Platet rich plasma or hyperbaric oxygen therapy as callus accellerator in aseptic tibial non union. Evaluate of outcomes

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Summary. Background and aim of the work: The incidence of long bone non-unions has been estimated to range between 5-10%. Nonunion of fracture is a delayed complication of fracture. A large bone resection, associated with Ilizarov’s osteo-distraction technique, is commonly used in these cases. The war experience was very important for dealing with these injuries. The purpose of this study is to report whether the use of Platelet Rich of Plasma(PRP) or Hyperbric Oxygen Therapy(HOT) as an adjuvant to the osteogenic distrac-
tion of Ilizarov with respect to the classical method has advantages. Methods: From 183 tibial non union, we
enrolled 50 patients suffering by Type B according ASAMI non union classification. We divided the patients
into two groups. The first group was a retrospective group of patient treated by Ilizarov Tecnique plus PRP.
Instead the second group, patients were treated by Ilizarov Tecnique associated with HOT. The chosen crite-
rria to evaluate the two groups during the clinical and radiological follow-up were: the complication after the
surgery in the two groups; the duration of surgery; the objective quality Bone results and functional results
were evaluated according to ASAMI classification while the subjective quality of life correlated with Ilizarov
frame function by the Short Form 12 Health Survey (SF-12); The correlation between bone regenerate/bone
healing and X-rays. The evaluation endpoint was set at 12 months from the remotion of Ilizarov’s frame for
both groups. Results: In comparing the complications of the two populations, there were a significant statisti-
cally difference(p<0.05) in the local skin inflammation and Dockin Point Skin retraction for HOT group
while in refracture p<0.05 was for group PRP. From the SF-12 we discovered not statistically differences
p<0.05. The average correlation between Bone Regenerate-Bone Healing/ X-rays is absolutely in the PRP as
in the HOT, p>0.05. The average Time for remove Ilizarov’s Frame in months was 15.37(±7.34; range 9–32)
in PRP while in HOT was15.22(± 7.83; range 9–31), p>0.05. Conclusions: From our study we can conclude
that the association of HOT and PRP with the Ilizarov technique does not improve the functional outcomes
but allows a more rapid healing of the regenerated bone and therefore an early removal of the device and a
responding improvement in the quality of life. (www.actabiomedica.it)

Keywords: Tibial Nonunion; Ilizarov; Paltlet Rich Plasma; Hyperbaric Oxigen Therapy; Outcomes; ASAMI;
Limb Reconstruction; Limb Savage.
Introduction

The epidemiology of fractures of the tibial shaft has been addressed in a number of studies (1–8). The incidence of tibial shaft fractures is reported in the literature with variation over years and between different countries and cultures (1). From our trauma database, in South Italy, Tibial fracture is one of the most common in the long bone of the body and high-energy collisions, such as road traffic accidents or motorcycle crashes and gunshot injuries, are common causes of tibial shaft fractures. Non-union or delayed union is a common complication of tibial fracture and indicates that the fracture did not heal in a timely fashion (2,3). Nonunion rates in tibial shaft nonunion were >10%, Tibia (14%), than others long bones(4). Univariate analysis assumes that the response variable is influenced only by one other factor, while multivariate analysis assumes that the response variable is influenced by multiple factors or combinations of these factors(5). There are local or general factors because a fracture don’t not heal(3). Non-unions are difficult to treat and have a high financial impact(3). Indirect costs, such as productivity losses, are the key driver for the overall costs in fracture and non-union patients(3). The Ilizarov method is the one most commonly used for the treatment of aseptic nonunion(3). According to the diamond concept(6), the use of biological stimulants such as platelet-rich plasma (PRP) (7) and hyperbaric oxygen therapy(HOT)(8) can be diaphanous for rapid healing. The purpose of the study is to compare the use of PRP with HOT combined with the Ilizarov Method in treating aseptic tibial shaft nonunions.

Materials and Methods

From January 2006 to December 2017, at the six Trauma Level I Center: Vito Fazzi Hospital, Lecce, Italy; Italy; Department of Orthopaedics and Traumatology, University Hospital “Gaetano Martino” Messina, Italy, AO G Rummo, Benevento, Italy; Santa Maria della Misericordia, Perugia, Italy; Clinic of Traumatology, University Hospital Clinical Center Banja Luka, Bosnia and Herzegovina; University of Valencia, “La Ribera” Hospital, Valencia, Spain Division of Orthopedics and Trauma Surgery; we treated 183 tibial non union. From 183 tibial non union, we enrolled 50 patients suffering by Type B according ASAMI non union classification (9).

