Clinical manifestations of allergic reactions and immunoglobulin E levels on allogeneic freeze-dried platelet-rich plasma (PRP) application

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ABSTRACT

Background: Several studies have reported that modern wound dressings offer better results. Allogeneic PRP products in the form of injections and gels are available in the market, which helps accelerate wound healing without triggering an immunological reaction. Meanwhile, this study was carried out to investigate the Allogeneic Freeze-Dried PRP form, which is more practical, efficient, and safer. This study aims to determine the effect of Allogeneic Freeze-Dried PRP on human split-thickness skin graft donor wounds on clinical manifestations of allergic reactions and levels of Immunoglobulin E (IgE).

Methods: A randomized Pre- and Post-test Control-Trial Group Design was conducted on patients with split-thickness skin graft donor wounds using Allogeneic Freeze-Dried PRP dressings at Dr. Soetomo General Hospital, Surabaya. Allergic manifestations were reviewed during the observation period of up to 4 days, and IgE levels were measured pre-test and 60 minutes post-application. A statistical comparison of results was carried out between the trial and control groups that used paraffin dressings.

Results: A total of 36 patients were used as subjects, with 19 (52.78%) in the trial group and 17 (47.22%) in the control group, and their parameters were generally homogeneous. There were no clinical manifestations of allergic, changes in vital signs, and significant differences in IgE levels before and after application statistically in all study subjects (p>0.05).

Conclusion: Application of Allogeneic Freeze-Dried PRP to human split-thickness skin graft donor wounds did not generate clinical manifestations of allergic reactions and an increase in IgE.

Keywords: allogeneic freeze-dried PRP, allergic reaction, immunoglobulin E (IgE).

INTRODUCTION

Disruption in the healing process can make wounds difficult to manage.¹ Reports have shown that chronic wounds affect 3-6 million people in the United States each year, with 85% being patients over 65 years old, resulting in an annual expenditure of approximately 3 billion USD.² Chronic wound problems can cause significant issues such as an increased risk of infection, pain, impaired quality of life, high expenses, depression, and mortality.³ Various modern dressings, such as Platelet Rich Plasma (PRP), promise better treatment outcomes due to several growth factors for wound healing compared to conventional wound care. However, autologous PRP therapy cannot be performed on patients with platelet deficiency or dysfunction, healthcare facilities with limited resources, and patients who lack the courage.⁴ Therefore, the use of Allogeneic PRP derived from another individual within the same species is needed as an alternative in modern dressing.⁵

Several studies conducted worldwide have investigated the immunological side effects of Allogeneic PRP administration. According to Rachmawati,⁶ Allogeneic Freeze-Dried PRP did not elicit an immunological response in rabbit test subjects. Similarly, the study by Zhi-Yong Zhang et al.⁷ on Allogeneic PRP injection into the gluteus maximus muscle of rabbits did not result in local clinical signs of inflammation, changes in tissue immunological structure, or differences in CD4+ and CD8+ levels. Gubina et al. and Jo et al. showed that the administration of Allogeneic PRP did not cause local or systemic complications.⁸⁹ Although platelet is cells that lack nuclei, platelet membranes still contain several antigen molecules, namely immunoglobulin, HLA, ABO antigen, HPA, and polysaccharide blood group antigens.⁹ This can trigger an immunological response in patients receiving Allogeneic PRP. To date, there has been no study regarding the safety of this product in human subjects. This makes it necessary to determine whether the administration of Allogeneic Freeze-Dried PRP can elicit an immunological response in terms of allergic manifestations and changes in immunoglobulin E (IgE) levels in humans. This study is expected to be the first to assess the immunological response to Allogeneic Freeze-Dried PRP administration, thereby increasing the safety of using the product in clinical practice.
METHODS

