Is Peripheral Blood Cell Balanced Altered by the Use of Fresh Frozen Bone Block Allografts in Lateral Maxillary Ridge Augmentation?

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ABSTRACT

Background: The relationship between the immune response and red and white blood cell homeostasis is cited in literature, but no studies regarding the balance of these cell populations following maxillary bone-graft surgeries can be found.

Aim: The aim of this study was to evaluate the possible impairments in the blood cell balance following fresh–frozen allogeneic bone-graft augmentation procedures in patients who needed maxillary reconstruction prior to implants.

Material and Methods: From 33 patients elected to onlay bone grafting procedures, 20 were treated with fresh–frozen bone allografts and 13 with autologous bone grafts. Five blood samples were collected from each patient in a 6-month period (baseline: 14, 30, 90, and 180 days postsurgery), and the hematological parameters (erythrogram, leukogram, and platelets count) were accessed.

Results: All evaluated parameters were within the reference values accepted as normal, and significant differences were found for the eosinophils count when comparing the treatments (30 days, $p = .035$) and when comparing different periods of evaluation (allograft-treated group, baseline × 180 days, $p \leq .05$ and 90 × 180 days, $p \leq .01$; autograft-treated group, 30 × 90 days, $p \leq .05$ and 30 × 180 days, $p \leq .05$).

Conclusions: Both autologous and fresh–frozen allogeneic bone grafts did not cause any impairment in the red and white blood cell balance, based on quantitative hemogram analysis, in patients subjected to maxillary reconstruction.

KEY WORDS: atrophic maxillae, autologous bone graft, blood cells, bone allograft, leucocytes, lymphocytes

INTRODUCTION

Adequate bone volume is one of the most important requirements to allow and maintain the osseointegration of implants throughout time and is directly connected to high success rates in Implantology.[1] Several techniques have been used in an attempt to correct bone defects in the maxillo/facial complex because the availability of an adequate bone volume is normally diminished by local and systemic factors[2], which lead to the necessity of bone grafting procedures, in order to rehabilitate patient's stomatognathic system.[3]

Among these techniques, the autologous bone graft,[4] both intra-[5] and extraoral[6], remains as the most predictable and best documented (and therefore considered as the gold standard) method for correcting this type of defect, showing advantages such as a rapid incorporation and consolidation, allied with a lack of immunologic considerations.[7,8] This technique, however, is associated to some disadvantages, such as donor site morbidity, postsurgical pain, increased blood loss, paresthesia, hypersensitivity, infection, increased operative time, and sometimes deficiencies in the quality and

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quantity of available bone – providing an insufficient biomaterial volume[9], which lead to the necessity of using other biomaterials to treat the bone defects.

One of the possible alternatives to autologous bone grafting is the use of fresh–frozen bone allografts because it provides a reasonable source for grafting material.[10] Advantages such as ease of obtaining, reduced surgical time, and the absence of a second surgical area makes this biomaterial, obtained from bone–tissue banks, a viable way to achieve the desired bone volume prior to rehabilitation with dental implants.[11]

Since the first clinical report of allograft use in humans, there is a range of almost 130 years, and during the last two decades, its use has increased significantly.[12] In comparison with the autologous bone, fresh–frozen bone allografts remodeling is slower, and the union between the recipient bed and the graft is achieved consistently given that this biomaterial acts as a platform for new bone formation, preferentially as a osteoconductive scaffold.[13,14] The increased use of allogeneic bone is directly linked to the establishment of severe guidelines for bone processing, which defined the protocols to work with this biomaterial[15,16] and augmented the safety of its use, with no reports of cross-contamination, mainly considering diseases such as hepatitis or human immunodeficiency virus (HIV).[17]

Another concern with bone allograft is its antigenicity, but there is still limited information regarding this issue.[18] Some studies tried to state the relationship between the immune system and the fate of bone grafts. Eventhough intuition demanded and experimentation confirmed that bone allografts, like any other tissue and organ allografts, are immunogenic, the magnitude and the consequences of this information is still unclear.[19,20] According to literature, when antigen-matched grafts are used, more bone-forming surface can be seen, while the amount of bone resorbing surface is not altered.[21] Allied to this information, there is the fact that, although the relationship between the immune response and red and white blood cells homeostasis is cited in literature,[22,23] no studies regarding the balance of these cell populations following maxillary bone-graft surgeries can be found.

