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CLÁUDIO ANDRÉ CORTESÃO DE ALMEIDA

Platelet-Rich Plasma in Burns – a Scoping Review

SCOPING REVIEW

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Trabalho realizado sob a orientação de:

PROFESSOR DOUTOR JOSÉ LUÍS DE ALMEIDA CABRAL
DOUTOR GONÇALO LIMA TOMÉ DA COSTA FERREIRA

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Platelet-Rich Plasma in Burns – a Scoping Review

Cortesão-de-Almeida C.[1], Tomé G[2], Cabral L.[3]

[1] Master's student of Medicine, Faculty of Medicine, University of Coimbra, Portugal

[2] Plastic Surgery Consultant at Coimbra University Hospital (CHUC), Coimbra, Portugal

[3] Invited Professor of Plastic and Reconstructive Surgery, Faculty of Medicine, University of Coimbra, Portugal; Plastic Surgery Consultant at Coimbra University Hospital (CHUC), Coimbra, Portugal

Adress:

Área de Medicina da Faculdade de Medicina da Universidade de Coimbra

Avenida Bissaya Barreto, Bloco de Celas

3000-075 Coimbra, Portugal

Endereço eletrónico: claudiocortealmeida@gmail.com

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Abbreviations

ABA - American Burns. Association

Ach - Acetylcholine

DSD - Deep Second-Degree

EGF - Endothelial Growth Factor

FGF -Fibroblast Growth Factor

GF - Growth Factor

Il-1 - Interleukin -1

Il-6 – Interleukin-6

JBI -Joanna Brigs Institute

KLC - Keratine Like Cells

LPRP - Lyophilized Platelet-rich Plasma

MCP - monocyte chemoattractant protein-

PBD - Post Burn Days

PDGF - Platelet-derived Growth Factor

PICO - Population/Intervention/Comparison/Outcome

PLRG - Platelet Leukocyte Rich Gel

P-NFkB – Nuclear Factor kB antibody

P-p38 – p38 Antibody

PPP - Platelet-Poor Plasma

PRF - Platelet-Rich Fibrin

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PRP - Platelet-Rich Plasma

PRGF - Platelet-rich in growth factors

RCT - Randomized Control Trial

SVF - Stromal Vascular Fraction

SYRCLE - Systematic Review Center for Laboratory animal Experimentation

TBSA - Total Body Surface Area

TGF-ß - Transforming Growth Factor Beta

TNF – Tumor Necrose Factor

VAT- Visual Analogue Thermother

VEGF - Vascular Endothelial Growth Factor

VSS - Vancouver Scar Score

Resumo

Introdução: O plasma rico em plaquetas (PRP) foi recentemente proposto e utilizado em doentes queimados devido ao seu potencial de aceleração do processo de cicatrização e possivelmente diminuição da incidência de complicações nas queimaduras. Contudo, a eficácia clínica e segurança do PRP são ainda controversas.

Objetivo: Os autores propõem-se a analisar o impacto, identificar falhas de conhecimento, clarificar conceitos e examinar o modo como a investigação tem sido realizada relativamente ao papel e segurança do PRP em queimados.

Materiais e métodos: Procedeu-se à elaboração de uma revisão em *scoping*. A pesquisa da literatura baseou-se nas três bases de dados da *Pubmed*, *Scopus* e *Embase*. Os artigos foram sujeitos a uma avaliação metódica seguindo as normas *Preferred Reporting Items for Systematic Reviews and Meta-Analyses*-(PRISMA) para *Scoping-Reviews*, as de Joanna Briggs Institute e as do Systematic Review Center for Laboratory animal Experimentation SYRCLE para qualidade. Apenas artigos centrados na avaliação do efeito do PRP em doentes queimados foram incluídos.

Resultados: Quarenta e oito artigos foram incluídos, tendo estes uma significativa heterogeneidade quanto ao tipo de estudo e também, quanto aos protocolos para a produção do PRP. Como principais achados dos estudos experimentais, destacam-se os efeitos positivos do PRP na cicatrização de feridas, redução de dor, regressão de cicatrizes, na recuperação da função esofágica após queimaduras e na recuperação após queimaduras oculares alcalinas. Não obstante, resultados obtidos relativos à reepitelização não foram totalmente consensuais. No que concerne à segurança do PRP, não foram referidos efeitos secundários major.

Discussão e Conclusão: O PRP destaca-se pelo seu grande potencial de utilização terapêutica e pelo impacto positivo que tem vindo a ser demonstrado na cicatrização das queimaduras. No entanto, a significativa heterogeneidade dos estudos existentes sobre a produção de PRP e os métodos de análise dos seus resultados, limitam a avaliação cabal do efeito deste concentrado plaquetar. Uma estandardização metodológica e a realização de mais ensaios clínicos randomizados são fundamentais para a melhor compreensão do papel do PRP em doentes queimado e, para a garantia da segurança da sua aplicação.

Palavras-Chave: Plasma Rico em Plaquetas, Queimaduras, Cicatrização de Feridas

Abstract

Introduction: Platelet rich plasma (PRP) has recently been proposed and used in burn patients due to its potential to accelerate the healing process and possibly decrease the incidence of burn complications. However, the clinical efficacy and safety of PRP are still controversial.

Objective: The Authors propose to analyze the impact, identify knowledge gaps, clarify concepts and examine how research has been conducted regarding the role and safety of PRP in burn patients.

Materials and methods: A scoping review was performed. The literature search was based on the three databases Pubmed, Scopus and Embase. The articles were subjected to a methodical evaluation following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards for Scoping-Reviews and those of Joanna Briggs Institute and the Systematic Review Center for Laboratory animal Experimentation SYRCLE for quality. Only articles focusing on the evaluation of the effect of PRP in burn patients were included.

Results: Forty-eight articles were included, these having a significant disparity of study type as well as protocol level in PRP production. As the main findings of the experimental studies, the positive effects of PRP on wound healing, pain reduction, scar regression, in the recovery of esophageal function after burns and with a positive impact on recovery after alkaline eye burns stand out. Nevertheless, results obtained regarding reepithelization are not totally consensual. Regarding safety, PRP proved to be a therapeutic option free of side effects.

Discussion and Conclusion: PRP stands out for the vast potential and positive impact it has been shown to have on burn healing. However, the significant heterogeneity in PRP production and methods of analysis of results, may limit the evaluation of the effect of this platelet concentrate. Methodological standardization and further randomized clinical trials are fundamental to better understand the role of PRP in burn patients, as well as to ensure the safety of its application.

KeyWords: Platelet-Rich Plasma, Burns, Wound Healing

Introduction

Burns are amongst the most common and devastating forms of trauma worldwide representing a leading cause of morbidity and important mortality.[1] They most frequently involve the skin, but in severe cases can also injure the subcutaneous tissue, mucous membranes, muscle and bone tissue, or internal organs, and may ultimately lead to death. According to World Health Organization 2018 fact sheet, burns are estimated to account for 180,000 deaths a year.[2,3] More recently, there is a tendency for a decrease in burn incidence, mortality and length of hospital stay, mainly on higher income countries[3], including Portugal.[4] Thermal energy from extreme heat of hot liquids flames or contact with overheated object as well as electrical, chemical and radiation energy can cause this type of tissue damage associated. Besides the skin, another body organs may also be primarily affected, including the eye and esophagus.[5]

Burns severity can be determined by many factors. According to Converse-Smith scale, there are five significant variables:

1) lesion depth
2) burns extension, expressed in percentage of Total Body Surface Area (TBSA) affected
3) injury localization
4) patient age
5) associated comorbidities.

Deeper and extensive burns, particularly in aged patients are correlated with worse prognosis.[6] Moreover, inhalation injury, increases morbidity and mortality.[7–9]

In Europe, burns are generally classified as first (superficial thickness), second (partial or intermediate thickness), third (full thickness) degrees according to its depth. Burns can additionally be classified using the American Burns Association (ABA) or using the Benaim classification, that allows the comparison between the degree of burn and the stratum that is affected.[5,6] (Table 1). If the skin germinal layer is well preserved, healing occurs without sequels. Second-degree burns are divided into superficial, which affect the epidermis and superficial dermis, and into deep second-degree burns (DSD), if the lesion reaches the deep dermis.[10] Third-degree burns affect the full-length epidermis, dermis and its appendages and the damage may also involve deeper tissues like

ligaments, fascia, muscles, bone and even viscera, what in the past was sometimes classified as fourth-degree burns, a terminology abandoned nowadays.[10,11] The extension of a burn lesion is calculated by the sum of all second and third degree areas, and expressed as a percentage of the total body surface area (%TBSA). Major burns may be grossly defined as having more than 20% TBSA, while minor ones are below 10% TBSA. However, these definitions are not consensual. [12]

Table 1 – Comparison between the Converse-Smith scale and The American Burns Association (adapted from Rossani et al.(2014). [6]

Converse-Smith	American Burns Association	Benaim
First Degree	Epidermic	A type
Superficial Second Degree	Superficial Dermic	AB-A type
Deep Second Degree	Deep Dermic	AB-B type
Third Degree	Total Length	B type

According to the Jackson Burn Wound Model there are three concentric zones resulting from the burn injury: the coagulation zone, the stasis zone and the hyperemia zone.[13,14] The first damage of skin burns results from direct coagulation necrosis, which occurs through contact with the heat source and will progressively evolve into ischemia-related damage, leading to cell death in the next 48-72 hours.[15] Around there is a zone of relative blood stasis that will evolve to necrosis if adequate fluidotherapy and topical care were not achieved. Another process that increases cell death is apoptosis, mediated by dysfunction of the stasis zone and can start from the 30th minute and last up to 48 hours after the burn. Cell autophagy, activated by stasis and inflammation is also present at this during the first 24 hours after the burn.[16] The third, external zone, is formed by the hyperemia of the peripheral tissue, induced by the normal inflammatory reaction the injury, being temporary and not subjected to necrosis.[16]

Burns treatment approach will depend on the depth, extension and type of the burn.[11] Deep burn wounds may necessarily have its necrotic tissue debrided because of its associated increased risk of infection, which is one of the most common complications with burns along with sepsis.[2,10,17,18] Autologous split-thickness skin graft is the mainstay for burn coverage, particularly important for deep and extended burns. Possible and utterly important clinical consequences of major burn injuries include the development of

hypertrophic scar and contractures.[19] Recent studies lean on the importance of growth factors (GF) and their re-epithelialization properties. [20]

Platelet-rich Plasma (PRP) is an autologous platelet concentrate in a small volume of plasma.[21] PRP first application dates back to the eighties when it was first used as an adjuvant in various oral and maxillofacial surgical procedures.[22] PRP was found to stimulate various cell types, and its use has been extended to the orthopaedic field (in bone, cartilage and tendon regeneration), plastic, general and gynaecological surgeries, dermatology (in wound healing, reepithelization and regeneration, revascularization and hair follicle regeneration) and in ophthalmology (corneal reepithelization and revascularization).[23]

PRP is obtained from the blood collection into a tube containing anticoagulant and posterior centrifugation.[22,24] This process separates blood components by density. At the centrifugated tube from the top to the base described different components are described: [18,22]

1) platelet-poor plasma (PPP) with lower density.
2) Platelet-rich plasma (PRP)
3) buffy coat
4) red blood cells, with the highest density

PRP must be activated to have a clinical effect and this can be achieved by adding calcium chloride, calcium gluconate, or thrombin, from either animal or human source.[22] Platelets start degranulating in ten minutes and the secretion of multiple bioactive agents accumulated in platelet alpha granules, including growth factors (Table 2)[25] and cytokines, occur within one hour. [22,26]

Table 2 – Principal functions of PRP growth factors

Growth Factor	Function
Platelet-derived growth factor (PDGF)	-Angiogenesis -Reepithelization -Central nervous system development
Vascular endothelial growth factor (VEGF)	-Angiogenesis -Vasculogenesis -Chemotaxis of macrophages and granulocytes -Neuroprotection and neurogenesis
Fibroblast growth factor (FGF)	-Collagen production -Chemotaxis promotor -Formation of granulation tissue
Transforming growth factor-beta (TGF-ß)	-Angiogenesis -Reepithelization -Extracellular matrix promotor
Epidermal Growth Factor (EGF)	-Angiogenesis -Epithelial repair

PRP use have been gradually proposed to be include burns treatment, considering potential beneficial hemostatic, wound healing and reepithelization effects. However, PRP studies on burns are still limited and burns are associated with an altered systemic physiological response and status, which may simultaneously evoke some concerns on its use. In fact, these patients have variations in platelet count, showing a nadir at post-burn day (PBD) 3 followed by a maximum level at PBD 15 and a gradual descending towards normal values at PBD 24. This evolution depends on TBSA and the patient.[27]

Even though no consensus yet exists on PRP application in burn patients, its potential effects have been pressing on its clinical use.[18,23] In this scoping review, we aimed to map the research and studies developed in the most recent years, to identify the types of available evidence and clarify key concepts centered on the PRP applicability and safety in burn patients.

