	14	97,11%
2	38133	F	11	97,61%
3	39588	F	16	76,47%
4	38586	M	11	100%
5	39375	M	23	100%
6	39126	M	12	63,36%
7	38652	M	09	78,2%
8	35204	F	16	72,12%
9	33526	M	12	100%
10	35744	M	20	92,6%
11	35901	F	17	82,78%
12	35107	F	16	49,77%
13	35415	M	09	14,16%
14	35398	F	23	74,12%
15	35420	M	13	100%

This analysis showed that none of the factors: group (control and experimental), sex, age (ideal and nonideal) have statistical significant difference over the digital measurement variable in a confidence level of 95%

Table 2: Data from the control group (number of charts, sex and age) and the measurements of the ideal bone augmentation

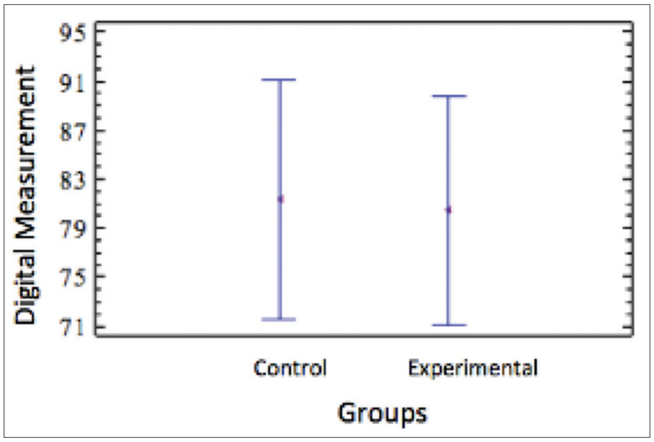
	Chart	Sex	Age (years)	Digital measure
1	38247	M	23	34,12%
2	40103	F	12	100%
3	35426	M	13	40,71%
4	37988	F	16	87,07%
5	33210	M	14	100%
6	33527	F	22	53%
7	35346	M	11	100%
8	33506	M	11	100%
9	33526	M	18	87,72%
10	33644	F	16	65,21%
11	33592	M	14	85,42%
12	35416	F	11	89,35%
13	34735	M	18	58,93%
14	36912	M	13	100%
15	35368	F	21	96,99%

Table 3: The Student ´*t* test was applied for independent groups. Where t = 1.703391E-03 (P = 0.9986)

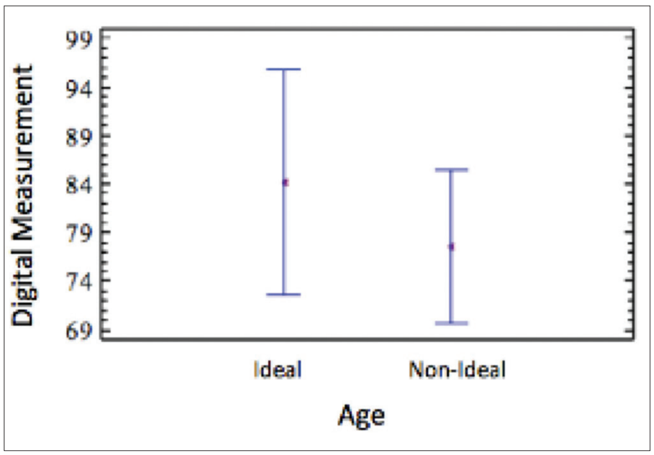
	Control Group	Experimental Group
Total patients	15	15
Mean value	79.90	79.89
Standard Deviation	23.23	23.29
Median value	87.72	82.78

these values are not significantly different. Analyzing the confidence intervals in 95% of the digital measurement variable under influence of the sex factor [Graph 3] there was no statistically significant difference.

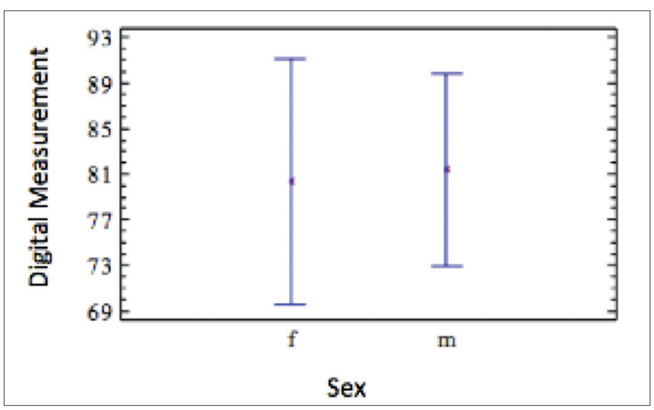
Summarizing the general statistical analysis of the digital measurement variable in function of the factors: group, age and sex; we can conclude that none of these factors, in any level, presented statistically significant differences (P = 0.05) comparing to any other value in any level [Tables 3 and 4]. Showing that in this study the clinical bone augmentation result was independent of the parameters evaluated.



