PROTOCOL

Use of Autologous Platelet-Rich Plasma to Treat Hypertrophic Burn Scars; A Randomized Controlled Double-Blinded Trial

NCT # NCT03935594

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Background

Severe burn injury is associated with hypertrophic scarring, which occurs in up to 70% of burn patients (Finnerty et al., 2016). Burn scars are particularly troublesome because they cause debilitating neuropathic pain and itching, joint contractures and stiffness that limit range of motion, inability to sweat, and physical disfigurement of cosmetically sensitive areas such as the hands and face.

Autologous platelet-rich plasma (PRP) is plasma with a higher concentration of platelets and is prepared by drawing up a small amount of a patient's blood, centrifuging it, and collecting the platelet-rich layer. Many automated machines exist for doing this process. Platelets contain a multitude of growth factors and other small molecules that have been shown to promote wound healing and tissue regeneration in a variety of contexts. PRP, which is rich in these healing growth factors, has been studied extensively and has proved to be both a safe and effective treatment modality for a wide range of applications, including acne scars and hair loss (Elghblawi et al., 2018). It has been shown to be a safe and effective treatment for some types of surgical and traumatic scars and has been safely applied to acute burn wounds where it has been shown to improve healing and subsequent scarring (Venter et al., 2016). Despite these known uses of PRP, its role in reducing the extent and severity of mature hypertrophic burn scars after they have already healed is notably lacking in the literature.

The purpose of this study is to assess whether intradermally-injected autologous platelet-rich plasma improves the size, texture, color, elasticity, contour, and neuropathic pain associated with mature burn scars.

Finnerty CC, Jeschke MG, Branski LK, Barret JP, Dziewulski P, Herndon DN. Hypertrophic scarring: the greatest unmet challenge after burn injury. *Lancet*. 2016;388(10052):1427-1436.

Elghblawi, E. (2018). Platelet-rich plasma, the ultimate secret for youthful skin elixir and hair growth triggering. *Journal of cosmetic dermatology*, *1 7*(3), 423-430.

Venter, N. G., Marques, R. G., dos Santos, J. S., & Monte-Alto-Costa, A. (2016). Use of platelet-rich plasma in deep second-and third-degree burns. *Burns*, *42*(4), 807-814.

2. Rationale and Specific Aims

Rationale:
Hypertrophic burn scars are experienced by more than 70% of burn victims. They are a major source of decreased quality of life in burn patients due to pain, decreased range of motion, and poor cosmetic appearance. Current treatment strategies (including fat grafting and laser resurfacing) are either highly invasive, prohibitively costly, or painful. Autologous PRP does not

require anaesthesia, and is an inexpensive, safe, fast, and less painful alternative that has been recognized for its role in reducing scars associated with acne, among other things. While PRP has not been studied specifically in burn scars, there is sufficient theoretical and practical evidence that it will improve the appearance and feel of these debilitating scars, representing a large potential benefit for these patients with minimal associated risk. The evidence supporting our proposal can be seen in section 3 of the protocol.

Specific Aims:

(1) Compare the effectiveness of intradermally-injected autologous platelet-rich plasma versus normal saline to improve burn scars, as measured by both clinician-reported (Vancouver scar scale; VSS) and patient-reported measures (Patient and Observer Scar Assessment Scale; POSAS).

(2) Compare the effectiveness of intradermally-injected autologous platelet-rich plasma versus normal saline as a control to improve neuropathic pain associated with burn scars.

(3) Understand the mechanism of action of PRP in improving burn scars via histological analysis of collagen content in the burn scar tissue biopsy specimens.

3. Animal Studies and Previous Human Studies

i. Cervelli, V., Nicoli, F., Spallone, D., Verardi, S., Sorge, R., Nicoli, M., & Balzani, A. (2012). Treatment of traumatic scars using fat grafts mixed with platelet-rich plasma, and resurfacing of skin with the 1540 nm nonablative laser. Clinical and Experimental Dermatology: Experimental dermatology, 37(1), 55-61.

