Study Protocol: Autologous Platelet-Rich Plasma (PRP) Therapy in the Treatment of Pyoderma Gangrenosum: A Prospective, Open-Label, Randomized, Split-Ulcer Trial to Evaluate the Efficacy of Platelet-Rich Plasma Therapy in the Treatment of Chronic Pyoderma Gangrenosum

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Abstract

BACKGROUND Pyoderma gangrenosum (PG) presents as an ulceration of the lower extremities with deep, undermining, and violaceous borders. The disease is an orphan disease without any FDA-approved treatments. **OBJECTIVE** This study aims to evaluate the efficacy and safety of intralesional injectable and topical platelet-rich plasma (PRP) therapy in the treatment of chronic pyoderma gangrenosum ulcerations. **METHODS** Methodologically, this study will be conducted as a prospective, randomized, open-label split-ulcer study in patients with confirmed PG by PARACELSUS score. In each patient, up to three separate ulcerations will be randomized to receive either monthly intralesional PRP injections or a topical PRP solution at 0, 4, 8, and 12 weeks, while the third target lesion will receive standard wound care only. Patients will be followed up with at 16 weeks, one month after the completion of treatment. The primary endpoint for this study will be the composite proportion of the target ulcers achieving either complete resolution or a 50% reduction in the surface area at week 12. **CONCLUSIONS** This study intends to demonstrate the efficacy of an intralesional injectable or topical PRP therapy in the treatment of PG. If successful, PRP treatment may offer a new and effective option for patients with this debilitating disease.

Keywords: pyoderma gangrenosum; platelet-rich plasma therapy; intralesional therapy; chronic cutaneous ulcers; topical therapy

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INTRODUCTION

Pyoderma gangrenosum (PG) is a chronic inflammatory condition, characterized by rapidly progressive, painful ulcers, mostly on the lower extremities.[1] The classical PG ulcer starts as a tender nodule or pustule, rapidly expanding into a sharply marginated ulcer with violaceous and undermined borders.[1-3] Treatment of PG is often challenging and involves a combination of topical and systemic immunosuppressive agents, wound care, and management of the underlying condition. However, to date, none of the treatments have been proven to be universally effective,[2] and there are no FDA-approved therapies for this indication.

Platelet-rich plasma (PRP) is the portion of plasma derived from whole blood, which has a higher platelet concentration compared to the baseline.[4,5] The platelets contain several growth factors, such as platelet-derived growth factor, fibroblast growth factor, and vascular endothelial growth factor, which promote wound healing.[4,5] Reportedly, these growth factors accelerate wound healing by epithelization and neovascularization. PRP may suppress cytokine release and inflammation, thus promoting tissue regeneration.[6] PRP therapy has been used extensively for its regenerative properties for various indications in oral and maxillofacial surgery, orthopedic and trauma surgery, plastic surgery, and burns.[6-13] Furthermore, several studies have demonstrated the effectiveness of autologous PRP therapy for the treatment of chronic skin ulcers of different origins, mainly diabetic, vascular, and surgical or traumatic ulcers.[4,14]

Previously, a few case studies have successfully reported use of this therapy in the treatment of recalcitrant pyoderma gangrenosum ulcers.[15-17] The purpose of this study is to evaluate the efficacy and safety of intralesional injectable and topical PRP therapies in the treatment of chronic pyoderma gangrenosum ulcerations.

METHODS

Research Hypothesis

We hypothesize that autologous PRP therapy containing a high concentration of platelets, which are rich in growth factors, will activate the wound-healing cascade in PG ulcers, stimulating neo-angiogenesis, granulation tissue formation, collagen deposition, reepithelization, and wound contraction, leading to a reduction in the size of the ulcer(s), symptomatic improvement, and the healing of PG ulcerations.

Primary Objective

- Proportion of the target ulcers achieving either complete resolution or a 50% reduction in surface area at week 12 after treatment with either intralesional injectable or topical platelet-rich plasma therapy as compared to standard treatment.