Materials and Methods

The Authors conducted a scoping review on the application of PRP for the treatment of burn patients. Available evidence in the literature related with its applicability, efficacy, safety, and preparation protocols on burns was retrieved and reviewed.

Research Question

A PICO (Population, Intervention, Comparison, Outcome) approach (Table 3) was used for the research question elaboration and literature analysis. The Authors formulated an objective scientific question on which the scoping review was centered and based, and consisted of the following: “Is PRP application in burn patients a practical and secure therapy?”

Eligibility Criteria

All available studies on PRP treatment for burn patients were collected. Different types of studies were evaluated including meta-analysis, systematic reviews, randomized controlled trials, observational studies, and experimental animal studies. We included studies published between the period of 2011-2021, written in English, Portuguese, or Spanish.

The burn patients included were adults with eighteen or more years old regardless of their nationality, sex or burn location. Animal experiments were also included.

Intervention included PRP or other similar platelet aggregates, with isolated or combined application and comparison with conventional treatment. The indicators consisted of report efficacy findings which included wound healing time and rate; associated graft take; time of hospitalization; neovascularization; wound infection; scar formation and complications rate. Additional information on preparation protocols, safety application and types of studies were also contemplated and analyzed.

Articles were excluded if they did not fit into the study conceptual framework and when they were duplicate or not available (data cannot be obtained, or if published as abstract or conference paper).

Table 3 – PICO Criteria

PICO	Description
Population	Adults (humans > 18 years old); animals;
Intervention	PRP application in electric/thermal/friction/chemical Burns (including ocular/corneal, esophageal and dermal).
Comparison	PRP vs Placebo; PRP vs other biological treatment options; PRP associations vs Placebo;
Outcome	**Primary –** efficacy **Secondary –** safety

Bibliographic search

To identify relevant documents, the EMBASE, SCOPUS and PUBMED databases were used in July 2021. Search strategies were planed and drafted by the authors, refining the search-key through team discussion. PUBMED search Medical Subject Headings search terms used were: (Burns) AND ((Platelet-Rich Plasma) OR (Platelet-Rich Fibrin)). In the other databases equivalent terms were used. Additional search was conducted by screening of the cross-references of relevant studies and links suggested by the database search engines.

DATA screening extraction

The initial search was independently conducted by two authors (CCA, GT), who performed a first screening through the titles and abstracts, removed the duplicate records and classified the articles as to be included, uncertain and excluded. The uncertain articles were fully analyzed and any disagreements regarding this division were discussed by all the authors, and a consensual decision was taken.

Second screening allowed to conduct a perceptible information analysis and important data on article general characteristics was retrieved including: year, first author, search database, language and specific characteristics such as type of study, species of participants, type of injury (classification of burns and the mechanism of injury), the main article objective, control/experimental groups, PRP protocol, days of observation, main results, secondary results and the tools used in quality assessment.

Quality Assessment of included studies

Methodological quality assessment of the studies was made using various bias risk evaluating tools, according to the study type. Since there was a huge diversity in study types, the Joanna Brigs Institute evaluation methods were used to assess the quality of articles.[28–33] Animal studies were evaluated using the Systematic Review Center for Laboratory animal Experimentation (SYRCLE) risk of bias tool.[34]

Results

Research Results

The initial search retrieved 459 articles. After removing the duplicates, 357 articles were analyzed by title and abstract content, and 225 were excluded. Thus, 132 records were selected for second screening, where full texts were reviewed. A total of 48 studies were included in the scoping review. The process flowchart is presented in Figure 1.

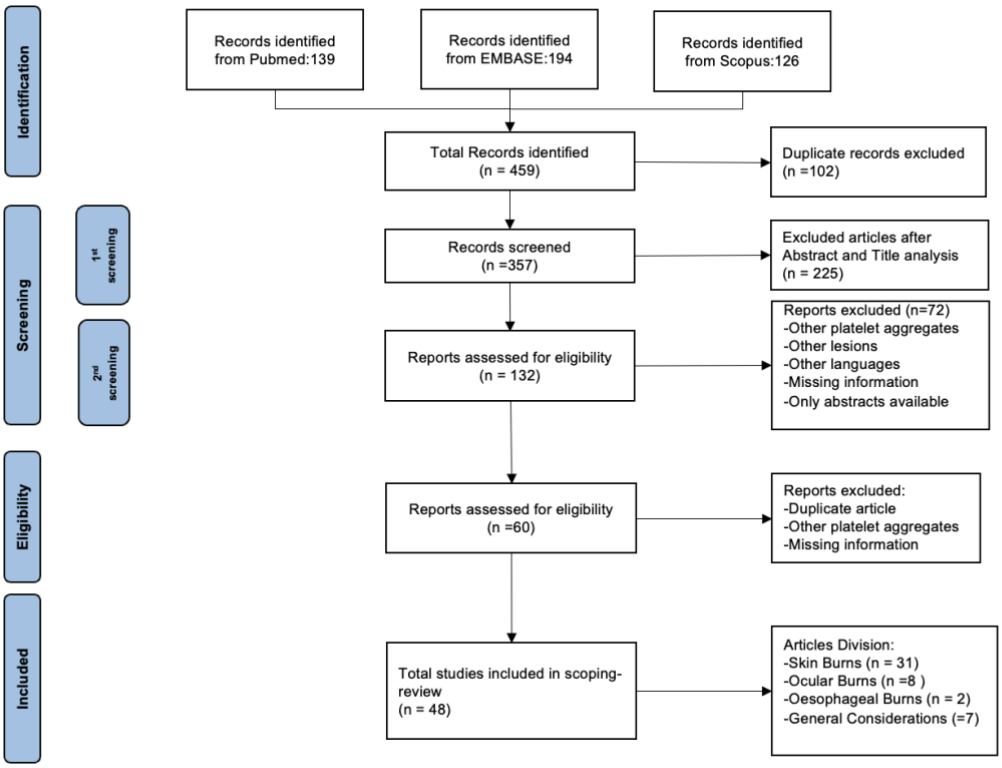

Figure 1 – Flow chart of study selection process

The studies were organized into four main topics according to type of burn injury, study design, outcomes and findings related with PRP application: 1. dermal burns, 2. ocular burns, 3. oesophageal burns and 4. general considerations.

The latter group addresses articles with general information that do not consider a specific organ burn. It also includes opinion texts/letters and a meta-analysis protocol.

For each topic, the information was organized and synthetized in correspondent information summary tables, summary PRP protocol tables and quality assessment figures.

Platelet-Rich Plasma variations and designations

PRP is a platelet concentrate obtained from blood centrifugation. However, the generically used PRP term may not always specifically or correctly designate its true content or structure. The terms found through the articles that have more specific connotations, indicating a different (higher) platelets concentration or structural changes, are resumed in Table 4.

Table 4 - PRP designations

Concept	Equivalent
Platelet Concentrate	Platelet-rich plasma
Platelet gel	Activated PRP
PRGF – Plasma rich in growth factors	Platelet-rich plasma poor in leukocytes
Platelet lysate	Obtained through PRP dilution, centrifugation and freezing to induce platelet lysis and PRGF release
LPRP	Lyophilized PRP
PRF	Platelet-rich fibrin

PRP production may have some inherent limitations which include room temperature variations, significant contamination rates and short half-life. The associate need for compounds with greater stability have led to the development of lyophilized PRP (LPRP). This compound is formed through multiple steps where vacuum freeze-drying and gamma-ray sterilization are contemplated.[35]

Platelet Rich Fibrin (PRF) is a second-generation platelet concentrate formed without adding of an anticoagulant and without the addition of an activating agent. When applied, PRF acquires a more three-dimensional structure, while PRP has a more liquid conformation.[36]

General description of included studies

The retrieved and included articles vary on their type and study design which includes systematic review and meta-analysis, case-reports, reviews, quasi-experimental, randomized control trials, cohort studies, letters of opinion, and protocols of PRP preparation and use.

Dermal Burns represent the majority of the literature found (Table 5.1 – Annex I). Three systematic reviews were included on this topic which reviewed the role of PRP in 1391 human subjects.[2,18,20] Of the most relevant findings, the study by Huang *et al.* (2021) shows no significant difference between delivery routes.[2] On the other hand, Wang *et al.* (2020) demonstrates that PRP accelerates the healing process, but does not affect the graft take rate, degree of epithelialization and the rates of adverse reactions and infections.[18] The authors suggested possible antibacterial activity of PRP. Kao *et al*. (2021), addressing wound healing, demonstrated positive results in the role of PRP. [20]

Marck *et al.* (2016), studied the effect of PRP on graft take rate on humans, concluding that there is a higher rate of acceptance and epithelialization in this group. [37]

Recent articles studied angiogenic potential of PRP in different animal models. The results of Singer *et al*. (2018) exploring PRP effect in swine model (in domestic pigs), did not support the PRP application in burns.[38] On the other hand Sun *et al.* (2020) shown positive results in Bama Pigs.[21]

Yeung *et al.* (2018) tried to establish a relationship between the application of LPRP in humans. The findings supported an improvement in wound healing.[35] Two years later, Laidding *et al.* (2020), using the association of Stromal Vascular Fraction (SVF) and PRP, compared topical and invasive application, not having concluded improvement from one form to another.[39] Liao *et al.* (2020) studied the effect of PRP on chronic wounds caused by burns and found a decrease in inflammatory effects.[40] Marck (2) *et al.* (2019) compared the constitution of the PRP obtained from burn patients and healthy patients, concluding that the PRP is similar between the groups. The study also confirmed an intra-individual variation of constituents and a correlation between GF and the number of platelets.[41]

In this section, eight Quasi-Experimental Studies were included, in which only two had humans as the population of the study. Klama-Baryla *et al.* (2011), applied PLRG with keratinocytes cultured *in vitro* accelerating the process of epidermization of the burn wound.[42] The second study to use a human model is a study conducted in Italy, and they applied Stromal Vascular Fraction (SVF) with PRP. From the positive results from this combination, Gentile *et al.* (2014) hypothesized that PRP created a suitable microenvironment, allowing the adequate architectural distribution of adipocytes.[43]

A case series by Teodoreanu *et al.* (2014) describes the PRP application on electrical burns, acknowledging the increasing of granulation in the treated area.[44] Whereas, Kakudo *et al.* (2011) reported a single case of third degree burn injury, where PRP was first gel applied and over time it was sprayed, showing signs of epithelization and formation of new vessels.[45] Both concluded that PRP promotes wound healing, improves vascularization and increases granulation.

Narrative reviews allowed a broader view of the literature. Within this kind of review, Picard *et al.* (2015), reported 17 cases of anemia.[24]

Ozcelik *et al.* (2016) studied the levels of hydroxyproline, concluding that there was an increase related to PRP, as well as an increase in collagen deposition.[46] Hosni *et al.* (2017) evaluated the expression of angiogenic genes, concluding that there was an increase in it but not statistically significant.[47]

A diabetic model on albino Wistar rats was used by Mansoub *et al.* (2018) to prove the increase in the effectiveness of keratinocyte like-cells (KLC- differentiated cells from adipose tissue that behave like keratinocytes) potentiated by PRP.[48] Venter *et al.* (2016) used a similar diabetic model, but studied PRP positive effects on re-vascularization and wound healing.[49]

Maclel *et al.* (2012), employing an equine model, studied the effect of PRP in gel and taking serial biopsies, they studied its effect on the bacterial population. As a primary outcome, using electron microscopy, they found that in some cases PRP showed signs of fibrosis.[10] Huang *et al.* (2018), evaluated the effect of PRP on neuropathic pain, one of the consequences of burns.[50] The authors found a decrease in the expression of inflammatory mediators, confirming the efficacy of PRP. The most recent quasi-experimental studies evaluated wound healing. Orhan *et al.* (2019) studied autophagy

processes, demonstrating that PRP has decreased the number of apoptotic cells in the stasis zone.[16] These findings were also found in the study by Uraloğlu *el al.* (2019) using an experimental model in rabbits.[15]

Ren *et al.* (2021) presented favorable results in terms of mechanical response to pain and an increase in the expression of angiogenic molecules.[51] However, a study by Karina *et al.* (2021) showed no significant effect of PRP on revascularization.[52] Josh *et al.* (2021) suggested a model to measure the effect of the combination of SVF and PRP, measuring levels of oxidative stress.[53]

Eight of the included studies assessed the PRP effect on ocular burns. One quasi-experimental study was included. Khaksar *et al.* (2013) measured the clinical impact of subconjunctival PRP injections in mice, finding no signs of statistical difference on corneal opacity.[54] Panda *et al.* (2012) were the only ones to study the application of PRP in humans as a RCT. The authors assessed the effect in moderate to severe chemical ocular burns, finding that PRP can reduce corneal epithelialization time.[55]

The remaining articles evaluated the usage of PRP in corneal alkali burns in animals. Charalambidou *et al.* (2018) applied PRP intrastromal, while other authors studied different delivery routes.[56] Ebrahim *et al.* (2017) proposed PRPs potentiating effect in mesenchymal stem cells.[57] The literature reviews harbored a larger quantity of human eyes, although Sharma *et al.* (2018) did not specify the sample number.[58] All reviews reported a positive effect of PRP on corneal clarity, as well as a decrease in recovery time.