Graph 1: Confidence intervals in 95% for the digital measurement variable in function of the group (control and test) factor



Graph 2: Confidence intervals in 95% for the digital measurement variable in function of the age (ideal and not ideal) factor



Graph 3: Confidence intervals in 95% for the digital measurement variable in function of the sex (female and male) factor

Discussion

The results demonstrated that the age was a fundamental influencing factor, whereas the success rates of the control (receiving ATBG) and test (receiving ALBG-PRP) groups were higher for the patients that were operated in the ideal age. Among the 9 patients from the experimental group that were operated on during a nonideal age, 5 reached total success and 4 reached partial success or failure. In the control group, 6 patients, among the 11 patients operated on during a nonideal age, reached total success and 5 patients reached partial success or failure. This data show the importance of the treatment being conducted on the correct age group.

Table 4: Result of the ANOVA Analysis for the digital measurement variable. This analysis decomposes the variability of the digital measurement in contributions according to the factors: group, sex and age. Once the sum of the type III squares was chosen, the contribution of each factor was measured with the effect of the other factors being removed. The probability values tested the statistical significance of each factor. Since the P values on the statistical analysis are smaller than 0.05, no factor showed a significant statistical effect on the digital measurement variable on a confidence level in 95%.

	Sum of the squares	Liberty degree	Medium square	F rate	P value
Group	5.76436	1	5.76436	0.01	0.9218
Age	272.945	1	272.945	0.47	0.5013
Sex	8.06939	1	8.06939	0.01	0.9076
Residue	15261.3	26	586.975		
Total (corrected)	15562.4	29			

The analysis of success or failures of the bone graft procedure performed in CLP patients, depend on the investigation criteria. All patients benefit from the treatments provided; however, according to the criteria used, success should be evaluated individually. Among the success criteria we can quote:

- Oral-nasal cleft closure;
- Bone support for the adjacent teeth and for impacted teeth;
- Bone bridge formation and stabilization of the maxillary segments; and
- Nasal alar base support and nasolabial contour.

The oral-nasal cleft closure stands out as being the most important outcome.

Many authors[9-11] suggest the use of mucoperiosteal flaps when performing these types of ridge augmentation. Studies[9,12] suggest vertical incisions directed to the buccal vestibule and incision of the periosteum on the base of the flap to facilitate graft coverage, optimizing the mobility, and reducing suture tension.

In the present investigation, the mucoperiosteal flap was used to maintain the keratinized gingiva.[11] The dental gingival tissue can also be preserved, reducing the need of free gingival grafts, and resulting in better conditions for teeth to erupt in the site where teeth will receive orthodontic force or for prosthetic anchorage using dental implants.

The first studies using ALBG showed success in this technique.[13-15] Many investigations[16-18] show reduced morbidity, less blood loss, and less hospital time when using this technique.

ALBG[19] showed the absence of growth factors, therefore, are not considered an ideal bone to support teeth eruption.

The aim of this investigation was to find an alternative bone graft for treating CLP patients, which promotes less morbidity with similar efficiency to the ATBG. The excellent amount of newly formed bone that was obtained with the ALBG was confirmed with X-ray analysis, and the efficacy observed was confirmed by the possibility of the canine to erupt into the newly formed bone site, in addition to the possibility of using orthodontic forces in the grafted sites. In addition, the difference between the groups, in relation to the success rate of the treatments, was not considered statistically significant; therefore, the clinical results between both procedures used in this investigation were very similar. The literature[15,16,18] approaching allogeneic bone grafting procedures state that they show promising results. The results obtained from this investigation are in accordance with the reviewed literature.

Negative immune responses to the ALBG[14,16,17] were not observed in the group that received it.

The allogeneic bone is a bone conductor, which is less efficient when compared to the autogenous bone that is a bone conductor and inductor. To increase the properties of this graft, we mixed PRP, which contains growth factors, rendering the allogeneic bone also a bone inductor.

