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Comparison of treatment efficacy between 100% platelet-rich plasma and 100% serum eye drops in moderate-to-severe dry eye disease: a randomised controlled trial protocol

Passara Jongkhajornpong ,[1,2] Pawin Numthavaj,[2] Thunyarat Anothaisintawee ,[2,3] Kaevalin Lekhanont,[1] Gareth McKay ,[4] John Attia ,[5] Ammarin Thakkinstian[2]

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For numbered affiliations see end of article.

Correspondence to
Dr Thunyarat Anothaisintawee; Thunyarat.ano@mahidol.ac.th and
Dr Pawin Numthavaj; pawin.num@mahidol.edu

ABSTRACT

Introduction Dry eye disease (DED) is a common eye problem. Although the disease is not fatal, it substantially reduces quality of life and creates a high economic burden, especially in patients with moderate-to-severe DED. Several biological tear substitutes (eg, autologous serum (AS), autologous platelet-rich plasma (APRP) and autologous platelet lysate) could effectively improve dry eyes. However, evidence on their comparative efficacy is controversial. This study aims to compare the efficacy of 100% APRP with 100% AS eye drops in patients with moderate-to-severe DED.

Methods and analysis The study is a single-centre, double-blinded randomised, parallel, non-inferiority trial. One hundred and thirty patients with moderate-to-severe DED, aged 18–70 years will be recruited from outpatient clinic, Department of Ophthalmology, Ramathibodi Hospital, Bangkok from February 2021 to January 2023. Patients will be randomised to receive either 100% APRP or 100% AS eye drops (1:1 ratio) for 4 weeks. The primary outcomes are ocular surface disease index (OSDI) and ocular surface staining (OSS) evaluated using the Oxford scale. Secondary outcomes are fluorescein break-up time, Schirmer's I test, meibomian gland parameters and adverse events. Other measured outcomes include best-corrected visual acuity, intraocular pressure and compliance.

Ethics and dissemination The study protocol and any supplements used in conducting this trial have been approved by the Ethics Committee of Faculty of Medicine, Ramathibodi Hospital, Mahidol University (MURA2020/1930). Informed consent will be obtained from all patients before study entry. Results will be presented in peer-reviewed journals and international conferences.

Trial registration number NCT04683796.

Strengths and limitations of this study

- First randomised controlled trial that directly compares autologous serum with platelet-rich plasma eye drops.
- Active control arm will be used to avoid unethical issue and avoid breaking blinding.
- A double-blinded, parallel, non-inferiority trial design.
- Apart from intention-to-treat analysis and per-protocol analysis, a counterfactual approach using instrumental variable analysis will be applied to estimate actual treatment effects received.
- Only short-term efficacy of biological tear substitutes will be evaluated.

BACKGROUND AND RATIONALE

Dry eye disease (DED) is a multifactorial ocular surface disease characterised by an imbalance of the tear film homeostasis accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular inflammation and neurosensory abnormalities play key roles.[1] The prevalence of DED increases by age, ranging from 5% to 50%.[2 3] DED costs have been estimated at US$3.84 billion from a payer's perspective to as high as over 50 billion from a societal perspective.[4]

Patients with moderate-to-severe DED account for approximately 30% or over of all DED patients,[5] in which initial treatments, such as lifestyle modification and artificial tears were unsuccessful.[6] Biological tear substitutes derived from blood products have demonstrated good efficacy in reducing dry eye symptoms and ocular surface staining (OSS) in patients with moderate-to-severe DED.[7–9] These products, including autologous serum (AS) and platelet-rich plasma (PRP), contain several bioactive ingredients (eg, epidermal growth factor; insulin-like growth factor; transforming growth factor beta; and platelet-derived growth factor) that are vital for maintaining homeostasis of the

ocular surface similar to natural tears.[10] AS is prepared from clotted blood[11] in contrast to PRP, which is prepared from unclotted blood.[12] Several randomised controlled trials (RCT) have shown that treatment of patients with moderate-to-severe DED with either AS and autologous PRP (APRP) significantly improve the ocular surface disease index (OSDI) score, OSS, and tear break-up time (TBUT) compared with artificial tears.[13–16] However, direct comparisons of these two agents is lacking. This RCT has been developed with the aim of comparing dry eye symptoms and OSS between PRP and AS in patients with moderate-to-severe DED. In addition, other clinical outcomes, such as fluorescein break-up time (FBUT), Schirmer's test (ST), meibomian gland parameters, and adverse events (AEs) will be compared.

