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Evaluation of the effectiveness of oral treatments and accuracy of oral diagnostics using in silico methodology

Ph.D. Thesis

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List of abbreviations

AE – adverse events

ALFS – Allergan Lip Fullness Scale

ANZCTR – Australian New Zealand Clinical Trials Registry

AUC-ROC – area under the receiver operating characteristic curve

BOP – bleeding on probing

CI – confidence interval

Cochrane CENTRAL - Cochrane Central Register of Controlled Trials

CTR – common treatment-site responses,

GAIS – Global Aesthetic Improvement Scale

GRADE - Grading of Recommendations Assessment, Development and Evaluation

HA – hyaluronic acid

HR – hazard ratio

I^2 – I square test

IF – implant failure

IGA – Investigator's Global Assessment

ISRs – Injection site responses

SRCTN - International Standard Randomised Controlled Trial Number

MA – meta-analysis

MBL – marginal bone level

MD – mean difference

MLFS – Medicis Lip Fullness Scale,

NOS - Newcastle Ottawa Scale

OCS – Oral Commissure Severity Scale

OR – odds ratio

OSF - Open Science Framework

PICO – population, intervention, control, and outcome

POL/POLSS – Allegran Perioral Severity Scale

POLM – Allergan Perioral Lines at Maximal Contraction scale

PPD – probing pocket depth

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses (a guideline)

QUADAS-2 – Quality Assessment of Diagnostic Accuracy Studies 2

RCT – randomized controlled trial

RoB – risk of bias

RR – risk ratio

S_a – arithmetical mean height values of implant surfaces

SEM – standard error of mean

SP – Standardized photography

TEAE – treatment-emergent adverse events

Ti – titanium

VAS – Visual Analogue Scale

WASULL – Wrinkle Assessment Scale of Upper Lip Lines

WMD – weighted mean difference

1. Introduction

As a result of the evolvement of healthcare, evidence-based medicine become the basis of modern clinical practice. Evidence-based clinical practice integrates the patient values with clinical expertise and research evidence (Sackett et al., 1996). As a consequence, the need for practice-driven research increased. As a result, the number of new publications in the last decades increased exponentially.

Researchers and healthcare providers are overwhelmed with the enormous amount of information being produced. Systematic reviews can provide efficient interpretation, integration and evaluation of already available information to provide results for coherent decision making. A systematic review attempts to find and collect all experimental evidence that fulfils a predefined eligibility criteria with the aim of answering a particular research question (Antman et al., 1992; Oxman & Guyatt, 1993). Systematic reviews assess consistency and generalisability of scientific findings. It also investigates the variability over various subsets (Mulrow, 1994).

Several systematic reviews include meta-analyses (MAs). Meta-analysis is the utilization of statistical methods to synthesize new data from the results of included individual studies (Glass, 1976; Higgins JPT, 2019). Meta-analysis particularly, can increase statistical power. In simpler terms, power is the likelihood of finding a significant result when there is truly an effect or association in the population (Dell et al., 2002). Some studies are too small to detect effects, however when many small studies are combined in a meta-analysis there is a higher probability of detecting an effect. Additionally, meta-analysis may improve the estimation of an intervention effect as it is based on more data (Higgins JPT, 2019).

In addition, meta-analysis with a selection of studies for sub-group analyses may help to answer questions not proposed by individual records. For example to evaluate the effect of intervention in various sub-group populations.

Moreover, meta-analyses may help to resolve controversies caused by opposing study results or aid formulating new hypotheses (Higgins JPT, 2019).

Furthermore, methods used in a meta-analysis are explicit, as a result, they aid in increasing accuracy, limiting bias, improving the reliability of conclusions (Higgins JPT, 2019; Mulrow, 1994).

1.1. Types of meta-analysis

1.1.1. Meta-analyses of the effect of interventions

Most meta-analyses include studies on the effect of health interventions. A meta-analysis of the effects of interventions is a systematic review and statistical technique used to synthesize and analyze data from multiple studies that evaluate the effectiveness of different interventions. It provides a comprehensive evaluation of the available evidence by combining the results of individual studies and synthesizing an overall estimate of the treatment effect. These meta-analyses focus mostly on randomized clinical trials as it has the highest level of evidence among primary studies. On the other hand, in some cases, non-randomized studies are also included. Such as in the case of adverse effects of specific treatments (Higgins JPT, 2019).