Using a quantitative evaluation of the red and white blood cell lineage count in patients who needed maxillary reconstruction prior to implants placement, the purpose of this study was to evaluate if fresh–frozen allogeneic bone grafts would invoke impairments in the blood cell balance when compared with autogenous bone grafts.

MATERIALS AND METHODS

This research protocol was approved by the Araraquara School of Dentistry Ethics Committee and by the National Research Ethics Committee under the protocol number 36/08, and it is in accordance to the World Medical Association Declaration of Helsinki (2002).

Patient Selection

The sample of this study is composed of 33 patients, 12 male and 21 female, with an average age of 47 years (ranging from 27 to 69), who presented for oral rehabilitation with titanium implants at the Department of Periodontology from Araraquara Dental School (UNESP – Universidade Estadual Paulista), Araraquara, São Paulo, Brazil, between May 2009 and December 2009. Patients with habits or systemic conditions that could confessedly interfere in bone grafts remodeling or in the implants osseointegration, such as smoking, alcoholism, drugs usage, or clinical and tomographic signs of maxillofacial lesions that contraindicate these elective surgeries were automatically dismissed.

Patients presenting severe bone deficiencies (width inferior to 4 mm in the sites which implants were planned, evaluated using computed tomography examination) were elected to onlay bone grafting procedures prior to implant placement (Figure 1). According to ethical guidelines, the patient treated with allogeneic bone grafts need to be informed and consent with the treatment; groups were not randomized. As the autologous bone grafting is the actual gold standard, only patients who presented an absence of a convenient amount of donor bone, which forbid the utilization of autologous bone grafts, or in cases that the cost-benefit effect of an autograft does not meet the willingness of the patient were treated with allografts. In this way, 20 patients were treated with allogeneic bone grafts and 13 with autologous bone grafts in a total of 64 and 22 bone blocks, respectively. In both groups, approximately 85% of the patients had the grafts made in the maxilla.

At the presurgical phase, when the patient's indication to each of the proposed protocols was already determined, all the documents regarding the biomaterial request were filled and sent to the registered bone bank that supplied the patients from allograft group (UniOss, Marília, Brazil). The allograft bone was processed

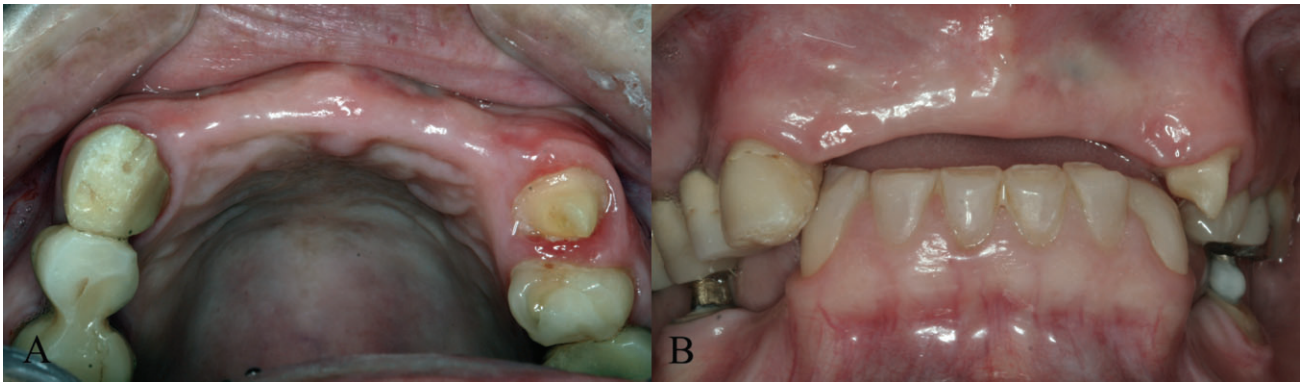

Figure 1 Initial clinical situation, occlusal (A) and buccal (B) view, showing the absence of an adequate bone volume to allow the installation of dental implants.

according to AATB guidelines[24] and delivered the day before the surgeries were made.