METHODS

Study design

This study is a randomised, double-blinded, parallel, non-inferiority trial of APRP and AS in patients with moderate-to-severe DED. This protocol conforms with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and the RCT will comply with the Consolidated Standards of Reporting Trials (CONSORT) statement, which has been registered in ClinicalTrial.gov.

Participants

Patients with at least one eye diagnosed with DED according to Tear Film & Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II)[17] will be invited to participate in the study if they meet the following eligibility criteria.

Inclusion criteria

- Aged 18–70 years.
- Have OSDI scores ≥23 or Oxford staining grade ≥2.
- Do not have following conditions:
  - Uncontrolled systemic diseases, active infection or advanced cancer.
  - Pregnant or nursing women.
- Have not recently used the following medications/interventions/surgery:
  - Anticoagulants or anti-platelets.
  - Topical undiluted blood products within 3 months.
  - Punctal plug or contact lenses.
  - Ocular surgery within 6 months.
- Do not have active ocular infection/inflammation, abnormal eyelid function or severe meibomian gland dysfunction (MGD stage 4).
- Have no contraindication for blood donation:
  - Positive HIV, hepatitis B or C, or syphilis.
  - Anaemia (haemoglobin <110 g/L) or platelet concentration <150*10^9/L.
- Being able to stop current dry eye treatment for 48 hours before staring trial intervention.
- Willing to comply with the 4-week study protocol and provide informed consent.

Patients will be withdrawn from the study by the research team at any follow-up visit based on the two following criteria: (1) intolerable ocular adverse effects or allergic reactions from topical eye drops, and (2) worsening OSDI score of ≥10 points or worsening Oxford scale of ≥2 grades.

Recruitment procedure

The study flow is outlined in figure 1. Patients visiting the outpatient clinic at the Ophthalmology Department, Ramathibodi Hospital, between February 2021 and January 2023 will be invited to participate in the study. The RCT protocol has been communicated to all residents, clinical/research fellows, staffs and nurses who will be involved in patient recruitment. Training on the patient recruitment procedure will be provided before enrolment commences.

Study information will be provided to all patients in a quiet room. They will be encouraged to take at least 30 min to consider the information provided before deciding on study participation. If they agree to participate, the primary investigators (PJ and KL) will undertake eye examination to evaluate eligibility criteria, and phlebotomy will be undertaken to screen for blood diseases. Finally, written informed consent will be obtained.

Randomisation and allocation concealment

Patients will be randomly allocated to receive either 100% APRP or 100% AS (the same treatment for both eyes). Block randomisation with varying block sizes of 4–8 and a ratio of 1:1 will be generated by an independent statistician, using STATA V.16. All randomisation sequences will be sealed in opaque envelopes by the same person. Sealed opaque envelopes will be kept in a locker at the outpatient clinic and will be opened just before phlebotomy under the responsibility of an independent research nurse.

Blinding

Treatment package will be labelled as randomisation number, thus, the ophthalmologists (PJ and KL) and clinical and research staffs involved in the outcome assessments will be blinded to the treatment allocation. In addition, participants will be blinded to the treatments they receive. Finally, data analysts will be blinded during the analysis process.

Interventions

There are two interventions of interest, APRP and AS. Patients will be asked to cease current artificial tears and topical medication for DED (ie, topical secretagogues, cyclosporine A and steroids) for at least 48 hours before their first visit (washout period); only artificial tears (dextran 70 0.1%, hydroxypropyl methylcellulose 0.3%; Tear Naturale Free) provided by the research team will be allowed. At the initial appointment, the patients will undergo ophthalmic assessment in the following order: best-corrected visual acuity (BCVA) and intraocular pressure by a nurse, OSDI score, FBUT, OSS (Oxford scale), ST, and meibomian quality and expressibility by

