Autologous bone grafting with platelet-rich plasma for alveolar cleft repair in patient with cleft and palate

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Giuseppe Giudice*, Daniela Anna Cutrignelli*, Sara Leuzzi*, Fabio Robusto**, Pasquale Sportelli*, Eleonora Nacchiero*

**Department of Emergency and Organ Transplantation, Complex Operating Unit of Plastic Aesthetic and Reconstructive Surgery, "Aldo Moro" University, Bari, Italy*
*** "Mario Negris Sud" Institute, Santa Maria Imbaro, Chieti, Italy*

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PURPOSE: Bone grafting of the alveolus has become an essential part of the contemporary surgical management of the oral cleft. The aim of this retrospective study was to evaluate the results of bone grafting in association with PRP (platelet-rich plasma) to enhance osteogenesis and osteointegration.
PATIENTS AND METHODS: *The study included 16 patients, aged between 9 and 11, affected with unilateral residual alveolar clefts, who underwent bone grafting using secondary alveoplasty. The eight patients belonging to the control group were administered autologous bone graft alone while the study group, consisting of 8 patients, underwent autologous bone grafting in association with PRP. All patients had pre and post surgery orthodontic treatment. The statistical analyses included Student's t test, 2 test and Kaplan-Meir time to event analysis. The p-value was considered significant if p<0.05. All statistical analyses were performed using SAS Software release 9.3 (SAS Institute, Cary, Nc).*
RESULTS: *The control group (M 50%, mean age 10.2±2.3) underwent simple autologous bone graft while the study group (M 62.5%, mean age 9.9±2.2) was treated with a combination of autologous bone and PRP. No statistically significant differences were found between the two groups as regards age, gender and labial-palatal cleft clinical characteristics. 6, 12, 24 month follow-ups were performed by means of clinical and radiographic investigations. None of the study group developed oronasal fistulas or experienced bone height, bone bridging and bone quality loss; only two patients developed mild periodontal problems. The study group was able to undergo a significantly (p<0.001) earlier and shorter orthodontic treatment.*
CONCLUSIONS: *In our experience, the use of PRP enhances the quality of osteoplasty, accelerates "creeping substitution" and bone healing and favours earlier orthodontic treatment.*

KEY WORDS: Alveolar cleft, Bone grafting, Palate, Platelet-rich plasma

Background

The labial and palatal cleft is a congenital malformation, which affects 1/750 live births with different levels of severity and requires an integrated diagnostic and therapeutic approach.
Alveoloplasty can be defined as primary if performed simultaneously with cheiloplasty within the first two years of life. Secondary alveoloplasty, performed during the period of mixed dentition (between 9 and 11 years), can be further classified as early secondary alveoloplasty when performed after the complete eruption of the deciduous dentition and before the onset of permanent dentition, and as transitional secondary alveoloplasty when performed during the period of mixed dentition. This period is considered the most appropriate for the closure of the alveolar process which, if carried out before

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Correspondence to: Eleonora Nacchiero, Department of Emergency and Organ Transplantation, Complex Operating Unit of Plastic Aesthetic and Reconstructive Surgery, "Aldo Moro" University, P.zza Giulio Cesare, 11, 70124 Bari, Italy (e-mail: eleonora.nacchiero@uniba.it)

the eruption of the canine, has a higher rate of success regardless of the severity of the cleft [1]. Finally, tertiary alveoloplasty is when bone grafting is performed after the complete eruption of permanent dentition in patients who have not undergone an adequate diagnostic and therapeutic approach.
The use of traditional surgical techniques such as the autologous bone graft, in association with the most recent findings in regenerative surgery, represents the most innovative approach to this problem.
Platelet-rich plasma (PRP) constitutes a valid aid in the field of tissue regeneration and is widely used in the treatment of burns, chronic ulcers, the reconstruction of ligaments of the knee, etc ...
It is defined as a high concentration of autologous platelets in a small volume of autologous plasma [2,3].
Physiologically, platelets are the first cells to rush to the site of tissue damage and, in addition to having a pro-coagulant effect, they provide a wide range of growth factors involved in the various stages of the regeneration of hard and soft tissue [4,5].
The aim of this study was to compare the use of autologous bone graft on its own and in association with PRP in the treatment of alveolar cleft, in order to assess the utility of PRP in bone regeneration and soft tissue healing.

