Histomorphometrical Evaluation of Fresh Frozen Bone Allografts for Alveolar Bone Reconstruction: Preliminary Cases Comparing Femoral Head with Iliac Crest Grafts

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ABSTRACT

Purpose: In the past few years, the use of fresh frozen bone (FFB) grafts has significantly increased. The aim of this study was to evaluate the reconstruction of alveolar bone using femoral head and iliac crest FFB grafts.

Materials and Methods: The study included 10 patients who need endosseous implant insertion in severe atrophic maxillae. The patients were treated with FFB grafts collected from the femoral head or iliac crest. Bone regeneration was evaluated 6 months after surgery by macroscopic and microscopic analyses.

Results: Our results showed good regenerative capacity, both with the FFB from the femoral head and iliac crest. In particular, similar percentages of new-bone formation and graft residual were observed, whereas differences between the percentage of total bone (higher for the iliac crest) and the percentage of non-mineralized tissue (higher for the femoral head) were present. A significantly higher percentage of CD34-positive vessels in the FFB allograft from the femoral head than in the iliac crest were observed.

Conclusions: These findings suggest that FFB allografts could represent a reliable option in oral and maxillofacial surgery. Nevertheless, differences between the use of femoral head or iliac crest bone allografts linked with their different structures should be considered for a more effective surgery.

KEY WORDS: allograft, alveolar bone remodeling, angiogenesis

INTRODUCTION

Bone availability is the key to successful implant placement, so several techniques for bone reconstruction before dental implant placement have been proposed.[1–8]

The technique of “bone regeneration” using bone grafts has been widely used to repair small- and medium-sized bone defects and represents an auxiliary medical therapy for the insertion of endosseous implants. Different bone grafting materials can be used: intraoral or extraoral autologous bone, homologous grafts, heterologous grafts, and alloplastic grafts that can be used alone or in combination with others.[9–11]

Among them, autologous bone has long been considered the ideal grafting material in bone reconstructive surgery; however, it presents some disadvantages, including morbidity, availability, and unpredictable graft resorption.[12,13]

A good alternative for the use of autologous bone is the homologous bone allograft, which is also available in different types: freeze-dried bone allograft (FDBA), decalcified FDBA, fresh bone, and fresh frozen bone (FFB).

In particular, in the past few years, the use of FFB significantly increased.[8,13,14] This fact is directly related to

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DOI 10.1111/cid.12028

the establishment of severe guidelines for bone processing, which define the donor selection, how the bone must be harvested, processed, and stored, together with record-keeping procedures that must be respected. Moreover, the absence of negative reports concerning its antigenicity and the demonstration of reduced immunological reaction in experimental models suggest that FFB could represent an adequate alternative to autografts.[15,16]

FFB is harvested aseptically from different anatomical areas of live or cadaveric donors and then immediately frozen and stored at −80°C; in the absence of contraindications emerging from the results of the screening procedures, it can be used for implantation.

To date, studies about the effect of FFB in oral and maxillofacial surgery are limited. Some literature data reported that FFB could be used alone or in combination with autologous bone graft for alveolar bone reconstruction and for large mandibular reconstructions after the resection of a large portion of the mandible for the complete removal of cysts and tumors.[8,13,14,17,18] The results were supported by observations showing the osteoinductive and osteoconductive properties of FFB[17,19] and the preservation of bone morphogenetic proteins.[20] Consistent with these findings, different recent clinical reports indicated that deep-frozen allogeneic bone grafts represent a reliable treatment option for bone reconstruction.[8,13,14,21–23] In disagreement with these results, other authors reported a higher percentage of nonremodeled bone in FFB-grafted bone with respect to autologous bone graft, where the majority of the grafted material was remodeled and showed characteristics of vital bone.[24]

Because the FFB grafts could be collected from different anatomical areas, the aim of this article was to evaluate and compare the reconstruction of alveolar bone defects using femoral head and iliac crest FFB grafts. Moreover, as neovascularization represents an early event closely associated with bone neoformation,[25,26] we analyzed the percentage of CD34-positive vessels in the regenerated bone.