This study in 60 human subjects found that the combination of PRP-enriched fat grafting underneath the scar along with nonablative laser resurfacing of the scar was more effective at reducing the severity of traumatic scars than either PRP-enriched fat grafting or nonablative laser resurfacing alone, as measured by the Manchester scar scale. Fat grafting is a recognized treatment to improve the appearance and feel of scars, but this study confirmed that fat grafts that are enriched with PRP also improve the appearance of scars, especially when combined with laser resurfacing. Unfortunately, this study did not directly compare the use of fat grafting without PRP to fat grafting with PRP. One drawback to fat grafting is that it frequently requires anaesthesia and operating room time in order to harvest the fat (e.g., from the abdomen or flanks), which significantly increases the time, cost and risks associated with the procedure. By contrast, when used alone, autologous PRP is fast and low-cost, can be done in the clinic, and poses minimal risk to the patient. Of note, in this study, the only side effect noted was mild transient erythema (duration of 2-3 days) and swelling (1-2 days).

ii. Prochazka, V., Klosova, H., Stetinsky, J., Gumulec, J., Vitkova, K., Salounova, D., ... & Ocelka, T. (2014). Addition of platelet concentrate to Dermo-Epidermal Skin Graft in

deep burn trauma reduces scarring and need for revision surgeries. Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia, 158(2), 242.

This study aimed to determine if platelet concentration in combination with autologous dermo-epidermal skin grafts (DESG) led to decreased pain and shorten hospital stays as compared to DESG alone. Eighteen patients with second or third degree burns received autologous platelet concentrate and were evaluated using digital photography, standardized scales, and laser Doppler imaging. The study determined that the combination of both APC and DESG led to decreased pain, use of analgesics, antipruritics, orthotic devices, and earlier discharges.

iii. Ruiz, A., Cuestas, D., Garcıa, P., Quintero, J., Forero, Y., Galvis, I., & Velasquez, O. (2018). Early intervention in scar management and cutaneous burns with autologous platelet‐rich plasma. Journal of cosmetic dermatology.

This study represents a case of a forty-year-old woman who presented with a second-degree burn to her right abdominal flank obtained 7 weeks prior secondary to liposculpture. On admission her scar was scaled as an 11 on the Vancouver scar scale (VSS) and a 49 on the Patient scar assessment scale (PSAS). Treatments of autologous platelet-rich plasma with platelet concentrations between 300% and 400% were applied to the edges of the burn on days 0, 30, 60, 90, 120, and 180. Ten months out from initial presentation, the patient's VSS had dropped to 2 and her PSAS had dropped to 10. Most notably, her pain had dropped from an 8 on initial presentation to a 0. This case report, in combination with the study done in rats by Huang et al, leads us to believe that PRP may be a potential treatment for reducing neuropathic pain in burn scar patients. Our study will be the first to investigate pain outcomes in relation to treatment of burn scars with PRP.

iv. Asif, M., Kanodia, S., & Singh, K. (2016). Combined autologous platelet‐rich plasma with microneedling verses microneedling with distilled water in the treatment of atrophic acne scars: a concurrent split‐face study. Journal of cosmetic dermatology, 15(4), 434-443.

This study aimed to investigate the efficacy of combining platelet rich plasma with microneedling in the treatment of atrophic acne scars. Fifty patients received microneedling on both halves of their faces. Each patient also received intradermal injections and topical applications of PRP on the right side of their face and intradermal injections of distilled water on the left side of their face. The patients received these treatments each month for three months, and were evaluated using the Goodman's Quantitative and Qualitative scales. Using the Quantitative scale, the right halves of patient's faces showed a 62.20% improvement compared to only 45.85% improvement on the left. Additionally, 40% of patients indicated that the right side of their face had an excellent response on the Qualitative scale compared to only 10% on the left side. This

study concluded that PRP in combination with microneedling was more effective at treating atrophic acne scars than microneedling alone.

v. Klosová, H., Štětinský, J., Bryjová, I., Hledík, S., & Klein, L. (2013). Objective evaluation of the effect of autologous platelet concentrate on post-operative scarring in deep burns. Burns, 39(6), 1263-1276.