Materials and Methods

Due to the retrospective nature of this study, it was granted an exemption in writing by the Institutional review board of the University of Bari. We reviewed 16 patients, aged between 9 and 11, who underwent secondary alveoloplasty at our institute from 2008 to 2012. All patients were operated on by the same surgeon following the technique described for the first time by Boyne and Sands in 1972 [6].
According to the treatment of choice, the patients were subdivided into a control group of 8 patients treated exclusively with autologous bone graft and a study group of 8 patients who were treated with a combination of autologous bone and platelet-rich plasma (PRP). The patients selected had no associated diseases. The exclusion criteria consisted of a lack of complete operative data and a clinical/radiological follow up of less than 36 months. The families were fully informed about the different treatment available and the possible benefits of the use of PRP in combination with autologous bone graft and were asked to sign an informed consent.
The PRP is prepared from 120 ml of whole blood or autologous plasma, using the "Vivostat" system. The blood is drawn into a closed system with the addition of 15 cc of citrate and the sampling kit is inserted into a processor (automatic biochemical process). This produces 6 ml of PRP in 25 minutes in a liquid form at pH 4.4, which, after being mixed with a neutralizing solution at pH 10, can be sprayed in a semi-solid form by means of a spray- pen (Fig. 1).

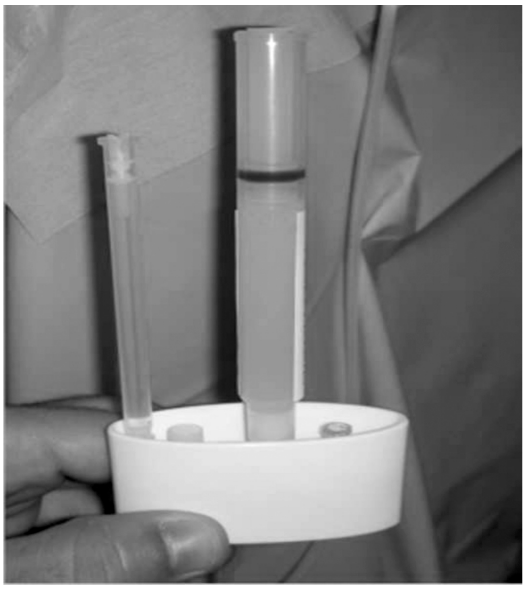


Fig. 1: The sampling kit containing whole blood or autologous plasma (a) is inserted into the "Vivostat" system (b) and produces 6 ml of PRP that can be sprayed by means of a spray-pen (c).

The minimum follow-up period after surgery was 36 months, during which the patients underwent a 6 monthly clinical evaluation and radiological investigations using intraoral radiographs and ortopantomographies (OPG) to investigate the height of the bone bridge and the extent of the bone trabeculae. As for the clinical evaluation, the results were assessed based on the presence or the absence of oro-nasal fistulas, bone thickness at the level of the previous alveolar cleft, state of the periodontium (gum attack, keratinization of the mucosa, formation of periodontal pockets) and state of the teeth (eruption, alignment and vitality of teeth).
All patients underwent pre and post surgery orthodontic treatment, as required by the protocol adopted.

Statistical Analysis

Patients' baseline characteristics were assessed using the 2 test to compare the results for specific subgroups with those of the rest of the patient population. Time-to-orthodontics was defined as the time between the bone graft and the first orthodontic treatment; duration-of-orthodontics was the time between the beginning and the end of the orthodontic treatment. Differences in time-to-orthodontics and duration-of-orthodontics between the control and study groups were evaluated by means of the T-student and Kaplan-Meier time to event analysis. The p-value was considered significant if $p<0.05$. All statistical analyses were performed using SAS Software Release 9.3 (SAS Institute, Cary, NC).

