

Comparative study of different anticoagulants and coagulants in the evaluation of clinical application of platelet-rich plasma (PRP) standardization

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Abstract

Background: Platelet-rich plasma (PRP) is a blood product obtained by concentration and separation of whole blood.

Aim: We aimed to evaluate anticoagulants and coagulants' efficacy in platelet-rich plasma preparation, aiming to standardize methods for optimized clinical applications by examining their impact on platelet yield and quality.

Materials and methods: We searched Electronic databases (PubMed, Web of Science, and the Cochrane library) with the following keywords: Anticoagulants, Coagulants, Clinical application, platelet-rich plasma standardization. Two authors independently screened the titles and abstracts of the search results. After deleting duplicate articles, the remaining studies were analyzed using inclusion criteria, obtaining full texts for assessment, and synthesizing the results.

Main findings: EDTA anticoagulation treatments yielded the highest counts of platelets. WBCs were not contaminated in all of the pure-PRP samples, and a significant difference in the WBC counts was observed between ACD-A and heparin-anti-coagulated samples. Over all pooled subgroup analysis comparing heparin and EDTA on RBCs MDs 95%CI: 0.008[0.012, 0.028], subgroup WBCs 0.066[0.97, 1.11] and platelets 522.09[0.26, 0.35], heterogeneity is detected among all pooled studies. **Conclusion:** Our meta-analysis highlights EDTA as the most effective anticoagulant for maximizing platelet counts in PRP preparation, with minimal WBC contamination. The superior platelet yield associated with EDTA underscores its potential as the anticoagulant of choice for PRP preparation, particularly in therapeutic applications requiring high platelet concentrations. These findings can guide clinicians in selecting anticoagulants to optimize PRP efficacy, thereby improving patient outcomes in regenerative medicine and other fields.

Key words: Anticoagulants, Coagulants, Clinical application, platelet-rich plasma (PRP) standardization

Introduction

Platelet-rich plasma (PRP) is a blood product obtained by concentration and separation of whole blood (1). It is characterized by platelet-rich plasma (PRP) platelet concentration which is more than four times higher than the whole blood platelets, containing high concentration of growth factors, such as platelet-derived growth factor PDGF, transforming growth factor TGF, Insulin-like growth factor IGF, vascular endothelial growth factor VEGF, epidermal growth factor EGF, epithelial cell growth factor for ECGF and IL-1 for bone tissue and soft tissue regeneration and repair (2).

Potential clinical applications of PRP include the repair of tendon and cartilage defects. PRP has a potential therapeutic effect on the repair of bone, muscle, ligament and tendon injuries. PRP injection can be used as an adjuvant treatment for cartilage defect surgery and a conservative treatment plan for OA (3).

Evidence from basic research, preclinical studies, and clinical trials has confirmed the effectiveness of PRP application in the repair of bone tissue injury. There have been studies demonstrating the effectiveness and safety of PRP in the treatment of joint degeneration (4).

However, there were few high-quality clinical studies and the sample size was small. Many clinical applications of PRP in the treatment of joint degeneration can easily lead to unstable therapeutic and a lack of standardized protocols for PRP in clinical applications (5). At present, it is difficult to control the biological effect of PRP because of the lack of evaluation on PRP quality. Concentrations of platelets, anticoagulants, coagulants, growth factor levels, PH value and hypoxia as well as leukocyte-rich may influence the quality components of PRP biological effects (6).

In a previous experiment of, **Wang KT et al.** (7) has reported that PRP with different concentrations of platelets exerted different effects on proliferation and differentiation of BMSCs, which indicated that preparing appropriate PRP may be a precise and efficient strategy for improving BMSC-based tissue regeneration.

In the current meta-analysis study, we aimed to conduct a comparative analysis of different anticoagulants and coagulants to evaluate their efficacy in platelet-rich plasma (PRP) preparation. By examining their impact on platelet yield, WBC contamination, and PRP quality, this meta-analysis seeks to provide evidence-based insights to standardize PRP preparation methods for optimized clinical applications.

Materials and methods

This systematic review and meta-analysis was conducted on 3 studies according to the guidelines by the Cochrane Collaboration reporting followed the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-analyses).