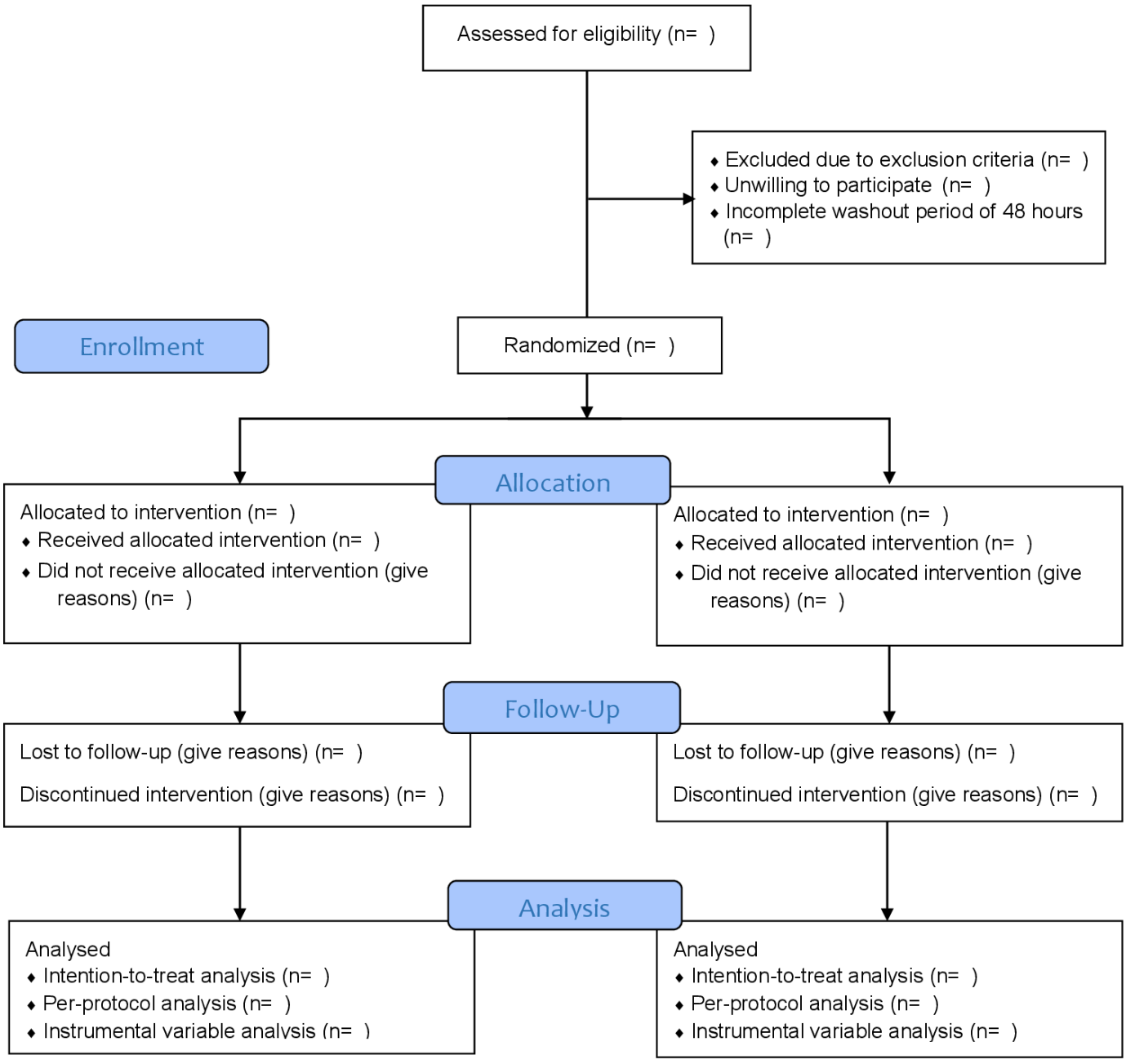

Figure 1 Consolidated Standards of Reporting Trials flow diagram of the study.

ophthalmologists (PJ and KL). Phlebotomy will require the collection of three 50 mL sterile centrifuge tubes (36 mL/tube). The patients' blood will be processed according to their allocated treatments.