Study design
This randomized true experimental study was conducted between 2021-2023, using a pre-test and post-test control trial group design to determine the immunological effects of Allogeneic Freeze-Dried PRP on human split-thickness skin graft donor wounds. The immunological effects assessed were clinical manifestations of allergic reactions and changes in serum IgE levels. The two groups used included the control (wound dressed with paraffin gauze dressing) and the treatment group (wound dressed with Allogeneic Freeze-Dried PRP). Clinical manifestations of type 1 hypersensitivity reactions, according to the Gell and Coombs classification, were observed in patients from after application until the 4th day. As we know, the Gells and Coombs type I hypersensitivity reaction consists of acute and late phases. This is known as the late-phase hypersensitivity reaction, which can last approximately 1-3 days and is caused by the release of additional mediators from the mast cells and basophils. Serum IgE levels were measured immediately before application and 60 minutes after application. Before dressing application, patients passed through split-thickness skin graft donor surgery using a dermatome with a skin thickness of 8-12/1000 inches.

The subjects of this study were patients treated at the Inpatient Unit of Dr. Soetomo Hospital Surabaya General Hospital who require split-thickness skin graft surgery. The inclusion criteria were patients aged 0-60 years who provided written consent to participate. Meanwhile, patients with a history of allergic, systemic diseases (vascular disorders, liver cirrhosis, diabetes mellitus, malnutrition, and sepsis), hemodynamic disorders, pregnant or breastfeeding patients, and smokers were excluded. Patients who decide to withdraw or refuse further treatment will be considered dropouts. Patients who lost to follow-up were also included as criteria of dropouts. This study used consecutive sampling with a single-blind randomization technique to determine the study subject groups. Based on Federer’s sample size formula, each group must have a minimum of 16 subjects. At least 10% of the sample size minimum for loss to follow up to still get a good result without any bias.

This study has been reviewed and approved by the Medical Ethical Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (Ref: No: 0129/KEPK/I/2021), following the guidelines of the Declaration of Helsinki. A certified Good Clinical Practice course has also been received, and all data used in this study were anonymous and information regarding patient data was kept confidential.

Materials
Allogeneic Freeze-Dried PRP is a blood component consisting of a thrombocyte concentrate centrifuged at a speed of 4,000 rpm for 10 minutes to obtain PRP. Subsequently, PRP was subjected to a Freeze-Dried process, lyophilization, and sterilization using gamma rays. The type of Freeze-Dried PRP used was “Allogeneic,” which indicated that the blood component material of PRP was obtained from other humans. We use any Freeze-Dried PRP provided by the Tissue Bank Installation - Dr. Soetomo General Hospital, Surabaya, with no specification of the range of age, gender, and blood type. This material is believed not to cause allergic responses based on animal model experiments before. Based on the procedure, Allogeneic Freeze-Dried PRP was applied to the split-thickness skin graft donor wound and covered with a transparent dressing for easy evaluation during the observation period. In the control group, the wound dressing used Paraffin Gauze, the standard care for donor split-thickness skin graft wounds.

Clinical manifestations of allergic reactions can include anaphylaxis and anaphylactoid reactions, urticaria-angioedema, dermatitis, allergic rhinitis, bronchial asthma, and gastrointestinal disorders. To identify clinical signs and symptoms of allergic reactions, medical professionals typically monitor the patient’s vital signs and observe the above manifestations. In this study, comprehensive management of these reactions was carried out by the standard protocols in the hospital. Serum IgE levels were measured twice, before dressing application and 60 minutes after application, using 3-5 mL of peripheral venous blood serum with an IgE-ELISA Kit by AccuDiag™ ELISA Total Human IgE – Diagnostic Automation, Inc. in the laboratory.

Statistic Analysis
The data were analyzed to examine clinical manifestations of allergic reactions, and comparative analysis was carried out on serum IgE levels among the subject groups. The SPSS (Statistical Program for Social Science) computer application/program version 25.0 (IBM SPSS Statistics for Windows, version 25.0 Armonk, NY: IBM Corporation) was used to process the data. Non-parametric comparative statistical analysis of two dependent and independent samples was performed using an Independent t-test, Mann Whitney U test, and Wilcoxon signed ranks test. Meanwhile, a p-value <0.05 was considered to indicate a significant difference in this study.

