Autologous serum eye drops for dry eye (Review)

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[Intervention Review]

Autologous serum eye drops for dry eye

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ABSTRACT

Background

Theoretically, autologous serum eye drops (AS) have a potential advantage over traditional therapies based on the assumption that AS serve not only as a lacrimal substitute to provide lubrication, but also contain other biochemical components mimicking natural tears more closely. The application of AS in dry eye treatment has gained popularity as a second-line therapy in the treatment of dry eye. Published studies on the subject indicate that autologous serum could be an effective treatment for dry eye.

Objectives

To evaluate the efficacy and safety of AS compared to artificial tears for treating dry eye.

Search methods

We searched CENTRAL (which contains the Cochrane Eyes and Vision Group Trials Register) (*The Cochrane Library* 2013, Issue 3), Ovid MEDLINE, Ovid MEDLINE In-Process and Other Non-Indexed Citations, Ovid MEDLINE Daily, Ovid OLDMEDLINE, (January 1950 to April 2013), EMBASE (January 1980 to April 2013), Latin American and Caribbean Literature on Health Sciences (LILACS) (January 1982 to April 2013), the *meta*Register of Controlled Trials (*m*RCT) (www.controlled-trials.com), ClinicalTrials.gov (www.clinicaltrials.gov) and the WHO International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/search/en). We also searched the Science Citation Index Expanded database (September 2013) and reference lists of included studies. We did not use any date or language restrictions in the electronic searches for trials. We last searched the electronic databases on 15 April 2013.

Selection criteria

We included randomized controlled trials (RCTs) in which AS was compared to artificial tears in the treatment of dry eye in adults.

Data collection and analysis

Two review authors independently screened all titles and abstracts and assessed full-text articles of potentially eligible trials. Two review authors extracted data and assessed the methodological quality and characteristics of the included trials. We contacted investigators for missing data. For both primary and secondary outcomes, we reported mean differences with corresponding 95% confidence intervals (CIs) for continuous outcomes.

Main results

We identified four eligible RCTs in which AS was compared with artificial tear treatment or saline in individuals (n = 72 participants) with dry eye of various etiologies (Sjögren's syndrome-related dry eye, non-Sjögren's syndrome dry eye and postoperative dry eye induced by laser-assisted in situ keratomileusis (LASIK)). The quality of the evidence provided by these trials was variable. A majority of the risk of bias domains were judged to have an unclear risk of bias in two trials owing to insufficient reporting of trial characteristics. One trial was considered to have a low risk of bias for most domains while another was considered to have a high risk of bias for most domains. Incomplete outcome reporting and heterogeneity in the participant populations and follow-up periods prevented the inclusion of these trials in a summary meta-analysis. For the primary outcome, improvement in participant-reported symptoms at one month, one trial (12 participants) showed no difference in participant-reported symptoms between 20% AS and artificial tears. Based on the results of two trials in 32 participants, 20% AS may provide some improvement in participant-reported symptoms compared to traditional artificial tears after two weeks of treatment. One trial also showed positive results with a mean difference in tear break-up time (TBUT) of 2.00 seconds (95% CI 0.99 to 3.01 seconds) between 20% AS and artificial tears after two weeks, which were not similar to findings from the other trials. Based on all other objective clinical assessments included in this review, AS was not associated with improvements in aqueous tear production measured by Schirmer's test (two trials, 33 participants), ocular surface condition with fluorescein (four trials, 72 participants) or Rose Bengal staining (three trials, 60 participants), and epithelial metaplasia by impression cytology compared to artificial tears (one trial, 12 participants). Data on adverse effects were not reported by three of the included studies. In one study, there were no serious adverse events reported with the collection of and treatment with AS.

Authors' conclusions

Overall there was inconsistency in the possible benefits of AS in improving participant-reported symptoms and TBUT and lack of effect based on other objective clinical measures. Well-planned, large, high-quality RCTs are warranted, in different severities of dry eye and using standardized questionnaires to measure participant-reported outcomes and objective clinical tests as well as objective biomarkers to assess the benefit of AS therapy for dry eye.

PLAIN LANGUAGE SUMMARY

Eyedrops made from autologous serum as a treatment for dry eye

Dry eye is a common disorder of the tear film, which is a layer of tears covering the surface of the eye. Dry eye affects many adults older than 40 years of age. One common treatment for dry eye is artificial tears, which provide lubrication to the surface of the eye. However, artificial tears lack the biologically active components found in natural tears that are critical to the maintenance of the tear film. Eye drops made by separating the liquid and cellular components of the patient's blood, known as autologous serum eye drops, have been shown to possess many of the same biological nutrients found in natural tears. Because of this, autologous serum eye drops are believed to be a better tear substitute and have become a common treatment for dry eye.

We conducted a wide range of searches for relevant trials in April 2013. We identified four randomized controlled trials with a total of 72 participants with dry eye from Chile, Australia and Japan. The trials compared autologous serum eye drops to traditional artificial tears for the treatment of dry eye. The results from the four trials could not be combined in analysis due to the variation in participant populations, follow-up intervals, and incomplete reporting of treatment outcomes. None of the included trials reported outcomes for the primary outcome of this review, the change in participant-reported symptoms after one month of treatment. Some improvements in participant-reported outcomes and tear film stability were seen in two trials after two weeks, but not in the other two trials or at longer follow-up periods. Autologous serum eye drops did not provide a benefit based on other clinical assessments of the surface of the eye compared to traditional artificial tears. Outcomes for quality or life and costs were not reported in any of the trials. One study reported that no serious harms were related to using autologous serum eye drops while the other studies did not discuss whether any adverse events occurred. Overall the results from these studies do not provide consistent information as to whether autologous serum eye drops are safe and effective for the treatment of dry eye. Future trials are needed using appropriate study designs to address participant-centered outcomes, to determine the effects of autologous serum eye drops in the treatment of dry eye.

BACKGROUND

Description of the condition

Dry eye is a common disorder, with an estimated 25% of patients in general ophthalmology or optometry clinics reporting dry eye symptoms (Doughty 1997). It is known that the incidence of dry eye increases with age and has a higher prevalence in women compared to men (McCarty 1998; Schaumberg 2003; Stern 2004). Recently, the Definition and Classification Subcommittee of the International Dry Eye Work Shop (DEWS), redefined dry eye as "a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort (including foreign body sensation, dryness or irritation, burning, light sensitivity, redness), visual disturbance, secretion with crusting on the eyelashes, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface" (DEWS 2007). An increased tear osmolarity, which causes ocular surface inflammation, is thought to be the central pathogenic mechanism of dry eye (DEWS 2007). The mechanistic classification of dry eye suggested by the DEWS defines two main subtypes: aqueous deficiency and evaporative dry eye, respectively corresponding to disorders of the lacrimal and meibomian glands (DEWS 2007). Disorders of the lacrimal and meibomian glands are usually secondary to either systemic diseases or local causes. One of the most common systemic diseases causing dry eye is Sjögren's syndrome. It presents as "sicca complex," a combination of dry eye and dry mouth (xerostomia) due to a T-lymphocyte mediated destruction of the exocrine glands (Fox 2006; Kumar 2005; Yamada 1990). Other systemic diseases, such as rheumatoid arthritis, diabetes, systemic lupus erythematosus, dermatological conditions such as acne rosacea, and Graves' disease have also been reported as causing clinically significant dry eye (Patel 2002). On the other hand, the leading causes for non-systemic disease-related dry eye include age-related lacrimal dysfunction (Demato 1984), hormonal changes, drug side-effects (e.g. systemic antihistamines, diuretics, topical beta blockers for glaucoma therapies) (Blomquist 2010; Baudouin 2001), surgical intervention (e.g. photorefractive keratectomy [PRK] and laser in situ keratomileusis [LASIK] (Campos 1992; Noda-Tsuruya 2006; Toda 2004), as well as long-term contact lens use (Lemp 1995).

The diagnosis of dry eye is made by validated patient symptom questionnaires and with a wide array of clinical assessments of the tears and ocular surface. Symptoms of dry eye have been standardized by use of questionnaires. The most common complaints described by patients include dryness or irritation, light sensitivity, foreign body sensation, red eyes, poor vision, daily life limitations, and symptom fluctuation in different environmental conditions. However, it has also been noted that there is no strong correlation between signs and symptoms, particularly in mild dry eye (Begley 2003; Viso 2012). Therefore, the clinical diagnosis of dry eye needs to incorporate objective tests such as tear osmolarity, tear production by Schirmer's testing, fluorescein clearance, fluorescein break-up time (BUT), and demonstration of ocular surface damage through dye staining (fluorescein and lissamine green) (Lemp 2011; Lemp 1995; Perry 2004). Although there is presently no gold standard diagnostic test to identify dry eye, a growing number of studies have suggested that tear osmolarity might be the best single metric for diagnosis and severity assessment of dry eye (Lemp 2011; Tomlinson 2006). According to Perry 2004, other authors also suggest tear film stability by BUT and delayed tear fluorescein clearance (Chodosh 1994; Marci 2000) as reliable ways to assess dry eye.

Currently there is no cure for dry eye. Common treatments are targeted to manage the symptoms. The mainstay of conventional therapy is the application of artificial tears that increase moisture on the ocular surface, and provide additional lubrication. A variety of artificial tear formulations differ from each other in their electrolyte composition, osmolarity, viscosity, the presence of preservatives, and compatible solutes (Lemp 2008). An unpreserved artificial tear containing 0.1% sodium hyaluronate was found to be effective in improving dry eye symptoms with a significant improvement in the mean tear film osmolarity, break-up times, and conjunctival and corneal staining scores (Nelson 1988). However, the use of artificial tears has some limitations. Natural tears have a complex composition of water, salts, hydrocarbons, proteins, and lipids that artificial tears cannot exactly substitute (Dogru 2011; Quinto 2008). Additionally, frequent application of artificial tears solutions containing chemical preservatives to prevent contamination has been found to induce toxic and allergic reactions, especially among those with sensitive eyes (Baudouin 2010; Dogru 2011; Quinto 2008).

Topical corticosteroids that target the inflammatory pathways associated with ocular inflammation have been shown to improve symptoms in people with dry eye (de Paiva 2008; Pflugfelder 2004), but their use is limited due to long-term side effects including cataracts and increased intraocular pressure (Blomquist 2010). In December 2002, the U.S. Food and Drug Administration (FDA) approved 0.05% solution of cyclosporine A (CsA) as an ocular therapeutic for people with dry eye (Meadows 2005). Several studies have shown an increase in tear production and conjunctival goblet cell density with few reported adverse effects following the topical application of CsA (Sall 2000; Stevenson 2000; Toker 2010; Wilson 2007).

Additional nutritional supplements such as essential fatty acids, including omega-3, linoleic acid, and gamma-linoleic acid, have been proposed as adjuvants in the treatment of dry eye due to their anti-inflammatory properties (de Paiva 2008). Increased water intake and reduced alcohol consumption are also recommended to improve dry eye symptoms (Dogru 2011). Environmental interventions designed to increase air moisture and reduce particles in the air, including indoor humidifiers and air filters or cleaners have been shown to reduce dry eye symptoms as well (Dogru 2011). For people in whom artificial tears are not sufficient, preservation

of the tear film can be achieved by inserting punctal plugs in the lacrimal ducts, designed to reduce the drainage of tears through the lacrimal ducts and increase lubrication on the ocular surface (Ervin 2010; Foulks 2003).

Description of the intervention

The composition of serum resembles that of tears; most concentrations are equivalent, with the exception of more vitamin A, lysozyme, transforming growth factor-β (TGF-β) and fibronectin, and less immunoglobulin A (IgA), epithelial growth factor (EGF) and vitamin C in serum than in tears (Bradley 2008; Joh 1986; Matsumoto 2004; Nelson 1992; Tsubota 1999). Since many of the essential components in tears are present in serum, the use of serum as a tear substitute for the maintenance of the ocular surface seems feasible (Imanishi 2000; Kojima 2005b). In 1975, autologous serum eye drops (AS) were initially applied for dry eye and reported by Ralph 1975. Since then, AS have become increasingly popular for treating ocular surface diseases, mainly dry eye.

Production of autologous serum eye drops

Currently, there are no commercially available forms of AS; AS must be compounded using autologous serum. Technological factors affect the product quality and properties of AS (Geerling 2004; Liu 2005). Even though there is large variability in the methodology for AS preparation, storage and administration, standards have been established to optimize therapeutic effectiveness and product safety (Geerling 2004; Liu 2005). In brief, blood is first drawn from the recipient and allowed to clot in the absence of an anticoagulant. Once a clot has formed, the supernatant is centrifuged to separate the serum from the solid components without inducing hemolysis. After centrifugation the serum is decanted into a sterile container and then may be diluted to the desired concentration. Autologous serum typically is administered in 20% concentration which is based on the concentration of the biological factors in actual tears, although higher concentrations (between 50% and 100%) have been used (Dogru 2011; Geerling 2004; Kojima 2008; Quinto 2008). There is always the possibility that serum may contain components that are detrimental to the ocular surface. TGF-β, for example, is known to have antiproliferative effects, and high concentrations of TGF-β may suppress wound healing of the ocular surface epithelium (Tsubota 2000). This was one of the reasons for using a diluted solution of serum in order to maintain TGF-β levels that are comparable with tears. Preservatives are usually not added to AS, thus reducing the risk of preservative-induced toxicity associated with other dry eye treatments. However, the lack of preservatives theoretically increases the risk of ocular infections. Autolgous serum can be stored for less than one month at 4°C while in use, and for up to three months at -20°C (Tsubota 1999). It is important that vials containing autologous serum be kept away from light to avoid degradation of vitamin A.

Indications

Autologous serum eye drops have been recommended for the treatment of several ocular surface disturbances, such as Sjögren's syndrome-related tear deficiency, non-Sjögren's tear deficiency associated with graft-versus-host disease, neurotrophic keratitis, persistent epithelial defects, superior limbic keratoconjunctivitis, and postoperative dry eye induced by LASIK. People who were treated with 20% to 50% AS four to eight times a day reported subjective improvement in dry eye symptoms; objective improvement based on fluorescein staining and break-up time tests also was observed (Chiang 2007; Hyon 2007; Kojima 2005b; Matsumoto 2004; Ogawa 2003; Poon 2001; Tananuvat 2001).