Surgical Procedures

Subjects were asked to wash their mouths using a 0.12% chlorhexidine rinse for 1 minute right before the procedure starts. Complimentary to this, a Povidone–iodine 10% solution was applied to the perioral skin to prevent contamination. During the first surgical phase and under local anesthesia, a total flap was attained in such a way to provide a full visualization of the bone defect. Any reminiscent of soft tissues were removed from bone surface, and delicate burs were used, always under intense saline solution irrigation, to etch the host cortical bone allowing the vascularization to occur in an easier way toward the grafts (Figure 2).

In the group treated with autologous bone, the cortico-cancellous grafts were retrieved from the mandible ramus according to the size of the defect to be treated. In the group treated with the allografts, at the time of the surgery, the cortico-cancellous bone blocks were removed from the freezer and put into sterile saline solution for 10 minutes allowing them to hydrate and get to room temperature gradually. Both types of blocks were then prepared with careful trimming using #700 cylindrical and maxi-cut burs, always under abundant sterile saline solution irrigation, and adjusted to the host bone. The blocks were fixated to the host bone using 1.5×10 mm or 1.5×12 mm screws (Neodent, Curitiba, Brazil) and covered by a collagen membrane (Genius Baumer, São Paulo, Brazil) prior to the suture, made with interrupted nylon 4-0 simple points (Figure 3). All patients were medicated using antibiotics (amoxicillin 500 mg three times daily for 7 days), nonsteroidal anti-inflammatory (nimesulide 100 mg two times daily for 5 days), and analgesics (acetaminophen 750 mg 4 times daily when there was pain), and the sutures were removed 14 days after surgery.

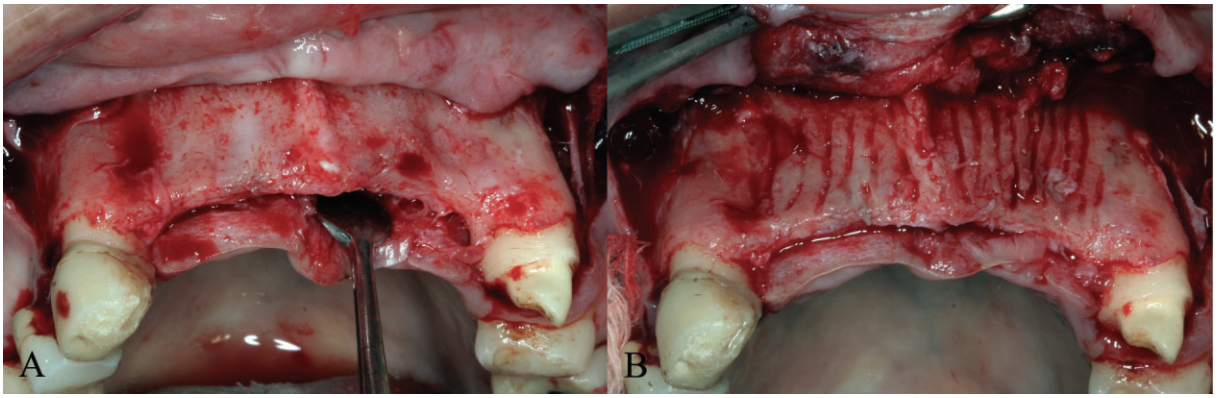

Figure 2 Surgical sequence used in the bone-graft procedure. (A) The bone berth after the total flap attainment and (B) cleaned from all soft tissues, scratched and prepared to receive the graft.