MATERIALS AND METHODS

Patients

This study included 10 patients (five women and five men), with a mean age of 49.7 ± 7.2 years (range 36–61 years), who need endosseous implant insertion in the atrophic maxillae. The patients selected for this study were treated at the Department of Oral Surgery, Fondazione IRCSS Ca' Granda Ospedale Maggiore Policlinico Milano, University of Milan, Milan, Italy. Written informed consent was obtained from all patients, and the study was in accordance with the Helsinki Declaration. None of the patients presented systemic diseases affecting bone turnover, were pregnant or lactating, or had habits that could interfere with treatments (i.e., smoking, alcoholism, and drug use). All patients had severely atrophic maxillae, classes V and VI or class IV extends on all mandibles and maxillae, according to Cawood and Howell,[27] evaluated by dental computed tomography scan. The patients were treated with FFB onlay grafts to increase the alveolar bone: four patients received FFB allografts collected from the femoral head (group I), and six patients received FFB allografts collected from the iliac crest (group II).

Graft Materials

FFB grafts were taken from femoral heads and iliac crest bones and were obtained from the Regional Skeletal Muscle Tissue Bank, Orthopaedic Institute "Gaetano Pini," Milan, Italy. The FFB graft was a mineralized, nonirradiated, only disinfected bone that was frozen at −80°C, without any cryoprotective solutions, and packed in double sterile casing. In this study, the FFB grafts were collected from the cancellous block of the femoral head or from the cortical-cancellous (monocortical, bicortical, or tricortical) of the iliac crest. The techniques used for the prepared femoral and iliac crest grafts were similar; nevertheless, for collecting cancellous block grafts from the femoral head, the cartilage surface was removed.

Surgical Procedure

All patients were treated by the same surgical team (first, second, and third surgeons), under general anesthesia with nasotracheal intubation.

To create the recipient site, a midcrestal incision (at the top of the edentulous alveolar crest) and two vertical releasing incisions were performed to provide a clear view of the surgical area. The bone defect was evaluated to determine the size and shape of the FFB block. The recipient site was prepared in order to make it suitable for receiving the graft. The FFB that had been deep frozen was restored in rifamycin solution according to the instructions provided by the reference bank and was adapted to the atrophic maxillae. The FFB onlay graft was inserted using ostheosynthesis screws (KLS Martin, Tuttlingen, Germany). The gaps between the graft and the alveolar bone were then filled with allogeneic bone (FFB) chips, and they were maintained in situ by resorbable collagen membranes. The wound was then sutured with a nonresorbable suture that was removed 10 to 14 days after surgery. Peripostoperative management was prescribed: all patients received 2 g of ampicillin intravenously at the time of induction of general anesthesia and 1 g of amoxicillin plus clavulanic acid three times a day for 7 days, as well as chlorexidine 0.2% mouthwash twice a day for 20 days; analgesics were administered if necessary. During the healing period, all patients were seen once a month until the time of implant placement.

After 6 months from surgery, the screws for the stabilization were removed, and the implant site was prepared using a 2.6-internal-diameter trephine (Komet Dental, Lemgo, Germany) under cold sterile saline solution irrigation; simultaneously, biopsy containing the grafted and native bone areas was collected for each patient for the microscopic evaluation.

Macroscopic Morphological Evaluation

The macroscopic evaluation was made by a qualitative evaluation of the bone resorption and of the grade of bleeding.

Microscopic Evaluation: Histomorphometric Analysis

All bone biopsies were fixed in 10% neutral buffered formalin pH 6.9 for 24 to 36 hours, decalcified in Osteodec (Bio-Optica, Milan, Italy) for 7 days at room temperature (RT), and finally stored in 70% ethanol and embedded in paraffin according to the standard procedures. Seven-micrometer-thick sections were cut by microtome and stained with hematoxylin and eosin (Bio-Optica). The histomorphometric analysis was performed blindly using an optical light microscope (Olympus, Hamburg, Germany) at a final magnification of ×100. Digitally fixed images of slices were analyzed using an image analyzer (Image Pro-Plus 4.5.1, Immagini e Computer, Milan, Italy). The measurements were made as the percentage of area in five random fields for each section. The percentage of the total bone (TB), non-mineralized tissue (n-MT), new bone (NB), and graft residual (GR) were quantified separately. In addition, the ratio between NB and TB was calculated.

Microscopic Evaluation: Immunohistochemical Analysis

Tissue sections were processed for immunohistochemical analysis to detect CD34-positive vessels and CD56-positive osteoblasts.