Complications

AS are usually well tolerated, and most recipients report improvement of discomfort. Occasionally, they may experience increased discomfort, slight epitheliopathy (drop-out of the corneal epithelial cells, akin to fluorescein staining of the surface of the eye), bacterial conjunctivitis, or eyelid eczema (Ogawa 2003; Rocha 2000; Tananuvat 2001). Fox 1984 reported no serious complications but mentioned that others had encountered scleral vasculitis and melting in people with rheumatoid arthritis. McDonnell 1988 described complications such as the deposit of immunoglobulins in the cornea and the presence of corneal peripheral infiltrates with 100% autologous serum treatment in one person.

Risk of infection

Because some of the serum's components may have bacteriostatic effects, for example, lysozyme, complement, and immunoglobulin G (IgG), the addition of a further bacteriostatic agent may not be necessary. It is reported that AS can be used safely in an outpatient as well as inpatient setting, under a strict protocol of preparation and storage (Langnado 2004; Partal 2011). However, even though AS are prepared under sterile conditions on an individual patient basis, there are risks for contamination, and consequent infection, during the preparation, storage, and use of the drops (Geerling 2004; Lee 2008).

Selection of people suitable for autologous serum

In the United States, the FDA and the American Association of Blood Banks (AABB) have specified criteria for autologous blood donors, which include a minimum hemoglobin concentration of 11 g/dL (hematocrit of 33%) and deferral for conditions presenting risk of bacteremia. Additional criteria may be applied by the individual blood collection facilities and medical providers; these often specify that the patient must be well enough to undergo venipuncture several times a year and withstand loss of blood (Noble 2004; Roback 2008). Blood collection facilities sometimes specifically defer people considered to be at greatest risk from blood donation such as those with unstable angina, recent myocardial

infarction or cerebrovascular accident, significant cardiac or pulmonary disease with chronic symptoms but who have not been evaluated by the treating physician, or untreated aortic stenosis. Children and pregnant women often are excluded (Roback 2008). To prevent the risk of viral transmission to others (e.g. production or nursing staff and children at home who may unintentionally use serum eye drops), it is strongly recommended that the donor be tested for blood-transmitted diseases (e.g. human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis), that hospital staff be cautious of serum production, and that the identity of the recipient be confirmed (Geerling 2004; Yoon 2007). Though there are significant legal ramifications due to the potential transmission of blood-based diseases to medical staff as well as serum recipients, there is no consensus as to whether people who have blood-transmissible diseases should be disqualified from donating serum for their personal use when medically indicated.

Legal regulations

AS are unique among ophthalmic therapies in that they are manufactured specifically for each individual and are made from that person's own blood. The regulations on autologous blood donation vary from country to country. In the United States the FDA's Center for Biologics Evaluation and Research (CBER) is responsible for the regulation of blood intended for transfusion, blood components and derivatives. In the European Union (EU), several directives on AS have been issued (1965/65, 1975/139, 1975/318) by the European Parliament and Council. However, these directives had to be taken into account in the laws of each member state of the EU (Geerling 2004). For example, the National Blood Service in England and Wales has supplied AS under a drug exemption certificate for the purposes of a clinical trial from the regulatory body in the United Kingdom, the Medicines and Healthcare Regulatory Agency (Noble 2004). Special regulations by the FDA and other regulatory agencies for using blood products must be taken into account when considering the integration of AS therapy into treatment regimens for AS (Geerling 2008; Noble 2004; Roback 2008).

How the intervention might work

Studies have shown that AS contain biochemical factors such as EGF, vitamin A, TGF-β, fibronectin, substance P, insulin-like growth factor 1 (IGF-1), nerve growth factor (NGF), and other cytokines that are essential for the proliferation, differentiation, and maturation of the normal ocular surface epithelium (Gordon 1995; Matsumoto 2004; McCluskey 1987; Nishida 1983; Nishida 1987; Phan 1987; Poon 2001). Therefore, a potential advantage of AS over traditional therapies is that AS serves as a lacrimal substitute to provide lubrication and other biochemical components of tears to assist in corneal and conjunctival epithelium maintenance with limited toxicity (Dogru 2011; Geerling 2004; Liu 2005; Poon 2001; Quinto 2008).

Why it is important to do this review

The use of AS in severe dry eye treatment has gained widespread acceptance in the past decade. However, it continues to be a restricted area because the preparation of serum eye drops requires a well-equipped laboratory and trained personnel. Studies conducted recently are controversial with regard to the effectiveness of AS for dry eye symptoms (Noble 2004; Tananuvat 2001). Therefore, we undertook a systematic review to determine the efficacy and safety of AS for the treatment of dry eye.

OBJECTIVES

The aim of this review was to evaluate the efficacy and safety of AS as compared to artificial tears in the treatment of dry eye in adults.

METHODS

Criteria for considering studies for this review

Types of studies

We included only randomized controlled trials (RCTs) for the purpose of this review. Given the stability of the condition of interest, we also considered cross-over studies in which the sequence of treatments was determined to have been assigned randomly.

Types of participants

We included in the review studies conducted in adults (age over 18 years old), with dry eye defined by the study investigators with no restrictions based on race or sex.

Types of interventions

We included studies in which the application of AS alone or in combination with artificial tears was compared to artificial tears alone, saline, placebo, or no treatment.

Types of outcome measures

Dry eye clinical tests generally do not correlate with participant-reported symptoms. There are a wide variety of participant-reported outcome scales that actually lead to the discrepancies between subjective symptoms and objective clinical tests (Chambers 1999; Fuentes-Paez 2011; Nichols 2004; Patrick 2011). Therefore we took into consideration both subjective data from participant-reported symptoms regardless of measurement scale and objective data obtained from clinical diagnostic tests to analyze fully their effect on the condition.

Primary outcomes

We defined symptom improvement as the change from baseline in participant-reported severity and/or frequency of dry eye-related symptoms based on validated patient symptom questionnaires at four weeks after initiation of treatment. Since trial design, frequency of AS administration, and timing of outcome assessment may vary, we considered all variations in frequency of AS use and other time points as reported by included studies.

Secondary outcomes

Objective data obtained from ophthalmic examinations and diagnostic tests (Behrens 2006; Tomlinson 2009) two to four weeks after treatment were recorded for the following tests:

- Tear hyperosmolarity: mean change in tear osmolarity.
- Ocular staining with fluorescein: mean change in total score from baseline to follow-up.
- Tear film break-up time: mean change in tear film break-up time in seconds.
- Schirmer's test: mean change in millimeters with or without anesthesia.
- Ocular staining with Rose Bengal: mean change in total score from baseline to follow-up.
- Corneal topography: mean change in tear film break-up time and the height of the tear meniscus by non-invasively assessing the tear film.
- Impression cytology: mean change in grades of epithelial metaplasia and goblet cell density.
- Tear fluorescein clearance: mean change in the speed of disappearance from the ocular surface of exogenously added fluorescein.
- Conjunctival biopsy: mean change in grades of squamous metaplasia of the conjunctiva.

Adverse effects

We tabulated adverse effects (e.g. bacterial and viral infection and eye irritation) reported in the included studies for both the AS and control groups.

Quality of life measures

We planned to record health-related quality of life data presented by any validated measure (e.g. activities of daily vision scale) in the included studies.

Economic data

We planned to documented cost analyses and other data on economic outcomes reported by the included studies.

Search methods for identification of studies

Electronic searches

We searched the Cochrane Central Register of Controlled Trials (CENTRAL) 2013, Issue 3, part of *The Cochrane Library*. www.thecochranelibrary.com (accessed 15 April 2013), Ovid MEDLINE, Ovid MEDLINE In-Process and Other Non-Indexed Citations, Ovid MEDLINE Daily, Ovid OLDMEDLINE, (January 1950 to April 2013), EMBASE (January 1980 to April 2013), Latin American and Caribbean Literature on Health Sciences (LILACS) (January 1982 to April 2013), the *meta*Register of Controlled Trials (*m*RCT) (www.controlled-trials.com), ClinicalTrials.gov (www.clinicaltrials.gov) and the WHO International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/search/en). We did not use any date or language restrictions in the electronic searches for trials. We last searched the electronic databases on 15 April 2013.

See: Appendices for details of search strategies for CENTRAL (Appendix 1), MEDLINE (Appendix 2), EMBASE (Appendix 3), LILACS (Appendix 4), *m*RCT (Appendix 5), ClinicalTrials.gov (Appendix 6) and the ICTRP (Appendix 7).

Searching other resources

We also searched the Science Citation Index-Expanded database (September 2013) and reference lists of included studies. We did not handsearch conference proceedings or journals.

Data collection and analysis

Selection of studies

Two review authors independently reviewed the titles and abstracts of all the reports identified from the electronic searches. We classified each study as 1) eligible for inclusion, 2) unsure, or 3) exclude. We obtained full-text copies of all potentially and definitely relevant articles. Two review authors assessed the full-text articles for final inclusion of studies in this review. For studies that we excluded at this stage, we documented reasons for exclusion (see

Characteristics of excluded studies). We resolved any discrepancies through consensus.

Data extraction and management

Two review authors extracted the data independently using the data extraction form developed by the Cochrane Eyes and Vision Group for this review. We resolved discrepancies by discussion and contacted study authors for additional necessary data. All data were entered into Review Manager 5 (RevMan 2012) by one review author and confirmed by a second review author.

Assessment of risk of bias in included studies

Two review authors assessed risk of bias independently according to methods set out in Chapter 8 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011a). Review authors were not masked to any trial details during the assessment. We considered the following risk of bias parameters for each of the included studies: sequence generation and allocation concealment (selection bias); masking (blinding) of participants and researchers during and after treatment as well as during outcome assessment (detection bias); completeness of follow-up for primary and secondary outcomes (attrition bias); and selective outcome reporting (reporting bias). We applied a judgment of 'low risk', 'unclear risk', or 'high risk' to each of the above parameters for each of the included studies.

For cross-over trials we considered additional methodological assessments of the risk of bias, including whether there was a washout period, the number lost to follow-up after each phase, and whether the data were reported for each phase or by treatment as described in Chapter 16 of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011b).

Measures of treatment effect

We did not conduct summary meta-analyses of the treatment effects in this review. If sufficient data are available in future updates we will calculate summary risk ratios (RRs) for dichotomous outcomes of interest (proportion of participants reporting improvement in dry eye related symptoms). We will summarize continuous data from objective ocular tests by calculating mean differences from baseline to follow-up between the treatment and control arms (ocular surface staining, Schirmer's test, and tear break-up time). For continuous scales of participant-reported outcomes, we will calculate standardized mean differences (SMDs) to account for the variation in measurement scales. We will dichotomize ordinal data to reflect varying degrees of symptom improvement ('some improvement') followed by sensitivity analyses using different cut points (Patrick 2011).

We will use the generic inverse variance method to summarize the treatment effects from studies that reported the computed measures of effect and variance estimates. We will not include quantitative data from cross-over trials which report only the first phase data, given the risk of bias for incomplete outcome reporting (Higgins 2011b).

Unit of analysis issues

The unit of analysis was the individual participants who were randomized to each treatment arm in two trials (Kojima 2005a; Urzua 2012). One trial used a paired-eye design in which each eye of the participant was evaluated and the eye was considered the unit of analysis. Another trial randomized participants to each intervention while the analyses included both eyes of each participant independently (Noda-Tsuruya 2006). We reported results using the unit of analysis reported by the studies.

Dealing with missing data

We contacted study authors of included trials for clarification or retrieval of missing primary and secondary outcome data. We did not conduct any imputations when study authors did not provide missing data and instead relied on data in the published reports. For future summary meta-analyses, when trial authors are unable to provide information on missing data, we plan to conduct the following sensitivity analyses: (a) assume all participants with missing data in the treated group had the worse outcome (if dichotomous); and (b) assume all participants with missing data in the treated group did not have the worse outcome.

Assessment of heterogeneity

We assessed clinical and methodological heterogeneity by examining the characteristics of study participants, treatment/control comparisons, and assessment of primary and secondary outcomes.

If future updates of this review include summary meta-analyses, we will examine consistency across studies with the I^2 test (Higgins 2003), with a value greater than 50% indicating substantial statistical heterogeneity. We will also inspect forest plots for the degree of overlap of the confidence intervals of the included studies. Little overlap is another indication of the presence of heterogeneity.

Assessment of reporting biases

We were not able to conduct summary meta-analyses and could not assess reporting bias through the inspection of funnel plots.

Data synthesis

There were insufficient data to conduct a meta-analysis. A narrative summary of results was used in place of statistical summary analyses.

For future updates we will conduct a random-effects meta-analysis when there is significant clinical, methodological, and statistical homogeneity among included studies. When fewer than three studies are included in a meta-analysis, we will use a fixed-effect

model. We will not combine studies in a meta-analysis when there is significant heterogeneity detected among included studies.

Subgroup analysis and investigation of heterogeneity

There were insufficient data to conduct a subgroup analysis in this review. If adequate data are present in future updates we will stratify by the underlying etiology of dry eye symptoms including tear deficiencies (Sjögren's syndrome), non-Sjögren's syndrome-related dry eye, evaporative dry eye (blepharitis or meibomian gland dysfunction (MGD)), and complications of LASIK.

Sensitivity analysis

We did not conduct a sensitivity analysis in this review. For future updates we will investigate the impact of studies with lower methodological quality (i.e. high risk of bias for random sequence generation or incomplete outcome data for primary or secondary outcomes) and unpublished studies through sensitivity analyses.