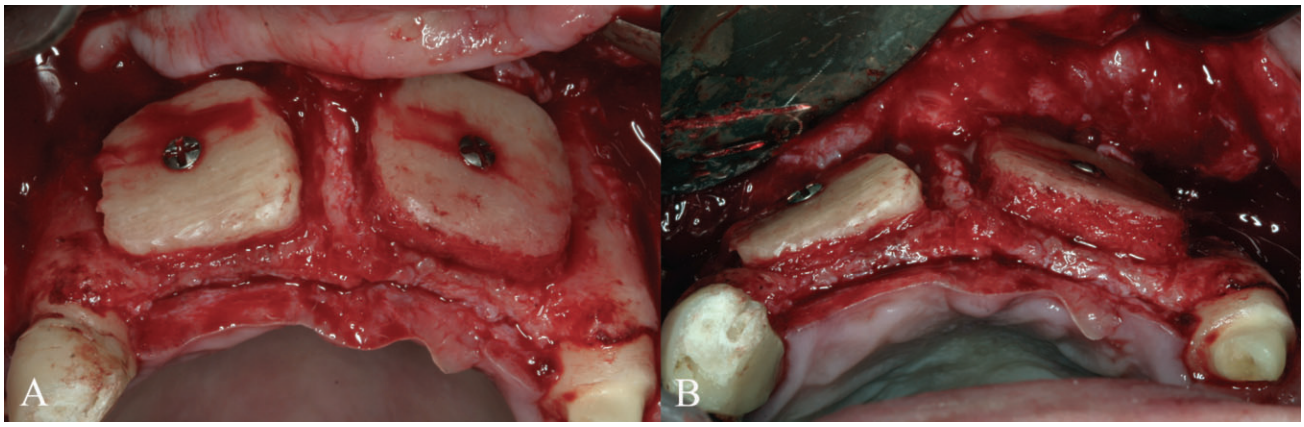

Figure 3 A buccal (A) and occlusal (B) view of the grafts after their installation with fixation screws and adjustments of the borders.

Blood Sample Collection and Processing

All 33 patients have five blood samples collected in a 6-month period. The first sample was collected 7 days prior to the grafting surgery (baseline), and the four subsequent were collected at the 14th, 30th, 90th, and 180th postsurgery. The last blood sample (180th day) was collected in order to evaluate the patient prior to the titanium implants placement.

Blood samples were collected under standardized conditions: 2.0 mL of total venous blood was collected in vacuum tubes containing EDTA/K3 to determine hematological parameters. Blood samples were collected in the morning after 12 hours of fasting in a seated position, transported at 4°C to the laboratory within 30 minutes, centrifuged under refrigeration at 1,800 g for 10 minutes immediately separated and protected from light.

Hematological analyses were conducted using a Cobra Micro automated hematology analyzer (Roche Pharma Ltd., Reinach, Switzerland). The analyses included red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, erythrocyte distribution width, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration. White blood cell, normal and abnormal lymphocyte, monocyte (M), eosinophils (E), segmented cells, and platelet (PLT) count were also performed using the same automated analyzer.

Data Analysis

Descriptive analysis of data was expressed as means and standard deviations. A commercially available software (GraphPad Prism 5.0 for Windows, GraphPad Software Inc., La Jolla, CA, USA) was utilized to compare means and draw the graphics. Data was subjected to normality test analysis (D'Agostino & Pearson). Parametric data was evaluated using analysis of variance followed by Bonferroni posttest for multiple comparisons, and *t*-test was used for paired comparisons. Evaluation of nonparametric data was made using Kruskal–Wallis followed by Dunns' posttest or Mann–Whitney test. Statistical significance was set at 5% ($p < .05$).

RESULTS

During the 180 days evaluation period, the problems related to the surgeries were one exposed graft, at the 30-day period in the allograft-treated group, and four loosened blocks, three in the allograft-treated group (4.69%) and one in the autograft-treated group (4.55%). The patient with the exposed block was instructed to apply chlorhexidine 1% gel over the exposed area twice a day for 14 days, and after that the graft was again covered by soft tissue. The four patients with loosened blocks removed these blocks at the moment of implant placement, and their treatment plan was changed, with new grafts being performed in those sites. These patients were maintained in the study sample because they all had more than one block grafted, and the samples used for the hematological evaluation had already been collected.