Two experimental studies focused on oesophageal burns are and evaluate different outcomes, consisting of wound healing and changes in oesophageal motility. Despite considering various aspects, they similarly conclude PRP positive effect on esophagitis recovery. PRP protocols are identical in terms of anticoagulant used despite having different concentrations.

No parameter can indicate with certainty the severity of caustic oesophageal burns. Oztan *et al.* (2018) used a distinct method to assess the effect of PRP, proposing to evaluate the impact on motility, assessing the impact on organ function. This study used biochemical parameters (IL-1, IL-6, TNF and MPC levels) and inflammation scores to measure the inflammatory response. One of the limitations mentioned in this article is that only oesophageal muscle contraction and not relaxation responses were measured.

As expected, there was a decrease in contractions in the burn group, but this reduction was minor in the PRP group, indicating that this positively affects wound healing. Oztan *et al.* (2018) assumed that the healing effect of PRP on the structural and neuronal level caused a better transmission. PRP may have the ability to repair damage from smooth muscle and mucosal damage.[59]

Seven articles address general considerations which includes comparisons between PRP production protocols, literature reviews that have less accuracy in information, opinion articles, and letters to the editor. All these articles were organized into this category because they include comparisons between PRP and GF application (Marck *et al.* (2014)).[36] The best example of that is the necessity of universal classification for PRP and the fact that standardization of methods is needed.[60]

Quality assessment

All articles were analyzed considering the JBI Critical appraisal tools and the SYRCLEs tool for assessing the risk of bias. The results were plotted in color-coded graphics for easy reading. The green color means that the answer is present, yellow has unclear information, red means it is absent, and the blue sign represent no applicability. The articles were evaluated according to the division mentioned above, with the exception for Chen *et al* (2020) which consists of a systematic review protocol, and therefore does not have an adequate assessment tool.

Graphs 1-8 (Annex III) present the quality assessment of the articles on dermis. Graphs 9-12 (Annex III) include the assessment of the quality of articles. Graphs 13 and 14 (Annex III)) have the assessment related to oesophagal burn articles. Graphs 15-18 (Annex III) have the quality assessment of articles of general considerations.

Platelet-Rich Plasma protocols

From the selected articles, PRP protocols were extracted and organized. For the articles where the PRP protocols could not be extracted, only the type of study was mentioned. The dermal burn protocols presented some omissions of information, mainly regarding the activating agent used (Table 5.2. – Annex II). Despite the heterogeneity, the main anticoagulant used was sodium citrate. There was a more significant omission of protocol data for the number of articles included in ocular burn protocols (Table 6.2. - Annex II) and in oesophageal burn protocols (Table 7.2. - Annex II). The protocols of the general considerations presented the most significant lack of information. (Tables 8.2. – Annex II)

Discussion

Several strategies have been developed and adopted as adjuvants to the conventional treatment of burns. Ever since the application of growth factors released by platelets was discovered, there has been a growing interest in their clinical use. Despite the increasing number of studies, there are still some concerns and divergences related with PRP universal use, particularly in burns.

Applicability

Dermal burn wounds were the most representative reported injuries in which PRP was applied and studied, having the higher number of articles included in this scoping review. This may be mainly due to the higher frequency of cutaneous burns. In randomized studies, most burns are found in exposed areas. There are even studies referring to the effect of PRP on scar regression. Of the experimental animal studies, the overwhelming majority tackled skin burns on the back of the models. The PRP was applied in different ways to understand whether the way it is administered influences the results, concluding that it does not affect.

Ocular chemical burns are responsible for 11.5%-22.1% of ocular injuries and require adequate diagnosis and fast treatment. [54,61] Alkali represents two-thirds of the agents, ammonia being the most common agent available in diverse industries, fertilizers and refrigerants.[62] These agents cause colliquative necrosis (cells transform into liquid), while acids cause coagulation necrosis (preserved tissue architecture).[11] One of the most relevant limitations on this topic is the lack of information on PRP elaboration protocols. Of the experimental studies, only Ebrahim *et al.* (2017) expressed the activating agent used. There is meaningful heterogeneity in the type of studies encountered, but the conclusions are globally similar - PRP is a safe agent with a beneficial effect on burns. However, more studies in humans as population are recommended.

More rarely seen, oesophageal burns associated with caustic ingestion can be potentially devastating, with perforation and consequent mediastinal infection.[63] Despite the extensive search, only two articles included in the scoping-review assess the impact of applying PRP topically to oesophageal burns.[59,64]

Primary Outcomes – Wound healing

Despite being the primary outcome, it turned out to be a very generic subgroup, because of the existing wide variety of assessment forms. In general, the studies evaluated the evolution of wound closure through macroscopy. Yeung *et al.* (2018) used a formula to more objectively evaluate wound closure rate [35], which was adapted by Sun et al. (2020), who evaluated the effect at several different times of PRP treatment.[21]

At the top of the evidence level are systematic reviews, meta-analysis, and randomized clinical trials. Even though they are particularly recent reviews, with different sample sizes, they reach the same conclusion: PRP is effective in healing and does not present data on having side effects. However, they raise essential questions regarding the lack of coherence in the literature[18,20]. Kao *et al.* (2021) also adds dressing choice as a factor of variability, referring to articles in which they discuss the questionable benefit of using silver-based dressings in preventing bacterial infection.[20]

The remaining articles preferred the histopathological study, evaluating the type of cells, as well as, the study of vessel proliferation, fibroblast activity, collagen deposition and epithelialization.

Other concept that gave rise to difficulties in interpretation is re-vascularization. Angiogenesis and vasculogenesis are different concepts, and they can often be misused. Angiogenesis concerns the formation of vessels formed through pre-existing vessels. In contrast, vasculogenesis defines by the formation of new vessels without pre-existing vessels.[65–68]

The articles present similar results in macroscopic terms and in the reduction of recovery time, but concerning re-epithelialization, some studies did not demonstrated a statistically significant improvement with PRP.[38,52]

Secondary Outcomes – Safety, pain, scar regression and infection

Although it was not initially considered as one of the primary outcomes to be assessed, there have been studies evaluating pain as one of them. Many others have also included pain in its secondary outcomes.[15,50] Because it is one of the vital signs and has an actual weight in burn patients, it was decided to be similarly assessed. The study conducted by Huang *et al.* (2018) stands out from the studies that evaluated this outcome, where the authors quantified specific markers of central sensitization and inflammation (p-p38 and p-NFkB, respectively), concluding that PRP is efficient in the treatment of neuropathic pain created by burns.[69]

Scars are one of the main sequels of burns in the context of dermal injury, which can have a significant and negative emotional impact. One of the included cohort studies objectively assessed the scar evolution by measuring viscoelasticity, noting that PRP decreased the time to healthy tissue-like properties.[70] A Spanish cohort study had a larger population sample, but it had not objectified a scar regression.[6] Recently, an experimental study demonstrated the efficacy of PRP in the regression of scars that were refractory to previous therapies.[2,71]

A recently published review by Merchán *et al.* (2019) presented a broader view of the role of PRP in Dermatology, however includes studies in humans that had not demonstrated a statistically significant difference in the quality of scars after one year.[22] Of the narrative revisions included, there is a vast disparity in the article structure. Picard *et al.* (2015) presented a complete literature review, being entirely transparent in writing and demonstrating the importance of platelet essays as a form of quality control.[24] Of the 1626 patients, the authors only found one case of adverse effect (anaemia).[22]
In several studies, samples were taken for microbiological study, considering that PRP has an antibacterial effect.[10,17,18,21,35] Bacterial contamination is only considered deleterious to the wound with values above 10^5 bacteria per gram of tissue.[72]

PRP associations

Several studies have been carried out to associate PRP with other groups of cells and related components SVF. An effect was found on the adipocyte microenvironment (by maintaining the three-dimensional structure) and, together with the positive effect of SVF on neovascularization and fibroblast stimulation, the combination of these two agents favored the acceptance of the fat graft.[39,43,73] In one of the most recent experimental studies, Josh *et al.* (2021) addressed the anti-oxidative response after administration of this association through subcutaneous injections.[53]

Studies encountered and included had a great variety in the models used (e.g. Wistar rats - with and without a diabetic model [48,49], Sprague Dawley rats [39,52,69], Domestic pigs[38], Bama pigs[21] and Humans), in the delivery route and also in the type of outcome studied and how it was assessed.

Platelet-Rich Plasma protocols

The analysis and comparison between PRP protocol descriptions used in the different studies found and included, as presented and organized on Tables 5.2, 6.2, 7.2 and 8.2 (Annex II) denotes a considerable disparity in their procedural elaboration and application. In fact, there are different forms even describing the same rotations, not counting the different laboratory machines.

A standard and universal method for defining the PRP and its components would allow the reproduction of the experimental findings. Some classifications have already been proposed, but up to this day an universally accepted classification has not yet been created.[60,74,75]

PRP Quality

One of the most relevant issues highlighted throughout the literature is related to the PRP quality assessment. In multiple articles, the quality of PRP is evaluated by measuring the number of platelets, with a standard agreement on the minimum number of platelets necessary for the effectiveness of PRP.[41] However, some articles state that the number of platelets may not be enough and address the need for the determination of growth factors. Studies that evaluated the constituents of PRP, mainly its amount of growth factors, confirmed the existence of variability in the concentrations of growth factors. Marck *et al.* (2016) evaluated the function of platelets and their ability to release growth factors in burn patients, showing that they maintain their capacity unchanged, which validates the use of autologous samples, even after the burn.[37]

Studies quality and types of study

Of the studies that address dermal burns, and besides the heterogeneity of typology, quality heterogeneity is also present. From the analysis of graphs 1-8 (Annex III), organized by the level of scientific evidence, it appears that Animal Experiments have a lower quality of study when compared to other types of study. However, this difference may be due to the different assessment tool used.

Strenghts and Limitations

The strength of this review is the systematic and extensive approach to identifying and analyzing the articles. To best of our knowledge, this is the first scoping-review that addresses the role of PRP in burns which analyzes conceptual disparities, appliance, practicality, effectiveness, safety, and the most recent research developed on PRP effects on burns.

The qualitative evaluation of most of the studies followed the JBI checklists, unlike animal experimental studies in which SYRCLE checklists were used. It was not possible to evaluate the systematic review protocol included, since there is no specific checklist for the effect. Even though two different quality assessment tools had to be used, the Authors believe that overall homogeneity and significance was not compromised.

Since there was certain search specification and particular criteria adopted on the research and studies selection, namely in terms of language, temporal space, and age of patients, some information may have not been covered. Furthermore, some studies did not mention methodological information regarding PRP protocols, or when present, the protocols varied among studies, which might also be a source of heterogeneity and thus affect the outcome. Another significant limitation is the absence of PRP quality analysis.

Conclusion

PRP use in burn patients is getting increasing interest with a growing number of studies being conducted in the most recent years, particularly due to its clinical applicability impending potential. In fact, PRP has reported benefits in pain control, wound healing, and angiogenesis. Although, effectiveness in reepithelization was not yet thoroughly demonstrated. The extraction of PRP protocols allows the assessment of the disparity in the description of the production process. Few articles reckon the quality of the PRP produced and some flaws in the study design, affecting the quality of information produced.