Exclusion criteria included: hematomal or oncological patients; Acute or Chronicle infections; ASAMI non union classification’s type A and C; not Ilizarov technique; not use of PRP or HOT; other treatments to treat aseptic nonunions; patients who did not adhere to a minimum follow-up of 12 months after the remotion of Ilizarov Frame.

We divided the patients into two groups. The first group was a retrospective group of patient treated by Ilizarov Tecnique plus PRP(PRP). Instead the second group, patients were treated by Ilizarov Tecnique associated with HOT(HOT). Patient division was done through the free choice of patients to adhere to one of the relative and absolute contraindications and treatments for hyperbaric therapy.

The population of the PRP group at the time of the nonunion had a mean age of 42.86 (±6.23;range 16–72), the relation between the sexes (M:F) was 2.57 (18:7). All patients had a closed tibial shaft fracture. The Type of closed Fracture according AO’s classification(1) were: A1 in 1(4%) patient; A2 in 1(4%) patient; A3 in 3 (12%) patients; B1 in 2 (8%) patients; B2 in 6 (24%) patients; B3 in 4 (16%) patients; C1 in 2 (8%) patients; C2 in 3 (12%) patients; C3 in 3 (12%) patients. The orthopedic trauma devices for the treatment of fractures were: Axial External Fixation in 8 (32%) patients; Circular external Fixation in 3 (12%) patients; Intramedullary nail in 10 (40%) patients; Plate in 4 (16%) patients.

The mean in months of nonunion after the trauma was 7.23(±1.58; range 4–8). The Type of nonunions according ASAMI’s classification were: B1 in 9 (36%) patients; B2 in 7 (28%) patients; B3 in 9 (36%) patients. The mean in months of nonunion after the trauma was 7.23(±1.58; range 4–8). The Type of nonunions according ASAMI’s classification were: B1 in 9 (36%) patients; B2 in 7 (28%) patients; B3 in 9 (36%) patients.

To understand and study the capacity of bone healing in patients, we used the Non-Union Scoring System (NUSS) in retrospective mode (3). The average point of the NUSS in Norm was 48.23(±7.45; range 36–72).

The population of the HOT group at the time of the nonunion had a mean age of 42.79 (±6.33; range 16–71), the relation between the sexes (m: f) was 2.12 (17:8). All patients had a closed tibial shaft fracture. The Type of closed Fracture according AO’s classification(1)
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were: A1 in 1(4%) patient; A2 in 1(4%) patient; A3 in 3(12%) patients; B1 in 2(8%) patients; B2 in 5(20%) patients; B3 in 5(20%) patients; C1 in 2(8%) patients; C2 in 3(12%) patients; C3 in 3(12%) patients. The orthopedic trauma devices for the treatment of fractures were: Axial External Fixation in 7(28%) patients; Circular external Fixation in 4(16%) patients; Intramedullary nail in 9(36%) patients; Plate in 5(20%) patients.

The mean in months of nonunion after the trauma was 7.21(±1.49; range 4-8). The Type of nonunions according ASAMI’s classification were: B1 in 9(36%) patients; B2 in 8(32%) patients; B3 in 8(32%) patients.

To understand and study the capacity of bone healing in patients, we used the Non-Union Scoring System (NUSS) in retrospective mode (3). The average point of the NUSS in Norm was 48.62(±7.39; range 35-74).

All patients were informed in a clear and comprehensive way of the two types of treatment and other possible surgical and conservative alternatives. Patients were treated according to the ethical standards of the Helsinki Declaration and were invited to read, understand and sign the informed consent form.

All patients of the two groups. They have undergone the same pre operative and post operative protocol according their treatment (see Patient case management).

The chosen criteria to evaluate the two groups during the clinical and radiological follow-up were: the complication after the surgery in the two groups; the duration of surgery; the objective quality Bone results and functional results were evaluated according to Association for the Study and Application of the Method of Ilizarov (ASAMI) classification(10) while the subjective quality of life correlated with Ilizarov frame function by the Short Form 12 Health Survey (SF-12) (11); The correlation between bone regenerate/bone healing and X-rays.