RESULTS
This study used 36 subjects, consisting of 19 (52.78%) assigned to the Allogeneic Freeze-Dried PRP group and 17 (47.22%) in the control group. All subjects understood and agreed to participate according to the applicable methods and ethics. There were no dropouts during this study. Regarding gender, 69.44% of the subjects were male, and 30.56% were female. Descriptive statistics of all subjects showed an average age of 28.92 years (±17.38 years). The average area of the split-thickness skin graft donor site used for treatment or control in all subjects was 133.43 cm² (±86.26 cm²). Of all study subjects, 24 (66.67%) were from the Plastic Reconstructive and Aesthetic Surgery Division, 6 (16.67%) were from the Oncology Surgery Division, and 6 (16.67%) from the Orthopaedic and Traumatology Surgery Division of Dr. Soetomo General Hospital. All of those data are shown in Table 1.

Comparative testing of the study subject characteristics was conducted using the Independent t-test on the age parameters and the Mann-Whitney test on the donor wound area. The results showed significant differences (p<0.05) between the two groups, except for the
There was a small difference in age between Allogeneic Freeze-Dried PRP and the control groups, with a mean age difference of 11.88 years. The size of the split-thickness skin graft donor area, which was the application site for the dressing in both groups, was also not significantly different (p>0.05). Based on the results, the subjects were homogenous, except for the age variable, which had a small difference only.

Clinical observations on all subjects in both groups showed no allergic manifestations, and the patient's vital signs were within normal limits during the observation period. There were also no cases of anaphylaxis accidents in all subjects after the dressing material application. The comparison of IgE1 (before application) and IgE2 (after application) values using the Wilcoxon signed ranks test in each group showed no significant difference in IgE levels before and after dressing application, both in Allogeneic Freeze-Dried PRP (p=0.520

Figure 1. Hypertrophic scar and hyperpigmentation on the subject with allogeneic freeze-dried PRP dressing. (a) on the 14th day after application, and (b) on the 28th after application.

Table 1. Statistics of Subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Allogeneic freeze-dried PRP Group (n=19)</th>
<th>Control Group (n=17)</th>
<th>Sign. (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td>Mean: 34.53</td>
<td>Mean: 22.65</td>
<td>0.039</td>
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<tr>
<td></td>
<td>Median: 35</td>
<td>Median: 24</td>
<td></td>
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<tr>
<td></td>
<td>SD: 15.90</td>
<td>SD: 17.25</td>
<td>Mean diff.: 11.88 y.o.</td>
</tr>
<tr>
<td></td>
<td>Minimum: 1</td>
<td>Minimum: 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum: 60</td>
<td>Maximum: 56</td>
<td></td>
</tr>
<tr>
<td>Donor Wound Area (cm²)</td>
<td>Mean: 139.46</td>
<td>Mean: 126.69</td>
<td>0.716</td>
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<tr>
<td></td>
<td>Median: 132</td>
<td>Median: 120</td>
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<tr>
<td></td>
<td>SD: 84.13</td>
<td>SD: 90.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimum: 38.50</td>
<td>Minimum: 14.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum: 332.50</td>
<td>Maximum: 300.00</td>
<td></td>
</tr>
<tr>
<td>Allergic Reaction Vital sign</td>
<td>There is no allergic reaction in all subjects</td>
<td>All subjects have a normal vital sign</td>
<td>-</td>
</tr>
<tr>
<td>IgE 1 – before application (IU/mL)</td>
<td>Mean: 180.04</td>
<td>Mean: 262.72</td>
<td>0.516</td>
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<tr>
<td></td>
<td>Median: 114.80</td>
<td>Median: 89.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD: 167.74</td>
<td>SD: 445.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimum: 6.90</td>
<td>Minimum: 7.80</td>
<td></td>
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<tr>
<td></td>
<td>Maximum: 672.00</td>
<td>Maximum: 1755.9</td>
<td></td>
</tr>
<tr>
<td>IgE 2 – 60 minutes after application (IU/mL)</td>
<td>Mean: 183.93</td>
<td>Mean: 222.31</td>
<td>0.496</td>
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<tr>
<td></td>
<td>Median: 143.90</td>
<td>Median: 86.60</td>
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<tr>
<td></td>
<td>SD: 177.41</td>
<td>SD: 348.22</td>
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<tr>
<td></td>
<td>Minimum: 4.51</td>
<td>Minimum: 7.20</td>
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<tr>
<td></td>
<td>Maximum: 666.90</td>
<td>Maximum: 1305.7</td>
<td></td>
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<tr>
<td>DlgE (IU/mL)</td>
<td>Mean: 3.90</td>
<td>Mean: -40.42</td>
<td>0.211</td>
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<tr>
<td></td>
<td>Median: 1.20</td>
<td>Median: -0.80</td>
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<tr>
<td></td>
<td>SD: 60.91</td>
<td>SD: 115.20</td>
<td></td>
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<tr>
<td></td>
<td>Minimum: -191.00</td>
<td>Minimum: -450.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maximum: 123.10</td>
<td>Maximum: 37.80</td>
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</table>
and the control (p=0.266) groups. The comparison of IgE values using the Mann-Whitney test between groups had similar results, with no significant difference in IgE1, IgE2, and ΔIgE values (p=0.516, 0.496, 0.211). These results indicated that the application of Allogeneic Freeze-Dried PRP and paraffin dressing on all subjects would not cause a statistically significant increase in IgE levels (p=0.05).