Search strategy

We searched Electronic databases (PubMed, Web of Science, and the Cochrane library) with the following keywords: Anticoagulants, Coagulants, Clinical application, platelet-rich plasma (PRP) standardization. Two authors independently screened the titles and abstracts of the search results. After deleting duplicate articles, the resulting studies were reviewed against the inclusion criteria. The full texts of all studies of possible relevance were obtained for assessment against the stated inclusion criteria. Only studies that fulfilled the criteria were further assessed to synthesize the results. The reference list of the included articles was assessed for any studies that fulfilled the inclusion criteria.

Inclusion criteria: Full text, English language, randomized controlled trials (RCTs), cohort, cross sectional, mixed-method articles were included if they compared analysis of different anticoagulants and coagulants to evaluate their efficacy in platelet-rich plasma (PRP) preparation.

Exclusion criteria: Investigations including reviews, books, or comments, studies without a clear focus on platelet-rich plasma, non-English articles, do not include evaluation of clinical application of platelet-rich plasma (PRP) standardization.

Risk of bias assessment: Our included clinical trials were assessed using ROB1 tool all of our studies were low risk except Aizawa et al., study which shows high risk regarding detection bias domain.

Data extraction: Two writers performed the information extraction independently. The collected information involved the author's name, publication year, location of research, research design, outcome measures and key findings. To maintain reliability and consistency, both reviewers operated independently during the extraction procedure, & any differences have been resolved by consensus & discussion.

Results

Our search across different databases yielded 545 articles upon removal of duplicate studies we have 200 articles submitted for title and abstract screening, 15 distinct articles were examined for full text, finally we have three articles met our inclusion criteria. (Figure 1)

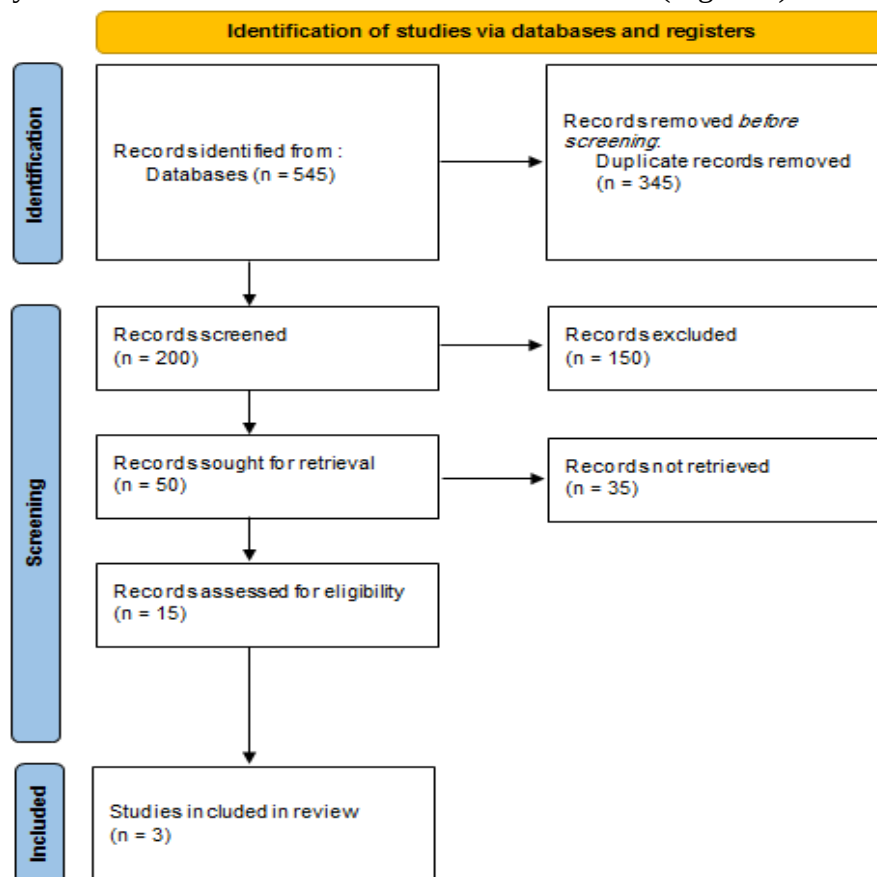


Figure 1: PRISMA flow chart for study selection process.