Before the immunohistochemical assays, the sections were deparaffined, hydrated, and heat treated in 0.05 M Ethylenediaminetetraacetic acid (EDTA) buffer pH 8.0 (Bio-Optica) for antigen unmasking at 98°C for 20 minutes and RT for 20 minutes. Endogenous peroxidase activity was blocked by incubation with a solution of 3% hydrogen peroxide. Sections were immunostained with the following monoclonal antibodies: CD34 (clone QBEND/10, Novocastra, Newcastle upon Tyne, UK) and CD56 (clone 123C3.D5, Neomarkers, Fremont, CA, USA). All sections were processed using UltraVision Quanto Detection System Horseradish Peroxidase (HRP; ThermoScientific, Bio-Optica), followed by development with diaminobenzidine (Amresco, Prodotti Gianni, Milan, Italy). A section incubated without the primary antibody served as a negative control.

Quantitative analysis of immunopositivity was performed to calculate the percentage of CD34-positive vessels. The analysis was performed blindly using an optical light microscope (Olympus) at a final magnification of ×200. Digitally fixed images of slices were analyzed using an image analyzer (Image Pro-Plus 4.5.1). The measurements were made as the percentage of area in five random fields for each section.

Statistical Analysis

The histomorphometric and immunohistochemical data were represented by the mean ± standard error of the mean (SEM). Appropriate analyses of variance corrected by the Bonferroni method were performed using statistical analysis software; $p < .05$ was considered to be significant.

RESULTS

Clinical Results

In all patients, no complications related to the grafting technique were observed over the study period. After 6 months from FFB grafts, good bone adaptation and integration, permitting the implant placement, was observed.

Nevertheless, differences between the groups (femoral head and iliac crest) were found. The femoral head allograft showed a greater bone resorption and

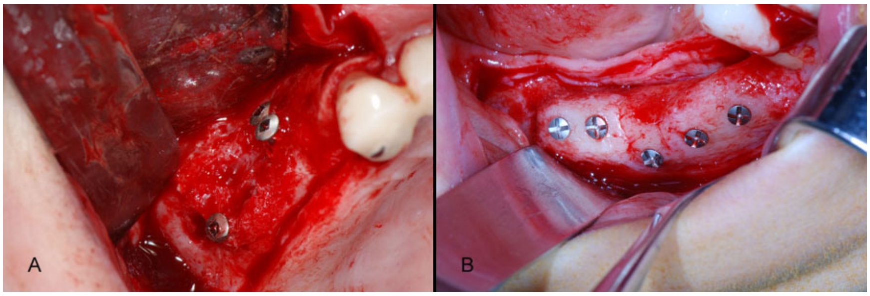

Figure 1 The grafted sites 6 months after the surgery. In the femoral head fresh frozen bone (FFB) allograft-treated group (A), greater bleeding and resorption were seen with respect to the iliac crest FFB allograft-treated group (B).

bleeding than the iliac crest bone allograft, as shown in Figure 1.

Histomorphometrical Results

Hematoxylin and eosin staining revealed bone remodeling and NB deposition for both femoral head and iliac crest FFB grafts after 6 months from graft placement (Figure 2).

Histomorphometrical analysis of group I samples (FFB from the femoral head) revealed that the mean percentage of TB was significantly ($p < .05$) less than in group II samples (FFB from the iliac crest;