This study sought to use an objective measurement to determine the effect of platelet rich plasma on post-operative scarring in patients with deep burns. Rather than using a subjective scale such as the Vancouver Scar Scale or the Patient and Observer Scar Assessment Scale, this study utilized the Cutometer MPA 580 to measure the viscoelastic parameters of the skin. Twenty-three patients with 38 scars were enrolled, and received treatment with either split thickness skin grafting (STSF) alone or STSF in combination with autologous platelet concentrate (APC). Treatment with APC in combination with STSF resulted in a more rapid return of viscoelastic properties to baseline (normal skin) as compared to treatment with STSF alone.

vi. Alser, O. H., & Goutos, I. (2018). The evidence behind the use of platelet-rich plasma (PRP) in scar management: a literature review. Scars, Burns & Healing, 4, 2059513118808773.

This review article summarizes findings from several studies on clinical applications of PRP for burn scars, surgical scars, and as adjunct treatment of scars with CO2 laser and fat grafting:

a. Lee, J. W., Kim, B. J., Kim, M. N., & Mun, S. K. (2011). The efficacy of autologous platelet rich plasma combined with ablative carbon dioxide fractional resurfacing for acne scars: a simultaneous split-face trial. Dermatologic Surgery, 37(7), 931-938.

Lee et al. used PRP as an adjunct to ablative fractional laser treatment in a split-face study of 14 Korean patients with analysis by blinded dermatologists. The experimental group received injections of PRP after treatment with ablative fractional laser and the control group received saline injections after treatment. It was found that the PRP adjunct group experienced shorter duration of post-treatment erythema and edema (6.1±1.1 vs. 7.1 ± 1.5 days and 5.9 ± 1.1 vs. 6.8 ± 1.0 days, respectively) and showed greater overall improvement on a quartile grading scale (2.7 ± 0.7 vs. 2.3 ± 0.5). Crusting was experienced by both treatment groups and no other adverse effects measured (petechiae, oozing, dyschromia, infection, scarring, or blistering) were experienced by any participant in either group.

b. Abdel Aal, A. M., Ibrahim, I. M., Sami, N. A., & Abdel Kareem, I. M. (2018). Evaluation of autologous platelet-rich plasma plus ablative carbon dioxide

fractional laser in the treatment of acne scars. Journal of Cosmetic and Laser Therapy, 20(2), 106-113.

Abdel et al. conducted a similar study to use PRP as an adjunct to ablative fractional laser treatment for atrophic acne scars but did not inject saline on the control side. This study also found better overall improvement and faster resolution of edema, it notably reported no post-inflammatory pigmentation on the PRP treated side and significantly lower incidence of post-treatment acneiform eruption. Patient satisfaction was higher on the PRP treated side as well.

c. Tehranian, A., Esfehani-Mehr, B., Pirjani, R., Rezaei, N., Heidary, S. S., & Sepidarkish, M. (2016). Application of autologous platelet-rich plasma (PRP) on wound healing after caesarean section in high-risk patients. Iranian Red Crescent Medical Journal, 18(7).

Tehranian et al. conducted a study examining use of PRP on scar healing from elective cesarian section. PRP was applied to subcutaneous tissue before closure in one group and the other group received saline irrigation before closure. Measurements included REEDA and VSS for scar healing and the PRP-treated group experienced greater reduction in these scores than the control group (85.5% reduction vs. 72% reduction in REEDA and 54% reduction vs. 18% reduction in VSS).

d. Gentile, P., De Angelis, B., Pasin, M., Cervelli, G., Curcio, C. B., Floris, M., ... & Insalaco, C. (2014). Adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical evaluation for cell-based therapies in patients with scars on the face. Journal of Craniofacial Surgery, 25(1), 267-272.