Surgical Technique

Using general anaesthesia, by means of endotracheal intubation, with the tube secured on the patient's median line, lidocaine and epinephrine are locally injected along the edges of the naso-labial fistula, along the labial and palatal side of the alveolar process and at the level of the anterior portion of the hard palate.
A mucosal incision is performed around the fistula on the oral vestibule and continued vertically, along the margins of the alveolar cleft (Fig. 2). On reaching the alveolar ridge, the incision assumes a horizontal direction along the labial side of the gingival sulcus of the teeth. The horizontal incision is then extended distally to the upper molars where it assumes an oblique direction towards the upper posterior vestibule. As regards unilateral clefts, the incision on gingival sulcus is extended only as far as the mesial side of the central incisor. Only a small amount of mucous tissue is mobilized at the level of the premaxilla, as this is not the primary source of soft tissue to be used to cover the bone graft.
A mucoperiosteal flap is then isolated on the labial side of the alveolar process. This dissection is extended up to the nasal floor, exposing the lateral side of the anterior nasal spine and the lower pyriform rim. Through the vertical incisions, two lateral and/or internal mucoperiosteal flaps are isolated, elevated off the bone wall of the cleft, and folded upwards to rebuild the nasal floor (Fig. 3).
When necessary, an incision is made at the level of the palatal side of the dental arch, on both sides of the cleft. The rear extension of the incision depends on the magnitude of the component of the palatal fistula. A palatine component requires that the incision in the posterior gingival sulcus continues up to the first molar to allow a more aggressive mobilization of the mucoperiosteal flap.
For fistulas with a wider palatal component, the fistula margins can be incised just before the dissection of the mucoperiosteal flaps, which are then separated laterally and sutured onto the paramedian line. Any excess of tissue in the raised flaps can be trimmed, being careful to avoid over-resectioning, which could lead to an under tension closure of the mucosa. The nasal plane is sutured postero-anteriorly, using an absorbable suture 4/0. The mucoperiosteal flaps on the palatal side are then fed forward medially and secured to each other using absorbable sutures 4/0.

Fig. 2: A mucosal incision is performed around the fistula, along the margins of the alveolar cleft.

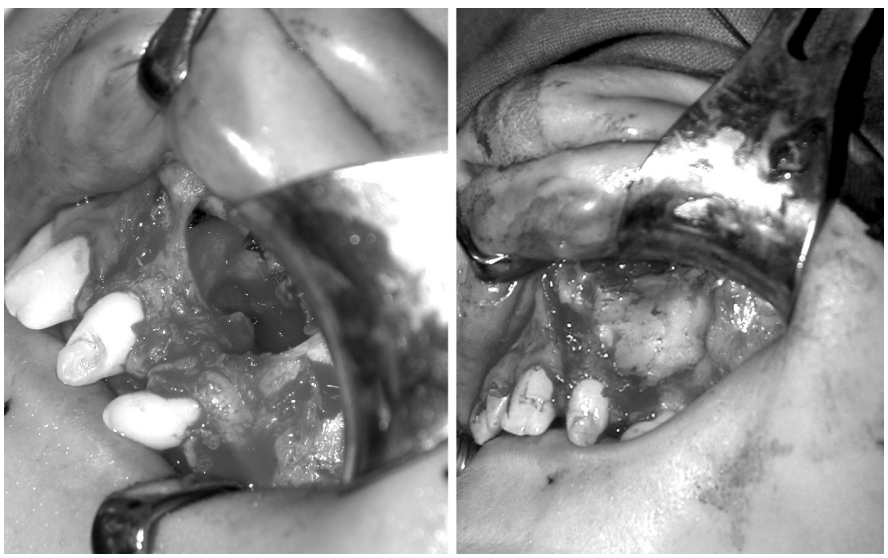


Fig. 3: Chips of autologous bone.