Baseline characteristics of the included population

Our three clinical studies included Blood samples from 45 humans and 30 Rabbits, geographical distribution included China and Niigata, mean age of included population in the studies is 26 years. (Table 1)

Table 1: Represents baseline characteristics of the included population.

Study ID	Study design	Site	Age (Y)	Sample Size	Study Population
Aizawa (8)	RCTs	Niigata	26 to 72	15	Human
Lei (9)	RCTs	China	26	30 (10M/20F)	Human
Zhang (10)	Clinical science	China	NR	30	Rabbit

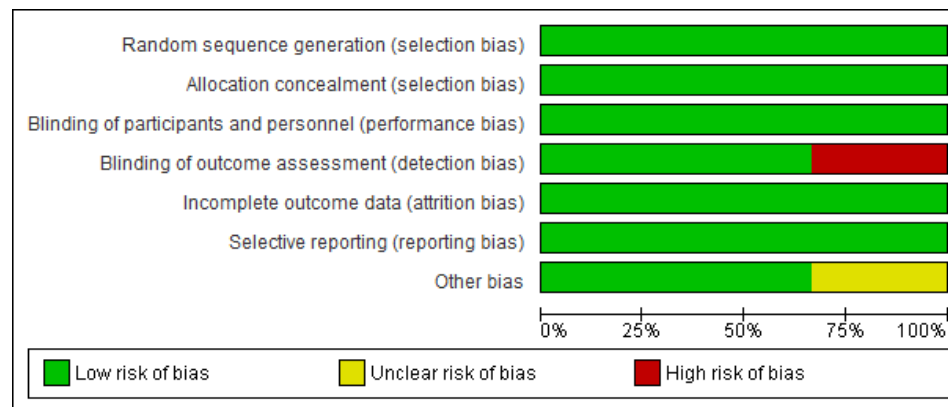


Figure (2): Risk of bias graph.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Aizawa 2020	+	+	+	-	+	+	+
Lei 2009	+	+	+	+	+	+	?
Zhang 2019	+	+	+	+	+	+	+

Table (2): Risk of bias summary.

Outcomes

EDTA anticoagulation treatments yielded the highest counts of platelets. WBCs were not contaminated in all of the pure-PRP samples, and a significant difference in the WBC counts was observed between ACD-A and heparin-anti-coagulated samples. Over all pooled subgroup analysis comparing heparin and EDTA on RBCs MDs 95%CI: 0.008[0.012, 0.028], subgroup WBCs 0.066[0.97,1.11] and platelets 522.09[0.26, 0.35], heterogeneity is detected among all pooled studies. (Figure 3)

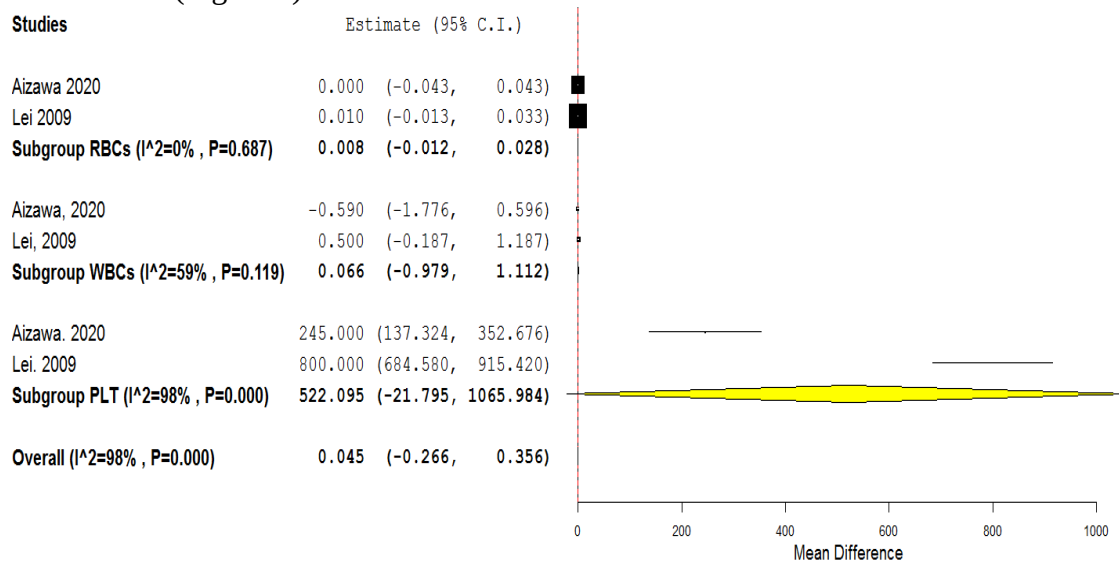


Figure (3): The forest plot comparing Heparin and EDTA anticoagulants on whole Blood components.