Secondary Objectives

- Analysis of the frequency of total closure of target/total ulcers from baseline to week 12 and week 16.
- Analysis of change in total surface area of target/total ulcers from baseline to week 12 and week 16.
- Analysis of change in Patient Global Assessment (PGA) from baseline to week 12 and week 16.
- Analysis of change in Investigator Global Assessment (IGA) x maximum wound dimension from baseline to week 12 and week 16.
- Analysis of change in patient pain perception using the 10-point visual analog scale (VAS) from baseline to week 12 and week 16.
- Analysis of change in patient quality of life using the dermatology life quality index (DLQI) from baseline to week 12 and week 16.

Study Design

This is a **prospective, randomized split-ulcer controlled trial** that will enroll 10 adult patients with pyoderma gangrenosum. Up to three lesions in each patient will be randomized into 3 comparative groups using a 1:1:1 ratio to either receive intralesional injectable or topical PRP therapy at 0, 4, 8, and 12 weeks, or no treatment. If only two ulcerations are present, they will be randomized using a 1:1 standard care vs. topical and intralesional PRP treatment. All other lesions, if any, will receive standard wound care during the study period. The **primary**

endpoint is the composite proportion of the target ulcers achieving either complete resolution or 50% reduction in surface area at week 12, as assessed by the treating physician.

Eligibility

Patients with a diagnosis of pyoderma gangrenosum for at least 3 months, on a stable dose of medications listed in the inclusion and exclusion criteria, may be enrolled in the study. Prisoners, children under 18 years of age, women who are breastfeeding, pregnant women, or women desiring to become pregnant will not be included. For eligibility, patients should meet full inclusion and exclusion criteria.

Inclusion Criteria

1. Have given written informed consent before participating in any study-specific activity.
2. Have a clinical diagnosis of classic PG as determined by the principal investigator based on results from clinical, histological, and laboratory assessments.
3. Have at least 2 PG ulcers characterized by 'Item a' AND 3/5 features in 'Item b' OR 2/5 features in 'Item b' with support from one of the conditions listed in 'Item c.'
    a. Stable or increasing size within 2 months preceding screening by patient report or documentation.
    b. Features such as violaceous border, undermining, cribriform scarring, pustules, or peristomal location.
    c. Identifiable secondary systemic condition, such as inflammatory bowel disease, arthritis, monoclonal gammopathy of unknown significance, noncancerous hematologic disease, streptococcal carriage, levamisole-tainted cocaine, or Bruton's agammaglobulinemia.
4. Have at least two PG target ulcers that have an area ≥ 2 cm^2 and ≤ 200 cm^2 at screening.
5. Age of at least 18 years at screening.
6. A negative pregnancy test (for females of childbearing potential) at both screening and Day 0.
7. PARACELSUS Score for pyoderma gangrenosum of 10 or greater.

Exclusion Criteria

1. Any condition (e.g., psychiatric illness, severe alcoholism, or drug abuse) or situation that may compromise the ability of the subject to give written informed consent, may put the subject at significant risk, may jeopardize the subject's safety after treatment, may confound the study results, or may interfere significantly with the subject's participation in the study.
2. History of malignancy within 2 years of screening other than carcinoma in situ of the cervix or adequately treated, non-metastatic, squamous, or basal cell carcinoma of the skin.
3. History of seropositivity for HIV antibody, active or carrier status of hepatitis B, or active hepatitis C (i.e., not treated or not cleared spontaneously, as confirmed by HCV PCR).
4. Patients with hemodynamic instability, bleeding disorders, and/or platelet dysfunction syndrome.
5. Serum hemoglobin concentration <11 g/dL, hematocrit <34%, or platelet count <1, 00000/ml at the time of screening.
6. Patients with uncontrolled secondary systemic disease in the opinion of the investigator.
7. Systemic infection or active local infection requiring oral antibiotics within 2 weeks of Day 0.
8. History of the following treatments:
    a. Patients taking anticoagulant medication.
    b. Changes (addition, discontinuation, or changes in dose) in immunosuppressive medication (including cyclosporine, azathioprine, methotrexate, mycophenolate mofetil, apremilast, dapsone, or corticosteroids) and biologics (Anti-TNF or other biologic therapies) within 2 months of Day 0.