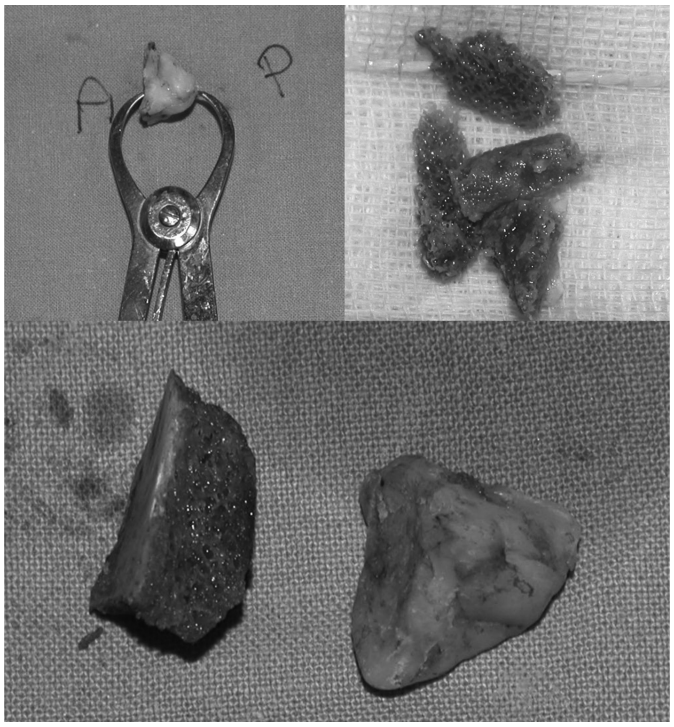


Fig. 4: The bone graft is positioned in the form of chips and blocks to fill the bone defect.

Fig. 5

For the harvesting of autologous bone, an incision of approximately 5 cm is made at the level of the anterior superior iliac spine. Once the ridge of the bone surface is exposed, using a chisel, a bone flap with a lateral base of approximately 4 cm is prepared and then raised. After a careful and delicate sub-periosteal dissection of the inner/or medial surface of the iliac crest, the internal cortical and cancellous bone is removed. Having been harvested, the superior bone flap is repositioned downwards to recreate the edge of the ridge and the donor site is sutured in layers. This method of harvesting reduces morbidity and improves the aesthetic result by avoiding the "step" effect at the level of the iliac crest and by retaining its curvature (Fig. 4).

The amount of bone to be taken in the alveolar cleft can be measured using a bone wax mould inserted into the alveolar cleft (Fig. 5). The bone graft is positioned in the form of chips and blocks to fill the bone defect from the nasal floor upwards, downwards to the palatine side and forwards to the alveolar rim (Fig. 6). Part of cortical and cancellous bone is positioned along the inferolateral piriform rim to rebuild the bone surface deficit and increase support to the nasal wing. It is imperative that the bone graft is placed down as far as the Cervical line (cement-enamel junction) of the teeth bordering the cleft to ensure optimal bone height. A fragment of cortical and cancellous bone closes the cleft anteriorly on the vestibule side. Overcorrection is useful, to a certain degree, to compensate for the normal processes of bone reabsorption, which occur physiologically in the post-operative period. Only patients in the study group underwent PRP infiltration using a "spray-pen" applicator to fill the dead space between the autologous bone graft and the cleft walls.

At this point, the anterior mucoperiosteal flap, which is fed forward medially, is sutured to cover the bone graft and the oro-labial side of the cleft (Fig. 7). Sutures at the level of the interdental papilla are positioned to stabilize the labial and palatal mucoperiosteal flaps towards each other, against the alveolar process.

Fig. 6

Fig. 7

Results

Eight patients (M 50%, mean age 10.2 ± 2.3) underwent simple autologous bone graft (control group) while 8 (M 62.5%, mean age 9.9 ± 2.2) were treated with a combination of autologous bone and PRP (study group). No significant statistical differences were found between the two groups as regards age, gender and labial-palatal cleft clinical characteristics.

As shown in Table I, no patient in the study group developed oro-nasal fistulas and the bone quality was satisfactory in terms of thickness, height and trabeculae (twice the height); only two patients belonging to the study group developed mild periodontal disease and transient dental disorders. In the control group, on the contrary, one patient developed a oro-nasal fistula, another showed a significant reduction in the height of the bone, one presented a reduction in bone thickness and two patients developed abscesses and periodontal disease with dental problems.