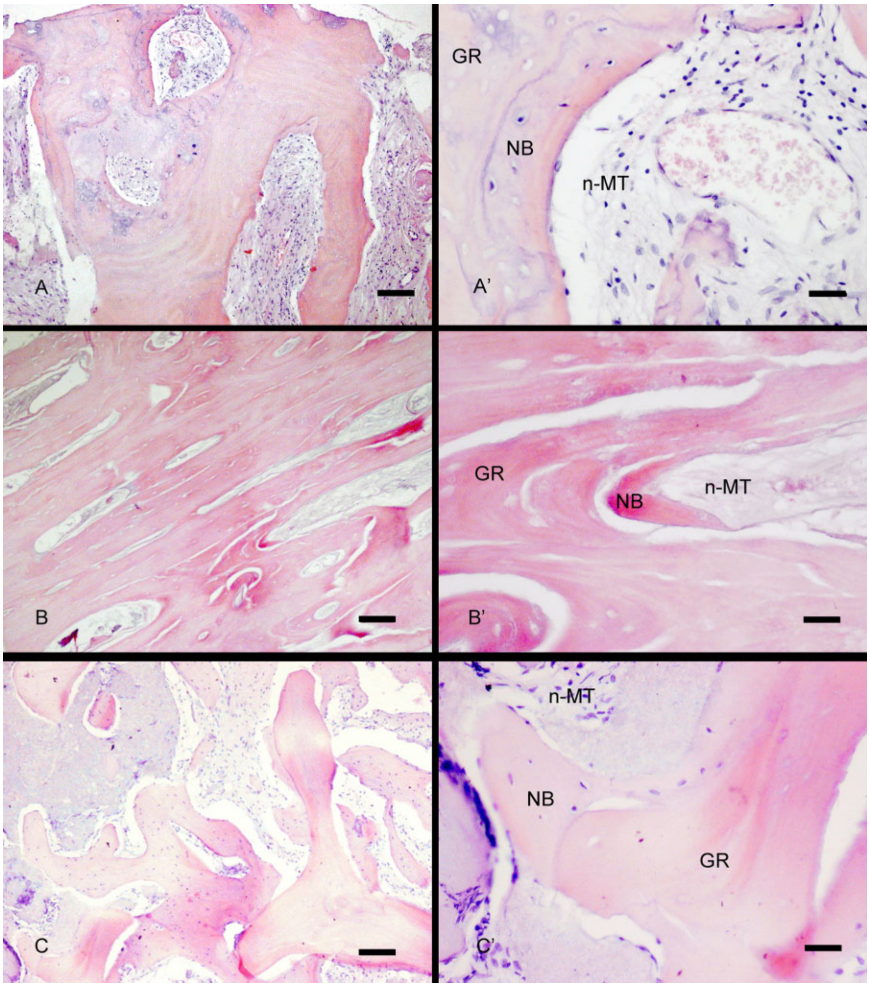

Figure 2 Photomicrographs of an overview of the biopsy area corresponding to the femoral head fresh frozen bone (FFB) allograft (A, A'), iliac crest FFB allograft (cortical bone, B, B'), and iliac crest FFB allograft (cancellous bone, C, C'). (A, B, C) Scale bar, 150 µm; (A', B', C') Scale bar, 30 µm. (GR = graft residual; NB = new bone; n-MT = non-mineralized tissue.)

TABLE 1 Histomorphometric Evaluation of the Bone Quality

	Femoral Head (Group I)	Iliac Crest (Group II)
Total bone (%)	44.85 ± 3.34*	55.63 ± 3.2
Non-mineralized tissue (%)	55.14 ± 3.34*	44.41 ± 3.2
New bone (%)	10.67 ± 1.89	12.56 ± 1.52
New bone/total bone (%)	25.04 ± 5.56	23.03 ± 2.5
Graft residual (%)	34.17 ± 3.31	43.02 ± 3.04

*$p < .05$ versus the iliac crest.

44.85 ± 3.34% vs 55.63 ± 3.2%), whereas the percentage of n-MT was significantly higher ($p < .05$) in group I than in group II (55.14 ± 3.34% vs 44.41 ± 3.2%).

No significant differences between the groups were observed in relation to the percentage of NB (10.67 ± 1.89% group I; 12.56 ± 1.52% group II), to the ratio between the NB and TB (25.04 ± 5.56% group I; 23.03 ± 2.5% group II), and to the percentage of GR (34.17 ± 3.31% group I; 43.02 ± 3.04% group II).

All histomorphometrical data (mean ± SEM) were reported in Table 1.

Immunohistochemical Results

At 6 months, only endothelial cells were CD34-positive in areas. A large number of CD34-positive vessels were observed in n-MT, and they were formed by vessels of different calibers (Figure 3).

Quantitative analysis of CD34-positive vessels revealed a significant difference between the groups, and the percentage of CD34-positive vessels was significantly higher ($p < .05$) in group I than in group II (3.2 ± 0.82% vs 1.78 ± 0.74%).

All immunohistochemical data (mean ± SEM) were reported in Table 2.