However, a more standardized PRP yield, and more experimental studies, mainly randomized controlled trials are much recommended for better PRP assessment and safer application on burn patients.

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Annex I – Articles Summary

Table 5.1. – Dermal Burns articles

1st Author/ Year	Type of Study	Type of Injury	Species	Method of use	What was measured	How it was measured	Outcomes	Secondary Outcomes
Zheng, W. /2020[18]	Systematic Review and Meta-analysis	Dermal Burns	Humans (n=393)	NA	-Area of Graft Take -Healing Rate -Healing time -Epithelialization -Adverse events -Scar assessment score	-Graft take in % -Week healing rate in % -% of epithelialization at different periods -Presence of keratitis -VSS	PRP accelerates the healing process and may improve scar quality. It does not affect the graft take rate, degree of epithelialization and the rates of adverse reactions and infections.	Theorical antibacterial activity.
Huang, H./ 2021[2]	Systematic Review and Meta-analysis	Deep dermal wounds	Humans (n=539)	NA	-Healing rate -Adverse events: infection, local inflammatory reactions, allergies.	-Adverse events -Pain -Scar score	PRP shortens the healing time. There is no significant difference between delivery route. Lack of information regarding the number of PRP applications.	PRP treatment reduces adverse events and reduces pain level and scar hyperplasia.
Kao, Y./2021[20]	Systematic Review and Meta-analysis	2nd and 3rd Degree burns	Humans (n=449)	NA	-Wound Healing	-Wound closure -Time to complete epithelialization	-PRP accelerated wound closure.	May improve graft take when treated with a skin graft.
Marck, K./2016[37]	Randomized Control Trial	Full Thickness Burns	Humans (n=52)	Single wound bed application	-Graft take rate -Pain response -Pruritus	-Macroscopy photographic records -Graft take and epithelialization -VAT score	PRP was shown to have a significant positive effect on graft take and epithelialization.	No significant difference in Pain, Itch or Scars.
Yeung, C./2018[35]	Randomized Control Trial	Deep 2nd Degree Burns	Humans (n=27)	Liquid/Sprayed	-Wound healing -Infection incidence	-Percentage of wound closure and bacteria picking out rate in the 2nd and 3rd week.	Lyophilized PRP powder shortened the duration of wound closure and decreased the bacteria picking out rate of deep 2nddegree burn wound	The comparison between liquid form and LPRP powder requires further studies.

Marck , R. (2) /2019[41]	Randomized Control Trial	Different Types of Burns	Humans (n=10)	Topical application	-PRP constituents	-Platelet quantification -Growth factors quantification	There is correlation between growth factor concentration and platelet count in PRP	There is a considerable individual variation in growth factor content.
Liao, X./2020[40]	Randomized Control Trial	Chronic Wounds (etiology included Burns)	Humans (n=60)	Injection	-Wound healing -Clinical evolution -Graft take rate	-Granulation Growth -Frequency of dressing replacement -Presence of infections or complications -Healing time -Skin graft survival	It shortens the operation time and reduces postoperative pain and swelling.	No secondary reaction. Allogeneic PRP release the same content of GF
Klama-Baryla, A./2011[42]	Quasi-Experimental	Thermal burns of III and IV degrees	Humans (n=5)	Administered as Fibroin Glue (PLRG) + autologous epidermal cells	-Wound healing	-Macroscopy – Photographic records -Histology	Accelerated the process of epidermization of burn	
Maclel, F./2012[10]	Quasi-Experimental	Deep second degree	Horses (n=4)	Gel application	-Wound Healing -Infection	-Electron microscopy- fibrose -Bacterial Profile	-PRP accelerated repair and induced fibrosis	Possible antibacterial activity
Gentile, P./2014[43]	Quasi-Experimental	Burns sequelae and post-traumatic scars	Humans (n=20)	PRP + Stromal Vascular Fraction	-Wound healing -Patient Self-evaluation	-Macroscopy – Photographic Records and Team evaluation -Magnetic Resonance Imaging (MRI) and ultrasound	The use of PRP during fat grafting improves adipose tissue maintenance and survival.	

1st Author/ Year	Type of Study	Type of Injury	Species	Method of use	What was measured	How it was measured	Outcomes	Secondary Outcomes
Huang, S./2018[50]	Quasi-Experimental	3rd Degree injury	Sprague-Dawley rat model (n=24)	Subcutaneous injections	-Pain response	-PTEN expression -Spinal cord mTOR p-p38, p-PTEN, p-mTOR, CCL2 and NFκB Immunofluorescence	PRP is effective in neuropathic pain induced by burns and may be used in clinical practice.	
Eldien,H./2019[76]	Quasi-Experimental	Full Thickness Burn	Mice (n=70)	Injection	-Wound Healing -Reepithelization -Re-vascularization	-Macroscopy - % of healing -Histology -Western Blot for MMP13 quantification -Angiogenesis – mRNA expression	It was visible a significant increase in MMP13, regenerated epidermis and incomplete healing of hair follicles. PRP	
Uraloğlu, M./2019[15]	Quasi-Experimental	Thermal injury	New-Zealand rabbits (n=20)	Subcutaneous injections	-Wound healing	-Histopathological Data – inflammatory cells proliferations and connective tissue apoptosis -Platelet count -Immunohistochemical analysis	PRP showed anti-inflammatory and antiapoptotic effects in the zone of stasis in acute burn. Greater reepithelialization and fibroblast proliferation.	Reduces pain and improves function in patients with tendinopathies.
Orhan, E./2019[16]	Quasi-Experimental	Comb burn model – full-thickness burns	Wistar Albino rats (n=72)	Intradermic injection	-Wound healing -Autophagy assessment	-Immunohistochemical analysis – Living areas, apoptotic, Nrf2, HO-, and autophagy assessment	The living area in the PRP was significantly higher. The number of apoptotic cells was lower. PRP administered in an inactive form, increased Nrf2, HO-1 levels and autophagy in the burn stasis zone.	Authors believe is an easy application, free from side effects and a low-cost clinical option for burns.
Laidding, S./2021[73]	Quasi-Experimental	Deep dermal burns	Wistar rats (*Rattus norvegicus*) (n=45)	Combination with Stromal Vascular Factors. (SVF+ PRP) PRP was administered individual and topically	-Wound healing -Re-vascularization	-Polymorphonuclear and fibroblast counts. -Thickness of granulation -Formation of epithelialization, collagen and capillary density	The combination showed better results than with the individual application. PRP stimulates undifferentiated stem cell proliferation and differentiation for tissue regeneration	

1st Author/ Year	Type of Study	Type of Injury	Species	Method of use	What was measured	How it was measured	Outcomes	Secondary Outcomes
Klosová, H. /2013[44]	Cohort	Thermal and electrical burns	Humans (n=23)	Topical application	-Scar regression	-Evaluation of the development of scar viscoelasticity by Cutmeter MPA 580 device	Shortening of time until normal viscoelastic properties to appear	Risk of blood transfer disease is virtually non-existent, and no systemic effects have been identified.
Rossani, G. /2014[6]	Cohort	Thermal II-degree burns	Humans (n=115)	Injection	-Scar assessment -Wound healing	-Macroscopy - skin firmness, skin uniformity and scars. -Hospitalization Time	PRP shortens recovery time for 2nd degree burns	No toxicity
Teodoreanu, R. /2014[6]	Case Series	High voltage burns	Humans (n=7)	Injection (mesotherapy or subcutaneous)	-Wound Healing -Re-vascularization	-Macroscopy- Photographic records -Biopsies (vascularization and infiltration cells assessment)	PRP-treated area showed increasing of granulation and shortened hospitalization by 7 days.	
Kakudo, N./2011[45]	Case Report	3rd degree burn	Humans (n=1)	Topical application and sprayed	-Clinical aspects -Wound Healing -Cytology -Re-vascularization	-Oedema -Immature fibroblasts and macrophages -New vessels in the dermal layer	PRP showed signs of epithelialization and angiogenesis in the formation.	
Picard, F./2015[24]	Review	Multiple Burns	Humans and Animals	Platelet Gel Injection/	-Efficacy of PRP -Safety	-Experimental Wound model -Skin graft reconstruction -Experimental burn model -Side effects	Mandatory uniformization of PRP methods;	Over 17 controlled studies found anaemia
Merchán, W./2019[22]	Review	NA	NA	NA	-Efficacy of PRP -Safety	NA	PRP is a safe and efficacious treatment option	
Ozcelik, U. /2016[46]	Animal Experiment	Partial thickness burn injury	Wistar albino rats (n=30)	Topical application	-Histopathologic examination -Re-vascularization	- Ehrlich-Hunt numeric scale -Collagen deposition -Hydroxyproline levels	Study group showed higher hydroxyproline levels and Vessel proliferation Excepting for inflammatory infiltration, there was no significant difference between the groups	PRP could benefit haemostasis and antimicrobial properties in wound healing

1st Author/ Year	Type of Study	Type of Injury	Species	Method of use	What was measured	How it was measured	Outcomes	Secondary Outcomes
Venter, N./2016[49]	Animal Experiment	Deep 2nd and 3rd degree burns	Wistar rats (diabetic model) (n=60)	Topical application	-Wound healing -Re-vascularization	-Macroscopy -Microscopy -Immunohistochemistry (CD31, CD68 and CD163, MM2) Collagen deposition	Reduction in CD21, CD68, CD163, MPO and in TGF-ß-positive cells and increase in MMP2-positive cells. Indicating that PRP can accelerate the healing process in dSDD but not in TD burns.	No complications and levels of glucose where consistently above 300mg/dL
Hosni, H./2017[47]	Animal Experiment	Thermal Burn wound	Albino Wistar Rats (n=72)	Intraregional	-Wound healing -Re-vascularization	-Histopathological assessment of skin tissue -Angiogenic gene expression of MMP-1, TIMP-2, Ang-1, Ang-2 and vimentin	Stimulated MSC proliferation; anti-inflammatory effects	Can be incorporated in tissue engineering
Mansoub, N./2018[48]	Animal Experiment	2ndDegree burn wounds	Wistar albino rats (diabetic model) (n=20)	Subcutaneous injections	-Wound healing	-Wound contraction -mRNA levels of wound healing markers e.g, EGF, FGF-2, TGF-ß1, COL1α2 and VEGF-α	PRP increases the effect of the keratinocyte-like cells (KLCs). PRP application led to a decrease level of MCP-1	
Singer, A./2018[38]	Animal Experiment	Partial Thickness Burns	Domestic Pigs (n=6)	Topical Application	-Wound Healing	-Scar Depth -Percentage Wound reepithelialization -	PRP showed similar rates of reepithelialization and scar depth when compared to standard topical antibiotics	
Sun, Y./2020[21]	Animal Experiment	Deep-Partial Thickness Burns	Bama Pigs (n=3)	Topical application	-Wound Healing -PRP constituents -Re-vascularization	-Histological quantification -Growth Factor assay (EGF, ß-FGF and VEGF)	Shorter time to wound re-epithelialization on PRP group. The levels of angiogenesis factors, examined by ELISA, were higher in the experimental group.	PRP has a crucial role in the subcutaneous wounds healing process due to the amount of GF released – showing antibacterial properties.
Laidding, S. (2) /2020[39]	Animal Experiment	Deep Dermal Wounds	Srapgue Dawley rats (n=64)	Combination with Stromal Vascular Factors. (SVF+ PRP) – injection and topical application	-GF monitorization	-Comparison between the Topical application and the Injection application. -TGF-ß levels monitorization.	There is no significant difference between routes of delivery	

1st Author/ Year	Type of Study	Type of Injury	Species	Method of use	What was measured	How it was measured	Outcomes	Secondary Outcomes
Ren, Z./2021[69]	Animal Experiment	3rd Degree Burns	Srapgue Dawley rats (n=24)	Subcutaneous Injection	-Wound Healing -Pain response -Re-vascularization	-Wound healing rate -Paw Withdrawal mechanical threshold -Paw Withdrawal Thermal Latency -ELISA – measuring the levels of. TNF-alpha, Il-1 ß, -RT-qPCR	The level of CD31 positive cells of PRP group was increased. Autologous PRP promotes expression of VEGF, MMP-9 and TGF-1ß in skin wound tissues.	Autologous PRP ameliorates mechanical pain caused by burns. It was not concluded if the composition of autologous PRP has a negative effect on the body-
Karina, K./2021[52]	Animal Experiment	Dermal Wounds	Sprague-Dawley Rats (n=30)	Intradermal Injection	-Wound healing -Re-vascularization	-Macroscopic evaluation -Real-time analysis of vascular endothelial growth factor expression -Microscopic evaluation	VEGF expression was 1.42 times higher than the control group – no statistical significance. PRP does not accelerate epithelialization duration of burns in the rat burn wound model. There is an improvement in wound vascularization and cell differentiation.	
Josh, F./ 2021[53]	Animal Experiment	Deep dermal wounds	Wistar Rats (n=36)	Subcutaneous injection of PRP associated with SVF	-Systemic stress and oxidative response	-Malondialdehyde and Nitric Oxide measurements	-Decrease in blood/tissue malondialdehyde level.	