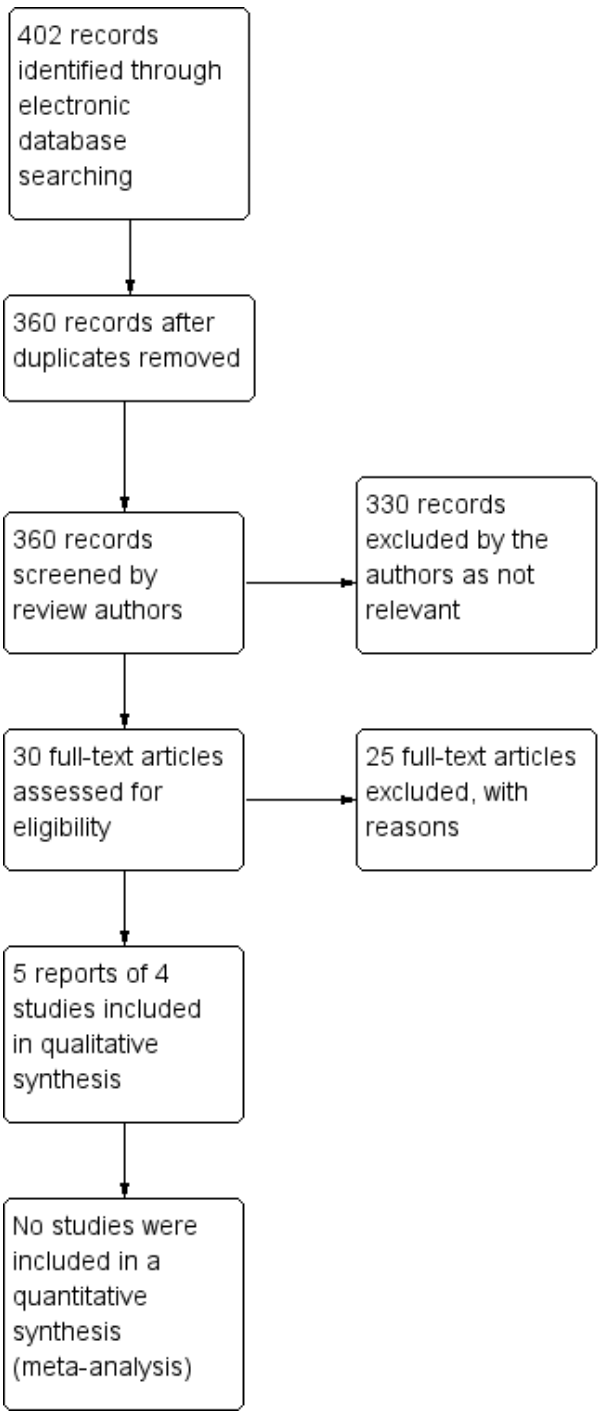
RESULTS

Description of studies

Results of the search

We identified a total of 402 titles and abstracts from the electronic searches as of April 2013 (Figure 1). After removing the duplicates, we screened 360 titles and abstracts. We identified 30 reports of 29 studies as potentially relevant for this review. After full-text review of the 30 reports, we included three full-text reports from three trials (Noda-Tsuruya 2006; Tananuvat 2001; Urzua 2012) and one full-text report and conference abstract reporting findings from another trial (Kojima 2005a) (see Characteristics of included studies). We found no other eligible trials or additional reports of the four included trials after searching other sources described above.

Figure 1. Results from searching for studies for inclusion in the review



Included studies

Participants

All study participants in the four trials included in this review had dry eye. The etiologies of dry eye were mainly post-LASIK, Sjögren's syndrome and non-Sjögren's syndrome. The number of participants in the studies ranged from 12 to 27 with the average age ranging between 30 and 60 years among three studies (Noda-Tsuruya 2006; Tananuvat 2001; Urzua 2012) and one with an overall age range from 50 to 75 years (Kojima 2005a).

Kojima 2005a included 20 participants with severe dry eye, 17 of whom had Sjögren's syndrome. None of the study participants had a history of ocular surgery or procedures, including punctal occlusion.

Noda-Tsuruya 2006 included 27 men who developed dry eye subsequent to LASIK and had not worn contact lenses before LASIK.

Tananuvat 2001 enrolled 13 study participants with severe dry eye including nine with a history of punctal occlusion. Five participants had Sjögren's syndrome, three had idiopathic dry eye, one had non-Hodgkin's lymphoma, one had graft-versus-host disease, one had Stevens-Johnson syndrome, and one had rheumatoid arthritis. One participant was excluded after enrollment due to an imbalance in the severity of dry eye between the two eyes.

Urzua 2012 enrolled 12 participants with severe non-Sjögren dry eye who had received prior treatment with artificial tears.

Interventions

All four trials evaluated 20% AS with instructions given to participants to apply drops four, five, or six times daily. Similar instructions for the storage of AS study vials were given to participants in all four trials. In one trial, participants were given 5 ml bottles of 20% AS in unpreserved normal saline solution or bottles of saline solution mixed with dilute fluorescein solution, which served as placebo. Participants were instructed to use the eye drops six times per day for two months, and to refrigerate the eye drop bottle in use while the rest were frozen. One bottle of eye drops was to be used for one week and then replaced. They were also instructed to continue use of preservative-free artificial tears as needed (Tananuvat 2001).

In the second trial, participants entered a "wash-out" phase where they used only preservative-free saline eye drops six times a day for two weeks. Subsequently, participants in the AS group used only 20% AS in saline six times a day for two weeks, and participants in the artificial tear group used only preservative-free artificial tears six times a day for two weeks. Participants were instructed to keep the vials they were using in a refrigerator at 4°C and were instructed to store the other vials in a freezer (Kojima 2005a). The specific formulation of the artificial tears used by the control group was not reported.

In the third trial, after LASIK, all participants used low-dose steroids (0.1% fluorometholone, Flumetholon, Santen); antibiotics (Tarivid, Santen); and 0.3% hyaluronic acid (Hyalein, Santen) eye drops five times per day for one week (Noda-Tsuruya 2006). Subsequently, the AS group used eye drops made of 20% AS diluted in sterile saline five times a day from one week to six months postoperatively and the artificial tear group used preservative-free saline-based artificial tears (Soft Santear, Santen), five times a day from one week to six months postoperatively. Participants were instructed to keep the bottle they were using in a refrigerator at 4°C and were instructed to store the other bottles in a freezer (-20°C). Each bottle of 20% AS was used for two weeks and then replaced.

In the fourth trial, Urzua 2012, participants were given 14 identical flasks containing either 20% AS or artificial tears (Systane) and were instructed to use one flask four times a day for the first two weeks. After the first two weeks, all study participants used 0.9% NaCl for a one-week wash-out, and were then given another 14 flasks containing the second study intervention (either 20% AS or artificial tears), opposite of the intervention they received in the first two-week period.

Outcome Assessment Measures

Each of the four included trials used a different method to evaluate participant-reported symptom improvement at different follow-up times. In each method used, higher values represented more severe symptoms/discomfort in which a decrease in values from baseline would suggest improvement in symptoms. Only one study reported participant-report symptoms at one-month follow-up, the primary outcome for this review. In the other three trials, participant-reported symptoms were reported at additional follow-up periods between two weeks and six months follow-up. Tananuvat 2001 assessed study participants at baseline and on three follow-up visits at one week, one month, and two months. Symptoms of dry eye (discomfort, foreign-body sensation, dryness, and photophobia) were graded as grade 0, no symptoms; 1, mild; 2, moderate; and 3, severe. In Kojima 2005a, visual analog pain symptom scores were assessed at baseline and at two weeks. In Noda-Tsuruya 2006, a written questionnaire was used to assess dry eye symptoms; the participants graded "typical dry eye symptoms" 0, none; 1, mild; 2, moderate; 3, strong; and 4, very strong. In Urzua 2012, the Ocular Surface Disease Index (OSDI), recommended by the International Dry Eye Workshop (Ozcura 2007), was used to evaluate participant-reported improvement in dry eye symptoms.

Although all of the studies included TBUT, tear secretion

(Schirmer's test) and fluorescein staining, the investigators of these studies did not all follow the same procedures with additional variation in the time points at which all outcomes were evaluated. We do not believe the variation in procedures used to evaluate the objective clinical tests would influence the ability to compare treatment effects across studies. In Kojima 2005a and Noda-Tsuruya 2006 TBUT was observed after instilling 2 μl of 1% Rose Bengal mixed with 1% fluorescein and saline into the cul-de-sac; in Tananuvat 2001, a fluorescein strip moistened with saline was placed into the lower cul-de-sac. In Urzua 2012, no additional description was given of how the investigators evaluated TBUT. Tananuvat 2001 and Noda-Tsuruya 2006 specify that the Schirmer test was done with anesthesia and without anesthesia in Kojima 2005a. Scoring of fluorescein staining of the ocular surface in Kojima 2005a and Noda-Tsuruya 2006 was carried out by dividing the cornea into upper, middle, and inferior compartments and grading each one on a scale of 0 to 3 points (maximum: 9 points). Tananuvat 2001 did not divide the cornea into thirds, and fluorescein staining of the cornea was graded from 0 to 3. Urzua 2012 used the OXFORD scale (six categories) to evaluate fluorescein staining (Bron 2003). Details of the procedures used to evaluate Rose Bengal staining were not reported by the three trials measuring this outcome (Kojima 2005a; Noda-Tsuruya 2006; Tananuvat 2001). Conjunctivel impression cytology and frequency of other topical lubricants were evaluated in only one trial (Tananuvat 2001).

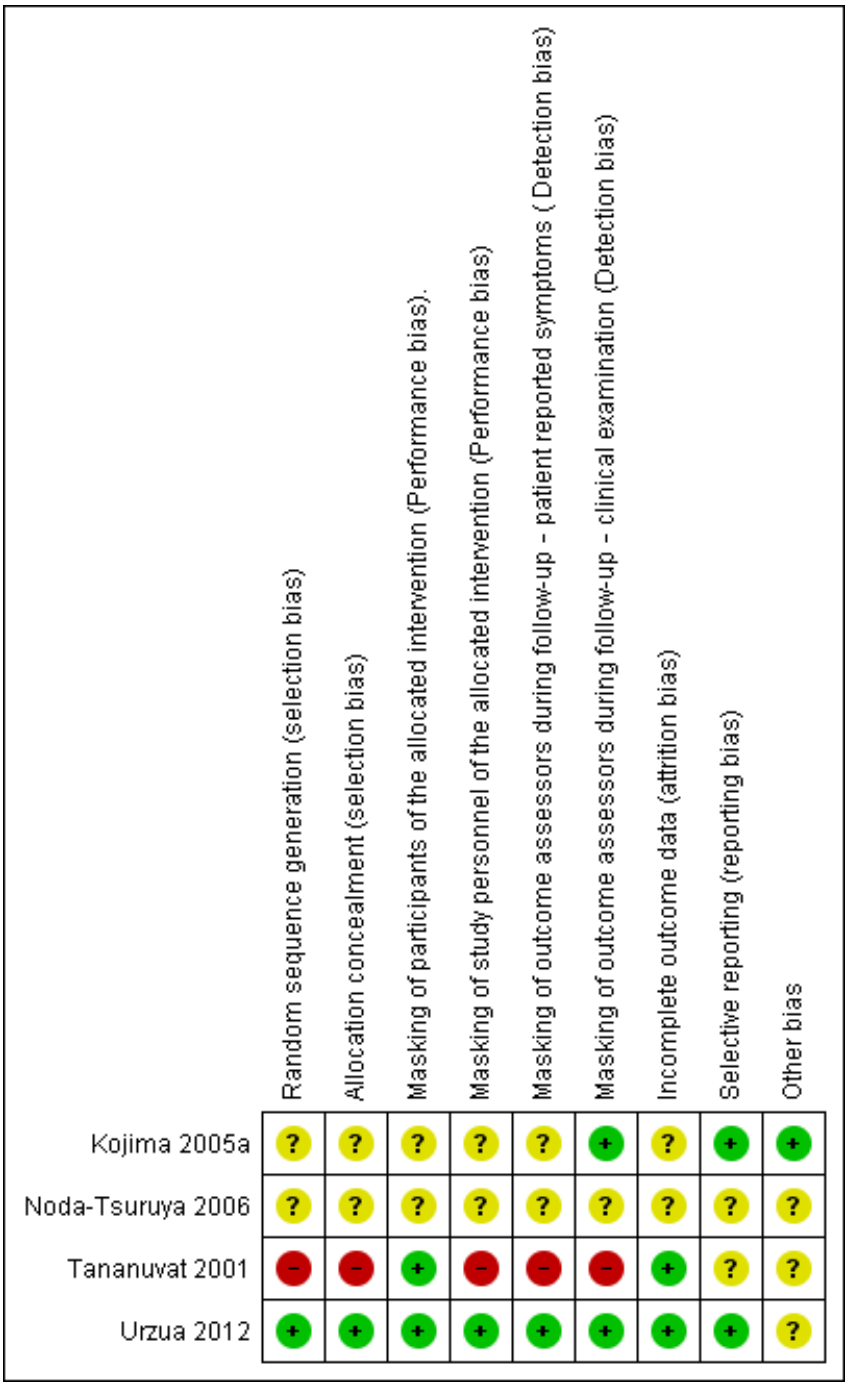
Excluded studies

We excluded 25 references after full-text review (see Characteristics of excluded studies). Two references were from conference abstracts (Harritshoj 2011; Jaksche 2005), and the remainder were from full-text publications. A majority of the excluded studies were non-randomized studies or reviews. We excluded three RCTs because the investigators did not compare AS to artificial tears or placebo (Jaksche 2005; Noble 2004; Yoon 2007).

Risk of bias in included studies

Figure 2 presents a summary of the risk of bias for the included studies. For two studies (Kojima 2005a; Noda-Tsuruya 2006) a majority of the risk of bias domains were unclear due to insufficient description in trial reports.

Figure 2. Methodological quality summary: risk of bias review authors' judgements about each risk of bias item for each included study.



Allocation

Sequence generation and allocation concealment

The risk of bias domains for sequence generation and allocation concealment were judged to be at low risk of bias in one study (Urzua 2012) and at unclear risk for two included studies (Kojima 2005a; Noda-Tsuruya 2006). Although randomization of participants was specified in the later two trials, none of the published reports described the methods used to generate the allocation sequence or how they implemented the treatment allocation in sufficient detail. One study used block randomization with block sizes of two resulting in alternating treatment assignment which we judged to have a high risk of bias for sequence generation. As the investigators were unmasked and assignments in a block size of two could be known we judged this study have a high risk for allocation concealment (Tananuvat 2001).

Masking (performance bias and detection bias)

Masking of participants and study personnel

Masking of participants and study personnel to the allocated intervention was judged to be at low risk of bias in one study (Urzua 2012) and at unclear risk in two studies (Kojima 2005a; Noda-Tsuruya 2006). A full description of the measures used to achieve masking was not included in the published reports for Kojima 2005a and Noda-Tsuruya 2006 which specified a prospective randomized design without clearly reporting masking of participants or study personnel. The third trial was conducted as a single masked study with participants masked to their treatment assignments and investigators unmasked (Tananuvat 2001). We judged this trial to be at low risk of bias for masking participants and at high risk of bias for masking study personnel.