Gentile et al. conducted a study on burn and traumatic scars comparing the efficacy of fat graft treatment enhanced by stromal vascular fraction or PRP. A control group of centrifuged fat without adjunct therapy was also used. In fat grafting supplemented with PRP, there was 69% maintenance of contour vs only 39% of the control group. Less resorption of injected fat was also observed compared to control.

vii. Huang, S. H., Wu, S. H., Lee, S. S., Lin, Y. N., Chai, C. Y., Lai, C. S., & Wang, H. M. D. (2018). Platelet-Rich Plasma Injection in Burn Scar Areas Alleviates Neuropathic Scar Pain. International journal of medical sciences, 15(3), 238.

This study in rats found that burn-induced neuropathic pain associated with burn scars was reduced after PRP was injected subcutaneously into mature burn scars, as measured by behavior tests and immunohistochemistry staining of burned skin and spinal cord dorsal horns. Specifically, PRP alleviated allodynia in burn-induced neuropathic pain four weeks after PRP treatment, and modulated several neuroinflammatory markers and pathways including PTEN, mTOR, and TNF-α in such a manner as to attenuate neuropathic pain. The role of PRP in

reducing burn scar neuropathic pain in humans has yet to be studied, and because of this work in rats, it is one of the aims of this present study.

viii. **Venter, N. G., Marques, R. G., dos Santos, J. S., & Monte-Alto-Costa, A. (2016). Use of platelet-rich plasma in deep second-and third-degree burns. Burns, 42(4), 807-814.** This study in rats found that deep second-degree burn wounds that were treated with topical autologous PRP at the time of the burn (immediately after) displayed a greater percentage of wound closure at 21 days when compared to control (burn without PRP). They also found that on histological analysis, the PRP treatment was associated with larger neo-epidermis thickness in deep second-degree burns. In sum, these findings indicate that PRP can hasten wound healing when applied to an acute burn wound. Two important differences exist between our model and this rat model: (1) we will be using the PRP on mature burn scars which have already re-epithelialized, and because of this re-epithelialization, (2) we will be injecting the PRP subcutaneously to allow the PRP to interact directly with the collagen and cells beneath the epidermis.

4. Inclusion/Exclusion Criteria

Inclusion Criteria:

- 18-70 years old, all sexes
- Thermal burn scar on the trunk or abdomen ≥ 1 year old that meets ONE of the following criteria:
    - Vancouver Scar Scale score ≥ 7
    - Patient and Observer Scar Assessment Scale (POSAS) score ≥ 35
    - Neuropathic symptoms
        - POSAS score ≥ 5 on question evaluating itchiness
        - POSAS score ≥ 5 on question evaluating pain

Exclusion Criteria:

- Initial burn injury was less than 1 year old
- History of chemical or electrical burn
- History of CO2 treatment to the scar of interest
- Genetic or acquired conditions that severely affect systemic wound healing or collagen formation (vasculitis, diabetes, Ehlers-Danlos syndrome, radiation therapy to the scar site or use of immunosuppressive medications within the last year, active cancer)

5. Enrollment/Randomization

Prior to enrollment, all sixty potential study ID numbers will be randomly assigned to a treatment group (Right/Left). The randomization will be performed by coin flip (heads is right, tails is left). This will be logged in a secure REDCAP form only accessible by the researcher not conducting scar evaluations (unblinded REDCAP). There will be a secondary REDCAP form used for logging VSS/POSAS scores that does not contain this information (blinded REDCAP).

Potential candidates will be identified by the attending surgeon at the Burn Reconstruction Clinic at Vanderbilt. All patients meeting preliminary inclusion/exclusion criteria will be contacted, via phone or in person at the clinic, and given a brief description of the study. If interested in participating, inclusion/exclusion criteria will be reviewed with the patient, and the patient will be scheduled for their first appointment. Patients will be assigned to a study ID number (with associated treatment group) based on order of enrollment.