% - Percentage; COL1α2 – collagen Type I Alpha 2; GF – growth factor; ELISA – Enzyme-Linked Immunosorbent assay; EGF – endothelial growth factor; KLC – keratinocyte-like cell; rt-qPCR – real time – polymerase chain reaction; VAT – Visual Analogue Thermother; VEGF-vascular endothelial growth factor; VSS – Vancouver Scar Score; TGF-ß – Transforming growth factor Beta
;

Table 6.1. – Ocular Burns articles

1st Author/ Year	Type of Study	Type of Injury	Species	Method of use	What was measured	How it was measured	Outcomes	Secondary Outcomes
Panda, A./2012[55]	Randomized Control Trial	Grade III to V chemical injury	Humans (n=20)	Platelet-rich eyedrops	-Visual acuity -Safety	-Follow-up and percentage of improvement -Complications	PRP can be effective in moderate to severe chemical burns, with reduction in corneal and conjunctival epithelialization	PRP has no ophthalmological side effects.
Khaksar, E./2013[54]	Quasi-experimental	Alkali wounds	New Zealand Albino Rabbits (n=20)	Subconjunctival injection	-Clinical Outcome	-Evaluation of epithelial defects, corneal opacity, duration of blepharospasm, corneal vascularization, duration of ocular discharge and wound area diameter measurement,	No significant statistical difference on corneal opacity. PRP groups had significantly lower degree of corneal vascularization	PRP groups showed less inflammation and vascularization. No signs of side effects.
Hamweyah, K./2017[77]	Review	Alkali wounds	Human eyes (n=297)	Platelet-rich eyedrops	Different treatment options	NS	Greater improvement in corneal clarity from baseline	
Giannaccare, G./2017[78]	Review	Chemical injuries, corneal ulcers resulted from burns	Human eyes (n=430)	Platelet-derived eye drops	Comparison between treatments	-Clinical evolution -Findings in vivo -Confocal microscopy	Significant corneal transparency Higher healing rate Reduction of inflammation and ocular pain	
Sharma, N./ 2018[58]	Review	Corneal Alkali Burns	Humans (n=NS)	NS	Was performed a review on different treatment options for ocular chemical burns	NS	PRP demonstrated faster healing of epithelial defects	

Ebrahim, N./ 2017[57]	Randomized Control Trial	Corneal Alkali Burns	Wistar Albino rats (n=50)	Gel application	-Corneal transparency -Angiogenesis	-Histological examination -RNA extraction for rt-PCR	PRP is recommended as a vehicle of MSCs	PRP promote the healing mechanism of Mesenchymal Stem Cells through microenvironment
Charalambidou, G./ 2018[56]	Randomized Control Trial	Corneal Alkali Burns	New Zealand rabbits (n=36)	Intrastromal Injection	-Angiogenesis -Wound healing	-Histopathological evaluation -Corneal opacity -% of corneal healing -% of severity of uveitis	Lower signs of oedema PRP treated eyes showed smaller mean defect.	The intrastromal administration can be related to the reduction of the corneal oedema.
Eldin, H./2019[79]	Randomized Control Trial	Corneal Alkali Burn	Albino rats (n=30)	Subconjunctival injection	-Wound healing with PRP administration at different times.	-Immunohistochemistry for Ki67.	PRP showed effective use on alkali-induced burns. The PRP injection after 2 hours was more efficient than passed 48h.	

PRP – Platelet-rich Plasma; MSCs – Mesenchymal Stem Cells; PCR – Polymerase chain reaction; rt-PCR – real time- Polymerase chain reaction; NS – Not specified;

Table 7.1 -Oesophageal Burns Articles

1st Author/ Year	Type of Study	Type of Injury	Species	Method of use	What was measured	How it was measured	Outcomes	Secondary Outcomes
Karaca, G./ 2017[64]	Quasi-Experimental	Corrosive oesophagitis	Wistar Albino rats (n=45)	Topical	-Wound healing	-Histopathological examination – Il-1, TNF, IL-6, MCP and Inflammation score measurements	PRP reduces oxidative injury. It has positive effect on recovery in oesophagitis	
Oztan, M./ 2018[59]	Animal Experiment	Corrosive oesophagitis	Wistar Albino rats (n=21)	Topical	-Changes in oesophageal motility	-Concentration-Response on the cholinergic, non-adrenergic non-cholinergic and Rho-kinase pathways	PRP treatment significantly increased KCl and Ach-induced contraction responses. PRP improves mucosal and smooth muscle damage, and increases the effects of inhibiting neuromediators	

PRP – platelet-rich plasma; Rho IL-1 – Interleukin 1; IL-6 – Interleukin-6; MCP- monocyte chemoattractant protein; KCl – Potassium chloride; Ach – acetylcholine;

Table 8.1. -General Considerations Articles

1st Author/ Year	Type of Study	Type of Injury	Species	Method of use	What was measured	How it was measured	Outcomes	Secondary Outcomes
Chen, Z./ 2020[17]	Protocol for Review and Meta-analysis	NA	NA	NA	NA	NA	-PRP has a pain-relieving effect. -PRP can repair the wound and reduce bacteria invasion/infection	
Marck, R./ 2014[36]	Systematic review	Deep dermal wounds	NA	NA	-Comparison between PRP yields -Comparison between GF application and PRP	NA	-Burn patients have altered physiological state, and it could affect the platelet quality	-So far there is no data on PRP being responsible for causing hypertrophic scars
Karakol, P./ 2021[71]	Quasi-Experimental	Burns contractures	Humans (n=29)	Subcutaneous injection of PRP associated with SVF	-Scar regression	-Scar measurements -Histopathological analysis – collagen deposition/organization, vascularization and melanocytic activity	-PRP may be used as an useful treatment option in cases with resistant and fibrotic contractures.	-Minor complications. -scaffold failure and hematoma.
Achora, S./ 2014[80]	Literature Review	Burns and superficial wounds	Humans (n=NS)	NA	NA	NA	-The addiction of PRP can reduce the healing time -Topical PRP when used prior to graft application may improve healing time	-No adverse reactions were observed.
Sharun, K./ 2020[74]	Letter to the Editor	Burns	NA	NA	NA	NA	PRP production vary in diverse aspects, so it is proposed a universal coding system for defining PRP based on its components concentrations.	

Sharun, K./ 2021[60]	Letter to the Editor	Burns	NA	NA	NA	NA	There should be a universal classification for PRP, to adequately assess the outcomes of burns
van der Bijl, I. /2021[75]	Opinion Paper	Burns	NA	NA	-Activation status of PRP -GF application and release systems -Platelet and GF concentration -Leukocytes -Timing of application	NA	High number of variables increases the difficulty of standardizing PRP research.

GF – Growth factor; NA – Not applicable; NS. -Not specified; PRP – Platelet-Rich Plasma

Annex II – PRP protocols

Table 5.2. – Dermal Burns PRP protocols

1st author/Year	PRP protocol	Type of anticoagulant	Activation	Centrifugation Protocol
Zheng,W./2020	Multiple protocol review			
Kao, Y./2021	Multiple protocol review			
Huang, H./2021	Multiple protocol review			
Marck, K./2016	PRP was prepared with the Gravitational Platelet Separation System (GPS-III system). Prior to surgery blood was drawn by a venous puncture. 54mL of blood was drawn and mixed with 6mL of citrate. PRP was centrifugated and then, was stored at 4ºC until further use. PRP and Thrombin were drawn up into a two syringes assembly component, ensuring that the PRP and autologous clotting factors were mixed in the correct 10:1 ratio.	6mL citrate	NS	1st centrifugation: 3200rpm for 15min
Yeung, C./2018	Human blood was obtained from Taipei Blood Center A batch of LPRP was separated from platelet concentrate and protection steps were processed. LPRP powder was dissolved in 50mL of sterile water. LPRP was diluted according to wound size. (concentration of 1.0 x 10^7 platelets/cm^2)	NS	NS	NS
Marck, R./2019	PRP was prepared with the Gravitation Platelet Separation System. From 27mL blood from 5 patients it was prepared. Activated PRP with autologous thrombin and then centrifugated.	Ethylene di-amine tetra-acetic acid (EDTA)	NS	Activated PRP was centrifugated (10000g at 4ºC for 15min)
Liao, X./2020	60mL venous blood was collected in a sodium citrate centrifuge tube. After the 1st centrifugation, it was obtained three layers. The uppermost supernatant (PPP) was aspirated and the intermediate layer to 2mm from the bottom layer were pippeted out.	Sodium citrate	Activated by calcium gluconate at a ratio of 10:1	1st centrifugation:400g for 10min 2nd centrifugation: 1200g for 20min

	The collected layer was centrifugated a second time. And the liquid was separated into two layers – the sediment (PRP) was collected.			
Klama-Baryla, A./2011	Platelet leukocyte Rich Gel is obtained by the gravitational platelet-rich plasma separation system (GPSIII). It is obtained from 120ml of patient's blood: 20ml of thrombocyte mass, 10mL of thrombin and 60mL of serum.	Sodium citrate	NS	NS
Maciel, F./2012	Collected 10ml of blood. After a 1st centrifugation, 500 µL of plasma was transferred to a Tube A – used to obtain autogenous thrombin. The remain plasma and intermediate zone were transferred to a Tube B, maintained at room temperature. 300µL of 10% calcium gluconate was added to tube A and the suspension was mixed and incubated at 15min at 37ºC. Tube A and Tube B had a 2ndcentrifugation. Tube A contained a thrombin-rich substrate, and the entire volume was used. Half of the volume of Tube B was discarded, homogenized and Tube A thrombin was added to Tube B at a 2:1 ration.	10% sodium citrate	NS	1st centrifugation: 300g for 10min 2nd centrifugation: 640g for 10min at room temperature
Gentile, P./2014	Followed the method of Cascade-Selphyl-Aesthetic Factors system, with modifications – PRP was prepared from 18mL of blood. PRP was then activated	Sodium citrate	Calcium	Single centrifugation at 3300rpm for 10min
Huang, S./2018	12mL was extracted by cardiac puncture and placed in a PRP centrifuge tube 50mL containing 1.8mL of acid citrate dextrose solution and centrifugated. 1.5mL of PRP was collected at the middle range of the tube, and all the samples were mixed.	1.8mL acid citrate dextrose solution (AesMed Co., Ltd., Taipei, Taiwan)	1.5mL of calcium chloride (100mg/mL) was added to activate the 1.5mL PRP.	Single centrifugation at 3000rpm for 4min
Eldien,H./2019	20mL of peripheral blood was obtained from healthy individuals and collected into tubes containing citrate-dextrose-acid. The PRP was further centrifugated to obtain Platelet Concentrate (PC). PC was kept sterile, and it was added human thrombin (0.2mL/1mL PC) and calcium gluconate.	Citrate-dextrose-acide	NS	1st centrifugation: 1000g for 10min 2nd centrifugation: 1500g for 10min