For 100% APRP, the preparation has been performed according to the well-established protocol originally described by Alio *et al*.[18–20] Two important points suggested in the original protocol were as follows: (1) the choice of speed and time of centrifugation could vary depending on the characteristics of each centrifuge and the size of tubes used, and (2) a haemocytometer is needed to quantify the number of platelets in whole blood after the centrifugation in order to obtain the maximum enrichment.[19] Due to limited equipment in our laboratory, the centrifugation speed, time and temperature in our protocol are slightly adjusted to achieve the optimal platelet enrichment based on their recommendations. Briefly, the collection tubes will contain 4 mL of 3.2% sodium citrate for anticoagulation (ratio of blood to sodium citrate=9:1). Tubes will be centrifuged at 350 *g* for 10 min at 20°C in a Sorvall Legend Mach 1.6R benchtop centrifuge (Kendro Laboratory Products, North Carolina, USA). Ninety per cent of plasma obtained after centrifugation will be collected in a sterile manner under a laminar air flow hood and used as the final product.[19] With this APRP preparation, the final product is expected to yield a 1.5–2.5-fold enrichment of platelets compared with whole blood.

For 100% AS, the collection tubes will be left standing in an upright position for 1–2 hours to enable blood clot formation at room temperature (18°C–25°C). The

tubes will be centrifuged at 3000 *g* for 15 min at 20°C. The supernatant serum will be aseptically transferred into a sterile syringe to enable filtration through a 0.2 µm pore size membrane filter under a laminar air flow hood.

The final blood products will be transferred into identical opaque eye drop bottles to protect the products from ultraviolet light (1.5 mL/bottle, 30 bottles/patient), labelled name, hospital number, dated and sealed. The leftover final blood products will be collected and stored at −80°C for future use in ancillary studies. Patients will be instructed to instil the assigned eye drops in both eyes, every 2 hours between 08:00 and 22:00 (eight times per day). Patients will be required to store the currently used bottle at 4°C for 24 hours (one bottle per day) and the remaining bottles at −20°C in a freezer until day of use. All participants will be allowed to use the artificial tears provided by the research team at least 30 min after the administration of 100% AS or 100% APRP if severe dry eye or irritation is experienced during the instillation intervals. Participants will be asked to record the number of eye drops (both intervention and artificial tears) administered each day and to return their report along with used eye drop bottles to the research team at 2 and 4 weeks post intervention.

Data collection procedure

Data will be collected at baseline, 2 and 4 weeks post treatment. All dry eye parameters will be recorded by two cornea specialists (PJ and KL) using the same measurement standard. The timeline of data collection is presented in table 1.

Table 1 Overview of data collection

Outcomes	Baseline	2 weeks	4 weeks
Baseline characteristics			
Age	X		
Gender	X		
Education level	X		
Occupation	X		
Smoking status	X		
Systemic disease	X		
Ocular comorbidities	X		
Previous ocular surgery	X		
Current medications (systemic and topical)	X		
Ocular surface disease index	X	X	X
Ocular examination			
Best corrected visual acuity	X		X
Intraocular pressure	X		X
Fluorescein break-up time	X	X	X
Ocular surface staining (Oxford scale)	X	X	X
Schirmer's test I	X	X	X
Meibomian gland quality and expressibility	X		X
Adverse events	X	X	X
Compliance	X	X	X

Outcomes

Primary outcomes

The primary outcomes are OSDI and OSS, evaluated using the Oxford scale measured at 4 weeks post treatment. The OSDI is a patient-reported outcome questionnaire assessing ocular symptoms and ability of function related to chronic DED.[21] The questionnaire comprises 12 questions divided into 3 domains, including ocular symptoms (5 questions), vision-related function (4 questions) and environmental triggers (3 questions). Each item is graded with a total score ranging between 0 and 100 and classified as normal (0–12 points), mild (13–22 points), moderate (23–32 points) and severe (33–100).

The Oxford scale will assess the total OSS and the corneal and conjunctival fluorescein staining pattern will be graded as zero to five in accordance with previously published criteria.[22] This scale has a good correlation with DED severity, with a higher score indicative of more severe DED. Before assessment, a fluorescein-impregnated strip will be dampened by a single drop of saline gently placed on the lower tarsal conjunctiva.[23] Staining will be recorded under cobalt blue illumination at 2 min after dye instillation and several blinks.