Participants allocated to the AS group had to undergo blood extraction as part of the serum production process. Specific instructions for the preservation and storage of AS were reported in all four studies (Kojima 2005a; Noda-Tsuruya 2006; Tananuvat 2001; Urzua 2012). It is not clear at what point in the randomization process participants were subjected to serum collection procedures and whether the same storage instructions were provided to all participants regardless of their treatment assignment. One study (Urzua 2012) implemented a cross-over study design which maintained participant masking whereby all participants underwent venous blood draw for preparation of their AS. Additional measures taken in Urzua 2012, including the use of opaque flasks and instructions to keep all study medication frozen at -20°C helped ensure participant masking.

Masking of outcome assessors

Outcome assessments were considered in two main categories: 1) assessment of participant-reported symptoms and 2) assessment of objective clinical examination. For two studies, the masking of outcome assessors for participant-reported symptoms was judged to be unclear (Kojima 2005a; Noda-Tsuruya 2006). Neither study provided a full description of how the participant-reported outcomes were recorded and whether the study personnel collecting this information were aware of the participant's treatment assignment. Participants were asked to complete either a written questionnaire or an analog pain scale in two studies (Kojima 2005a; Noda-Tsuruya 2006). In one study, participants were asked to report symptoms at each visit, and this information was then recorded by an unmasked study investigator and was judged to be at high risk of bias (Tananuvat 2001). Masking of outcome assessors for the objective clinical examination was judged as unclear for one study (Noda-Tsuruya 2006) and one study was judged as being at low risk of bias (Kojima 2005a). Study investigators were unmasked in one study which was determined to be at a high risk of bias for the objective clinical outcome assessment (Tananuvat 2001). Although these were objective clinical tests, there is potential detection bias if investigators conducting the test and interpreting the results were aware of the participant's treatment assignment. Urzua 2012 was the only study found to be at low risk of bias for both participant-reported outcomes and objective clinical tests.

Incomplete outcome data

The domain for incomplete outcome data was judged to be at low risk of bias for two studies (Tananuvat 2001; Urzua 2012). There was no loss to follow-up or missing data reported as confirmed by a review of the number analyzed after initial inclusion/exclusion in the results section. One study (Noda-Tsuruya 2006) reported the number of eyes for each outcome at all time points across both treatment arms, but the investigators did not provide reasons for missing outcome data; the number of analyzed eyes was variable throughout the intervention and resulted in a judgment of unclear risk of bias. Two eyes were excluded from the analyses in the full-text report from one trial (Kojima 2005a) compared to the conference abstract for the same trial, without an explanation for the discrepancy.

Selective reporting

We found two studies to be at low risk of reporting bias (Kojima 2005a; Urzua 2012); the other two studies were judged to have an unclear risk of reporting bias (Noda-Tsuruya 2006; Tananuvat 2001); the investigators reported all outcomes at all time points as

described in the methods, although reported information was insufficient to extract usable data for quantitative summary analyses. For one study we were able to confirm the prespecified outcomes described in the ClinicalTrials.gov record with the corresponding publication (Urzua 2012), but did not have access to study protocols or other related materials for the other three studies, and were unable to confirm the reported outcomes with the intended outcomes for each study.

Other potential sources of bias

We were unable to fully assess other potential sources of bias for two studies that were judged to have an unclear risk of bias. In one study (Tananuvat 2001), participants in both groups were able to use artificial tears lubricants. The estimated treatment effect of AS may be influenced if the additional lubricants had a perceived therapeutic effect on the outcomes of interest and were used in different frequencies in each group. In another study (Noda-Tsuruya 2006), there was a discrepancy between the unit of randomization (individual) and unit of analysis (eyes), which can lead to biased treatment effects by not considering the within participant correlation. For one study we found sufficient information (appropriate study design, proper ethical conduct, no involvement from industry) to establish a low risk for other potential bias (Kojima 2005a). On review of the predefined inclusion criteria, we identified a discrepancy with the inclusion criteria listed in the published report for one study which led us to judge this as an unclear risk of bias (Urzua 2012).

Effects of interventions

Included studies could not be combined in summary meta-analyses due to heterogeneity in the time points at which primary and secondary outcomes were reported and insufficient reporting of descriptive statistics (means and standard deviations) necessary for computing treatment effect estimates. One study implemented a cross-over design but did not report the necessary summary statistics from a paired analysis (i.e. mean difference from paired t-test and corresponding confidence interval or P value) to account for the participant-level differences in AS and artificial tears (Urzua 2012). We can therefore provide only a narrative description of the reported findings.

Subjective measurements

None of the included trials reported results for the primary outcome of this review, the change from baseline in participant-reported symptoms at one month follow-up.

At one month follow-up, the mean composite symptom score was 5.36 and 6.45 for both the 20% AS and control group (saline solution with diluted fluorescein) respectively, and 5.3 and 5.9 at the two-month follow-up visit for the 20% AS and the control group respectively (Tananuvat 2001). Without the reported number of participants and SD estimates for each treatment group we could not generate an overall variance estimate (95% CI) and corresponding P value for the difference between 20% AS and the control group for either follow-up interval (Analysis 2.1). The authors reported the mean symptom scores were not statistically different (P > 0.05) between the 20% AS and control groups over a two-month treatment period.

Among 10 participants in both the 20% AS group and the artificial tear control group, the mean change and SD from baseline at two weeks follow-up measured with the visual analog scale was -19.2 ± 8.8 and -7.2 ± 9.8 and respectively, resulting in a difference in the mean change from baseline of -12.00 (95% confidence interval (CI) -20.16 to -3.84 (Analysis 1.1; Kojima 2005a). This difference suggests there was a greater decrease in the pain/dry eye symptoms in the 20% AS group compared to the artificial tear group after two weeks. The mean and SD in the OSDI scale in the 20% AS and artificial tear groups was 59 ± 10 and 51 ± 7, respectively, among 12 participants at baseline and 30 ∓ 8 and 41 ± 8, respectively, at two weeks (Urzua 2012). From these data, the trial investigators reported there was a 51% decrease in the OSDI scale in the 20% AS group and a 22% decrease in the artificial tear group.

Descriptive statistics (mean and SD) were not reported for 27 post-LASIK participants (54 eyes) as measured by a five-point questionnaire (Noda-Tsuruya 2006). However, in a narrative description, the trial investigators reported that there was no statistically significant difference (P > 0.05) in the participant-reported symptoms between the 12 participants (24 eyes) in the 20% AS group and the 15 participants (30 eyes) in the artificial tear group before and after LASIK surgery through six months of follow-up.

Ocular surface staining

Rose Bengal staining

The mean changes and SDs from baseline in Rose Bengal at two weeks follow-up for the 20% AS and artificial tear groups were -2.3 ± 0.8 and -0.1 ± 0.3 respectively, resulting in a difference in the mean change from baseline of -2.20 (95% CI -2.73 to -1.67; P < 0.00001) (Analysis 1.2) across 20 participants (10 in each treatment group) (Kojima 2005a). The difference in the decrease in Rose Bengal scores suggest the 20% AS group had greater overall improvement compared to the artifical tear group after two weeks.

The means and SDs were 0.3 ± 0.7 in 20 eyes in the 20% AS group and 0.9 ± 0.8 in 15 eyes in the artificial tear group, resulting in a mean difference in Rose Bengal staining score of -0.60 (95% CI -1.11 to -0.09; P = 0.02) one month after LASIK (Analysis 1.3), and 0.1 ± 0.3 (16 eyes) and 0.8 ± 1.1 (11 eyes), respectively, three months after LASIK resulting in a mean difference of -0.70 (95% CI -1.37 to -0.03; P = 0.04) (Analysis 1.3; Noda-Tsuruya 2006).

The reported means for Rose Bengal in the 20% AS group at one week, one month and two months of follow-up were 3, 4.22 and 3.7, and 3.67, 4.22 and 3.8 in the control group respectively

(Tananuvat 2001). Again, the number of participants and SDs were not reported, although the trial investigators indicated a non-significant difference (P > 0.05) between 20% AS and artificial tears across all time points (Analysis 2.2).

Fluorescein staining

One study reported fluorescein staining greater than 1 as abnormal (Kojima 2005a). In 10 participants, the mean change and SD in fluorescein staining from baseline to two weeks follow-up was -1.1 ± 0.7 in the 20% AS group and -0.2 ± 0.6 in the artificial tear group, resulting in a difference in the mean change from baseline of -0.90 (95% CI -1.47 to -0.33; P = 0.002) (Analysis 1.4; Kojima 2005a). Noda-Tsuruya 2006 reported the mean and SD among 20 eyes at one month follow-up for the 20% AS group (0.5 ∓ 0.7), but the mean from the 23 eyes in the control group was not reported. Among 12 participants, the reported means at one week, one month and three months follow-up were 1.6, 1.55 and 1.33 in the 20% AS group and 1.7, 1.55 and 1.42 for the control group respectively (Analysis 2.3; Tananuvat 2001). The mean OXFORD scale for fluorescein staining decreased from baseline to two weeks from three to two in the 20% AS group, and from four to three in the artificial tear group (Urzua 2012). The trial investigators reported this was a non-significant difference (P > 0.05).

Aqueous tear production: Schirmer's test

Schirmer's test was performed without anesthesia in one of the included studies (Kojima 2005a) and with anesthesia in two of the included studies (Noda-Tsuruya 2006; Tananuvat 2001). At two weeks follow-up, the mean and SD for the 20% AS group was 3.3 ± 2.6 mm compared to 3.7 ± 3.1 mm in the artificial tear group, resulting in a mean difference of -0.40 (95% CI -2.91 to 2.11 mm; P = 0.75) (Analysis 1.5; Kojima 2005a). Tananuvat 2001 reported means of 2.83 mm in the 20% AS group and 3.25 mm in the control group after two months of follow-up, compared to 0.92 mm and 1.83 mm for both groups at baseline respectively (Analysis 2.4). Discriptive statistics (mean and SD) were not reported by Noda-Tsuruya 2006 for the 20 eyes in the 20% AS group or the 15 eyes in the control group evaluated for this outcome at one month. The trial investigators concluded that there was not a significant difference between the two groups.

Tear film stability: tear break-up time (TBUT)

Kojima 2005a reported a mean change and SD from baseline to two weeks follow up of 2.1 ± 1.1 seconds in the 20% AS group and 0.1 ± 1.2 seconds in the artificial tear group, resulting in a mean difference of 2.00 (95% CI 0.99 to 3.01) seconds (P = 0.0001) between 10 participants in each treatment group (Analysis 1.6). Among the 12 participants in Urzua 2012, the baseline mean TBUT was 4 ± 1.9 seconds in the 20% AS group and 3 ± 2.2 seconds in the artificial tear group. After two weeks, the mean was 6 ± 1.2 seconds in the 20% AS group and 4 ± 2.3 seconds in the artificial tear group (P > 0.05). Tananuvat 2001 described means (in seconds) of 0.8 at one week, 0.55 at one month and 0.83 at two months for the 20% AS group and 0.7 at one week, 0.64 at one month and 1.17 at two months for the control group (Analysis 2.5). The six-month mean TBUT for the 20% AS groups was 6.3 ± 2.6 seconds among eight postoperative LASIK eyes in the 20% AS group and 3.8 ± 1.9 seconds in 10 control eyes, leading to a mean difference of 2.50 (95% CI 0.35 to 4.65) seconds (P = 0.02) (Analysis 1.7; Noda-Tsuruya 2006).

Impression cytology

Only one study reported results from impression cytology at baseline and at two months follow-up according to conjunctival differentiation separated into six stages scored from 0 to 6 (Tananuvat 2001). At baseline, the mean score was 2.67 in the 20% AS group and 2.42 in the artificial tear group followed by means of 1.57 in the 20% AS group and 2.17 in the control group after two months of follow-up. The trial investigators reported that the mean difference at two months follow-up was non-significant (P > 0.05).

Complications and infections

Tananuvat 2001 reported two participants with signs of conjunctivitis with negative culture; in both cases symptoms resolved later with proper treatment. It was not stated whether the eye assigned to the AS or control group, or both eyes showed signs of conjunctivitis. Microbiologic culture of serum stored at -20°C for up to two months showed no growth. All returned used serum bottles underwent culture, and only one sample exhibited mixed organisms, including yeast. No infectious conjunctivitis or other adverse reaction was detected. Adverse events were not reported by the investigators of the remaining trials (Kojima 2005a; Noda-Tsuruya 2006; Urzua 2012).

None of the included trials evaluated tear osmolarity, corneal topography, fluorescein clearance, or conjunctival biopsy, specified as secondary outcomes for this review. Quality of life and cost or economic analyses were not reported by any of the included studies as well.

DISCUSSION

The use of autologous serum eye drops (AS) to treat people with dry eye has been described in a number of studies (Fox 1984; Kojima 2005a; Noda-Tsuruya 2006; Tananuvat 2001; Tsubota 1996; Tsubota 1999; Tsubota 2000). Our aim in performing this systematic review was to analyze the highest quality evidence from randomized controlled trials (RCTs) to determine the efficacy and safety of AS in treating people with dry eye. However, the majority of the published literature is limited to retrospective case reports or non-randomized case series.

Summary of main results

We identified four RCTs that investigated the effects of AS compared to artificial tears in participants with a variety of dry eye etiologies (Kojima 2005a; Noda-Tsuruya 2006; Tananuvat 2001; Urzua 2012). Kojima 2005a evaluated the effectiveness of 20% AS after a two-week treatment interval (six times a day) in participants with severe Sjögren's and non-Sjögren's syndrome dry eye. Urzua 2012 used a cross-over design to compare two week treatment intervals with 20% AS and artificial tears in 12 adult participants with severe non-Sjögren's syndrome dry eye. Tananuvat 2001 investigated efficacy of 20% AS in 12 participants with bilateral severe dry eye over a two-month treatment interval (six times daily). Noda-Tsuruya 2006 assessed the efficacy of 20% AS (five times daily) for post-LASIK dry eye from one week to six months.

Although precise measurement of symptoms is an important part of dry eye diagnosis, there is no universally accepted standardized method for recording participant-reported symptoms; it is commonly observed that participant-reported symptoms do not correlate with objective clinical tests (Alfonso 1999; Lin 2003; Schein 1997; Viso 2012). In this review, each study applied different methods to measure participant-reported symptoms. After taking into consideration the wide array of subjective questionnaires and scales used to measure participant symptoms and differences in the length of follow-up, improvement in participant-reported symptoms was not consistently observed in the four trials comparing AS to artificial tears. This might be due to the variety in the type and severity of dry eye among the participants in the studies included in this review.