TABLE 2 Quantitative Evaluation of CD34-Positive Vessels (%)

	Femoral Head (Group I)	Iliac Crest (Group II)
CD34-positive vessels (%)	3.2 ± 0.82*	1.78 ± 0.74

*$p < .05$ versus the iliac crest.

In addition, immunohistochemical analysis for osteoblasts showed CD56-positive osteoblasts next to the margin of the newly formed trabecular bone (Figure 4).

DISCUSSION

FFB is considered a good opportunity in oral and maxillofacial surgery.

The aim of this work was to compare the alveolar bone regeneration with respect to the use of FFB bone grafts from the femoral head and iliac crest.

The clinical findings showed good regenerative capacity, both with the cancellous FFB from the femoral head and the corticocancellous FFB from the iliac crest at 6 months after surgery. Nevertheless, the femoral head allograft showed greater bone resorption and bleeding than the iliac crest bone allograft.

The good regenerative capacity of both grafts was also confirmed by histomorphometric analysis. We observed, in fact, a similar percentage of NB formation and a nonsignificant difference in the percentage of the residual grafts; on the contrary, significant differences were evident for the percentage of TB and the percentage of n-MT, which resulted higher in the iliac crest and femoral head, respectively.

These discrepancies could be explained by considering the different structures of the two types of FFB

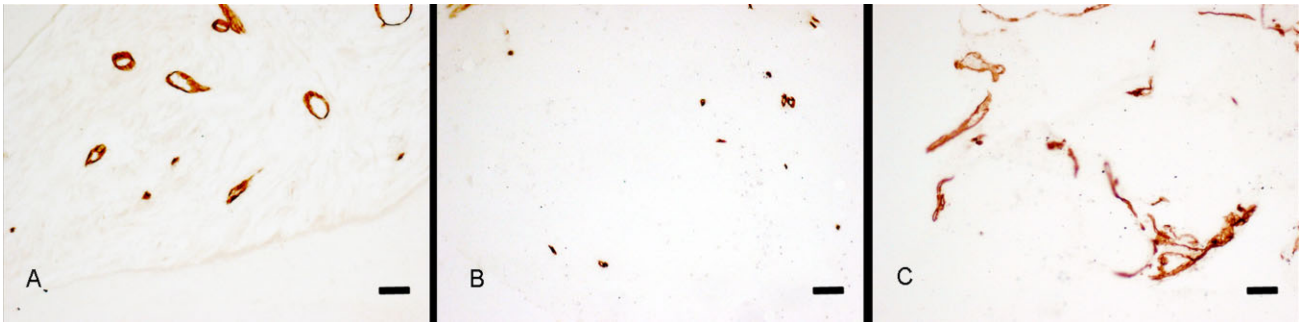

Figure 3 Photomicrographs show CD34-positive vessels (brown) in the femoral head fresh frozen bone (FFB) allograft (A), iliac crest FFB allograft (cortical bone, B), and iliac crest FFB allograft (cancellous bone, C). Scale bar, 50 μm.

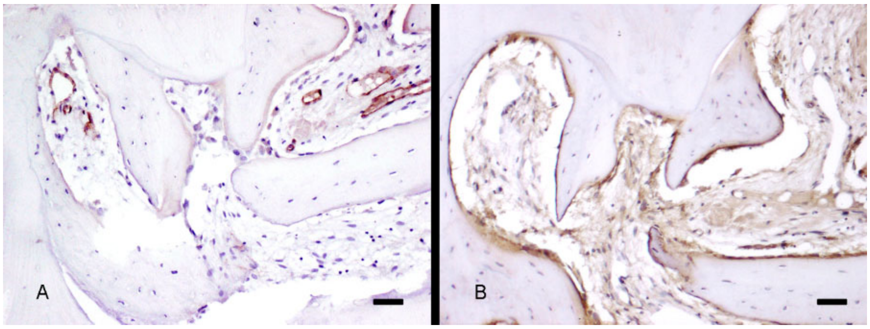

Figure 4 Photomicrographs show CD34-positive vessels (brown, A) and CD56-postivie osteoblasts (brown, B) in serial section from the iliac crest fresh frozen bone allograft hematoxylin counterstaining. Scale bar, 50 μm.

allografts. The femoral head is characterized by the presence of a high density of the cancellous bone without the cortical bone, in which a large amount of n-MT is present, whereas the iliac crest bone presents a well-evident cortical bone and offers a limited cancellous bone and n-MT.