Secondary outcomes

Secondary outcomes include FBUT, ST, meibum quality and expressibility, and AE.

FBUT will measure the stability of the tear film.[17 22] The time period from the complete opening of the eyelid to the first tear break up will be recorded on 3 occasions and a mean value used.

ST will measure tear volume of both basic and reflex tears using a strip of filter paper 35 mm long and 5 mm wide without anaesthesia.[17] The strip will be folded and placed in the lateral canthus away from the cornea. The wet strip length at 5 min post placement will be recorded in millimetres, with higher values indicative of less severe DED.

Meibum quality and expressibility at 4 weeks after treatment will be assessed by applying pressure on each of the eight glands of the central one-third of the lower lid on a scale of 0–3 for each gland: 0, clear; 1, cloudy; 2, cloudy with debris (granular); and 3, thick, like toothpaste (total score range, 0–24). Expressibility is assessed on a scale of 0–3 in five glands in the lower or upper lid, according to the number of glands expressible: 0, all glands; 1, three to four glands; 2, one to two glands; and 3, no glands.[24] A higher score indicates more severe MGD.

AE is defined as any undesirable experience, which occurs during eye drop application or post treatment. It is classified as severe AE (SAE) if it causes hospitalisation, is life-threatening or leads to permanent disability. All AE will be recorded across the study period following discussion with the participants about potential AEs using both topical biological tear substitutes. The following additional covariables will also be assessed:

Demographic data, including gender, age, educational level (ie, university, high school or pre-high school), occupation, smoking status, systemic disease (eg, rheumatic disease, diabetes, hypertension and thyroid disease), ocular comorbidities (eg, glaucoma, cataract, limbal stem cell deficiency and pterygium), previous ocular surgery (type and date of surgery) and current medications (systemic and topical drugs).

BCVA will be measured in decimal units at baseline and 4 weeks post treatment using Snellen charts.

The number of eye drops administered per day will be recorded to determine participant compliance. Non-compliance will be defined whereby participants miss more than 30% of the expected total applications of the assigned treatment per day (>2 drops/day).[16] The number of artificial tears used per day will also be recorded.

Data management

Written case record forms (CRFs) of all participants will be checked for completion by the research nurse or primary investigator at the outpatient clinic on the same day of recording. Personal information of patients will be kept separate from the outcome data set and stored securely to protect confidentiality. All data will be entered by two independent staff members of the research team in a database created using EpiData V. 3.1. Any unclear, missing or nonsensical information will be cross-checked against the CRFs. All data will be automatically backed up using Google Drive to mitigate potential data loss.

Data monitoring

The study does not require a formal data and safety monitoring board as both treatment arms are widely used for dry eye patient management with very low reports of AE.[25 26] Any unexpected SAE will be managed under the responsibility of the trial committee, including all authors of this protocol. The committee will also monitor recruitment and retention rates, and any protocol violations.

Patient and public involvement

No patients were involved in protocol development. The burden of the intervention was assessed by the investigator team. The results of the study will be submitted to a peer-reviewed academic journal and disseminated to all participants after the research has ended via the letters or emails.

Sample size calculation

The sample size estimation is based on a non-inferiority trial comparing OSDI between 100% APRP and 100% AS. The mean OSDI and SD of 20.89 (6.15) in the 100% AS group was estimated by pooling data from four previous RCTs.[13–15 27] The 100% APRP will be judged non-inferior to 100% AS if the OSDI does not exceed 20% of the AS OSDI, that is, a non-inferiority margin of 4.18 or lower. To detect this difference, given a power of 0.9 and a one-sided alpha of 0.025, 46 patients per group are required. Taking account of loss to follow-up of 30%, a total sample size of 130 participants is estimated. The choice of non-inferiority margin is guided by the minimal clinically important difference of OSDI of 7–9.9, as reported by Miller *et al*.[28]

Stopping rule

The study will end when the target sample size of 130 participants is reached or at the conclusion of the 24-month enrolment period, if the proposed sample size cannot be reached.