Based on the reported data from the included studies, 20% AS were not associated with a significant improvement in aqueous tear production as measured by Schirmer's test or improvement in the condition of the ocular surface as measured by fluorescein or Rose Bengal staining compared to preservative-free artificial tears. Tananuvat 2001 further found that 20% AS did not significantly change impression cytology among participants with severe bilateral dry eye. Regarding tear film stability as measured by tear break-up time (TBUT), only Kojima 2005a showed a clinically meaningful difference between 20% AS and artificial tears for Sjögren's and non-Sjögren's syndrome dry eye participants after two weeks of treatment.

Three of the included studies did not report outcomes for adverse events or complications. One study (Tananuvat 2001) reported conjunctivitis in two participants with cultures showing no growth followed by resolution of symptoms. All used AS containers returned by study participants were cultured, with one sample showing mixed organisms, including yeast, but no infectious conjunctivitis or adverse reaction was detected in the study participant.

Overall completeness and applicability of evidence

A major difficulty in summarizing results from the included studies was the heterogeneity in the participant populations, interventions and comparisons**,** as well as variations in the procedures for preparing AS. Summary meta-analyses could not be conducted due to additional differences in follow-up intervals as well as incomplete descriptive statistics in the reported treatment outcomes. Thus, we were able to draw conclusions based on qualitative assessment of the trial reports.

Participant characteristics

The etiologies of dry eye described for included participants may not be representative of all people with dry eye who potentially may benefit from AS. Previous punctal occlusion was reported as an exclusion criterion in one study (Kojima 2005a), while people with previous punctal occlusion were eligible for another (Tananuvat 2001). One trial included participants with post-LASIK dry eye (Noda-Tsuruya 2006). Two trials enrolled participants with both severe Sjögren's and non-Sjögren's syndrome dry eye (Kojima 2005a; Tananuvat 2001), while participants with severe non-Sjögren's syndrome dry eye only were enrolled in another (Urzua 2012).

Interventions and comparisons

It is worth noting that participants of the intra-individual study (Tananuvat 2001) in which participants used AS in one eye and placebo in the fellow eye were instructed to use non-hyaluronan and unpreserved saline-based artificial tears as needed. Prior punctal occlusion was also reported in 75% of participants at the beginning of the study, adding further to the heterogeneity among included studies (Tananuvat 2001).

Preparation and storage of AS

Currently there are neither regulatory guidelines nor standard protocols for the manufacturing of AS for dry eye. The critical steps in the production of AS, such as clotting time, centrifugation, and dilution can influence the biochemical properties of AS and may lead to variable efficacy and treatment outcomes. In this review, only one study reported a clotting time of two hours following venipuncture (Urzua 2012) while clotting time was not reported in the other three studies. Geerling 2004 in Germany proposed two hours of clotting time at room temperature followed by optimal centrifugation of whole blood at 3000 × *g* for 15 minutes. Variation in the centrifugation speed and time were reported across included trials ranging from 1500 revolutions per minute (rpm) for five minutes (Kojima 2005a), 2200 rpm for 20 minutes (Noda-Tsuruya 2006), 4200 rpm for 15 minutes (Tananuvat 2001), and 3500 rpm for five minutes (Urzua 2012). It has been demonstrated that higher concentrations of EGF and lower concentrations TGF-b are obtained at higher centrifugation speed (Liu 2005; Pancholi 1998; Phasukkijwatana 2011). Although none of the trials measured the concentration of the biologically active components within AS, it is possible that the concentration of epithelial growth factor (EGF), transforming growth factor-β (TGF-β) or other biologic factors might be different across the included studies due to variation in the rpm used in

centrifugation. Interestingly, all of the four included trials compared 20% AS to non-hyaluronan and unpreserved saline-based artificial tears in this review. Although results from in vitro studies show the greatest cell proliferation with serum concentrations ranging from 12.5% to 25% (Geerling 2004; Liu 2005), 20% AS was the only concentration evaluated in the four trials included in this review.

In addition, the instructions given to study participants for storing AS were similar across included studies. Specifically, they were instructed to keep vials containing AS in the freezer (-20°C) for up to three months and in a refrigerator at 4° C for two weeks after thawing. The AS storage instructions given to study participants have been shown to be effective in preventing contamination and deterioration of biological growth factors (Geerling 2004). Kojima 2005a reported an additional precaution to protect serum vials from ultraviolet light because vitamin A is easily degraded by light. A study from Thailand (Phasukkijwatana 2011) demonstrated that the stability of biologically active components within AS could be maintained for up to six months when stored at -20°C. However, the U.S. Food and Drug Administration has not approved a standard procedure for preparing AS.

Quality of the evidence

Only four small RCTs in single-center settings have been identified; they included 72 total participants with severe Sjögren's-related dry eye, non-Sjögren's dry eye and post-LASIK dry eye. The small number of participants is insufficient to detect or rule out meaningful beneficial or harmful effects of AS or to provide precise estimates of individual outcomes.

Two studies were found to have an unclear risk of bias for masking of participants. Given the primary outcome (i.e. change in participant-reported symptoms), the results of individual trials could have been influenced if participants were aware of their treatment assignment. However, complete masking may not be feasible given the necessary venipuncture involved in AS production. Additional variation in the instructions reported for the proper storage of AS compared to artificial tears may also have led to participants knowing their treatment assignment.

Potential biases in the review process

We employed a comprehensive search strategy to identify potentially eligible trials in order to minimize selection bias. Throughout the review process, two review authors assessed all potentially eligible studies and completed data extraction independently to minimize errors. Although we sought unpublished data from investigators of all the included trials to supplement the data provided in the published reports, we were unable to conduct quantitative synthesis for any of the outcomes specified for this review. Based on the limited number of included trials we could not evaluate potential publication bias through examination of funnel plots.

Agreements and disagreements with other studies or reviews

In the 2011 Prefered Practice Patterns, the American Academy of Ophthalmology (AAO) suggested autologous serum drops improve ocular symptoms and conjunctival and corneal staining in severe dry eye (AAO 2011). The AAO's conclusions were described as level "A III" and did not incorporate the findings or conclusions from any of the four RCTs included in our review. Another evidence-based review (Akpek 2011) found II B evidence for serum eye drops in Sjögren's syndrome dry eye which reflect an absence of reliable evidence to support treatment decisions. None of the four trials included in our review were discussed in the review by Akpek et al. (Akpek 2011) which was focused on individuals with Sjögren's syndrome dry eye only and evaluated two studies excluded from our review (Noble 2004; Yoon 2007).

AUTHORS' CONCLUSIONS

Implications for practice

Based on the current evidence, 20% AS may provide some benefit in improving participant-reported symptoms in the short term (two weeks), but improvement was not observed through longer periods of follow-up. No effect was seen based on objective clinical measures of the ocular surface.

AS preparation, manufacturing and storage require a well-established, specialized service with strict aseptic processing. Procedures for AS production (clotting time, centrifugation and concentration), including the proper solute for making the AS should be optimized for the clinical application of AS in people with dry eye. In addition, all applicable legislative restrictions should be carefully considered and well documented, and informed consent should be obtained from each participant.

Implications for research

Well-planned, large scale, high-quality randomized controlled trials are needed, stratified by age and severity of dry eye, comparing AS to artificial tears (or other treatments), as well as evaluating additional concentrations of AS. These studies must have a random sequence generation protocol as well as appropriate concealment of the treatment assignments before allocation. Future studies should make attempts to ensure both participants and study investigators (clinical staff and outcome assessors) are masked to the treatment assignments in order to limit potential bias in participant-reported outcomes. We recommend that randomization

in such trials be stratified by participant's age and the severity of dry eye-related symptoms. Any future studies should utilize standardized and validated scoring systems of dry eye clinical severity and symptom questionnaires. Objective biomarkers, which have been reported as a parallel index of the dry eye severity scale, such as tear osmolarity, tear cytokines and HLA-DR expression by ocular surface cells (Lemp 2011; Tomlinson 2006; Versura 2012), should be applied as outcomes in conjunction with participant symptoms. Analyses should include both short-term (two to four weeks) and long-term (six to 12 months) outcomes. Data on adverse outcomes, including complications, infection, and tolerance of AS should be documented in future trials.

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The views expressed in this publication are those of the authors and not necessarily those of the NIHR, NHS or the Department of Health.

REFERENCES

References to studies included in this review

Kojima 2005a ***{published and unpublished data}***

* Kojima T, Ishida R, Dogru M, Goto E, Matsumoto Y, Kaido M, et al.The effect of autologous serum eyedrops in the treatment of severe dry eye disease: a prospective randomized case-control study. *American Journal Ophthalmology* 2005;**139**(2):242–6.

Kojima T, Ishida R, Goto E, Matsumoto Y, Dogru M, Tsubota K. The efficacy of autologous serum eye drops for Keratoconjunctivitis Sicca - A prospective study. *Investigative Ophthalmology and Visual Science* 2004;**45**: ARVO E-abstract 3894.

Noda-Tsuruya 2006 ***{published data only (unpublished sought but not used)}***

Noda-Tsuruya T, Asano-Kato N, Toda I, Tsubota K. Autologous serum eye drops for dry eye after LASIK. *Journal of Refractive Surgery* 2006;**22**(1):61–6.

Tananuvat 2001 ***{published data only (unpublished sought but not used)}***

Tananuvat N, Daniell M, Sullivan LJ, Yi Q, McKelvie P, McCarty DJ, et al.Controlled study of the use of autologous serum in dry eye patients. *Cornea* 2001;**20**(8):802-6.

Urzua 2012 ***{published data only (unpublished sought but not used)}***

Urzua CA, Vasquez DH, Huidobro A, Hernandez H, Alfaro J. Randomized double-blind clinical trial of autologous serum versus artificial tears in dry eye syndrome. *Current Eye Research* 2012;**37**(8):684–8.

References to studies excluded from this review

Albegger 1972 ***{published data only}***

Albegger KW, Tilz GP. Sjogren's syndrome and its therapy. A therapeutic attempt using antilymphocyte serum [Zum Sjogren–syndrom und seiner behandlung. Ein therapieversuch mit antilymphozytenglobulin]. *Zeitschrift Fur Laryngologie, Rhinologie, Otologie Und Ihre Grenzgebiete* 1972;**51**(7):429–37.

Alvarado 2004 ***{published data only}***

Alvarado Valero MC, Martinez Toldos JJ, Borras Blasco J, Alminana Alminana A, Perez Ramos JM. Treatment of persistent epithelial defects using autologous serum application [Tratamiento de defectos epiteliales persistentes mediante suero autologo]. *Archivos De La Sociedad Espanola De Oftalmologia* 2004;**79**(11):537–42.

Anderson 2004 ***{published data only}***

Anderson NG, Regillo C. Ocular manifestations of graft versus host disease. *Current Opinion in Ophthalmology* 2004;**15**(6):503–7.

Badami 2009 ***{published data only}***

Badami K, McKellar M. Allogeneic serum eye drops-a useful alternative for those unable to be autologous donors. Transfusion. Proceedings of the AABB Annual Meeting and TXPO; 2009 Oct 24 -27; New Orleans (LA). 2009.

Bradley 2008 ***{published data only}***

Bradley JC, Bradley RH, Mccartney DL, Mannis MJ. Serum growth factor analysis in dry eye syndrome. *Clinical and Experimental Ophthalmology* 2008;**36**(8):717–20.

Brown 2005 ***{published data only}***

Brown SM, Bradley JC. The effect of autologous serum eye drops in the treatment of severe dry eye disease: a

prospective randomized case-control study. *American Journal of Ophthalmology* 2005;**140**(3):565.

Chiang 2007 ***{published data only}***
Chiang CC, Lin JM, Chen WL, Tsai YY. Allogeneic serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. *Cornea* 2007;**26**(7): 861–3.

Craig 2008 ***{published data only}***
Craig JP. A natural solution to dry eye?. *Clinical and Experimental Ophthalmology* 2008;**36**(2):109–10.

Fuchsluger 2005 ***{published data only}***
Fuchsluger TA, Steuhl KP, Meller D. Neurotrophic keratopathy-a post-LASIK case report. *Klinische Monatsblatter fur Augenheilkunde* 2005;**222**(11):901–4.

Geerling 2002 ***{published data only}***
Geerling G, Hartwig D. Autologous serum-eye-drops for ocular surface disorders. A literature review and recommendations for their application [Autologe serum–augentropfen zur therapie der augenoberflache eine ubersicht zur wirksamkeit und empfehlungen zur anwendung]. *Ophthalmologe* 2002;**99**(12):949–59.

Geerling 2004 ***{published data only}***
Geerling G, Maclennan S, Hartwig D. Autologous serum eye drops for ocular surface disorders. *British Journal of Ophthalmology* 2004;**88**(11):1467–74.

Geerling 2008 ***{published data only}***
Geerling G, Unterlauft JD, Kasper K, Schrader S, Opitz A, Hartwig D. Autologous serum and alternative blood products for the treatment of ocular surface disorders [Eigenserum und alternative Blutprodukte zur Behandlung von Augenoberflachenerkrankungen]. *Ophthalmologe* 2008; **105**(7):623–31.

Harritshoj 2011 ***{unpublished data only}***
Harritshoj LH, Hansen MB, Julian HO. Ready-made allogenic serum eye drops for severe dry eye disease. Vox Sanguinis. Proceedings of the 21st Regional Congress of the International Society of Blood Transfusion; 2011 Jun 18-22; Lisbon, Portugal. 2011.

Hyon 2007 ***{published data only}***
Hyon JY, Lee YJ, Yun PY. Management of ocular surface inflammation in Sjogren syndrome. *Cornea* 2007;**26**(9 (Suppl 1)):S13–5.

Jaksche 2005 ***{unpublished data only}***
Jaksche A, Sbeity Z, Domeier E, Fimmers R, Holz F, Loeffler KU. Undiluted versus diluted Autologous Serum Eye Drops (ASED): A prospective, randomized, double-blind study in patients with refractory dry eye-syndrome. *Investigative Ophthalmology and Visual Science* 2005;**46**: ARVO E-abstract 2045.