The evaluation endpoint was set at 12 months from the remotion of Ilizarov’s frame for both groups.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of the study group and subgroups, including means and standard deviations of all continuous variables. The t test was used to compare continuous outcomes. The Fisher, in this groups are smaller than 10 patients, exact test were used to compare Categorical variables. The statistical significance was defined as p<0.05. We used Pearson correlation coefficient (r) was used to compare the predictive score of outcomes and quality of life. Statistical analyses were performed with SPSS v.15.0 (SPSS Inc., an IBM Company, Chicago, IL, USA). Mean ages (and their standard deviations) of the patients were rounded at the closest year. The predictive score of outcomes and quality of life and their standard deviations were approximated at the first decimal while at the second decimal was approximated Pearson correlation coefficient (r). The reliability and validity of the correlation between bone regenerate/bone healing and X-rays were determined by the Cohen's kappa(k).

Patient Managements

Preoperative Evaluation and Planning

All patients were assessed in a multidisciplinary clinic, comprising orthopaedic and plastic surgeons, radiologist, anesthesiologist, hematologic physicians, and a specialist Ilizarov nurse practitioner. A preoperative plan was performed by drawing on paper or on radiographic plate.

Surgical Management and Microbiologic Sampling

Surgical excision and tissue sampling were performed according to a previously described protocol(Figure 1, Figure 2). The medullary canal were reamed. The excision was complete when only healthy bleeding bone remained. After debridement, an assessment of the stability of the nonunion was made. The nonunion was regarded as “stiff” if it had angular bending of less than 7 degrees and axial movement of less than 5 mm on manual testing.

Stabilization and Realignment

Monofocal distraction: performed in cases with stiff nonunions with no major bone loss. A 4-ring frame
was used, and a distal fibular osteotomy was made. Hinges were used to gradually correct angular deformity if present. Distraction was commenced at 1 mm per day for 2 weeks, or until deformity was corrected.

Postoperative Care

Vancomycin and meropenem were given intraoperatively, Samples and cultures were done on bone resection used to detect possible infections, all of which were negative. Knee and Ankle Joints mobilization were started on day 2 and early full-weight-bearing encouraged. Radiographs were reviewed every 2 weeks during the distraction period and monthly during the consolidation period. The Ilizarov external fixator was removed when radiographs showed solid docking-site union and the regenerate area had a minimum of three complete cortices.

Platelet Rich Plasma Injection Protocol

For each patient in the PRP Group, 60 ml of whole blood was drawn from an antecubital vein. The 30-ml syringe was primed with 8 ml of anticoagulant citrate dextrose solution (ACD-A) before 52 ml of whole blood was obtained from each patient using standard phlebotomy procedures. On reaching the final volume of 60 ml, the syringe was loaded into centrifuge. The centrifuge was programmed to produce 6 ml of PRP from the 60 ml volume. The local injection of PRP was done on the docking site at first day of docking point compression.

Hyperbaric Oxygen Therapy Protocol

For patients in the HOT treatment group, the aim was for them to receive a total of 20 HOT sessions over
results

The mean Follow up in years were in PRP 2.1(±0.62; range 1-5) as in HOT 1.62.2(±0.58; range 1-5), p>0.05.

The average duration of surgical time in the PRP group was 126.1 (±33.2; range 89-169) minutes; in the HOT group was 126.6(±28.7; range 87-172) with a p> 0.05.

The average bone resection in cm in the PRP group was 6.3(±1.75; range 3.6-10.9)as in the HOT group was 6.5(±1.72; range 3.8-11.2)with a p> 0.05.

The average Time of bone transport took in days was 92.7(±9.27; range 69-92) in the PRP while in HOT was 92.4(±9.31; range 67-90), p>0.05.

Figure 2. The X-rays of a 34-year-old woman with a closed tibial shaft fracture 42C3 according AO, treated with an axialexternal fixator and K-wire in the fibula, 7-month after the trauma(A;B). Tumor-type resection and Ilizarov frame(C) implant. X-ray showed the start of bone transport(D, E) and after hyperbaric oxygen therapy protocol. X-ray showed excellent results post removal Ilizarov’s frame(F).
The average External Fixation Index in months/ cms was 0.88(±0.47; range 0.52–1.88) in the PRP while in HOT was 0.86(±0.53; range 0.48–1.86), p>0.05.