Statistical analysis

Descriptive statistics will define baseline characteristics and participant outcomes between both intervention groups. Mean (SD) or median and range will be used for continuous data (ie, age, BCVA, OSDI score, FBUT, ST and meibum quality), and frequencies and percentages for categorical and ordinal data (ie, gender, education, systemic disease, current medication, Oxford scale, meibum expressibility and AEs). Imputation will be performed if missing data from any of the primary outcomes (ie, OSDI and Oxford score) exceeds 10%, under the assumption that data are missing at random using a multiple imputation with chained equations (MICE) method.[29 30] Truncated and logistic regression will be used for modelling continuous and dichotomous outcomes, respectively.

Between-group comparisons of mean outcome values (ie, OSDI score, FBUT and ST) at the 4-week follow-up will be analysed using linear mixed-effects models with participants considered as random effects, and visit (2 and 4 weeks) and treatment arm (100% AS and 100% APRP) as fixed effects. For the ordinal Oxford scale, ordered logistic regression accounting for repeated measurement will be used. The occurrence of AEs will be compared between both groups at the 4-week follow-up using Poisson regression models or negative binomial regression models. If randomisation fails to equally distribute proportions of baseline characteristics between both groups, the imbalanced factors will be fitted for adjustment within the model in sensitivity analyses. Outcomes for the Oxford scale, FBUT and ST from all eligible eyes will be included in the analysis accounting for within-subject correlations.[31 32]

To assess non-inferiority of the OSDI score, 95% CIs for the mean difference between both treatment groups (APRP group–AS group) will be estimated. We will conclude the APRP is non-inferiority relative to AS if the upper limit of the mean difference does not exceed the prespecified margin for the OSDI score of 7.

The main statistical analyses will be performed according to the intention-to-treat principle, which includes randomisation of all participants, regardless of compliance, actual treatment received, subsequent withdrawal of treatment, and/or deviation from the protocol, as illustrated in the CONSORT flow diagram (figure 1). Per protocol (PP) analysis (ie, inclusion of patients who completed the assigned treatment) will also be reported according to the CONSORT guidelines.[33] In addition, a counterfactual approach using instrumental variable analysis[34 35] will assess actual treatment effects received (participants initially allocated to APRP, but are switched to AS instead or vice versa). A treatment model will be constructed by fitting instrumental variables (randomised intervention) against the received intervention using a logit equation. The OSDI outcome model will be constructed using linear regression equations. All analyses will be performed using STATA V.16. P value <0.05 will be considered as significant.

Protocol violation

A protocol violation will be recorded under the following conditions:

- All participant inclusion criteria are not met (ie, missing informed consent, non-moderate/severe DED).
- Diagnosis of corneal infection or severe active systemic disease during trial participation, and/or pregnancy after recruitment.
- Loss to follow-up since enrolment.
- Incorrect medication storage (eg, not keeping eye drops at the recommended temperature).
- Non-compliance to interventions, defined as missing more than 30% of the expected applications .
- Having co-interventions (ie, other dry eye treatments apart from the assigned intervention during the study period).
- Incorrectly allocated interventions.

The reasons for all protocol violation will also be recorded.

Ethics and dissemination

The study is approved by the Ethics Committee of Ramathibodi Hospital, Mahidol University (MURA2020/1930) and will be conducted in agreement with the Helsinki declaration. Written informed consent (online supplemental file 1) will be obtained from all patients at study commencement. Any substantial protocol amendments will be reported to the institutional ethics committee, registered at ClinicalTrial.gov, and declared in the study report. Data will be recorded anonymously using assigned study identification numbers instead of hospital number. Computer-based data will be stored with secure password protection with access limited to authorised staff only.