Koffler 2006 ***{published data only}***
Koffler BH. Autologous serum therapy of the ocular surface with novel delivery by platelet concentrate gel. *Ocular Surface* 2006;**4**(4):188–95.

Kojima 2005b ***{published data only}***
Kojima T, Dogru M, Matsumoto Y, Goto E, Tsubota K. Tear film and ocular surface abnormalities after eyelid tattooing. *Ophthalmic Plastic and Reconstructive Surgery* 2005;**21**(1):69–71.

Kojima 2008 ***{published data only}***
Kojima T, Higuchi A, Goto E, Matsumoto Y, Dogru M, Tsubota K. Autologous serum eye drops for the treatment of dry eye diseases. *Cornea* 2008;**27**(Suppl 1):25–30.

Messmer 2005 ***{published data only}***
Messmer EM. Management of keratoconjunctivitis sicca in Sjogren's syndrome. *Aktuelle Rheumatologie* 2005;**30**(1): 59–65.

Movahedan 2006 ***{published data only}***
Movahedan H, Ghassemifar V. Treatment of persistent corneal epithelial defect with autologous serum. *Asian Journal of Ophthalmology* 2006;**8**(6):236–41.

Noble 2004 ***{published data only}***
Noble BA, Loh RS, MacLennan S, Pesudovs K, Reynolds A, Bridges LR, et al.Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular surface disease. *British Journal of Ophthalmology* 2004;**88**(5):647-52.

Ogawa 2003 ***{published data only}***
Ogawa Y, Okamoto S, Mori T, Yamada M, Mashima Y, Watanabe R, et al.Autologous serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. *Bone Marrow Transplantation* 2003;**31** (7):579–83.

Poon 2001 ***{published data only}***
Poon AC, Geerling G, Dart JK, Fraenkel GE, Daniels JT. Autologous serum eyedrops for dry eyes and epithelial defects: clinical and in vitro toxicity studies. *British Journal of Ophthalmology* 2001;**85**(10):1188–97.

Watson 2010 ***{published data only}***
Watson S L, Daniels JT, Geerling G, Dart JK. Clinical trials of therapeutic ocular surface medium for moderate to severe dry eye. *Cornea* 2010;**29**(11):1241–6.

Yoon 2007 ***{published data only}***
Yoon KC, Heo H, Im SK, You IC, Kim YH, Park YG. Comparison of autologous serum and umbilical cord serum eye drops for dry eye syndrome. *American Journal of Ophthalmology* 2007;**144**(1):86–92.

Additional references

AAO 2011
American Academy of Ophthalmology Cornea/External Disease Panel. Preferred Practive Pattern Guidlines. Dry Eye Syndrome - Limited Revision. San Francisco, CA: American Academy of Ophtalmology; 2011; Vol. Available at: www.aao.org/ppp.

Akpek 2011
Akpek EK, Lindsley KB, Adyanthaya RS, Swamy R, Baer AN, McDonnel PJ. Treatment of Sjögren's Syndrome-associated dry eye. *Ophthalmology* 2011;**118**:1242–1252.

Alfonso 1999
Afonso AA, Monroy D, Stern ME, Feuer WJ, Tseng SC, Pflugfelder SC. Correlation of tear fluorescein clearance and Schirmer test scores with ocular irritation symptoms. *Ophthalmology* 1999;**106**(4):803–10.

Baudouin 2001
Baudouin C. The pathology of dry eye. *Survey of Ophthalmology* 2001;**45**(Suppl 2):S211–20.

Baudouin 2010
Baoudouin C, Labbe A, Liang H, Pauly A, Brignole-Baudouin F. Preservatives in eyedrops: the good, the bad and the ugly. *Progress in Retinal and Eye Research* 2010;**29**(4):321–34.

Begley 2003
Begley CG, Chalmers RL, Abetz L, Venkataraman K, Mertzanis P, Caffery BA, et al.The relationship between habitual patient-reported symptoms and clinical signs among patients with dry eye of varying severity. *Investigative Ophthalmology and Visual Science* 2003;**44**(11):4753–61.

Behrens 2006
Behrens A, Doyle JJ, Stern L, Chuck RS, McDonnell PJ, Azar DT, et al.Dysfunctional tear syndrome: a Delphi approach to treatment recommendations. *Cornea* 2006;**25**(8):900–7.

Blomquist 2010
Blomquist PH. Ocular complications of systemic medications. American Journal of Medical Science 2011; Vol. 342, issue 1:62–9.

Bron 2003
Bron A, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea* 2003;**22**(7):640–50.

Campos 1992
Campos M, Hertzog L, Garbus JJ, McDonnell PJ. Corneal sensitivity after photorefractive keratectomy. *American Journal of Ophthalmology* 1992;**114**(1):51–4.

Chambers 1999
Chambers CT, Giesbreacht K, Craig KD, Bennett SM, Huntsman E. A comparison of faces scales for the measurement of pediatric pain: children's and parent's ratings. *Pain* 1999;**83**(1):25–35.

Chodosh 1994
Chodosh J, Dix RD, Howell RC, Stroop WG, Tseng SC. Staining characteristics and antiviral activities of sulforhodamine B and lissamine green. *Investigative Ophthalmology and Vision Science* 1994;**35**(3):1046–58.

de Paiva 2008
de Paiva CS, Pflugfelder SC. Rational for anti-inflammatory therapy in dry eye syndrome. *Arquivos Brasileiros de Oftalmolgia* 2008;**71**(Suppl 6):89–95.

Demato 1984
Demato BE, Allan D, Murray SB, Lee WR. Senile atrophy of the human lacrimal gland: the contribution of chronic inflammatory disease. *British Journal of Ophthalmology* 1984;**68**(9):674–80.

DEWS 2007
Anonymous. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop. *Ocular Surface* 2007;**5**(2):75–92.

Dogru 2011
Dogru M, Tsubota K. Pharmacotherapy of dry eye. *Expert Opinion on Pharmacotherapy* 2011;**12**(3):325–34.

Doughty 1997
Doughty MJ, Fonn D, Richter D, Simpson T, Caffery B, Gordon K. A patient questionnaire approach to estimating the prevalence of dry eye symptoms in patients presenting to optometric practices across Canada. *Optometry and Vision Science* 1997;**74**(8):624–31.

Ervin 2010
Ervin A-M, Wojciechowski R, Schein O. Punctal occlusion for dry eye syndrome. *Cochrane Database of Systematic Reviews* 2010, Issue 9. [DOI: 10.1002/14651858.CD006775.pub2]

Foulks 2003
Foulks GN. The evolving treatment of dry eye. *Ophthalmology Clinics of North America* 2003;**16**(1):29–35.

Fox 1984
Fox RI, Chan R, Michelson JB, Belmont JB, Michelson PE. Beneficial effect of artificial tears made with autologous serum in patients with keratoconjunctivitis sicca. *Arthritis and Rheumatism* 1984;**27**(2):459–61.

Fox 2006
Fox RI, Liu AY. Sjogren's syndrome in dermatology. *Clinics in Dermatology* 2006;**24**(5):393–413.

Fuentes-Paez 2011
Fuentes-Paez G, Herreras JM, Cordero Y, Almaraz A, Gonxalez MJ, Calonge M. Lack of concordance between dry eye syndrome questionnaires and diagnostic tests [Falta de concordancia entre los cuestionarios y las pruebas diagnosticas en el sindrome de ojo seco]. *Archivos de la Sociedad Espanola de Oftalmologia* 2011;**86**(1):3–7.

Glanville 2006
Glanville JM, Lefebvre C, Miles JN, Camosso-Stefinovic J. How to identify randomized controlled trials in MEDLINE: ten years on. *Journal of the Medical Library Association* 2006;**94**(2):130–6.

Gordon 1995
Gordon JF, Johnson P, Musch DC. Topical fibronectin ophthalmic solution in the treatment of persistent defects of the corneal epithelium. *American Journal of Ophthalmology* 1995;**119**(3):281–7.

Higgins 2003
Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**:557–60.

Higgins 2011a
Higgins JPT, Altman DG, Sterne JAC (editors). Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic

Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Higgins 2011b
Higgins JPT, Deeks JJ, Altman DG (editors). Chapter 16: Special topics in statistics. In: Higgins JPT, Green S (editors), Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Imanishi 2000
Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozone C, Kinoshita S. Growth factors: importance in wound healing and maintenance of transparency of the cornea. *Progress in Retinal and Eye Research* 2000;**19**(1):113–29.

Joh 1986
Joh T, Itoh M, Katsumi K, Yokoyama Y, Takeuchi T, Kato T, et al.Physiological concentrations of human epidermal growth factor in biological fluids: use of a sensitive enzyme immunoassay. *Clinica Chima Acta* 1986;**158**(1):81–90.

Kumar 2005
Kumar V, Abbas AK, Fausto N. *Robbins and Cotran Pathologic Basis of Disease*. 7th Edition. Philadelphia: Elsevier Saunders, 2005.

Langnado 2004
Lagnado R, King AJ, Donald F, Dua HS. A protocol for low contamination risk of autologous serum drops in the management of ocular surface disorders. *British Journal of Ophthalmology* 2004;**88**(4):464–5.

Lee 2008
Lee GA, Chen SX. Autologous serum in the management of recalcitrant dry eye syndrome. *Clinical and Experimental Ophthalmology* 2008;**36**(2):119–22.

Lemp 1995
Lemp MA. Report of the National Eye Institute/Industry workshop on clinical trials in dry eyes. *The CLAO Journal* 1995;**21**(4):221–32.

Lemp 2008
Lemp MA. Management of dry eye disease. *American Journal of Managed Care* 2008;**14**(Suppl 3):S88–101.

Lemp 2011
Lemp MA, Bron AJ, Baudouin C, Benitez del Castillo JM, Geffen D, Tauber J, et al.Tear osmolarity in the diagnosis and management of dry eye disease. *American Journal of Ophthalmology* 2001;**151**(5):792–8.

Lin 2003
Lin PY, Tsai SY, Cheng CY, Liu JH, Chou P, Hsu WM. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology* 2003;**110**(6):1096–101.

Liu 2005
Liu L, Hartwig D, Harloff S, Herminghaus P, Wedel T, Geerling G. An optimised protocol for the production of autologous serum eyedrops. *Graefe's Archive for Clinical and Experimental Ophthalmology* 2005;**243**(7):706–14.

Marci 2000
Marci A, Pflugfelder S. Correlation of the Schirmer 1 and fluorescein clearance tests with the severity of corneal epithelia and eyelid disease. *Archives of Ophthalmology* 2000;**118**(12):1632–38.

Matsumoto 2004
Matsumoto Y, Dogrou M, Goto E, Ohashi Y, Kojima T, Ishida R, et al.Autologous serum application in the treatment of neurotrophic keratopathy. *Ophthalmology* 2004;**111**(6):1115–20.

McCarty 1998
McCarty C, Bansal AK, Liningston PM, Stanislavsky YL, Taylor HR. The epidemiology of dry eye in Melbourne, Australia. *Ophthalmology* 1998;**105**(6):1114–9.

McCluskey 1987
McCluskey P, Wakefield D, York L. Topical fibronectin therapy in persistent corneal ulceration. *Australia and New Zealand Journal of Ophthalmology* 1987;**15**(4):257–62.

McDonnell 1988
McDonnell PJ, Schanzlin DJ, Rao NA. Immunoglobulin deposition in the cornea after application of autologous serum. *Archives of Ophthalmology* 1988;**106**(10):1423–5.

Meadows 2005
Meadows M. Dealing with dry eye. FDA Consumer Magazine 2005; Vol. 39, issue 3.

Nelson 1988
Nelson JD, Farris RL. Sodium hyaluronate and polyvinyl alcohol artificial tear preparations. A comparison in patients with keratoconjunctivitis sicca. *Archives of Ophthalmology* 1988;**106**:484–7.

Nelson 1992
Nelson JD, Gordon JF. Topical fibronectin in the treatment of keratoconjunctivitis sicca. Chiron Keratoconjunctivitis Sicca Study Group. *American Journal of Ophthalmology* 1992;**114**(4):441–7.

Nichols 2004
Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. *Cornea* 2004;**23**(3):272–85.

Nishida 1983
Nishia T, Ohasai Y, Awata T, Manabe R. Fibronectin. A new therapy for corneal trophic ulcer. *Archives of Ophthalmology* 1983;**101**(7):1046–8.

Nishida 1987
Nishida T, Nakamura M, Ofuji K, Reid TW, Mannis MJ, Murphy CJ. Synergistic effects of substance P with insulin-like growth factor-1 on epithelia migration of the cornea. *Journal of Cell Physiology* 1996;**169**(1):156–66.

Ozcura 2007
Ozcura F, Aydin S, Helvaci MR. Ocular surface disease index for the diagnosis of dry eye syndrome. *Ocular immunology and inflammation* 2007;**15**(5):389–93.

Pancholi 1998
Pancholi S, Tullo A, Khaliq A, Foreman D, Boulton M. The effects of growth factors and conditioned media on

the proliferation of human corneal epithelial cells and keratocytes. *Graefe's Archive for Clinical and Experimental Ophthalmology* 1998;**236**(1):1–8.

Partal 2011
Partal A, Scott E. Low-cost protocol for the production of autologous serum eye drops by blood collection and processing centres for the treatment of ocular surface disease. Transfusion Medicine 2011; Vol. 21, issue 4:271–7.

Patel 2002
Patel SJ, Lundy DC. Ocular manifestations of autoimmune disease. *American Family Physician* 2002;**66**(6):991–8.

Patrick 2011
Patrick D, Guyatt GH, Acquardro C. Chapter 17: Patient-reported outcomes. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org. The Cochrane Collaboration, 2011. Available from www.cochrane–handbook.org.

Perry 2004
Perry HD, Donnenfeld ED. Dry eye diagnosis and management in 2004. *Current Opinion in Ophthalmology* 2004;**15**(4):299–304.

Pflugfelder 2004
Pfludgfelder SC. Antiinflammatory therapy for dry eye. *American Journal of Ophthalmology* 2004;**137**(2):337–42.

Phan 1987
Phan TM, Foster CE, Bourochoff SA, Zagachin LM, Colvin RB. Topical fibronectin in the treatment of persistent corneal epithelial defects and trophic ulcers. *American Journal of Ophthalmology* 1987;**104**(5):494–501.