Uraloğlu, M./2019	10mL of blood was collected from rabbit's ears. 9mL were centrifugated in a citrate tube. 5 to 6mL of serum was obtained.	Citrate	PRP was activated by the addition of 0.5mL 5% to 10% calcium chloride enriched with adenosine, guanine, cytosine and thymine nucleotides for activation of 1mL PRP.	1st centrifugation: 2000rpm for 10min
Orhan, E./2019	The blood was obtained from 8 rats by cardiac puncture – 65mL. 60mL of blood was placed in a 3.8% sodium citrate containing tube and centrifugated. It was obtained a stratified tube and the middle layer, and the upper plasma layer were transferred to another tube not containing sodium citrate and a 2nd centrifugation was performed – resulting in a two-part divided tube – containing PRP and PPP.	3.8% sodium citrate	NS	Centrifugation: 2400rpm for 10min 2nd centrifugation: 3600rpm for 15min
Laidding, S./2021	NS	NS	NS	NS
H.Klosová/2013	Patient's peripheral blood was collected in two consecutive steps: from the 1stbatch was created the autologous thrombin; from the 2nd batch was created the Autologous Platelet-Concentrate. 9mL of blood volume enabled to produce 10mL of the APC	NS	NS	Centrifugation using the Harvest SmartPReP Platelet Concentrate System: 2400 rpm for 14min at room temperature
Rossani, G/2014	Venopunction of 3.5ml syringes and addition of 3.2% sodium cytrate it was obtained 40ml for each patient to produce 8-10ml of PRP 3-5x basal concentration	3.2% sodium citrate	For posterior platelet activation was used 0.1mL of calcium gluconate 10%.	Single centrifugation at 3200rpm for 12min
Teodoreanu, R./2014	Was used a ready to inject kit produced by RegnenLab.	NS	Calcium Gluconate and Thrombin (0.9mL of PRP was mixed with 0.1mL Calcium Gluconate or Thrombin, to obtain 1mL of activated PRP)	NS
Kakudo, N./2011	PRP was prepared from the patient's blood and activated by the Kakudo et al. protocol	NS	NS	Activated gelled PRP was centrifugated at 4000rpm for 10min
Picard, F./2015	Multiple protocol review			
Merchán, W./2019	Literature Review			
Ozcelik, U. /2016	Blood was collected from the rats in group three. After the 1stcentrifugation, the plasma fraction was separated from the red blood cells. Then the plasma	Thrombin (Diathrombin Diamed, Switzerland)	PRP was combined with thrombin and calcium chloride. The mixture ration was 1mL:	Two step centrifugation: 1st: short spin centrifugation: 1500rpm, 20ºC, 10min

	fraction was separated with a 2nd centrifugation into PRP and the plasma-poor platelets.		0.1mL: 1mL (PRP-Thrombin-CaChloride).	2nd hard spin centrifugation: 2000rpm,20ºC, 15min
Venter, N./2016	Blood (6.5mL) was collected by cardiac puncture into a syringe containing the anticoagulant. After the 1st centrifugation, a red cell component and a serum component were obtained. A point was market at 2mm below the line dividing the two fractions. All the content above was pipetted and transferred to another tube, in which a line corresponding to 0.72mL (10% of the total volume of whole blood + sodium citrate) was drawn from the tube's bottom. After the 2nd centrifugation, was obtained 2 layers; PRP and PPP, which was pipetted and discarded.	0.7mL of 10% sodium citrate	The PRP fraction was activated with 0.05mL of 10% calcium chloride solution per 1mL of PRP	1st centrifugation 160g for 20min 2nd centrifugation: 400g for 15min
Hosni Ahmed, H./2017	10mL volume of blood was drawn from the retro-orbital vein into tubes containing 10% sodium. The first centrifugation resulted in two components: blood cell component (BCC) in the lower fraction and serum component (SEC) in the upper component. A second centrifugation was performed and the content above a 6mm mark below the line separating BCC and SEC was pipetted and transferred.	10% sodium citrate	NS	Two step centrifugation: 1st centrifugation: 160g for 20min at room temperature 2nd centrifugation: 400g for 15min
Mansoub, N/2018	Intracardiac blood samples of 5 Wistar Albino rats under anesthesia were withdrawn into anticoagulant containing tubes and then centrifugated. The PRP fraction was collected and then a 2nd centrifugation was performed. The supernatant was separated and discarded.	3.8% sodium citrate	100U/mL thrombin in 10% Calcium Chloride in a 1/0.15 ratio was used.	1st centrifugation: 300g for 10min 2nd centrifugation: 3000g for 15min
Singer, A./2018	Blood was collected from the ear veins in three 60mL syringes with 8mL anticoagulant, prior to application. The blood was processed by a device (Magellan, Arteriocyte, Hopkinton, MA) for 15 min.	Citrate dextrose formula A (ACD-A; Arteriocyte Medical Systems, Hopkinton, MA)	Combination of Calcium/Thrombin in a ratio of 10:1	NS
Sun, Y./2020	One hour after the burn injury, it was collected 18mL of blood sample from the ear vein into a tube containing sodium citrate. The 1st centrifugation was performed – and three layers were obtained. The middle and the top layers were transferred to another tube for the 2nd centrifugation. The plasma sample was divided into two layers.	2mL 3.2% sodium citrate	Addition of calcium chloride to the PRP in a 1:9 ration (0.2mL 10% calcium chloride was added to 1.8mL of PRP)	Double-spin method: 1st centrifugation: 900g for 5min 2nd centrifugation: 1500g for 15min

Laidding, S./2020	The blood was drawn and transferred to a tube containing citrate. Blood was centrifugated The supernatant plasma with a buffy coat was collected and was performed a 2nd centrifugation. The top layer was discarded until 5mL remained at the bottom	3.8% sodium citrate.	NS	1st centrifugation: 2400rpm (450g) for 10min 2nd centrifugation: 3600rpm (850g) for 15min
Ren, Z./2021	12 mL of venous blood was collected to tubes containing 3.8% sodium citrate. The samples were centrifugated to collect plasma. And a 2nd centrifugation was performed. Then, the upper layer of plasma was discarded. The 2mL at the bottom represented the PRP.	3.8% sodium citrate	2mL of 10% calcium gluconate and mixture with PRP activator at a ratio of 10:1	Centrifugation: 800g for 10min 2nd centrifugation: 1000g for 5min
Karina, K./2021	Blood collection was done using sodium citrate tubes. Four tubes containing 4mL of blood were centrifugated. Plasma was transferred into a new 15mL tube, and it was centrifugated a second time. The upper layer was discarded until the last 5cc remained at the bottom with the aggregate of the platelet. Platelet and plasma were mixed and activated	Sodium citrate	Calcium activator (H-Remedy)	1st centrifugation: 300g for 5min 2nd centrifugation: 1000g for 5min
Josh, F./ 2021	Was followed the Juntendo University Tokyo PRP protocol. Blood was collected from cardiac puncture into tubes containing anticoagulant. 30mL of donor blood was collected and was processed the first centrifugation. The supernatant plasma and buffy coat were collected, and a second centrifugation was performed.	Ethylenediaminetetraacetic (EDTA)	10% calcium chloride	1st Centrifugation: 2400rpm (450g) for 10min 2nd Centrifugation: 3600rpm (850g) for 15min

%- percentage; EDTA - Ethylenediaminetetraacetic, LPRP- lyophilized platelet-rich plasma; mL – milimiter; NS- Not specified; PC- platelet concentrate; PRP – Platelet-Rich Plasma; rpm – rotation per minu

Table 6.2. - Ocular Burns PRP protocols

1st author/Year	PRP protocol	Type of anticoagulant	Activation	Centrifugation Protocol
Panda, A./2012	NS	NS	NS	NS
Khaksar, E./2013	The blood was collected from intracardiac puncture from ten rabbits to tubes containing anticoagulant. The samples were centrifugated, and it was obtained a three-layer divided tube. The 6mL plasma layer was centrifugated a second time. The lower part was PRP and it was transferred to a sterile tube.	Citrate Dextrose	NS	1st centrifugation 72g for 15min at 4ºC 2nd centrifugation: 1006g for 5min
Hamweyah, K./2017	NS	NS	NS	NS
Giannaccare, G./2017	NS	NS	NS	NS
Sharma, N./ 2018	NS	NS	NS	NS
Ebrahim, N./ 2017	30cc of venous blood was obtained by venopunction. It was added anticoagulant. It was initiated the centrifugation protocol. After the first centrifugation – the supernatant plasma was transferred into tubes without anticoagulant. It was performed the second centrifugation, where the lower third was considered PRP.	Acid citrate dextrose	Thrombin and Calcium Chloride	1st centrifugation (soft spin): 1480rpm for 6min 2nd centrifugation (hard spin): 3400rpm for 15min
Charalambidou, G./ 2018	In a 10mL syringe, it was collected blood and added 1.3mL of anticoagulant. The samples passed through a two-step centrifugation protocol and was removed the upper lawyer (PPP). PRP was aspirated in a sterile 1mL syringe and kept at 4ºC.	1.3mL of Citrate dextrose solution	NS	1st centrifugation:72g for 15min at 4ºC 2nd centrifugation:1006g for 5 min at 4ºC
Eldin, H./2019	Blood was collected from intra-cardiac aspiration, into tubes containing citrate dextrose.	Citrate dextrose	NS	1st centrifugation 3000rpm for 7min at 20ºC

	It was performed the first centrifugation. The supernatant plasma was aspirate and centrifugated a second time. To pellet PRP.			2nd centrifugation: 4000rpm for 5min at 20ºC

ºC – Celsius degree; mL – milliliter; NS- Not specified; PPP – platelet-poor plasma; PRP – platelet-rich plasma; rpm – rotation per minute;

1st author/Year	PRP protocol	Type of anticoagulant	Activation	Centrifugation Protocol

Karaca, G./ 2017	Blood from 5 female rats was aspirated from the heart. After it was performed the first centrifugation. To the upper portion was performed a second centrifugation. The upper two-thirds were discarded. The lower one-third portion was considered PRP	3.2% Sodium citrate	NS	1st Centrifugation: 400g for 10min 2nd Centrifugation: 800g for 10min
Oztan, M./ 2018	2mL was drained from the tail vein of each rat into anticoagulated tubes. These samples were centrifugated. The upper layer, (containing red blood cells), was separated. The suspension was centrifugated. A major part of the supernatant was discarded	3.8% sodium citrate	NS	1st Centrifugation: 1500 rpm for 10min 2nd Centrifugation: 3000rpm for 10min

Table 7.2.- Oesophageal Burns PRP protocols

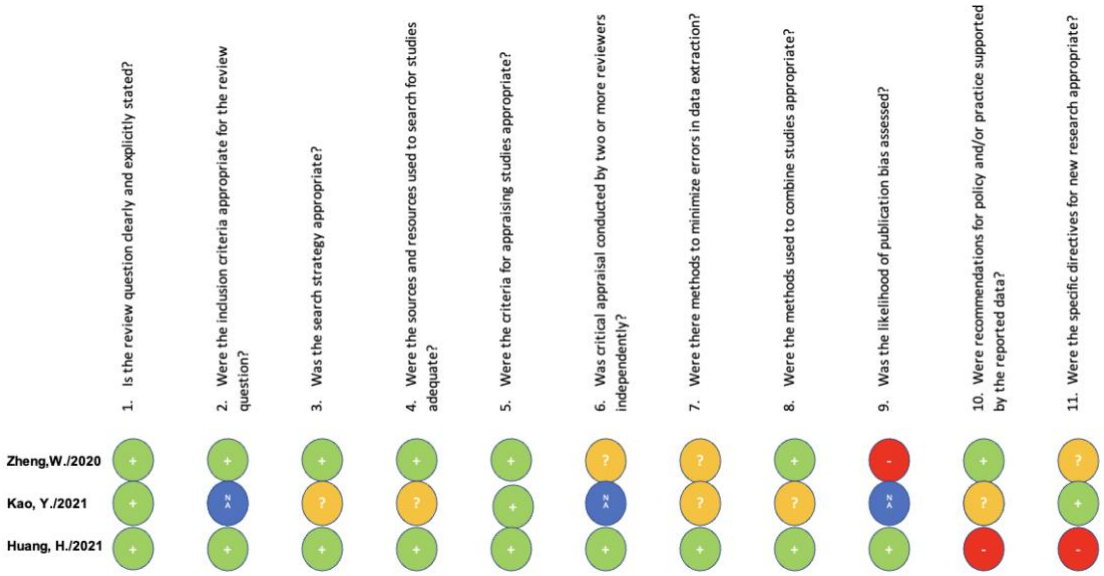
%- percentage; g- G force; mL – milliliter; NS- Not specified; PRP – platelet-rich plasma rpm – rotation per minute