Discussion

The development and clinical use of platelet-rich plasma (PRP) is undoubtedly an outstanding innovation and it has markedly impacted regenerative medicine. PRP has been increasingly used in various fields of regenerative therapy to date since the first report appeared in 1998 (11). In parallel, several in-vitro studies have provided evidence to support its clinical application (12, 13). Our meta-analysis demonstrated that EDTA anticoagulation yielded the highest platelet counts among the tested anticoagulants, with no WBC contamination observed in pure PRP samples. Significant differences in WBC counts were noted between ACD-A and heparin-treated samples, and pooled subgroup analysis highlighted variability in RBC, subgroup WBCs and platelet outcomes. These findings emphasize the influence of anticoagulant choice on PRP composition. Consistent with our findings, study by **Zhang N et al., (10)** revealed that anticoagulants had an effect on the structure and function of platelets. Different anticoagulants caused different degrees of lysis and spontaneous activation of platelets, which lead to different quality of PRP. Compared with heparin sodium (HS) and sodium citrate (SC), EDTA could maintain the structural integrity of platelets, reduce their spontaneous activation, and increase the release of PRP growth factors for a longer period of time, thus ensuring the biomass of PRP.

Additionally, **Aizawa H et al., (8)** examined the effects of three anticoagulants, ACD-A, EDTA, and heparin, on pure-PRP quality, i.e., platelet and growth factor contents. They demonstrated that EDTA is more efficient than ACD-A (or citrate) for inhibiting platelet aggregation and subsequent

platelet collection in PRP. The preparation of well-suspended and homogenous PRP is much easier with EDTA than with other anticoagulants, which should be considered to be an advantage of EDTA use over other anticoagulants.

However, study of, **Lei H et al., (9)** suggested alternative anticoagulants such as ACD and CTAD may also be viable for PRP preparation. These anticoagulants were shown to maintain platelet structural integrity and enhance the release of growth factors such as TGF- β 1, which promote cell proliferation. While our findings highlight EDTA as the optimal choice, these results indicate that ACD and CTAD could be suitable alternatives depending on specific clinical requirements.

The superior performance of ACD in some studies is attributed to its glucose and low citrate concentrations, which support platelet energy metabolism and mitigate pH-related viability loss during storage **(14-17)**.

On the other hand, **Golański J et al., (18)** reported that heparin is not used in coagulation studies because it activates platelets in vitro, which potentially interferes with the determination of hemostatic parameters. However, heparin is usually used in collecting whole blood for PRP production **(19, 20)**.

Strengths of the Analysis

A key strength of our analysis lies in its adherence to the PRISMA framework, ensuring a systematic and transparent review process. Additionally, the inclusion of both human and animal models provided a comprehensive perspective on PRP preparation. The low risk of bias across most included studies further enhances the reliability of our findings.

Limitations of the study

Despite its strengths, our analysis was limited by the small sample size and geographical concentration of studies, which may affect the generalizability of findings. Furthermore, the high detection bias observed in the **Aizawa (8)** study highlights the need for improved reporting in PRP research. Variability in preparation techniques among studies also contributed to heterogeneity, potentially influencing outcome measures.

Conclusion

Our meta-analysis highlights EDTA as the most effective anticoagulant for maximizing platelet counts in PRP preparation, with minimal WBC contamination. The superior platelet yield associated with EDTA underscores its potential as the anticoagulant of choice for PRP preparation, particularly in therapeutic applications requiring high platelet concentrations. These findings can guide clinicians in selecting anticoagulants to optimize PRP efficacy, thereby improving patient outcomes in regenerative medicine and other fields.

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