After the patients were discharged from the hospital, the side effect was found in one patient of the Allogeneic Freeze-Dried PRP group (5.26%). Those patient-reported changes in wound appearance in the form of a hypertrophic scar accompanied by excessive hyperpigmentation on the donor area 14 days after the procedure (Figure 1a). The patient did not complain of local allergic reactions during the previous observation period or after being discharged from the hospital. The patient only complained about the appearance of the scar, which was aesthetically displeasing and made the patient uncomfortable due to the scar. During outpatient care, these patients were regularly observed and evaluated at the clinic. Treatment was given using anti-keloid cream and topical depigmentation cream for two weeks. The therapy response on day 28 after the procedure was quite good, and the patient’s complaints were resolved. No other side effects were found in all study subjects (Figure 1b).

DISCUSSION

Several studies have reported the benefits of PRP. Saputro et al. stated that in full-thickness wounds, PRP could accelerate epithelialization without causing allergic reactions in animal models (p<0.05). Platelets are a source of various growth factors that play a role in cells’ healing process, differentiation, and replication. It was discovered that on the 7th day, the wound healing process occurred better in the treatment group based on epithelium closure. PRP functions as a tissue protection and growth stimulator that initiates the appearance of growth factors from alpha granules. PRP also suppresses inflammation, interacts with macrophages, and triggers regeneration, capillary growth, and epithelialization in chronic wounds. Another study stated that PRP could act as an anti-inflammatory agent in cases of knee joint osteoarthritis. PRP injection has been shown to reduce inflammation markers (IL-1 and TNFα) in joint inflammation compared to control with Hyaluronic Acid injection in vivo. Liao et al. investigated the use of PRP in chronic wounds and discovered that wound closure occurred faster after 3 weeks of using Allogeneic PRP in 85% of the treatment group compared to only 55% of the control group subjects. There were also no allergic reactions or rejections in the use of this material. Garbin et al. reported that Allogeneic Freeze-Dried plasma lysate had a similar effect to frozen autologous plasma lysate. This confirmed that the application of Allogeneic materials would not differ in efficacy compared to Autologous materials derived from the patients.

For almost over a decade, experts have been investigating the potential of PRP as a regenerative therapy, but there are limitations to its use in clinical application. The preparation of PRP can affect its efficacy, and the production of a high-quality product requires a good technique. Autologous PRP, which is derived from patients, can take up to 30 minutes and may be inefficient for some clinicians in their daily practice. The technique of preparing PRP mixture from different organism types, such as bovine, is also suspected of transmitting previously unknown diseases. Therefore, the use of proper techniques and equipment for PRP preparation will impact the availability of expensive facilities.