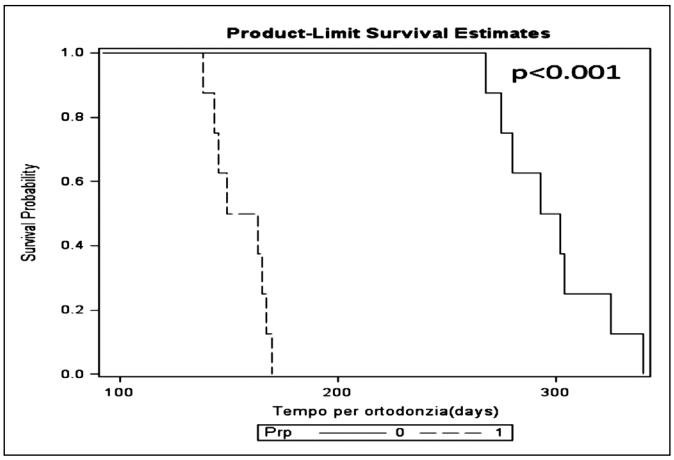
Patients in the study group were able to undergo a significantly earlier and shorter orthodontic treatment (Table II). In fact, the average wait was of 155 days in the study group, against 298 days in the control group. The use of autologous bone graft together with PRP also affected the average duration of the orthodontic treatment which lasted 294 days in patients treated with PRP, compared to 356 days in those in the control group.

TABLE I - *Complications after surgery*

	Control Group	Study Group
Oro-Nasal Fistulas	1	0
Periodontal Diseases	2	2
Dental Diseases	2	2
Bone Thickness Loss	1	0
Bone Height Loss	1	0
Reduction Of The Bone Trabeculae	0	0

TABLE II - *Differences in the mean times to orthodontic treatment and its duration in each group: t-student and time-to-event analysis.*

	N	Mean time to orthodontics (days)	p
Control group	8	298.4 ± 8.8	<0.001
Bone graft + PRP	8	155.0 ± 4.4	
	N	**Mean duration of orthodontics (days)**	**p**
Control group	8	356.0 ± 12.2	<0.001
Bone graft + PRP	8	294.5 ± 5.1	



Discussion

In recent years the use of PRP in association with autologous bone graft has found numerous applications in oro-maxillofacial surgery [7,8].

As a matter of fact, several authors have successfully used the PRP together with autologous bone in the post-traumatic and post-oncological reconstruction of the mandible [9-13].

Recently, the use of PRP has also been suggested for the reconstructive treatment of jaw bone osteonecrosis following therapy with bisphosphonates [14-20] and for the treatment of avascular necrosis caused by radiation therapy, to accelerate the healing of soft tissue and bone maturation.

However, these procedures are considered elective in the elderly, who, from a dental perspective, present special needs and require a specific approach. Age is, in fact, a major factor in the development of periodontal diseases, which are the prime cause of tooth loss in adults. Moreover, elderly patients are much more susceptible to

systemic diseases that can adversely affect their response to surgical treatment in terms of clotting and tissue repair. The application of PRP during surgical procedures has reduced these problems [7,8].

A radiographic study of Marx et al. [21], shows an increased rate of bone maturation from 1.62 to 2.16 times higher in patients treated with autologous bone graft in association with PRP compared to patients treated with the traditional technique.

On the contrary, Lee et al. [22], on analysing both the patients undergoing secondary alveoloplasty in association with PRP and those undergoing surgery with simple bone graft, and having measured the bone density in equivalents of aluminium (Al Eq), conclude that there is no significant difference in terms of the rate of bone reabsorption between the two groups. According to this study, the PRP stimulates bone remodelling in the early stage, but its effects are not long lasting and are insufficient to prevent the natural course of bone graft reabsorption in the postoperative period.

According to several studies, the PRP would be more useful if used in combination with biocompatible bone substitutes such as porous hydroxyapatite [23,24]. This consideration is based on the concept that autologous bone, already rich in growth factors, does not need an "osteoinductor" like PRP, which would be crucial for the osseointegration of an inert "osteoconductive" such as porous hydroxyapatite. Bearing this in mind, the benefits of PRP in association with autologous bone would not justify the cost the hospital would have to sustain for the preparation kit.

Despite this diversity of views, most of the studies examined agree on one key point: the PRP, used together with autologous bone or other bone substitutes has a positive effect on soft tissue, improving the tropism and thus accelerating the healing process [13,21-26].

In the present study, the latter played a vital role. The best soft tissue tropism in association with a more rapid postoperative recovery and fewer complications, incidentally, allowed the study group to begin the post-operative orthodontic treatment sooner than the control group, so achieving better aesthetic and functional results.