c. Systemic corticosteroids > 20 mg per day (prednisone or prednisone equivalent) within 8 weeks of Day 0 or change in dose within 4 weeks of Day 0. Steroids may be tapered (although not increased above the Day 0 dose) during the trial as determined by the principal investigator.

d. Intralesional corticosteroids within 8 weeks of Day 0; topical immunomodulators are also not permitted.

e. Systemic antibiotics within 2 weeks of Day 0.

f. Hyperbaric treatment within 4 weeks of Day 0.

g. Investigational drug or investigational device within 4 weeks of Day 0.

h. Other treatments not described above should be maintained at a stable dose and frequency throughout the study as best as possible.

9. Major, general surgery within 3 months of screening, or anticipated general surgery during the study period.
10. Pregnancy, plans to become pregnant during the study, delivery within 3 months of screening, or breastfeeding.
11. If previous use of cyclosporine or systemic corticosteroids, failure to have any stabilization/response is exclusionary. This potentially indicates the disease is not PG.

Recruitment

Patients will be recruited for this trial based on the diagnosis of PG from the OSU outpatient dermatology clinic, as well as through the use of the OSU Wexner Medical Center patient database for patients who have been seen in the past four years with an encounter diagnosis code of 686.01 or L88. After identification through records, the investigators will review the patient's chart from multiple encounters to ensure that the patient has the diagnosis of PG (per chart review) and documented PARACELSUS criteria.

Randomization

Following consent from the patients who fulfill the selection criteria, ulcerations (up to three) in each patient will be randomized to receive either the intralesional injectable, topical PRP therapy, or no treatment in a 1:1:1 ratio. If more than three ulcerations exist, the three smallest ulcerations by area will be selected for randomization. If only two ulcerations are present, both PRP methods will be utilized on the one randomized ulceration. Randomization will be performed using a random number generator after assigning each ulceration a target number.

Treatment Groups

Up to three target lesions in each patient will be randomly assigned to receive either intralesional injectable therapy, topical PRP therapy, or no treatment. There will be three comparative groups with two treatment arms, one receiving injectable PRP therapy, the second receiving topical PRP therapy, and the third control group with standard wound care only.

Study Interventions

Before study entry, throughout the study, and at the follow-up evaluation, various clinical evaluations, including a physical examination, will be performed. Vital signs will be recorded, and medical history, disease history, and a concomitant medication list will be reviewed. A urine pregnancy test will be performed in women of reproductive age at all visits.

Autologous PRP Preparation and Administration

A double spin method will be used for PRP preparation.[18] As non-steroidal anti-inflammatory drugs (NSAIDs) can interfere with the platelet function, patients will be instructed to avoid NSAIDs a week before the procedure.

***Blood collection*:** Using an aseptic technique, a 30 ml sample of the patient's blood will be drawn from a peripheral vein in ACD (acid citrate dextrose) tubes.

***First centrifugation step*:** Whole blood from the patient will be centrifuged at 2800 rpm for 5 minutes at 16°C to separate it into different layers: the topmost layer containing mostly platelets and some WBCs; the middle buffy coat layer, rich in WBCs; and a lowermost layer consisting of RBCs. The topmost layer, along with the superficial buffy coat, will be extracted into a sterile tube with no anticoagulant.

***Second centrifugation step*:** To concentrate platelets, the tubes with the supernatant plasma will then be centrifuged again at 3000 rpm at 16°C for 10 minutes. The second spin will result in the formation of soft pellets of RBCs and platelets at the tube bottom. The lower 1/3 portion of plasma thus obtained is PRP, while the upper 2/3 portion is platelet-poor plasma (PPP). PPP will then be separated, to be applied to the dressings for topical use. The platelet pellets formed at the bottom will be used to make PRP by suspending them in 2-4 ml of plasma and gently shaking the tube.