In the latter case, the compact, lamellar, cortical bone with a porosity ranging around 10%[28] permitted good osteointegration of the limited cancellous bone and simultaneously offers a good surface for the insertion of the ostheosynthesis screws. The use of the femoral head FFB allograft, on the contrary, represented certainly an adequate scaffold with osteoinductive and osteoconductive properties, but it also determined greater and fast bone resorption that could be not associated with fast and simultaneous NB deposition. The imbalance between bone resorption and NB deposition was also suggested by our histomorphometrical results, which showed a higher tendency in bone resorption for FFB from the femoral head (GR: 34.17 ± 3.31%) with respect to the FFB from the iliac crest (GR: 43.02 ± 3.04%) and a higher tendency in bone deposition for the FFB from the iliac crest (NB: 12.56 ± 1.52%) than the femoral head (NB: 10.67 ± 1.89%). Although future studies that will consider more patients are necessary to confirm the tendencies reported, the results obtained are encouraging.

In the literature, there are different data regarding the percentages of NB formation, n-MT, and residual grafts, depending on the type of bone substitute utilized and the type of surgical protocol used. Galindo-Moreno and collegues,[26] using a mixture of anorganic bovine bone and autogenous cortical bone, revealed a mean of 34.88 ± 15.2% vital bone, 32.02 ± 15.1% n-MT, and 33.08 ± 25.4% of residuals in maxillary sinus augmentation. A lower percentage of the vital bone (20.47 ± 18.25%) was observed by other authors using a mineralized bone allograft in maxillary sinus augmentation.[29]

Recently, some studies about the use of FFB from different anatomical sites (i.e., tibia and iliac crest) have been published, supporting its success when used as graft material for the extensive bone reconstruction of atrophic maxillae. Several authors reported that none or very few implants were lost.[8,10,13,14,23,30] From a histological point of view, Contar and colleagues reported that all samples showed signs of active remodeling and the bone samples removed during the reentry procedures showed a similar collagen pattern to that of the autogenous bone[10] and that of living bone, showing feature characteristic of mature and compact osseous tissue surrounded by marrow spaces.[8,30] In disagreement with these results, other authors reported a large amount of necrotic bone surrounded by few spots of newly formed bone using FFB allografts with respect to an advanced stage of bone remodeling associated with the use of autogenous bone graft.[24]

The data obtained in this study confirmed that FFB grafts represent a biologically acceptable alternative for bone reconstruction, although associated with a slower remodeling (percentage of NB: 10.67 ± 1.89% for the femoral head and 12.56 ± 1.52% for the iliac crest).

The presence of n-MT was reported to be important for graft remodeling, considering the close direct correlation among the density of vessels, the amount of n-MT, and the bone neoformation.[26,31] The percentage of n-MT observed in this study (55.14 ± 3.34% for the femoral head and 44.41 ± 3.2% for the iliac crest) could be considered appropriate, comparing with the percentage observed using other types of bone substitutes.[26,29]

Adequate vascularization was reported to be important for appropriate levels of oxygen and nutrients for bone proliferating cells,[32] supporting the concept about the existence of a strong sequential link between angiogenetic and osteogenetic processes.[26] This relationship was confirmed by CD56 and CD34 immunohistochemical results that showed an intense osteoblast activity surrounding the bone trabeculae close to vascularized n-MT (see Figure 4).

According to these data, our results showed a significantly higher percentage of CD34-positive vessels in the FFB allograft from the femoral head than in the iliac crest. Nevertheless, it is important to consider that these data were certainly influenced from the different percentage of n-MT calculated for femoral head and iliac crest bone allografts, respectively. Therefore, the grade of the angiogenetic process could be linked with the greater bone resorption and bleeding observed when the FFB allograft from the femoral head (characterized by the presence of high density of cancellous bone) was used.

CONCLUSION

The findings of our study showed that FFB allografts represent a reliable option in oral and maxillofacial surgery, consistent with findings reported about the use of autologous bone. Nevertheless, some differences between the use of femoral head or iliac crest bone allografts linked with their different structures should be considered by the surgeon for a more effective surgery.

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