Regarding the data from the hematological analyses, only the M and E counting returned a nonparametric data distribution. All other evaluated data were considered as parametric. In the erythrogram, all evaluated parameters were within the reference values accepted as normal, and no statistically significant differences

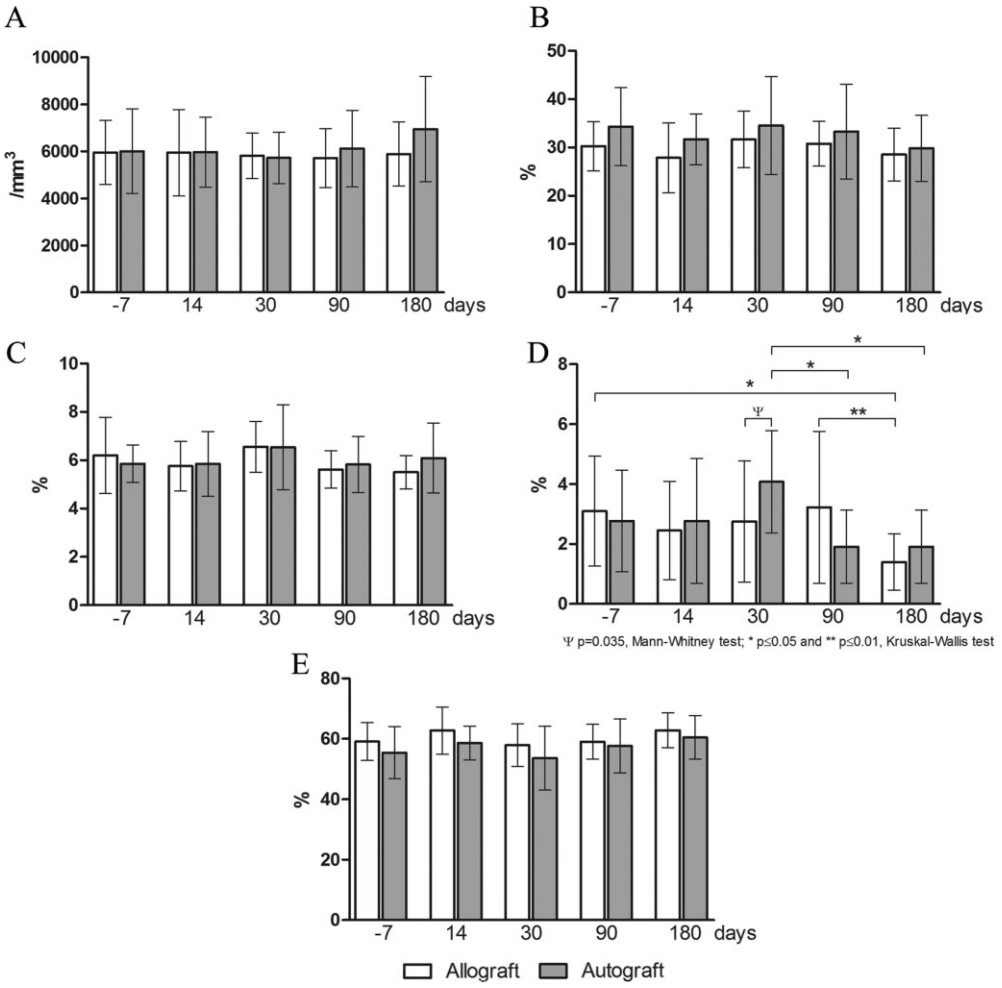

Figure 4 Leukogram data expressed in means and standard deviations: (A) white blood cells (/mm^3); (B) normal lymphocytes (%); (C) monocytes (%); (D) eosinophils (%); and (E) segmented cells (%).

between autograft and allograft groups, in all observed periods, could be found (Table 1).