***Confirmation of PRP preparation success*:** The platelet count will be measured in the formed PRP. Clinically valuable PRP contains at least one million platelets per microliter in 5ml of plasma.[5] The PRP will be used immediately and will not be stored.

***Wound bed preparation*:** Target ulcers will be cleaned with sterile normal saline followed by mechanical or surgical debridement if > 50% fibrinous slough is present on the wound bed (under local anesthesia).

***Administration of intralesional PRP therapy*:**

Target Ulcer Group 1 (Injectable PRP therapy): Under aseptic conditions, 1-3 ml of 1% lidocaine with epinephrine using a ratio of 1:100,000 will be administered in the target ulcer prior to intralesional PRP injections for local anesthesia and to obtain vasoconstriction (to reduce bleeding and to concentrate the PRP in the infiltration). Then, 2 mL of autologous PRP will be injected with a 30 G needle at multiple sites in and around target the ulcer approximately 1.5 cm apart at 0, 4, 8, and 12 weeks.

Target Ulcer Group 2 (Topical PRP therapy): Under aseptic conditions, 2 mL of autologous PRP will be applied topically followed by a PPP solution-soaked dressing on the second target ulcer at 0, 4, 8, and 12 weeks.

Target Ulcer Group 3 (No treatment): The target ulcer in the control group will receive standard wound care only.

Outcome Measures

***IGA*:** Assessment will be done by a single physician. All lesions will be examined and measured during each study visit. The length and width of target ulcers will be measured at each study visit, and the total surface area will be calculated.

***Photography*:** Ulcers will be imaged by photography at Screening, and at the week 0, 4, 8, 12, and 16 visits.

***DLQI, PGA, and VAS*:** The patient-reported outcomes will be collected using the DLQI, PGA, and VAS on 0, 12, and 16-week visits.

***Sample size calculations*:** No sample size calculations will be performed, as this trial is a preliminary study testing the feasibility of PRP therapy use for PG.

Statistical Analysis

Patient characteristics and outcomes will be summarized with descriptive statistics. The primary objective of a composite proportion of the target ulcers achieving either complete resolution or 50% reduction in the surface area at week 12 after treatment will be analyzed as the difference from baseline, with the null hypothesis that no patients will obtain the specified improvement if PRP therapy does not have efficacy. The endpoint will be analyzed using the Fisher Exact test. The secondary objectives will be analyzed in comparison to baseline measurements and at each visit afterwards to evaluate for efficacy using either the Fisher Exact test/Chi-square test or the Wilcoxon Rank Sums test, as appropriate.

RESULTS

We will begin recruiting patients for this study in November 2023. The last patient visit is anticipated in July 2024, and results are expected to be published in December 2024.

DISCUSSION

The beneficial effects of autologous PRP therapy in enhancing wound healing in chronic cutaneous ulcers of different etiologies have been shown in a systematic review and metanalysis.[14] However, there are no definitive data available about the use of PRP therapy in PG besides a few case studies that have shown benefits.[15-17] This clinical trial aims to provide valuable information on the safety and efficacy of PRP therapy in the treatment of chronic PG ulcers. If successful, this treatment may offer a new and effective option for patients with this debilitating disease, and another prospective study with a larger sample size, involving multiple ulcerations and with a longer follow-up period, can be undertaken in the future.

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Abbreviations

PRP	platelet-rich plasma
PG	pyoderma gangrenosum
DLQI	Dermatology Life Quality Index
PGA	Patient Global Assessment
VAS	Visual Analogue Scale
IGA	Investigator Global Assessment
HIV	human immunodeficiency virus
HCV	hepatitis C virus
PCR	polymerase chain reaction
TNF	tumor necrosis factor
NSAIDs	non-steroidal anti-inflammatory drugs
ACD	acid citrate dextrose
RBCs	red blood cells
WBCs	white blood cells
Rpm	rotations per minute
PPP	platelet poor plasma

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