6. Study Procedures

Day 1:

When the patient first arrives, they will be given a copy of the patient POSAS, the study information sheet, and the demographics sheet to review and fill out while in the waiting room. The demographics sheet will collect the following demographic information: MRN, date of birth, sex, %TBSA, comorbidities (diabetes, smoking, hypertension, history of radiation or chemotherapy, history of cancer), presence of skin graft and type (xeno- or allograft) and location, size and donor site of skin graft used, race, Fitzpatrick skin type, mechanism of injury (e.g., flame, scald, radiator, etc.), current medications (in particular, any anti-inflammatories, anti-neoplastics, or steroids), and prior scar treatments, including over the counter creams or lotions.

Once roomed, a provider will administer both the VSS and the observer POSAS to confirm that the patient meets inclusion criteria. A secondary provider will also administer a VSS and POSAS.

After determining that the patient meets inclusion criteria, the consent form will be reviewed in depth to describe the purpose and procedures associated with the study. The consent form, demographics form, and VSS/POSAS scores will be logged into the blinded REDCAP.

For the procedure itself, 6mL of anticoagulant citrate dextrose A (ACD-A) will be drawn up in a 60mL syringe, after which 44 mL of the patient's own venous blood will be drawn into the same 60mL syringe using standard venipuncture techniques (Bellus Medical, 2019). While patient is waiting in the examination room, the sterile tubes of blood will be centrifuged by the ELMI CM-7S centrifuge which takes approximately 10-15 minutes. While waiting for the PRP to undergo centrifugation the scar to be treated will first be wiped with an alcohol swab, then measured and marked out with a marking pen such that the entirety of the scar will fit into a rectangle that is drawn, and the rectangle is filled entirely with the scar tissue. A ruler will be used to measure the scar in its greatest horizontal span, and the midway point will be marked with a vertical line. The length and height of the rectangle will be recorded. The vertical and horizontal distance from the nearest point-like anatomic landmark (e.g., xiphoid process) to the two edges of the rectangle will be documented so that the rectangle can be recreated at a later date. A photo of the outlined scar(s) will be obtained with a digital camera, with a ruler in view placed against the skin adjacent to the scar for reference as well as a "L" and "R" to designate laterality. The area inside the rectangle will then be subdivided with a marking pen into 1cm x 1cm square boxes, with the plan of injecting 1mL of either PRP or normal saline into each 1 square cm box.

The unblinded researcher who does not participate in the scar assessments will review the unblinded REDCAP containing the pre-designated treatment group (termed "Right and Left") and will inject the PRP and saline into the scar. The syringes will be covered with a white sticker and labeled such that the patient is unable to infer which syringe contains PRP (PRP is yellow

with a red hue and normal saline is clear). The unblinded researcher will then log this treatment into the unblinded REDCAP.

One Month Status-Post First Treatment (Day ~30):

At the one-month follow-up visit, the patient's treated scar will be identified with the assistance of old photos and measurements, and the rectangle will be re-marked out. The patient's blood will be collected in the same manner as Day 1 and spun down in the ELMI centrifuge. The patient and blinded providers will complete the VSS and POSAS while waiting, and the scar will then be marked into 1cm squares for subsequent treatment. The two different halves of the scar will be treated with the same substance that they were in the first round by the unblinded researcher.

Two Months Status-Post First Treatment (Day ~60):

The two-month follow-up visit will follow the same protocol as the one-month visit.

Three Months Status-Post First Treatment (Day ~90):

The three-month follow-up visit will follow the same protocol as the one-month visit.

Four Months Status-Post First Treatment (Day ~120):

The four-month follow-up visit will follow the same protocol as the one-month visit.