Studies[20-22] show the efficiency and benefit of PRP in the healing process. The main effect of the PRP is for optimization of the tissue healing processes mainly using the platelet-derived growth factor and the transforming growth factors-β1 and -β2 (TGFs-β1 and β2). Authors conducted a study[22] using polypeptides (growth factors) present in the blood plasma, PDFG e TGF-β1 and β2 that showed PRP to have essential activity in tissue repair.

There are divergences among authors regarding the various techniques used for attaining PRP. The type of centrifuge to be used, the number of rotations per minute, the need of thrombin use, and the location (in office or laboratory) to conduct these procedures, are debatable. PRP has shown success when attained in a dental office setting.[20,21] However, it is ideal to obtain the PRP in a specialized laboratory, preferably in hospital facilities, to avoid transport and contamination risks.

Author divergences[20-22] among PRP attainment did not alter the final results of the platelet concentrate. The literature shows no consensus on the quantity of PRP to be used for these types of surgical procedures. In the present study, the mean values obtained from the PRP were 864.000 ± 59.560 platelets/ml using one centrifugation. The authors conducted a study[23] where they reached 1.200.000 platelets/ml using a technique approach of two centrifugations but needing to add thrombin to facilitate blood clot. In the present study, the mean values obtained from the PRP were 864.000 ± 59.560 platelets/ml using one centrifugation and having better efficiency without needing thrombin. Therefore, only calcium chloride (3,3%) was used to revert the anticoagulant (calcium citrate 0.150 M).

Investigators[24] analyzed the magnification of conventional X-rays. In this study, they compared panoramic, bitewing, and periapical X-rays. The panoramic X-rays showed a 27% greater magnification. The other two X-rays showed an 8% magnification. This observation demonstrated that the panoramic X-rays are not indicated for bone graft follow-ups due to the presence of distortions. The periapical X-ray analysis offers better reliability of the images in addition to being a better assessment technique.

Authors[25] stated that the computerized tomography has an advantage of rendering in three-dimensional images, which permit evaluation of the volume of the graft. These authors criticize the conventional X-rays because they can show a difference in results 3 months after the procedure has been done, prolonging the clinical assay. In the computerized tomography, the images of graft incorporation can be obtained as early as 1 month after the surgical procedure.

Equipment availability and cost-benefit should dictate the method used to evaluate the results. The most precise images obtained were from the computerized tomography; however, the high cost and excess radiation should be taken into consideration. Among the conventional X-rays, the periapical examination is the most indicated, due to the ease of imaging and reduced degree of magnification compared to occlusal and panoramic X-rays. However, long-term storage can become a problem.

As an alternative, in computerized tomography, and in conventional X-rays, the digitalized image can be a very interesting option. In addition, it permits instantaneous visualization of the images. Some advantages of the digitalized image are storage in floppy discs and CD-ROM; maintaining image quality for a longer period; it is a more inexpensive alternative; and the patient is submitted to less radiation when compared to the computerized tomography technique. The disadvantage of periapical radiographs is that it is a two-dimensional image.

Considering the possibility of failure of the alveolar cleft grafting procedure, the literature[26,27] showed that oral infections (cavity and periodontal disease), nasal infection, suture dehiscence, split-thickness, and mucoperiosteal flap dehiscence, deciduous teeth extraction during surgery, insufficient maxilla immobilization and excessive surgeries in the site result in fibrosis and reduced vascularization.

Authors[28] stated that the complications with suture dehiscence and bone sequestration increase in proportion to the patients' age.

Authors[29] consider cleft size, mesial rotation of the adjacent teeth, permanent tooth eruption level, factors that can contribute to bone graft failure, or higher bone graft resorption. An investigator[10] emphasized the importance of dental hygiene before and after the surgical procedure. All these factors can influence the success rate of the treatment. To minimize these risks, it is important to work with a multidisciplinary team for treating CLP patients. Some problems can be avoided if the patient and their parents participate actively in increasing their hygiene level. However, some problems as tooth extraction and localized infections in the surgical site, in addition to choosing the correct flap design, depending on the surgeon's judicious evaluation. Other factors that could impose a negative effect on the treatment are cleft size, a need of greater bone quantity, reduced vascularization of the graft, and excessive surgeries in the site hindering the flap mobility.

Conclusion

The authors concluded that Allogeneic bone is an interesting alternative for alveolar cleft grafting procedures, with similar results when compared to the autogenous bone grafting procedures. In addition to having the advantage of reduced morbidity and surgery length, the PRP showed to be an important auxiliary in the tissue repairing process; optimizing ALBG and adjacent soft tissue healing.

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Conflicts of interest

There are no conflicts of interest.

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