In the leukogram (Figure 4A–E; see Table 1), all evaluated parameters were also within the reference values accepted as normal, and statistically significant differences were found only for the E count, between the autograft and allograft groups (only in the 30-day evaluation period, $p = .035$ – Mann–Whitney test), and in the same group, when comparing the different periods of evaluation. For the allograft group, differences were found when comparing baseline × 180 days ($p \leq .05$, Kruskal–Wallis followed by Dunn's posttest) and 90 × 180 days ($p \leq .01$, Kruskal–Wallis followed by Dunn's posttest), while in the autograft group, differences were found when comparing 30 × 90 days ($p \leq .05$, Kruskal–Wallis followed by Dunn's posttest) and 30 × 180 days ($p \leq .05$, Kruskal–Wallis followed by Dunn's posttest). No abnormal lymphocytes were found in any of the evaluated patients.

In the PLT evaluation, the values were also within the reference, and no statistically significant differences between both groups and periods of evaluation were found (Figure 5; see Table 1).

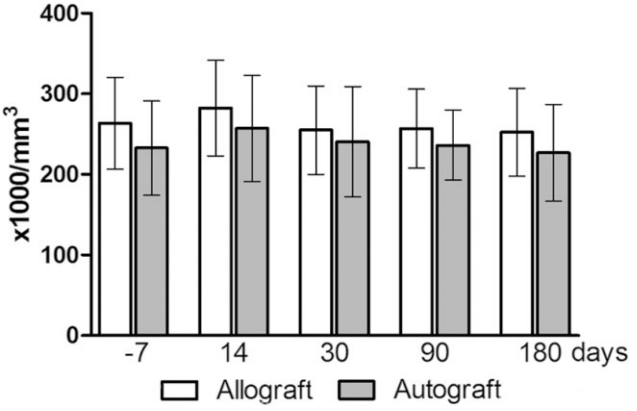

Figure 5 Platelets (×10^3/mm^3) expressed in means and standard deviations.

TABLE 1 Erythrogram, Leukogram, and Platelet Count Data, Expressed in Means and Standard Deviations

	Baseline		14 Days		30 Days		90 Days		180 Days	
Variable	Allograft	Autograft	Allograft	Autograft	Allograft	Autograft	Allograft	Autograft	Allograft	Autograft
Erythrogram										
RBC	4.65 ± 0.52	4.86 ± 0.40	4.63 ± 0.45	4.69 ± 0.46	4.67 ± 0.40	4.77 ± 0.47	4.58 ± 0.52	4.74 ± 0.38	4.62 ± 0.42	4.81 ± 0.48
Hb	13.49 ± 1.55	13.74 ± 1.44	13.53 ± 1.39	13.25 ± 1.23	13.60 ± 1.49	13.49 ± 1.50	13.34 ± 1.31	13.70 ± 1.56	13.58 ± 1.31	13.37 ± 1.76
Ht	41.01 ± 4.23	42.45 ± 3.57	40.83 ± 3.76	40.54 ± 3.44	41.25 ± 4.08	41.48 ± 4.12	40.10 ± 4.05	40.80 ± 4.44	40.87 ± 3.62	40.83 ± 1.34
MCV	88.35 ± 4.93	87.55 ± 5.57	88.26 ± 4.76	86.75 ± 5.43	88.44 ± 5.13	87.00 ± 4.91	87.80 ± 4.93	85.98 ± 5.21	88.60 ± 3.91	88.42 ± 4.68
RDW	13.24 ± 0.87	12.95 ± 1.08	13.11 ± 0.87	13.01 ± 0.98	13.15 ± 0.84	13.30 ± 1.01	13.27 ± 1.43	13.06 ± 0.98	13.13 ± 0.87	13.45 ± 1.08
MCH	29.08 ± 2.26	28.31 ± 2.23	29.23 ± 1.64	28.39 ± 2.55	29.13 ± 2.05	28.26 ± 1.81	29.24 ± 2.02	28.71 ± 2.20	29.41 ± 1.29	28.26 ± 2.04
MCHC	32.83 ± 0.98	32.32 ± 1.18	33.12 ± 0.83	32.70 ± 1.11	32.94 ± 1.19	32.52 ± 0.88	33.28 ± 0.91	33.40 ± 0.98	33.20 ± 0.76	32.68 ± 1.14
Leukogram										
WBC	5955 ± 1357	6008 ± 1796	5945 ± 1837	5969 ± 1486	5810 ± 971	5723 ± 1093	5711 ± 1251	6118 ± 1624	5890 ± 1363	6945 ± 2239
NL	30.25 ± 5.11	34.31 ± 8.10	27.85 ± 7.23	31.69 ± 5.27	31.65 ± 5.82	34.54 ± 10.13	30.78 ± 4.62	33.27 ± 9.78	28.50 ± 5.48	29.82 ± 6.84
M	6.20 ± 1.57	5.86 ± 0.77	5.75 ± 1.02	5.84 ± 1.34	6.55 ± 1.05	6.53 ± 1.76	5.61 ± 0.78	5.82 ± 1.17	5.50 ± 0.69	6.09 ± 1.45
E	3.10 ± 1.83	2.77 ± 1.69	2.45 ± 1.64	2.76 ± 2.09	2.75 ± 2.02	4.08 ± 1.71	3.22 ± 2.53	1.91 ± 1.22	1.40 ± 0.94	1.91 ± 1.22
SC	59.10 ± 6.28	55.46 ± 8.58	62.75 ± 7.78	58.62 ± 5.63	57.90 ± 7.05	53.62 ± 10.57	59.06 ± 5.79	57.64 ± 8.92	62.85 ± 5.74	60.45 ± 7.17
Platelets ($\times 10^3/mm^3$)	263.4 ± 57.0	232.7 ± 58.6	282.4 ± 59.6	257.0 ± 65.9	255.1 ± 54.7	240.5 ± 68.5	256.9 ± 48.8	236.3 ± 43.6	252.4 ± 54.3	226.9 ± 60.0