Five Months and 1 Year Status-Post First Treatment (Day ~150 and ~ 365):

At the five-month follow-up visit the attending will obtain a 2mm punch biopsy from two sites: one from the center of the control (saline-treated) scar, and one from the center of the PRP-treated side of the scar. The biopsy sites will first be numbed with local anesthetic (after confirming the patient has no allergies to the numbing medication). After the biopsy, the site will be closed with 5-0 fast absorbing gut suture using sterile instruments in the standard sterile fashion and dressed with a band-aid. The timing of the biopsy during the visit will be after administering the VSS and POSAS.

This scar tissue specimen will be collected and stored for further examination in the laboratory setting according to the following protocol: Punch biopsies from each scar will be obtained at five months and one year. The tissue samples will then be fixed in formalin and embedded in paraffin wax. The samples will be cut and stained with hematoxylin and eosin before then receiving Masson's trichrome stain to identify collagen fibers, Verhoeff's stain to identify elastic fibers, and toluidine blue stain to identify mast cells. The density of the collagen fibers and elastic fibers will be classified as either sparse or dense, and each individual fiber will be classified as either thin or thick. Under high power view, the thicknesses of the layers in the scars compared to normal tissue using a ruler and the number of mast cells will be counted.

7. Risks

This study is categorized as standard risk. Autologous platelet-rich plasma has been studied extensively and has been found to be extremely safe for human use, as it is derived from the patient's own body. The most severe side effects that have been observed from PRP treatment are erythema and swelling at the application site, which subsides within 1-3 days. All patients will receive a pre-screening survey to eliminate those who meet any of our exclusion criteria that may put them at any additional risk for complications. To decrease the chance of any injection-site-related infection, all sites will be cleaned adequately, and all procedures will be performed using proper sterile technique. Because this is an injection, the possibility of infection or minor blood vessel or nerve damage exists, but these are essentially theoretical risks, as these complications have not been observed in any studies, we are aware of. Nevertheless, in the event of any PRP-related complication, patients will have access to a team member through the clinic phone number or www.myhealthatVanderbilt.com and will be evaluated promptly. Regarding the skin biopsy, sterile technique will be used to minimize the risk of infection, and proper biopsy site closure performed by qualified persons will minimize the risk of wound breakdown. Further, 2mm punch biopsy sites are at very low risk of wound breakdown given their small size. If an infection were to occur at this site, it would be promptly treated with appropriate antibiotics and wound care.

8. Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Adverse events will be reported to the IRB immediately through standard reporting mechanisms. The primary contact for reporting:
Salam Kassis
Vanderbilt University Medical Center
Department of Plastic Surgery
D-4207 Medical Center North
Nashville, TN 37232-2345
Phone: (615) 936-0198
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9. Study Withdrawal/Discontinuation

There are no anticipated circumstances under which subjects would be withdrawn from research without their consent. If subjects withdraw, there will be no penalty or consequences.

10. Statistical Considerations

-Our proposed study is methodically similar to "Treatment of traumatic scars using fat grafts mixed with platelet-rich plasma and resurfacing of skin with the 1540 nm non-ablative laser" by Cervelli et al., and we expect to analyze a similar population and outcome. In this study, they

met power with 31 patients. We will plan to enroll 35 patients, but we will recruit 40 with an expected 10% attrition.
-In our study, data will be recorded in a spreadsheet (Excel; Microsoft Corp., Redmond, WA, USA) and analyzed with SPSS software for Windows (version 15.0; SPSS, Chicago, IL, USA). Descriptive statistics will include mean ± SD for parametric variables, and median and range for nonparametric variables. The median will be used to test the difference between the groups before and after treatment, and the Wilcoxon signed rank test will be used to examine differences within groups before and after treatment. $P<0.05$ will be considered significant.

11. Privacy/Confidentiality Issues

Private health information (PHI) will used in the study, but not disclosed. All PHI will be stored on a secure, password-locked server. All participants in the study have obtained Human Research certification to have the necessary authorization to PHI. When collecting patient data, only medical record numbers will be used to identify patients.

12. Follow-up and Record Retention

The study will last for approximately 1 year after initiation. At the study conclusion, all identifiable data will be destroyed, and de-identified data will be kept for analysis.