Table 8.2 – General considerations PRP protocols

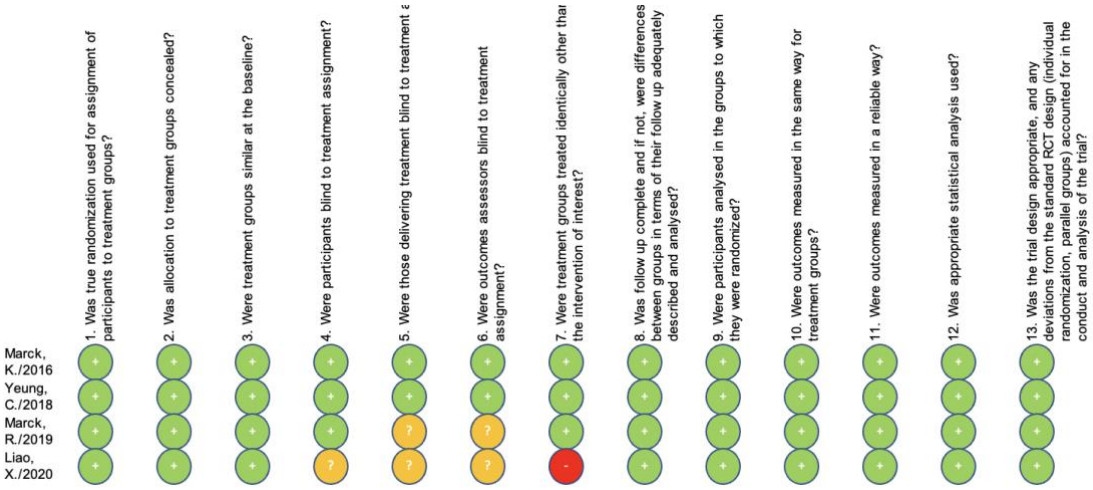
1st author/Year	PRP protocol	Type of anticoagulant	Activation	Centrifugation Protocol
Chen, Z./ 2020	Protocol to meta-analysis			
Karakol, P./ 2021	After collecting the blood, it was transferred to anticoagulated tubes. It was performed the first centrifugation. The plasma layer was transferred and was performed a second centrifugation	Citrate dextrose solution	Thrombin	1st Centrifugation: 1200g for 5min 2nd Centrifugation: 1200g for 10min
Marck, R./ 2014	Systematic Review			
Achora, S./ 2014	Literature Review			
Sharun, K./ 2020	Letter to Editor			
Sharun, K./2021	Letter to Editor			
van der Bijl, I. /2021	Opinion Paper			

g – G force; min. - minute

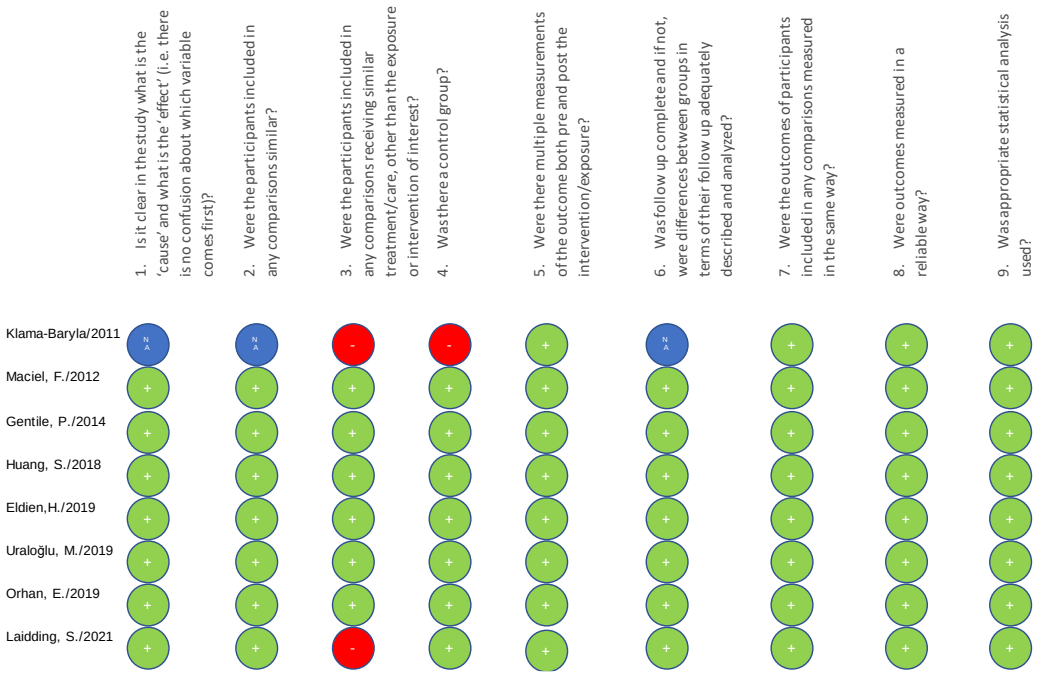
Annex III - Quality Assessments



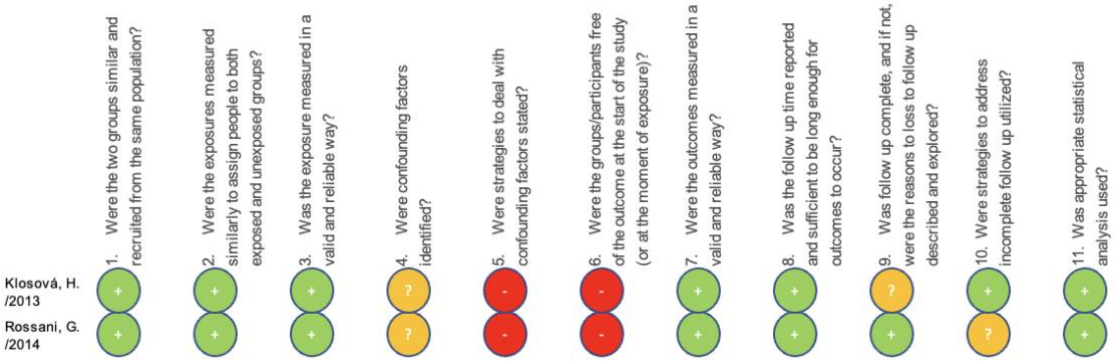
Graph 1 – Quality Assessment of Dermal Systematic Reviews



Graph 2 - Quality Assessment of Dermal Randomized Control Trials Articles.

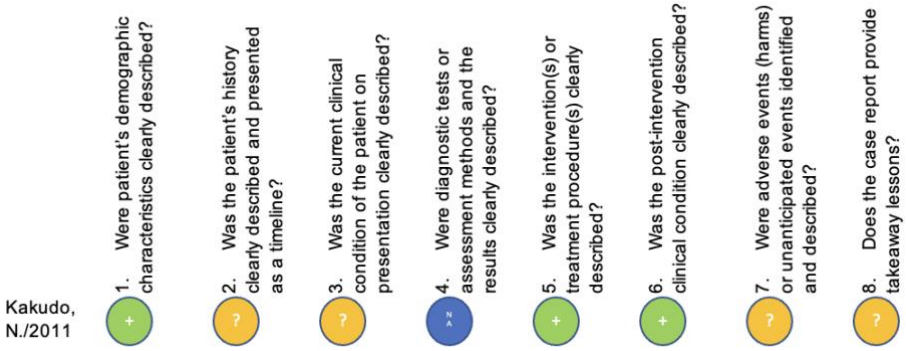


Graph 3 – Quality Assessment of Dermal Quasi-Experimental Articles.

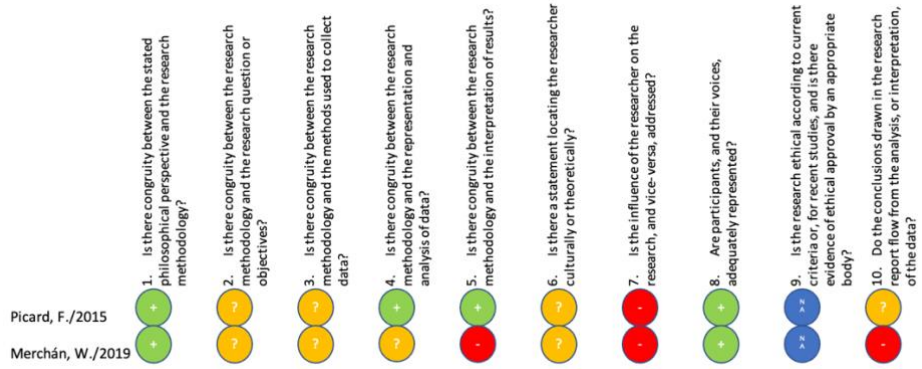


Graph 4 – Quality Assessment of Dermal Cohort Articles.

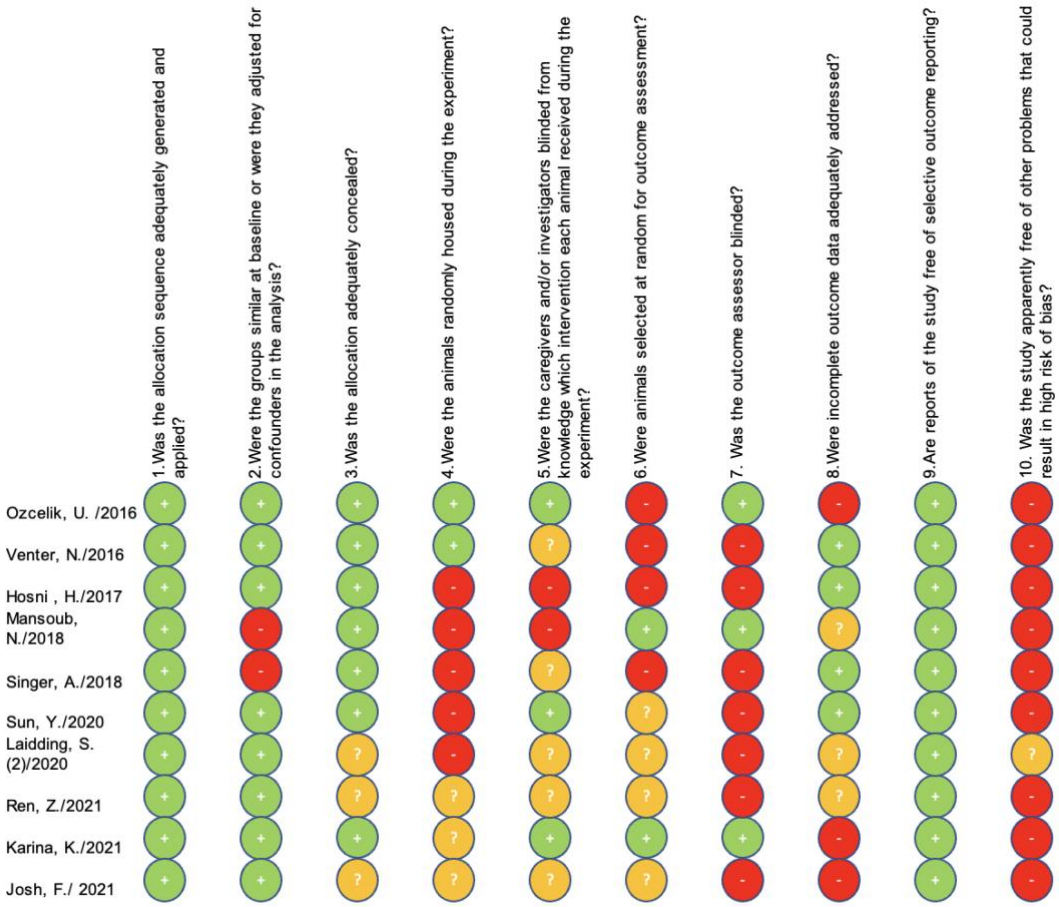
Graph 5 – Quality Assessment of Dermal Case Series Articles.



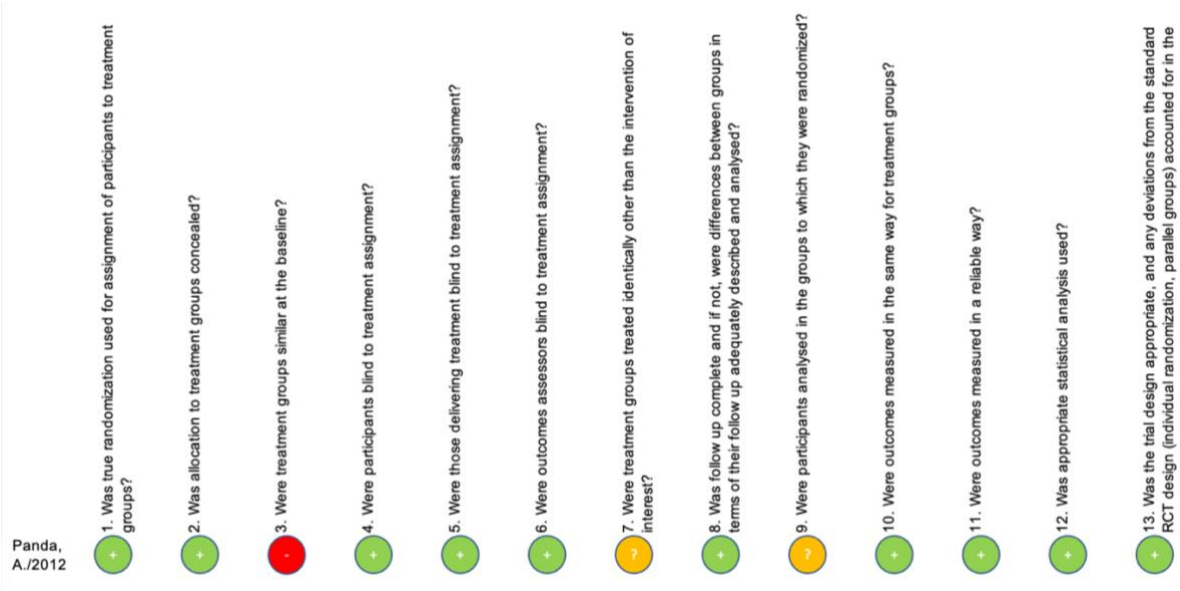
Graph 6 – Quality Assessment of Dermal Case Reports.