Phasukkijwatana 2011
Phasukkijwatana N, Lertrit P, Liammongkolkul S, Prabhasawat P. Stability of epitheliotrophic factors in autologous serum eye drops from chronic Stevens-Johnson syndrome dry eye compared to non-autoimmune dry eye. *Current Eye Research* 2011;**36**(9):775–81.

Quinto 2008
Quinto GG, Campos M, Behrens A. Autologous serum for ocular surface disease. *Arquivos Brasileiros de Oftalmologia* 2008;**71**(Suppl 6):47–54.

Ralph 1975
Ralph RA, Doane MG, Dohlman CH. Clinical experience with a mobile ocular perfusion pump. *Archives of Ophthalmology* 1975;**93**(10):1039–43.

RevMan 2012
The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). 5.2. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2012.

Roback 2008
Roback JD, Coombs MR, Grossman BJ, Hillyer CD. *Technical manual*. 16th Edition. Bethesda, MD: American Association of Blood Banks, 2008.

Rocha 2000
Rocha EM, Pelegrino FS, de Paiva CS, Vigorito AC, de Sourza CA. GVHD dry eye treated with autologous serum tears. *Bone Marrow Transplant* 2000;**25**(10):1101–3.

Sall 2000
Sall K, Stevenson OD, Mundorf TK, Reis BL. Two multicenter, randomized studies of the efficacy and safety of cyclosporine ophthalmic emulsion in moderate to severe dry eye disease. CsA Phase 43 Study Group. *Ophthalmology* 2000;**107**(4):631–9.

Schaumberg 2003
Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Ophthalmology* 2003;**136**(2):318–26.

Schein 1997
Schein OD, Tielsch JM, Munõz B, Bandeen-Roche K, West S. Relation between signs and symptoms of dry eye in the elderly. A population-based perspective. *Ohthalmology* 1997;**104**(9):1395–401.

Stern 2004
Stern ME, Gao J, Siemasko KF, Beurerman RW, Pflugfelder SC. The role of the lacrimal functional unit in the pathophysiology of dry eye. *Experimental Eye Research* 2004;**78**(3):409–16.

Stevenson 2000
Stevenson D, Tauber J, Reis BL. Efficacy and safety of cyclosporin A ophthalmic emulsion in the treatment of moderate-to-severe dry eye disease: a dose-ranging, randomized trial. The Cyclosporin A Phase 2 Study Group. *Ophthalmology* 2000;**107**(5):967–74.

Toda 2004
Toda I, Asano-Kato N, Hori-Komai Y, Tsubota K. Ocular surface treatment before last in situ keratomileusis in patients with severe dry eye. *Journal of Refractive Surgery* 2004;**20**(3):270–5.

Toker 2010
Toker E, Asfuroglu E. Corneal and conjunctival sensitivity in patients with dry eye: the effect of topical cyclosporine therapy. *Cornea* 2010;**29**(2):133–40.

Tomlinson 2006
Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: determination of a referent for dry eye diagnosis. *Investigative Ophthalmology and Visual Science* 2006;**47**(10):4309–15.

Tomlinson 2009
Tomlinson A, McCann L, Pearce EI. Comparison of OcuSense and Clifton Nanolitre Osmometers. *Investigative Ophthalmology and Vision Science* 2009;**50**:ARVO E-abstract 534.

Tsubota 1996
Tsubota K, Satake Y, Ohyama M, Toda I, Takano Y, Ono M, et al.Surgical reconstruction of the ocular surface in advanced ocular cicatricial pemphigoid and Stevens-Johnson syndrome. *American Journal of Ophthalmology* 1996;**122**(1):38–52.

Tsubota 1999

Tsubota K, Goto E, Fujita H, Ono M, Inoue H, Saito I, et al.Treatment of dry eye by autologous serum applications in Sjögren's Syndrome. *British Journal of Ophthalmology* 1999; **83**(4):390–5.

Tsubota 2000

Tsubota K, Higuchi A. Serum application for the treatment of ocular surface disorders. *International Ophthalmology Clinics* 2000;**40**(4):113–122.

Versura 2012

Versura P, Profazio V, Buzzi M, Stancari A, Arpinati M, Malavolta N, et al.Efficacy of standardized and quality-controlled cord blood serum eye drop therapy in the healing of severe corneal epithelial damage in dry eye. *Cornea* 2012; **32**(4):412–8.

Viso 2012

Viso E, Rodriguez-Ares MT, Abelenda D, Oubina B, Gude F. Prevalence of asymptomatic and symptomatic meibomian glad dysfunction in the general population of Spain. *Investigative Ophthalmology and Visual Science* 2012; **53**(6):2601–6.

Wilson 2007

Wilson SE, Perry HD. Long-term resolution of chronic dry eye symptoms and signs after topical cyclosporine treatment. *Ophthalmology* 2007;**114**(1):76–9.

Yamada 1990

Yamada K, Hayasaka S, Setogawa T. Test results in patients with Sjogren's syndrome defined by the Japanese criteria. *Acta Ophthalmogica* 1990;**68**(1):80–6.

* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Kojima 2005a

Methods	**Study design:** "prospective randomized case-control study" **Unit of randomization: (Individuals/eyes):** 20 individuals (37 eyes) **Unit of analysis (Individuals/eyes):** individuals **Number randomized** Total: 20 Per group: 10 **Number analyzed** Total: 20 Per group: 10 **Was an intention-to-treat analysis performed? (Yes/No):** No
Participants	**Country:** Japan **Age:** AS: 62.3 ∓ 12.5 artificial tears: 65.4 ∓ 9.7 **Gender:** AS: 2 men and 8 women artificial tears: 2 men and 8 women **Underlying conditions:** 9 of the 10 participants in artificial tear group and 8 of the 10 participants in the AS had Sjögren's Syndrome **Inclusion criteria:** All participants met the diagnostic criteria of the Japanese Dry Eye Research group: Schirmer 1 test < 5 mm, or tear film BUT < 5 seconds **Exclusion criteria:** History of punctal occlusion, ocular or systemic disease, or a history of drug or contact lens use that would alter the ocular surface
Interventions	**Treatment or Intervention 1:** 20% AS (saline) **Control or Intervention 2:** preservative-free artificial tears **Length of follow-up:** Planned: 2 weeks Actual: 2 weeks
Outcomes	**VISUAL ANALOG PAIN SYMPTOM SCORE:** Absence of any pain constituted a score of 0 points on the visual analog pain scales, and intense, unbearable pain was considered a full pain score of 100 points. **Tear function:** Tear film BUT was measured 3 times, and the mean value was calculated. The tear film BUT was considered abnormal if it was less than 5 seconds. Schirmer's test was considered abnormal if it was less than 5 mm. **Ocular surface:** The ocular surface was examined by the double vital staining method. 2 milliliters of a preservative-free combination of 1% Rose Bengal and 1% fluorescein dye was instilled in the conjunctival sac. "According to the study protocol, tear film BUT analysis was performed initially, followed by fluorescein and Rose Bengal vital staining of the ocular surface. The Schirmer 1 test

	was then performed. Tear film BUT, vital staining of the ocular surface, and visual analog pain symptom scores were compared before and after treatment"
Notes	**Type of study (published/unpublished) and journal of publication:** published in the American Journal of Ophthalmology Presented at the 28th Japan Cornea Congress, February 19 - 21, 2003, Yonago, Japan, and at the 2004 ARVO Meeting, Fort Lauderdale, Florida, April 26, 2004. **Source of Funding:** Japanese Ministry of Education and Science (Tokyo) and Hightech Research Center at Tokyo Dental College (Chiba, Japan) Study author provided additional information not included in published report

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"After washout, all patients were randomly assigned to two groups..."
Allocation concealment (selection bias)	Unclear risk	"After washout, all patients were randomly assigned to two groups..."
Masking of participants of the allocated intervention (Performance bias)	Unclear risk	The study described the collection and production of autologous serum including venipuncture as well as storage requirement, but it was unclear whether only the autologous serum group underwent the necessary collection procedures or received the same storage instructions.
Masking of study personnel of the allocated intervention (Performance bias)	Unclear risk	No information was provided in the published report to determine whether study personnel were aware of each participant's treatment assignment. Specific instructions were given to study participants regarding the proper care and storage of the autologous serum vials
Masking of outcome assessors during follow-up - patient reported symptoms (Detection bias)	Unclear risk	"patients were asked to check a point on the line corresponding to their degree of pain"
Masking of outcome assessors during follow-up - clinical examination (Detection bias)	Low risk	"The examiner who carried out the tear function and ocular surface evaluations was masked to the type of the eyedrops prescribed to the patients in this study"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	A total of 39 eyes from 20 participants were analyzed and reported in a conference ab-

Kojima 2005a (*Continued*)

		stract, while 37 eyes from 20 patients were analyzed in the full-text publication. No explanation given for eyes excluded in the full-text report
Selective reporting (reporting bias)	Low risk	All outcomes were reported as described in the methods.
Other bias	Low risk	No other potential sources of bias were identified.

Noda-Tsuruya 2006

Methods	**Study design:** Prospective randomized study **Unit of randomization: (Individuals/Eyes):** individuals **Unit of analysis (Individuals/Eyes):** eyes **Number randomized:** Total: 27 participants (54 eyes) Per group: AS: 12 participants (24 eyes) artificial tears: 15 participants (30 eyes) **Number of eyes analyzed:** **1 month** BUT: AS 20, artificial tears 23 Schirmer's: AS 20, artificial tears 19 Rose Bengal: AS 20, artificial tears 15 Fluorescein: AS 20, artificial tears 23 **3 months** BUT: AS 18, artificial tears 15 Schirmer's: AS 16, artificial tears 15 Rose Bengal: AS 16, artificial tears 11 Fluorescein: AS 18, artificial tears 15 **6 months** BUT: AS 8, artificial tears10 Schirmer's: AS 8, artificial tears 10 Rose Bengal: AS 6, artificial tears 10 Fluorescein: AS 8, artificial tears 10 **Was an intention-to-treat analysis performed? (Yes/No):** No
Participants	**Country:** Japan **Age:** 30.1 – 5.8 **Gender:** 100% men **Underlying conditions:** All participants had LASIK surgery one week prior to start of study. No others reported **Concurrent dry eye treatments:** One week after LASIK surgery all participants received topical steroids, antibiotics and hyaluronic acid eye drops 5 times per day and discontinued use at 1 week postoperatively **Inclusion criteria:** post-LASIK male participants, no others reported. "All patients revealed normal findings by routine preoperative ophthalmologic examina-

	tion including tear function and vital staining. None of the patients had worn contact lenses before LASIK."
Interventions	**Treatment or Intervention 1:** 20% AS (saline) **Control or Intervention 2:** artificial tears: unpreserved, saline-based (Softsantear, Santen) **Length of follow-up:** Planned: 1 week post-LASIK to 6 months post-LASIK Actual: 1 week post-LASIK to 6 months post-LASIK
Outcomes	**Participant questionnaire:** Dry eye symptoms were graded by the participants using a written questionnaire according to the following criteria: 0, none; 1, mild; 2, moderate; 3, strong; and 4, very strong **Tear function:** Schirmer test with anesthesia, tear clearance rate, and tear break-up time (BUT) **Ocular surface staining:** Fluorescein staining was graded from 0 to 3 for each of the upper, middle, and lower thirds of the cornea. Rose bengal staining was graded from 0 to 3 for the temporal conjunctiva, cornea, and nasal conjunctiva. The grading scale was decided according to the extent of staining; 0, negative; 1, minute scattering; 2, moderately spotty; and 3, diffuse blotchy staining. Total of scores in the 3 areas was defined as fluorescein or Rose Bengal score
Notes	**Type of study (published/unpublished) and journal of publication:** published in the Journal of Refractive Surgery **Source of Funding:** not reported **Reported subgroup analyses:** none reported Contacted author for additional information, but did not receive additional information not included in published report

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"All candidates for the study were selected, and patients were randomly divided into two groups"
Allocation concealment (selection bias)	Unclear risk	"All candidates for the study were selected, and patients were randomly divided into two groups"
Masking of participants of the allocated intervention (Performance bias)	Unclear risk	The study described the collection and production of autologous serum including venepuncture as well as storage requirement, but it was unclear whether only the autologous serum group underwent the necessary collection procedures or received the same storage instructions.