At the end of the treatment the complication in PRP were: Blood Loss was average 937±164.38 mls; Intra Operative Fracture in 0 patient; Loosening of wires or pins in 4(16%) patients; Local Skin inflammation in 8(32%) patients; Docking point skin retraction in 7(28%) patients; Retard to consolidation of bone regenerate in 2(8%) patients; Refracture in 0. while in the group HOT: Blood Loss was average 942±157.46 mls; Intra Operative Fracture in 0 patient; Loosening of wires or pins in 3(12%) patients; Local Skin inflammation in 4(16%) patients; Docking point skin retraction in 3(12%) patients; Retard to consolidation of bone regenerate in 2(8%) patients; Refractures in 2(8%) fractures.

In comparing the complications of the two populations, there were a significant statistically difference(p<0.05) in the local skin inflammation and Docking Point Skin retraction for HOT group while in refracture p<0.05 was for group PRP.

The average correlation between Bone Regenerate-Bone Healing/ X-rays is absolutely in the PRP $k=0.812204\pm0.099677$ (Figure 1) as in the HOT 0.812208±0.065343, p>0.05 (Figure 3).

The average Time for remove Ilizarov’s Frame in months was 15.37(±7.34; range 9–32) in PRP while in HOT was15.22(± 7.83; range 9–31),p>0.05.

In comparing the outcomes of the two populations, there were not a significant statistically difference(p>0.05).

According the ASAMI outcomes classification we had some different results in both two groups were: in PRP excellent results were in 9(36%) patients while in HOT 10(40%), p>0.05; patients in PRP good results were in 9(36%) patients while in HOT 8(32%) patients, p>0.05; fair results were 7(28%) in both groups, p=1; poor results in both groups in 0 patients, p=1.

The subjective quality of PRP’ life before the tibial shaft fracture, measured by SF-12, was about

![Figure 3](image_url). Comparison between the two Gaussian distributions. At the endpoint there was not statistically differences between the two groups, p>0.05.
92.3 points (range 82-100) while the quality of life before the tibial shaft fracture, measured by SF-12, was about 92.4 points (range 84-100) in HOT, p>0.05 (Figure 4). After Discovery of Non union the SF-12 score was in 68.3 (range 26-82) points in PRP and 67.7 (range 28-78) in HOT, (p>0.05). After 1 month of applied Ilizarov’s Frame the SF-12 score in PRP was 39.7 (range 24-68) points while in HOT was 39.6, p>0.05 (Figure 4). At the moment of total weight bearing the SF-12 score in PRP was 66.3 (range 40-84) while 66.4 (range 40-84) in HOT, p>0.05 (Figure 4). When ended each protocol group the SF-12 score was in 70.5 (range 52-90) while 70 (50-92) in HOT, p>0.05 (Figure 4).

At the moment to remove the Ilizarov’s Frame the SF-12 was 77.2 (range 66-92) point in PRP while in HOT was 77.3 (range 66-94), p>0.05 (Figure 4).

At 12 months after the removed Ilizarov’s Frame we had SF-12 score in PRP of 81.9 (range 64-92) as in HOT was 8.9 (range 64-92) points, p>0.05 (Figure 4).

From the SF-12 it is shown that the pain, the stiffness of the knee and/or the ankle have affected the patient’s quality life.

The SF-12 psychological scores show that women in the patients of both groups were the most affluent people. At the end of the therapeutic protocol for each group, we did not show any placebo effect or increase in anxiety and depression states for any re-interventions to free the docking site or prescription for antibiotic therapy for inflammation of the skin.

From a functional point of view, women complained about the size of the Ilizarov Frame in both groups while in the Hot Group they underwent five times a week for 4 weeks for 2 hours per day at hyperbaric therapy.

The men complained that they did not immediately have an effective benefit that could make them...
work for the work, sports and hobbies that they did before the trauma.