Graph 7 – Quality Assessment of Dermal Literature Reviews.

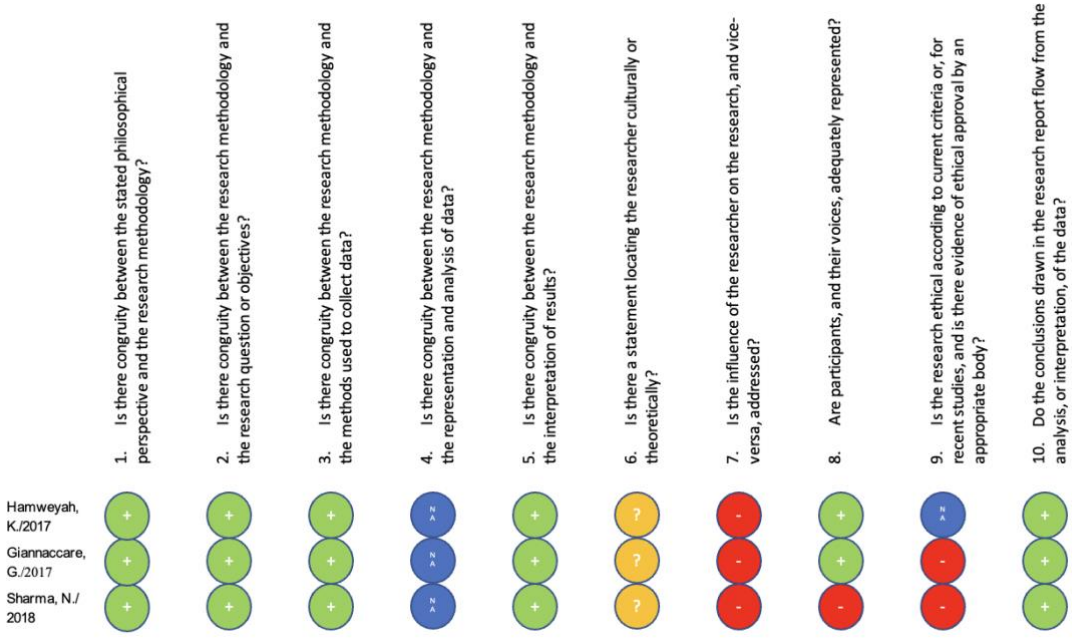


Graph 8 – Quality Assessment of Dermal Animal Experiments.

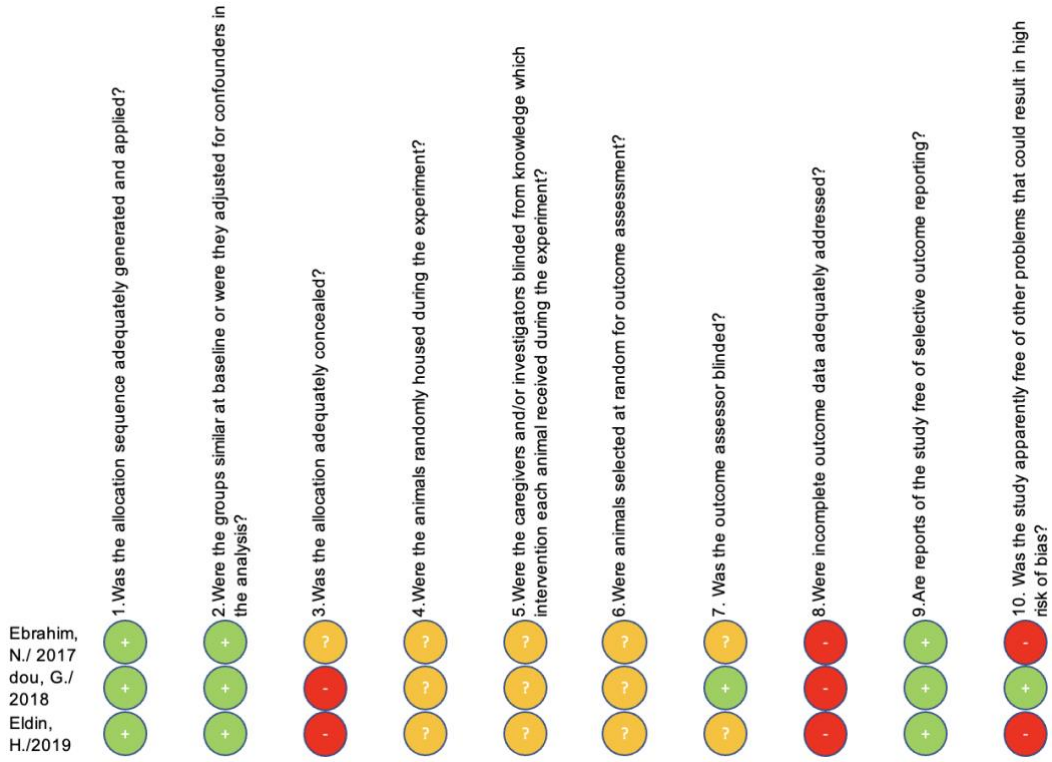


Graph 9 – Quality Assessment of Ocular Randomized Control Trial Article.

Graph 10 – Quality Assessment of Ocular Quasi-Experimental Article.

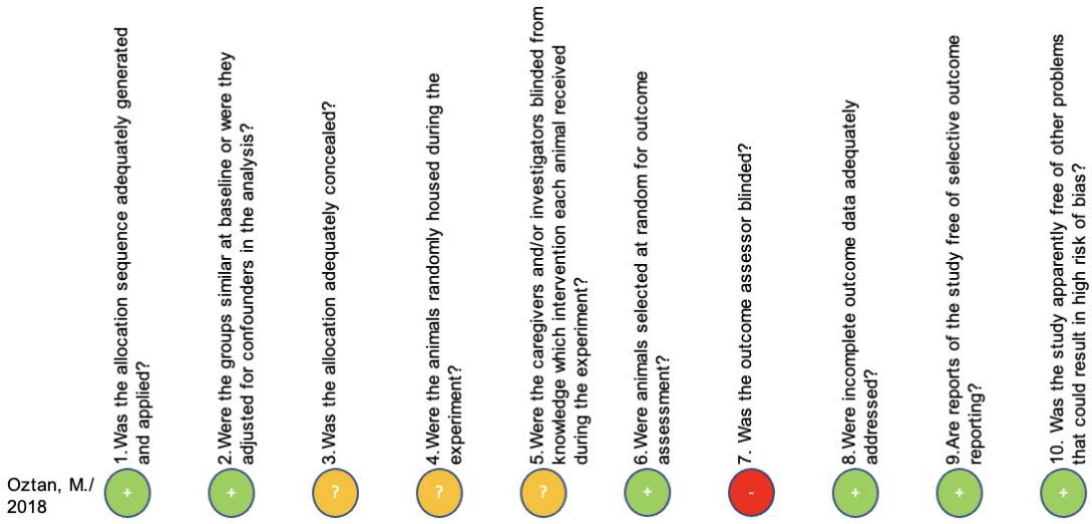


Graph 11 – Quality Assessment of Ocular Literature Reviews.

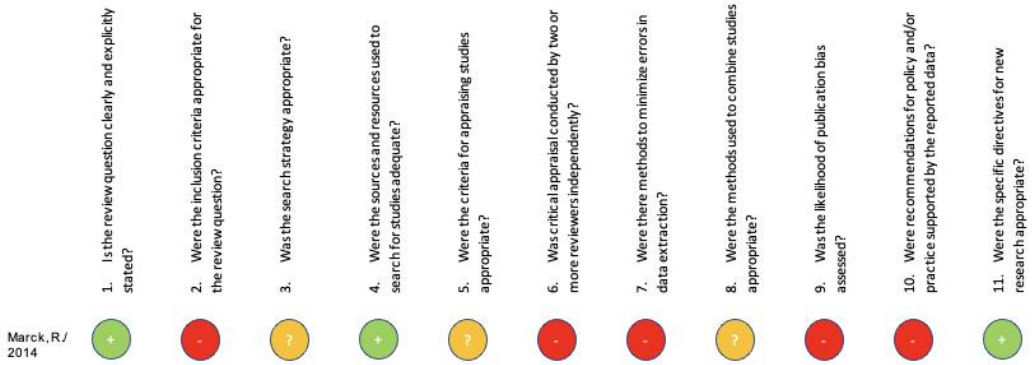


Graph 12 – Quality Assessment of Ocular Animal Experiments.

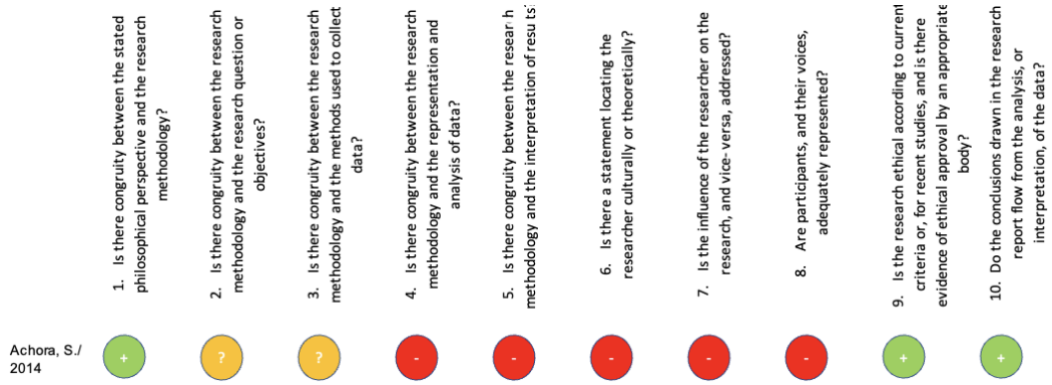
Graph 13 – Quality Assessment of Oesophageal Quasi-Experimental Article.



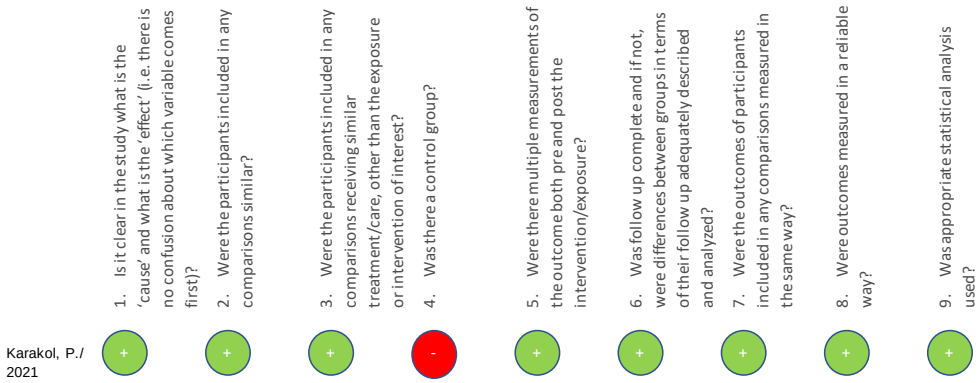
Graph 14 – Quality assessment of Oesophageal Animal Experimental Article.



Graph 15- Quality Assessment of General Considerations Systematic reviews



Graph 16– Quality assessment of General Considerations literature review.



Graph 17– Quality assessment of General Considerations Quasi-Experimental Studies.

Graph 18 -Quality Assessment of General Considerations Opinion Texts.