In the case of interventional MA, the focus is on assessing the effectiveness or efficacy of interventions, such as medications, therapies, surgical procedures, or behavioural interventions. The primary outcome measures are typically clinical outcomes or measures related to the effectiveness of the intervention, such as improvements in symptoms, disease progression, mortality rates, or adverse events. Interventional meta-analyses commonly involve pooling data on effect sizes, such as odds ratios (OR), mean differences (MD) or hazard ratios (HR) to estimate an overall treatment effect. Statistical methods, such as fixed-effects or random-effects models, are used to calculate summary measures (Higgins JPT, 2019).

1.1.2. Meta-analyses of diagnostic test accuracy

Diagnostic test accuracy meta-analysis focuses on evaluating the performance of diagnostic tests in correctly identifying the presence or absence of a particular condition or disease. It synthesizes data from studies that compare the results of a diagnostic test to a reference standard, aiming to assess the accuracy and reliability of the test (Cohen et al., 2016).

The main objective is to evaluate the accuracy and diagnostic performance of a specific test or set of tests in identifying a target condition or disease. The primary outcome measures are diagnostic accuracy measures, including positive predictive value, negative

predictive value, area under the receiver operating characteristic curve (AUC-ROC), specificity, sensitivity and likelihood ratios. Diagnostic test accuracy meta-analyses typically involve pooling data on sensitivity, specificity, or other accuracy measures to estimate summary measures of the test's performance. Weighted average estimates are commonly used, considering factors such as sample size or study quality (Cohen et al., 2016).

1.1.3 Other types of meta-analysis

Prognostic Meta-Analysis focuses on evaluating the ability of specific factors or variables to predict the future outcomes or prognosis of individuals. It synthesizes data from multiple studies that examine the association between prognostic factors and specific outcomes of interest. The primary outcome measures are typically related to the prognosis or predictive ability, such as hazard ratios, risk ratios, odds ratios, or predictive accuracy measures like the c-statistic or AUC-ROC curve (Riley et al., 2019).

Aetiology meta-analysis focuses on investigating the association between specific risk factors or exposures and the development of a particular condition or disease. It synthesizes data from multiple studies that examine the relationship between the exposure of interest and the outcome. It could involve evaluating the effect sizes, such as risk ratios, odds ratios, or hazard ratios (Dekkers et al., 2019).

Prevalence meta-analysis focuses on estimating the prevalence or occurrence of a particular condition or disease in a specific population. It synthesizes data from multiple studies that report prevalence rates or proportions of the condition of interest (Jan et al., 2013).

Meta-analysis of meta-analyses involves the synthesis of multiple meta-analyses that have been conducted on similar or related research questions. Instead of analysing primary studies, it examines the findings and results from existing meta-analyses. The outcome measures are the summary measures and results from the individual meta-analyses, such as pooled effect sizes, subgroup analyses, or heterogeneity assessments (Sigman, 2011).

1.2 Rationale for meta-analysis on the topic of dental implants

During the last decades, the focus from machined implants moved to implants with moderately rough surfaces such as sand-blasted surface, however, the scientific justification behind this shift is not well-grounded. No robust evidence exists for clinical practice to support the utilization of sand-blasted implants over implants with smooth surface in healthy people. The randomized controlled trials (RCT) conducted on the healing of sand-blasted implants utilized a relatively small number of participants, yielding weak evidence.

Performing a meta-analysis may eliminate the drawbacks of RCTs as it could increase the number of participants involved in the analysis and also improving the validity of the result by other means mentioned earlier. Numerous reviews are available on the topic (Doornewaard et al., 2017; Esposito et al., 2014; Papaspyridakos et al., 2014; Wennerberg et al., 2018), however, these did not conduct statistical calculations based on meta-analysis or included various studies with different methodological design, generating a high level of heterogeneity.

Ostensibly, there has been no meta-analysis performed including exclusively RCTs: investigating the effect of sand-blasted surface of titanium implants on osseointegration compared to machined titanium implants. Identifying all eligible records and performing a meta-analysis may overcome the limitations of individual RCTs and increase the level of evidence on the topic.

1.3 Rationale for meta-analysis on the topic of hyaluronic acid dermal fillers in the oral cavity

Lips are part of the oral cavity and its surrounding anatomical region. Lips have a fundamental functional role in mastication and vocalization. Furthermore, they have a crucial importance in the aesthetic appearance of the face (Larrabee & Moyer, 2017; Wollina, 2013). Specifically, lip fullness is essentially connected with youth, beauty and attractiveness (Stojanovič & Majdič, 2019; Wollina, 2013). Hereditary and several harmful factors promote the deterioration of the tissues related to the perioral region with age (Luthra, 2015; Wollina, 2013). As a result, the volume of the lips may shrink with various other signs of ageing, for

example, the flattening of the cupid bow and the development of marionette lines (Wollina, 2013).