DISCUSSION

Prior studies have shown that several kinds of biological tear substitutes significantly improve dry eye symptoms compared with artificial tears.[13 36–38] However, the blood product treatment modalities contain different bioactive ingredients with variable clinical efficacy.[39] APRP is one of the most commonly used blood derivatives in ophthalmology. Platelets compared with AS, contain significant quantities of alpha granules with more bioactive ingredients, which are essential for ocular surface homeostasis.[10] Although there is significant variation in the concentration of blood-derived products used in ophthalmological treatments, we previously reported favourable outcomes associated with 100% AS,[40 41] supporting previous findings from Cho *et al*, which suggested 100% AS was more effective in decreasing DED symptoms, corneal epitheliopathy and promoting fast wound closure.[27] To achieve the best clinical outcomes for both intervention arms, undiluted APRP and AS will be evaluated for participants in this study. Only a single RCT has shown more significant benefits of APRP on DED symptoms compared with artificial tears.[16] Garcia-Conca *et al*[16] used a commercial PRP preparation kit for processing APRP eye drops, which is costly and unavailable in several countries. In this current study, we will apply a single-spin protocol to produce 100% APRP eye drops to compare efficacy with 100% AS, which is considered a prototype biological tear substitute using a non-inferiority trial design. This study will provide evidence to support replacement of 100% AS with 100% APRP for treating patients with moderate-to-severe DED. Additionally, we will apply modern statistical approaches, including instrumental variable regression in the event of protocol violation to minimise potential bias from PP analysis and preserve the effect of randomisation. However, this study will be limited to the assessment of only short-term efficacy over a 4-week follow-up. Further studies will be warranted for evaluating long-term efficacy of 100% APRP.

In summary, we will conduct a single-centre, randomised, parallel, participant-assessor-blinded, non-inferiority trial to evaluate the comparative intervention efficacy of 100% APRP and 100% AS for the treatment of symptoms and clinical outcomes for DED. The findings from this study will inform treatment guidelines and indication for the use of biological tear substitutes in patients with moderate to severe DED.

Author affiliations
[1]Department of Ophthalmology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[2]Department of Clinical Epidemiology and Biostatistics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[3]Department of Family Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[4]Centre for Public Health, Faculty of Medicine Health and Life Sciences, Queen's University Belfast, Belfast, UK
[5]Centre for Clinical Epidemiology and Biostatistics, School of Medicine and Public Health, Faculty of Health and Medicine, University of Newcastle, and Hunter Medical Research Institute, Newcastle, New South Wales, Australia

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Contributors PJ is the principal investigator. PJ, KL, TA, JA and AT designed the study. PJ and TA drafted the manuscript. PJ, KL, TA, PN, GM, JA and AT critically revised the study protocol and the manuscript. The entire project will be supervised by KL, TA, GM, JA and AT.

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Competing interests None declared.

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ORCID iDs
Passara Jongkhajornpong http://orcid.org/0000-0001-8203-2353
Thunyarat Anothaisintawee http://orcid.org/0000-0003-1002-8536
Gareth McKay http://orcid.org/0000-0001-8197-6280
John Attia http://orcid.org/0000-0001-9800-1308

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FACULTY OF MEDICINE RAMATHIBODI HOSPITAL

CONSENT BY SUBJECT FOR PARTICIPATION IN A RESEARCH PROTOCOL

STUDY TITLE: COMPARISON OF THE EFFICACY BETWEEN 100% PLATELET-RICH PLASMA AND 100% SERUM EYE DROPS IN MODERATE TO SEVERE DRY EYE DISEASE: A RANDOMIZED CONTROLLED TRIAL

Investigators: Assoc. Prof. Passara Jongkhajornpong, M.D.
Assist. Prof. Pawin Numthavaj, M.D.
Assoc. Prof. Kaevalin Lekhanont, M.D.
Assoc. Prof. Thunyarat Anothaisintawee, M.D., Ph.D.
Prof. Ammarin Thakkinstian, Ph.D.

Name of participant ..... **Age**.....

Consent by participant

I, _____ (participant's name), have been clearly informed of the details of the research project, including benefits and risks of the participation. I am aware that I can contact the study investigators if questions or concerns arise. The participation is voluntary and I do not have to sign this form if I do not want to be involved in the study. The personal information collected will be kept confidential and will only be use for research publications or presentations. My name and other identifying information will be removed before this data is used. Identifying information may be reviewed by the institution in case of academic necessity only.

_____
Signature of participants

_____
Witness

Date_____

Consenting Investigator

I have explained and disclosed the nature and purpose of the study and the risks involved to the parent/guardian of the participant, with no undisclosed information.

_____

Signature of Investigator

Date_____