Noda-Tsuruya 2006 (*Continued*)

Masking of study personnel of the allocated intervention (Performance bias)	Unclear risk	No information was provided in the published report to determine whether study personnel were aware of each participant's treatment assignment. But specific instructions were given to study participants regarding the proper care and storage of the autologous serum vials
Masking of outcome assessors during follow-up - patient reported symptoms (Detection bias)	Unclear risk	"Typical dry eye symptoms were graded by the patients using a written questionnaire according to the following criteria: 0, none; 1, mild; 2, moderate; 3, strong; and 4, very strong."
Masking of outcome assessors during follow-up - clinical examination (Detection bias)	Unclear risk	"To evaluate tear function, Schirmer test with anesthesia, tear clearance rate, and tear break-up time (BUT) were measured as previously described." No other description discussing whether outcome assessment was done by a masked investigator
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Study reported the number of eyes for each outcome at all time point across both treatment arms, but did not give reasons for missing outcome data
Selective reporting (reporting bias)	Unclear risk	Reported all outcomes at all time points for outcomes described in the methods, although reported information was insufficient to extract usable data for quantitative summary analysis
Other bias	Unclear risk	Unit of randomization was the participant while the reported results were per eye

Tananuvat 2001

Methods	**Study design:** Prospective, single-masked, placebo-controlled, paired-eye study **Unit of randomization: (Individuals/Eyes):** eyes **Unit of analysis (Individuals/Eyes):** eyes **Number randomized** Total: 13 Per group: 13 **Exclusions after randomization and reasons for exclusion** "One patient with ocular cicatricial pemphigoid was excluded after enrollment because of asymmetry of the severity of dry eye between the two eyes" **Losses to follow-up and reasons for loss to follow-up** none reported **Number analyzed** Total: 12 Per group: 12 **Was an intention-to-treat analysis performed? (Yes/No):** No
Participants	**Country:** Australia **Age:** mean 59.5 (range: 33 - 80) **Gender:** Men: 5 Women: 7 **Underlying conditions:** 5 participants had Sjögren's syndrome, 2 participants had primary Sjögren's syndrome and 3 had secondary Sjögren's syndrome. The non-Sjögren's type dry eyes included non-Hodgkin's lymphoma (n = 1), graft-versus-host disease (n = 1), Stevens-Johnson syndrome (n = 1), rheumatoid arthritis (n = 1), and 3 idiopathic **Concurrent dry eye treatments:** artificial tears as needed **Inclusion criteria:** "Patients with bilateral severe dry eye were enrolled in this study. All had low Schirmer test scores and positive rose bengal staining and symptoms of dry eye despite frequent lubricants or previous punctal occlusion". **Exclusion criteria:** "Patients were excluded if they had active ocular infection or inflammation not related to dry eye, had ocular surgery within 3 months, were monocular, or had other conditions that may mimic dry eye symptoms such as allergic conjunctivitis or lid or lash abnormalities"
Interventions	**Treatment or Intervention 1:** 20% AS **Control or Intervention 2:** unpreserved saline solution and dilute fluorescein solution **Length of follow-up:** Planned: 2 months Actual: 2 months
Outcomes	**Participant questionnaire:** Symptoms of dry eye (discomfort, foreign-body sensation, dryness, and photophobia) were recorded at every visit and graded according to the severity as grade 0, no symptom; 1, mild; 2, moderate; and 3, severe **Tear function:** assessed by Schirmer's test with anesthesia at baseline and 2 months after treatment **Ocular surface:** examined with tear break-up time (TBUT) and vital dye staining with fluorescein andRose Bengal. Fluorescein staining was also rated from 0 to 3 but only on the cornea. For Rose Bengal staining the degree of staining was recorded separately for temporal and nasal conjunctiva and cornea on a scale of 0 to 3. The maximum score for each area was 3. The scores for each area were added together to obtain the total score for each eye. Therefore, the maximum score for each eye was 9. Conjunctival impression

	cytology and slit-lamp photography were also performed to document the change of ocular surface. Corrected visual acuity and slit-lamp examinations were also performed on each visit and the application of additional topical lubricants was recorded for both treatment groups
Notes	**Type of study (published/unpublished) and journal of publication:** published in the journal Cornea **Source of Funding:** not reported **Reported subgroup analyses:** no Study author provided additional information for assessing risk of bias not included in published report

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Investigators used blocks of 2 for randomization where the right eye of participant 1 was assigned to be the study eye and the fellow eye as the control eye. For participant 2, the left eye was assigned as the study eye and the fellow eye as the control. (correspondence from study investigator)
Allocation concealment (selection bias)	High risk	Investigators used blocks of 2 for randomization where the right eye of participant 1 was assigned to be the study eye and the fellow eye as the control eye. For participant 2, the left eye was assigned as the study eye and the fellow eye as the control. (correspondence from study investigator)
Masking of participants of the allocated intervention (Performance bias)	Low risk	Participants were masked to treatment assignment. (correspondence from study investigator)
Masking of study personnel of the allocated intervention (Performance bias)	High risk	The investigator who assessed the outcomes was not masked to the treatment assignments. (correspondence from study investigator)
Masking of outcome assessors during follow-up - patient reported symptoms (Detection bias)	High risk	The investigator who assessed the outcomes was not masked to the treatment assignments. (correspondence from study investigator)
Masking of outcome assessors during follow-up - clinical examination (Detection bias)	High risk	The investigator who assessed the outcomes was not masked to the treatment as-

Tananuvat 2001 (*Continued*)

		signments (correspondence from study investigator). Although these were objective clinical tests, there is potential detection bias if investigators conducting the test and interpreting the results were aware of the participant's treatment assignment
Incomplete outcome data (attrition bias) All outcomes	Low risk	Study reported one participant who was excluded after randomization. No other missing data were reported
Selective reporting (reporting bias)	Unclear risk	Reported all outcomes at all time points for outcomes described in the methods, although reported information was insufficient to extract usable data for quantitative summary analysis
Other bias	Unclear risk	Participants used lubricant artificial tears as needed during the study. This may have had an effect on results.Frequency and quantity of application of the drops in each participant was unknown

Urzua 2012

Methods	**Study design:** randomized two-period cross-over trial **Unit of randomization (Individuals/Eyes):** Individual (both eyes) **Unit of analysis (Individuals/Eyes):** Individual **Number randomized:** 12 **Exclusions after randomization and reasons for exclusion:** Not reported **Losses to follow-up and reasons for loss to follow-up:** No loss to follow-up reported **Number analyzed:** 12 **Was intension-to-treat analysis performed? (Yes/No):** Not reported
Participants	**Country:** Chile **Study period:** May to June 2008 **Age:** mean 52 (SD 6.3) **Gender:** 11 women; 1 man **Underlying conditions:** severe non-Sjögren dry eye **Concurrent dry eye treatments:** "all had used previous treatment with artificial tears with preservative" **Inclusion criteria:** • Age > 18 years • Severe Dry Eye, as defined by a OSDI score ≥ 40 • Tear Break Up Time (TBUT) < 5 seconds • Cornea-conjunctival epithelial defects measured by Fluorescein staining and evaluation using the Oxford score • Schirmer's score less than 5 mm/5 minutes

	Exclusion criteria: • ocular surface disease other than dry eye • inability to complete study protocol • severe anemia • previous use of autologous serum • concomitant use of other topical ocular drug (i.e. topical steroids or cyclosporine) • hypersensibility to any proposed interventions
Interventions	1. Autologous serum - Systane Cross-over arm starting with autologous serum for 2 weeks. After a 1-week wash-out with 0.9% sodium chloride, they continue with 2 weeks using artificial tears (Systane) 20% autologous serum solution used 4 times a day for 2 weeks. Then 0.9% sodium chloride 4 times a day for 1 week. Finally, Systane 4 times a day for 2 weeks 2. Systane - Autologous serum Cross-over arm starting with artificial tears (Systane) for 2 weeks. After a 1-week wash-out with 0.9% sodium chloride, they continue with 2 weeks using autologous serum Systane 4 times a day for 2 weeks. Then 0.9% sodium chloride used 4 times a day for 1 week. Finally, 20% autologous serum solution 4 times a day for 2 week **Length of follow-up:** 5 weeks
Outcomes	Primary Outcome Measures: • To compare the score reduction in the Ocular Surface Disease Index (OSDI) between participants treated with autologous serum and conventional artificial tears at 5 weeks. Secondary Outcome Measures: • To compare variations in objective eye measurements, such as Tear Break Up Time (in seconds), corneal-conjunctival staining according to the Oxford Score (6 categories), and best-corrected visual acuity in participants treated with autologous serum and conventional artificial tears at 5 weeks
Notes	**Type of study (published/unpublished) and journal of publication:** published in the journal Current Eye Research **Source of funding: N**ot reported **Reported subgroup analyses:** No ClinicalTrials.gov Identifier: NCT00779987

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Using random number tables method, a random treatment assignment code was created and every patient was given a sealed opaque envelope with the secret code."
Allocation concealment (selection bias)	Low risk	"Using random number tables method, a random treatment assignment code was created and every patient was given a sealed opaque envelope with the secret code.

		Then, the patient handed the envelope to the Cell Therapy Laboratory operator who delivered the treatment set.“
Masking of participants of the allocated intervention (Performance bias)	Low risk	”Both groups of treatment were given a set of 14 identical, opaque flasks (containing either AS or artificial tears) with instructions of keeping them frozen at -20°C.“
Masking of study personnel of the allocated intervention (Performance bias)	Low risk	”Both DES patient groups, clinical evaluators, and data analyst were masked to group intervention assignment through the whole completion of the protocol (double-masked design).“
Masking of outcome assessors during follow-up - patient reported symptoms (Detection bias)	Low risk	”Both DES patient groups, clinical evaluators, and data analyst were masked to group intervention assignment through the whole completion of the protocol (double-masked design).“ ”Clinical evaluation of each patient (OSDI, BCVA, TBUT,and OXFORD) was assessed at baseline, beginning and end of treatment by two researchers (Cristhian A. Urzua and Dario H. Vasquez) in a masked way.“
Masking of outcome assessors during follow-up - clinical examination (Detection bias)	Low risk	”Both DES patient groups, clinical evaluators, and data analyst were masked to group intervention assignment through the whole completion of the protocol (double-masked design).“ ”Clinical evaluation of each patient (OSDI, BVCA, TBUT,and OXFORD) was assessed at baseline, beginning and end of treatment by two researchers (Cristhian A. Urzua and Dario H. Vasquez) in a masked way.“
Incomplete outcome data (attrition bias) All outcomes	Low risk	No loss to follow-up reported
Selective reporting (reporting bias)	Low risk	Reported data for all outcomes described in CT.gov record: NCT00779987
Other bias	Unclear risk	Differences in eligibility criteria between the CT.gov record and published report including non-Sjogrens syndrome and Shrimer's score < 5 mm/5 min

Urzua 2012 (*Continued*)

		We felt the cross-over design was appropriate given the relative stability of dry eye eliminating the potential for a temporal treatment effect and that there was clearly a random order in which participants received their treatments. The use of a 1-week wash-out between treatment periods ensured there was no carry-over effect from one treatment period to the next. Although the study report described paired analyses to take advantage of the within-participant design, the outcome data were reported according to treatment group and thus we were not able to extract the paired data

DES - dry eye syndrome

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Albegger 1972	Not a randomized controlled trial
Alvarado 2004	Non-randomized case-series
Anderson 2004	Non-randomized case-series
Badami 2009	Non-randomized case-series
Bradley 2008	Non-randomized case series
Brown 2005	Non-randomized case-series
Chiang 2007	Non-randomized case-series
Craig 2008	Non-randomized case-series
Fuchsluger 2005	Non-randomized case report
Geerling 2002	This is an overview about efficacy and recommendations for autologous serum for dry eye disease. It is not a randomized controlled trial
Geerling 2004	Non-randomized case-series

(*Continued*)

Geerling 2008	This is a review of autologous blood products in the treatment of dry eye, it is not a randomized controlled trial
Harritshoj 2011	Retrospective study investigating allogenic (donor) serum
Hyon 2007	Retrospective study
Jaksche 2005	Randomized trial comparing 50% autologous serum drops with 100% autologous serum drops
Koffler 2006	Non-randomized case-series
Kojima 2005b	Non-randomized case-series
Kojima 2008	Non-randomized case-series
Messmer 2005	Not a randomized controlled trial.
Movahedan 2006	Non-randomized case-series
Noble 2004	Conventional treatment arm included different pharmacological agents for each participant
Ogawa 2003	Non-randomized case-series
Poon 2001	Non-randomized case-series
Watson 2010	Non-randomized case-series
Yoon 2007	Comparison group 'Umbilical cord serum' did not meet the criteria for our included studies. No publication found

DATA AND ANALYSES

Comparison 1. Autologous serum (20%) versus artificial tears

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Participant-reported symptoms (severe dry eye)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
1.1 Change from baseline at two weeks follow-up	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
2 Rose Bengal (severe dry eye)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
2.1 Change from baseline at two weeks follow-up	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
3 Rose Bengal (post-LASIK dry eye)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
3.1 Mean at one month follow-up	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
3.2 Mean at three months follow-up	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
4 Fluorescein (severe dry eye)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
4.1 Change from baseline at two weeks follow up	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
5 Schirmers I test (severe dry eye)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
5.1 Two weeks follow-up	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
6 TBUT (severe dry eye)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
6.1 Change from baseline at two weeks follow-up	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]
7 TBUT (post-LASIK dry eye)	1		Mean Difference (IV, Fixed, 95% CI)	Totals not selected
7.1 Six months follow-up	1		Mean Difference (IV, Fixed, 95% CI)	0.0 [0.0, 0.0]

Comparison 2. Autologous serum (20%) versus saline solution

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Participant-reported symptoms (severe dry eye)			Other data	No numeric data
1.1 One month follow-up			Other data	No numeric data
1.2 Two months follow-up			Other data	No numeric data
2 Rose Bengal (severe dry eye)			Other data	No numeric data
2.1 One week follow-up			Other data	No numeric data
2.2 One month follow-up			Other data	No numeric data
2.3 Two months follow-up			Other data	No numeric data
3 Fluorescein (severe dry eye)			Other data	No numeric data
3.1 One week follow-up			Other data	No numeric data
3.2 One month follow-up			Other data	No numeric data

3.3 Two months follow-up	Other data	No numeric data
4 Schirmers I test (with anesthesia) (severe dry eye)	Other data	No numeric data
4.1 Two months follow-up	Other data	No numeric data
5 TBUT (severe dry eye)	Other data	No numeric data
5.1 One week follow-up	Other data	No numeric data
5.2 One month follow-up	Other data	No numeric data
5.3 Two months follow-up	Other data	No numeric data

WHAT'S NEW

Last assessed as up-to-date: 15 April 2013.

Date	Event	Description
15 October 2013	Amended	Revisions made in accordance with MECIR reporting standard guidelines

CONTRIBUTIONS OF AUTHORS

Conceiving the review: AA

Designing the review: AA, QP

Coordinating the review: MM

Data collection for the review

- Designing search strategies: AA, CEVG Trials Search Co-ordinator
- Undertaking searches: CEVG Trials Search Co-ordinator
- Screening search results: QP, AA, AZ, MM, TH, LT
- Organizing retrieval of papers: MM
- Screening retrieved papers against inclusion criteria: QP, AA, AZ, MM, TH, LT

- Appraising quality of papers: QP, AA, AZ, MM

- Extracting data from papers: QP, AA, AZ, MM
- Writing to authors of papers for additional information: AZ, MM
- Providing additional data about papers: MM
- Obtaining and screening data on unpublished studies: AZ, MM

Data management for the review

- Entering data into RevMan: MM

- Checking data once entered into RevMan: QP

Interpretation of data

- Providing a methodological perspective: MM
- Providing a clinical perspective: QP, AA, AZ, YD, WS,TH, EKA
- Providing a policy perspective: QP, AA, YD, WS, EKA
- Providing a consumer perspective:

Writing the review: QP, AA, AZ, MM

Performing previous work that was the foundation of the current study: AA, EKA

Guarantor of the review: MM

DECLARATIONS OF INTEREST

No authors have conflicts of interest to report.

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INDEX TERMS

Medical Subject Headings (MeSH)

*Serum; Dry Eye Syndromes [*therapy]; Ophthalmic Solutions [therapeutic use]; Randomized Controlled Trials as Topic; Sodium Chloride [therapeutic use]

MeSH check words

Adult; Humans