RBC = red blood cells (millions/mm^3); Hb = hemoglobin (g/dl); Ht = hematocrit (%); MCV = mean corpuscular volume (u^3); RDW = erythrocyte distribution width (%); MCH = mean corpuscular hemoglobin (pg); MCHC = mean corpuscular hemoglobin concentration (g/dl); WBC = white blood cells (/mm^3); NL = normal lymphocytes (%); M = monocytes (%); E = eosinophils (%); SC = segmented cells (%).

DISCUSSION

Allogeneic bone grafts are one of the most frequently chosen bone substitutes and, in the last decade, its use as the surgeon's second option has increased 15-fold and already accounts for about one-third of bone grafts performed in the United States if we consider its use in dentistry and orthopedics.[25–27] Besides that, bone graft is the second most common transplantation tissue, with blood being the most common.[27] Associated to bone allografts, literature cites advantages such as convenience for the surgeon, decreased operative trauma for the patient, a theoretically unlimited supply of reconstructive material, allied to decreased blood loss, absence of donor site morbidity, and decreased operative time.[28]

The most cited disadvantages regarding the use of bone allografts are the different biological properties when compared with bone autografts[27] and its possibility of antigenicity and the risk of disease transmission[16] There is a wide range of studies stating that bone allografts represent a minimal risk to the patient regarding diseases transmission and cross-contamination, if we consider bone–tissue banks that are based into the AATB standard protocols.[4] According to the literature, the risk of viral transmission using unprocessed deep-frozen, non-irradiated grafts from screened donors is currently less than 0.0005% for HIV and hepatitis C.[29]

The antigenicity of these allografts is a major interest issue. The lack of information regarding specific characteristic shows up as a concern for clinicians and researchers seeking for the reasons of the different biological properties of allografts when compared with bone autografts.[18] It is important to highlight that bone allografts are frequently considered as a universal donor material, which has the immunologic potential diminished by the processing of the tissue (mainly the removal of the cells and the deep-freezing procedures), despite the lack of information on this issue.[20,30,31]

One evaluation method, which could bring information in this issue, would be the screening of the blood cell profile of patients who went through bone grafting surgery. The analysis of blood constituents allows the detection of various physiological or pathological states when their values are increased or decreased in relation to a well-defined reference group or to themselves if monitored longitudinally.[32] In this aim, special attention must be given to the white cell profile of the patients because cells such as the lymphocytes could be directly connected to any current foreign-body reaction toward the grafted material.[33]

Literature states bone allograft as a material that does not provoke severe marked immune responses and has acceptable compatibility with the recipient site, although there are no long-term studies regarding this issue.[34] However, experimental studies, mainly in animals, and using major-size grafts shows that transplanted fresh–frozen allografts can elicit an immunologic response in the host; although the clinical significance of this is still unclear.[20,21,30,31] In our study, and in agreement with current information, all treated patients presented good systemic health after the bone grafting procedures, without any signs of contamination or immunological incompatibility problems.