These results are explained by the concept of "functional graft." The bone is a dynamic and plastic tissue that modulates its structure thanks to both organic and mechanical stimuli through a continuous process of reabsorption and production of newly formed bone. A 2007 study[27] confirms that the "functional stress" exerted on the graft through orthodontics and chewing, exerts a decisive influence on the volume of the osteoplasty and on the extent of the bone support to the teeth adjacent to the cleft. The presence of dental elements adjacent to the grafted area and the orthodontics, the latter of which favours the movement of the same (whatever the width of the cleft and the material used), stimulate a functional remodelling of the grafted bone by reducing the phenomenon of "creeping substitution". On the contrary, in patients who are not having post-operative orthodontic treatment, the grafted bone undergoes stringent processes of reabsorption. Finally, it is important to note that differently from the control group, the study group benefited from a shorter orthodontic treatment with an advantage in terms of costs.

This suggests that the use of PRP in combination with autologous bone in the treatment of bone deficits and, particularly, in secondary alveoloplasty has several advantages.

Firstly, the PRP is prepared in an automated closed system, using autologous blood and is, therefore, an extremely safe product, which does not expose the patient to any risk of infection.

The high concentration of growth factors that characterizes the platelet-rich plasma has a positive effect on post-surgical bone regeneration in terms of quality, height and thickness of the graft. The PRP also improves intraoperative haemostasis (by reducing the risk of hematoma), has an anti-inflammatory effect, improves the soft tissue tropism and accelerates healing, thus reducing hospitalization.

These effects enable patients undergoing secondary alveoloplasty with autologous bone graft in combination with PRP to benefit from an earlier and shorter post-surgical orthodontics programme, with advantages in terms of compliance and costs.

The only drawbacks of this approach are surgical morbidity of the donor site of the bone graft, a longer surgery dictated by the harvesting time of the graft and the cost of the PRP preparation kit.

With reference to the morbidity of the donor site, this can often be avoided by improving the surgical technique as described above, on the other hand, the surgical times can be shortened by having two teams work simultaneously.

The essential conditions for the success of secondary alveoloplasty include a meticulous surgical technique and adequate pre- and post surgical orthodontic treatment. Indeed, it is necessary that tension free muco-periosteal flaps and keratinized gingival mucosa cover the bone graft.

The orthodontic treatment plays an essential role in the different phases of the bone engraftment, by exerting a functional stimulation on the graft.

Orthodontic stress has a decisive influence on the volume and quality of the osteoplasty and on the bone support of the teeth adjacent to the cleft [27]. This allows the physiological eruption of the lateral incisor and / or of the canine in a valid bone environment, giving support to the development of a regular periodontium at the level of the teeth adjacent to the cleft and permitting the formation of a well-aligned dental arch. The PRP allows an earlier and faster post-surgical orthodontic treatment, not only by promoting the apposition of newly formed bone tissue, but also by improving the condition of the soft tissue surrounding the wound.

We are of the opinion that PRP cannot be considered the gold standard for all bony regeneration and wound healing problems. However, in secondary alveoloplasty, it is a great help in obtaining graft integration and regeneration.

Riassunto

L'innesto osseo è essenziale nella terapia chirurgica della labiopalatoschisi (LPS), ma i tempi di osteogenesi ed integrazione ossea nella sede ricevente possono procrastinare i successivi interventi di ortodonzia. Nel nostro studio è stata valutata la capacità della PRP (platelet-rich plasma) associata al trapianto osseo nel diminuire il tempo d'attesa e la durata del trattamento ortodontico in una coorte di 8 bambini, confrontandola con 8 controlli sottoposti a semplice innesto osseo. I risultati hanno mostrato come i soggetti sottoposti a trapianto osseo + PRP abbiano avuto la possibilità di essere sottoposti ad un trattamento ortodontico più precoce (155 giorni vs 298) e più breve (295 giorni vs 356) rispetto al gruppo di controllo. Inoltre nel gruppo di controllo si sono verificate con maggiore frequenza complicanze post-chirurgiche (quali fistole oro-nasali, disturbi periodontali, malattie dentali, perdita di spessore, massa o trabecolatura ossea) rispetto al gruppo sottoposto a PRP. Perciò l'associazione della PRP (platelet-rich plasma) al trapianto osseo è una metodica che sembra associarsi ad una diminuzione statisticamente significativa dei tempi di integrazione ossea nei pazienti affetti da LPS, con conseguente anticipazione della ortodonzia e miglioramento degli outcome chirurgici.

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