Since 2001, Freeze-Dried technology have been introduced for the global application of PRP. This technology can extend PRP shelf life for several months without significant damage. Studies have shown that through the Freeze-Dried process, PRP efficacy is not significantly different from fresh platelet. This biomaterial is useful for clinical application in areas that lack sufficient resources, such as wound care in war zones. Therefore, healthcare centers and clinics with limited funds and resources can use Freeze-Dried PRP as a solution. The use of high-quality and well-prepared Allogeneic materials can be a good alternative for patients and clinicians who have problems with providing fresh autologous PRP. The application of Allogeneic materials compared to autologous ones has widely been discussed in previous investigations. It was suspected that the active PRP levels would be easier to obtain in well-prepared and high-quality Allogeneic materials than in autologous materials, whose quality was difficult to be guaranteed. High-quality control will also reduce the risk of disease transmission and side effects of Allogeneic materials. However, this high-quality assurance will certainly increase the production cost of Allogeneic PRP. The cost of cell therapy has shifted from autologous materials to Allogeneic materials for mass production. Kawase suggested that in the future, the use of Allogeneic PRP can become more widespread in society.

He et al. compared the benefits of Allogeneic PRP to autologous PRP for diabetic ulcer therapy and reported that both had equally faster wound healing abilities than the standard care (p<0.01). There were no significant differences between the two types of PRP and no adverse reactions such as fever, edema, pain, itching, redness/irritation, and others. The results showed that the use of Autologous and Allogeneic PRP had better healing abilities than the control and a low risk of adverse effects in chronic diabetic wounds. In situations where Autologous PRP is difficult to produce or in clinically unhealthy patients, the application of Allogeneic PRP can be a solution. Patients with chronic wounds usually have poor physiological conditions, making it difficult to ensure the quality of PRP from autologous material. Repeated blood draws in such patients can also burden the patient’s body functions due to periodic blood loss, which will hinder the healing process, and their primary disease will worsen. Allogeneic PRP can be a solution for such patients because of its ability to provide PRP material in large quantities, with quality guaranteed.

Currently, Allogeneic PRP is not widely available for clinical application due to concerns about the risk of immune reactions resulting from cross-contamination. According to He et al., the risk is not negligible and equivalent to that of autologous PRP material.

ORIGINAL ARTICLE

Immunological reactions may not occur in Allogeneic PRP due to the strict sterilization process and preparation of blood materials in each blood bank. The blood material is first screened to ensure that the blood is always sterile. According to He et al., Allogeneic PRP injection studies in rabbit muscle did not cause allergic reactions. On the efficacy and safety of Allogeneic material injection, Kandil et al. discovered that the administration of Allogeneic material to patients is safe when it is properly prepared. The blood base material should be prepared through a standardized screening process for infectious agents such as Hepatitis B, Hepatitis C, HIV and Syphilis. Inactivation of viruses using ultraviolet radiation can also reduce the risk of infection from the material. Microbiological safety is achieved through the techniques mentioned above. Zhang et al. stated that Allogeneic PRP has very low immunogenicity, good wound-healing ability, and no side effects. Rachmawati et al. also reported that the material did not increase IgM levels or cause local inflammatory reactions.

Latałski et al. reported that an allergic reaction occurred in a 14-year-old child with a bone cyst on the distal Os Tibia who passed through an autologous PRP injection. The patient developed a rash 24 hours post-injection, followed by symptoms of pharyngitis, tonsil enlargement, mucopurulent discharge from the esophagus, and eyelid swelling. Moreover, allergic management using standard antihistamine drugs resulted in a good response, and the patient was discharged from the hospital after 4 days of treatment. Long-term evaluation up to 6 years after the procedure revealed that there were no allergic reactions in the future. The report concluded that autologous PRP is safe but poor preparation may lower its safety profile. Although the material comes from the patient (autologous), the risk of allergic reactions can still arise, especially triggered by mixed materials that can act as a hapten.