Discussion

Fracture healing is a dynamic process governed by cellular and biochemical agents. Despite the investigation of many factors that may affect this process and the achieved progress on this issue, there are still problems in fracture healing(13). The basic principles of atrophic nonunion treatment involve resection of nonviable bone, autogenous grafting, and skeletal stabilization(3,14-16). However, extensive surgical approaches further endanger an already impaired blood flow at the nonunion site(3,14-16). The biomechanical properties inherent to circular external fixation and the techniques of compression-distraction and internal bone transport are important innovations that will help surgeons meet the challenges of this difficult problem(3-16). Cellular evidence of the potential applications of HOT therapy in fracture healing has previously been investigated. Wu et al. suggested that HOT enhances the differentiation of osteoblasts towards the osteogenic phenotype in vitro(17). Milovanova et al.(18) showed that HOT stimulated vasculogenic stem cell growth and differentiation in vivo. According two systematic reviews(19,20) failed to locate any relevant clinical evidence to support or refute the effectiveness of HOT for the management of delayed union or established non-union of bony fractures. Good quality clinical trials are needed to define the role, if any, of HOT in the treatment of atrophic nonunion. There are three randomised controlled trials provide some relevant clinical evidence to address this issue(21-23). Fracture union is promoted by the removal of non-viable bone through subperiosteal corticotomy and intermittent compression-distraction in most cases(3,14-16). However, this technique remains controversial due to the disadvantages of prolonged framing time, the need for good patient tolerance, the fact that frame application is a technically demanding procedure, and the need for close followup and frequent frame adjustment(11,12,14-16). Therefore, adjunctive HOT therapy can enhance fracture healing in an atrophic tibial nonunions in patients and can therefore be used as supplementary therapy. From the Tenth European Consensus Conference on Hyperbaric Medicine, the HOT is Strongly recommending treatment for traumatic bone ischaemias as the aseptic non union(24).

Two papers have reported that PRP can enhance the healing process in bone injuries(25,26). The bone regenerative effects of PRP have also been reported at early phases of distraction osteogenesis (27,28). Some research has indicated that the addition of MSC into a PRP scaffold would be beneficial for increased new bone formation, mineralization, and mechanical property (29,30).

Dehghan et al. (30) in their canine animal study reported a 30% lengthening of the left tibia (mean distance: 60.8 mm) was performed in ten adult male dogs by callus distraction after osteotomy and application of an Ilizarov fixator. Autologous bone marrow mesenchymal stem cells (BM-MSCs) and PRP as the treatment group (n=5) or PRP alone (control group, n=5) were injected into the distracted callus at the middle and end of the distraction period. At the end of the consolidation period, the dogs were sacrificed after which computerized tomography (CT) and histomorphometric evaluations were performed; From the radiographic evaluations revealed that the amount and quality of callus formations were significantly higher in the treatment group (P<0.05). As measured by CT scan, the healing parameters in dogs of the treatment group were significantly greater (P<0.05). New bone formation in the treatment group was significantly higher (P<0.05). Their conclusion the transplantation of BM-MSCs and PRP positively affects early bony consolidation in osteogenic distraction and their use might allow a shortened period of consolidation and therefore permit earlier device removal. The use of platelet-rich plasma (PRP) can augment the healing process of soft tissues following surgical interventions(31). Rodriguez-Collazo and Urso(31) used of DBM saturated with cBMA PRP and in combination with the Ilizarov technique to treat distal tibial fracture in patients with non union’s high rates. Their results in an 85% healing rate in approximately 4 months (31). Their conclude this strategy is safe,
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reliable, and effective with good clinical outcomes for the treatment for complex fractures in patients with significant comorbidities and nonunion rates(31).

If we compare to the meta-analysis and literature reviews of ordinary Ilizarov’s treatment with our results for outcomes, prognosis, complications, they do not differ but our data are better in the early removal of the device.

From our study we can conclude that the association of HOT and PRP with the Ilizarov technique does not improve the functional outcomes but allows a more rapid healing of the regenerated bone and therefore an early removal of the device and a corresponding improvement in the quality of life.

Limitations In Investigational Methodology

The limits of the current study was the limited number of patients, non-probability sample of convenience, due to few centric sample, Level 1 Trauma Center.

Another limit’s is is retrospective study.

Disadvantages of retrospective studies: inferior level of evidence compared with prospective studies; subject to confounding (other risk factors may be present that were not measured); cannot determine causation, only association; some key statistics cannot be measured.

Selection of patients may be biased, making generalization of results difficult. It may be unclear whether the confluence of findings is merely a chance occurrence or is truly characteristic of a new disease or syndrome. Another limitation was that the measurements and intervention were made without randomization of the researcher to the experimental groups, which have potential for bias. Finally other limiting factors of the study acknowledged by the authors can be: the potential for regression to the mean, the presence of temporal confounders and the mention of subjective score.

Human and animal right

For this type of study is not required any statement relating to studies on humans and animals. All patients gave the informed consent prior being included into the study. All procedures involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments.

Conflict of Interest

Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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