Although the relationship between the immune response and red and white blood cells homeostasis is cited in literature,[22,23] we could not find literature researches regarding the blood count profile through time in patients after bone grafting in the maxillo/facial region. In our study, all evaluated parameters were within the reference values accepted as normal, in erythrogram, leukogram, and PLT profile. Normally, these types of study mainly look for the antibodies that can be produced toward the grafts rather than the alterations that can be caused in the white blood cell count, such as the study of Friedlander,[35] in which graft-specific anti-human leukocyte antibodies were identified in the sera of nine of 44 patients who had received freeze–dried massive allografts but without present poor clinical results.

In our study, statistically significant differences comparing the groups were found only for the E count (30 days, $p = .035$, Mann–Whitney test). The concept that the eosinophil functioned in tissue repair was supported by results showing that E and their products neutralized mast cell-derived mediators of anaphylaxis.[36,37] However, a direct test of this hypothesis did not show any difference between the severity of hypersensitivity reactions in the presence and absence of the E.[36,38] Allied to that, our results regarding the comparison between the different periods in the same group also showed some differences in the autograft group, making clear that this statistical significant result is not direct related to any antigenicity miscarriage in the allografts. The high standard deviations that we had in this exam could have influenced this result.

The differences found for E count can also be connected to the bone healing and angiogenesis that was undergoing in the grafted region. The literature shows that E can interfere in angiogenesis through the eosinophil-derived major basic protein pathway. The production of vascular endothelial growth factor and of other pro-angiogenic factors, such as basic fibroblast growth factor, tumor necrosis factor alpha, granulocyte macrophage colony stimulating factor, nerve growth factor, and interleukin-8, has suggested a strong link between E and angiogenesis.[39]

The normal levels of lymphocyte and the absence of abnormal lymphocytes that were seen are also a good predictive that no significant ongoing inflammatory processes were related to the grafts; although more specific studies, based on interleukins detection, should be addressed to definitively clarify this point.[40] This goes toward the information that, like any other tissue allograft, fresh–frozen bone allografts should be immunogenic, but the real significance and impairments cause by these events are still unclear.[19,41] In this regard, it has been showed that the administration of immunosuppressive drugs in the host could improve bone allograft performance,[21,42] although the interference of these drugs usage on the systemic health from the recipient could be an major disadvantage.[31]

Important consideration about our results is the fact that the chosen periods of evaluation, beginning 14 days after the grafting surgery, did not include any evaluation of the "immediate" cellular responses to the grafts, which could have occurred earlier (e.g., in the first 7 days). Studies focusing on the first days of interaction between the host bone and the grafted material would be necessary to understand if our results represent the full of the cellular alterations that occur following the bone grafting procedures, or if there are other early variations that could be important to understand the fate of these grafts. So far, specific literature did not address to that.

Allied to that, further long-term studies are needed to elucidate biological events related to bone allografting in the maxillo/facial region, mainly because besides the potential consequences of local graft rejection, the implications of sensitization of the host to an expanded pool of human leukocyte antigen antigens also should be considered, even limiting the options for subsequent tissue transplants.[20] The remodeling process of these grafts and the success rates of implants and implant-supported prosthesis installed over them also have to be accessed to long term, providing evidence that could support this therapeutic option. Our study clarifies that peripheral blood cell balance is unaltered by the use of fresh–frozen bone allografts or autogenous bone block grafts in lateral maxillary ridge augmentation.

CONCLUSION

Considering these results and based on the limitations of the evaluation model that was used, it is concluded that both autologous and fresh–frozen bone allografts did not cause any impairment in the red and white blood cell balance, based on quantitative hemogram analysis, in patients subjected to maxillary reconstruction.

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