Various efforts have been made to reduce the potential immunogenicity of Allogeneic PRP. The use of gamma radiation rays was discovered to reduce the immunogens contained in Allogeneic PRP materials while sterilizing, as previously mentioned. Literature suggests that gamma radiation rays can change the structure of initially immunogenic proteins. According to Afify et al., the structure of albumin and globulin can change due to exposure to gamma radiation rays. Harder and Arthur stated that there is still uncertainty in the role of gamma radiation rays in reducing the immunogenicity of allergenic food ingredients. Gamma rays can cause covalent breaks and conformational changes in molecules, which irreversibly alter allergen structure and decrease its immunogenicity. In addition to maintaining the function of platelet and their cytokines, the Freeze-Dried process in PRP preparation can also reduce the risk of immunogenicity in Allogeneic PRP materials. Lyophilization, a process involving freezing, sublimation, and vaporization of water, has become a standard in PRP production. This process aims to ensure that the quality and efficacy of PRP are maintained.

After comparing the storage at room temperature, frozen, and Freeze-Dried forms, Shiga et al. concluded that the Freeze-Dried form can maintain PRP bioactivity and efficacy in the long term. The number of platelets can be maintained when it is stored in frozen or Freeze-Dried form even up to 1 year of storage. Additionally, flow cytometry examination showed that 80% of platelet levels were still maintained in frozen and Freeze-Dried forms and growth factors can still be detected in both forms. The preparation of Allogeneic Freeze-Dried PRP production is easy and simple, and the therapeutic effect and safety are well guaranteed. Meanwhile, this study showed that the application of Allogeneic Freeze-Dried PRP did not cause an allergic response in terms of clinical manifestations and changes in IgE levels before and after application. The United States Department of Health Research and Quality assessment on using PRP for wound care stated that using PRP is safe and can accelerate wound healing. This material is a future biological therapy, especially for patients who are clinically unable to donate blood.

Complications such as hypertrophic scar reactions and hyperpigmentation are common after split-thickness skin graft, affecting approximately 64% of patients. Hypertrophic scarring of the donor wound has also been reported to occur in 28% of patients, even up to 8-10 years postoperatively. Scars and pain in the donor area are common in every split-thickness skin graft procedure. Based on a systematic review using the Vancouver Scar Scale, a score to assess the degree of scars on a scale of 0-13, the incidence of scars on donor wounds within 1 year varies from a score of 0 to 10.9. Karlsson et al. stated that there was no association between dressing type and the incidence of hypertrophic scars in donor wounds as well as the healing period. Therefore, subjects with hypertrophic scars and hyperpigmentation were not directly affected by Allogeneic Freeze-Dried PRP. Any split-thickness procedure still carries the risk of hypertrophic scarring in the donor wound, but it cannot be ascertained whether other subjects will experience similar long-term side effects due to the limited study period. Other reports suggested that the incidence of hypertrophic scars in split-thickness skin graft donor wounds was affected by the variables of young age, female gender, black/dark skin color, location on the inferior extremities, and re-epithelialization time.

We have several limitations of this study. This study did not evaluate the efficacy of Allogeneic Freeze-Dried PRP on wound healing speed. However, there is a need to investigate the long-term effects of other hypersensitivity types on the use of Allogeneic Freeze-Dried PRP, especially for chronic wounds. Similar studies with variable measurements of hypersensitivity mediators such as cytokines, growth factors, complement, and others can also be conducted to determine the biomolecular immunological response that occurs from administering Allogeneic freeze-dried PRP.

CONCLUSION

The administration of Allogeneic Freeze-Dried PRP in human split-thickness skin graft donor wounds did not cause allergic reactions regarding clinical manifestations and changes in IgE levels.
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ETHICAL CLEARANCE

This study has been reviewed and approved by the Medical Ethical Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (Ref. No.: 0129/KEPK/I/2021), following the guidelines of the Declaration of Helsinki.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest or financial considerations involved in the writing of this manuscript.

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AUTHOR CONTRIBUTION

All authors have made the same contribution in writing the report on the results of this study, from the stage of proposal preparation, data search, and data analysis, to the interpretation of research data and presentation of the final report.

REFERENCES