In theory, several treatment modalities exist to rebuild deteriorated tissues. Such as biocompatible artificial scaffolds (Hegedűs et al., 2019; Juriga et al., 2016) or gene therapy and stem cells with methods of tissue engineering (Farkas et al., 2010; Földes et al., 2016; Grimm et al., 2011; Racz et al., 2014; Rakonczay et al., 2008), however, their utilization is not well established in everyday clinical practice.

Within the non-surgical treatment modalities, hyaluronic acid (HA) dermal filling is among the most often used treatment techniques (Chung et al., 2020; Taylor et al., 2019). The use of non-animal-based HA was first approved in the early 2000's (Cohen et al., 2013). Since that time various clinical trials targeted to show the true capabilities of HA dermal fillers. However, short follow-up times and small sample sizes of clinical trials investigating the effectiveness yielded findings with weak evidence and a high level of uncertainty.

With the methodological approach of meta-analysis, one could overcome the limitations of individual clinical studies i.e. to increase precision and power of the estimated effect of HA dermal fillers on lip augmentation. Additionally, a systematic review may help to explore the type and frequency of rare adverse effects (AEs) associated with HA dermal fillers.

1.4 Rationale for meta-analysis on the topic of oral diagnostics

As the population exponentially increases on the globe and most of the people live and work in crowded areas, the risk of air-born pandemics drastically increases. Such as COVID-19, caused by the SARS-CoV-2 virus. Globally, as of 19 July 2023, there have been 768,237,788 confirmed cases, including 6,951,677 deaths (World Health, 2023). Fast diagnosis and early and quick quarantine of infected individuals may play a crucial part in halting the future spread of any disease.

At present, the gold standard for diagnosing COVID-19 is nasopharyngeal swabbing. However, it requires trained medical staff (World Health, 2020), consequently exposing medical personnel to a high risk of infection (Kim et al., 2017).

Mass screening with this technique is expensive and poses an extra risk on the healthcare system as more medical personnel are needed (Kim et al., 2017). Furthermore, nasopharyngeal swabbing has several contraindications, such as anticoagulant therapy, coagulopathy, significant septum deviation (Li et al., 2020; Sri Santosh et al., 2020).

To overcome these limitations diagnosis from saliva specimens is under continuous development. Multiple studies were published on the potential use of saliva samples for detecting COVID-19. However, the published papers use small sample sizes, resulting in a low level of evidence, high uncertainty and weak statistical power to detect significant differences. Conducting a meta-analysis on the topic could overcome the limitations of a small sample size, and also reveal regional or age differences among populations.

2. Objectives

In the last decade, the number of published papers on clinical trials exponentially increased. The large number of original studies became more than that clinicians and research groups can comprehensively assess and process to implement these findings into practice.

Conducting a meta-analysis on a given topic helps to elevate the level of evidence and clarify questions within a research area. This is achieved by systematically finding and assessing each eligible record in the topic. The present scientific work aimed to utilize the methodology of meta-analysis in order to assess the available evidence and clarify practice-related issues and questions in clinically important dental areas which were not assessed by the means of meta-analysis.

A. The first objective of the present scientific work was to conduct a meta-analysis to investigate the performance and healing potential of two different types of commercially pure Titanium implant surfaces, smooth (machined) and moderately rough, sand-blasted surface.

B. The next aim was to investigate the effectiveness of HA for lip augmentation and to verify its long-term aesthetic results using the methodology of meta-analysis. Additionally, the nature and number of adverse effects of HA in the literature were also reviewed. systematically.

C. Finally, a meta-analysis was conducted to estimate the diagnostic sensitivity of saliva-based detection of the SARS-CoV-2 virus. Additionally, various factors related to the methodological differences used in COVID-19 tests were explored.

Thus, the present scientific work aimed to apply the methodology of meta-analysis in three important topics of dentistry lacking strong evidence and to overcome the limitation of small sample sizes and high uncertainty, as well as weak statistical power, to increase the level of evidence aiding clinical and research decision in these particularly important themes.

3. Methods

3.1. Protocol and registration

The present work was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and guideline (Moher et al., 2015). The methodology for each dental topic was registered prior to conducting the meta-analysis in the database. For the MA in the topic of dental implants registration was on 07.02.2018. (PROSPERO registration number: CRD42018084190.). For the MA on the topic of HA dermal fillers, the registration was performed on 10.12.2018. (PROSPERO registration number: CRD42018102899.) For the MA in the topic of oral diagnostics registration was performed in the Open Science Framework (OSF) database on 23.04.2020 (registration ID: https://osf.io/3ajy7)). Deviation from the registered protocol: Eligible studies did not provide sufficient data on true negative, true positive, false negative, false positive values. These were not available. Hence 2x2 contingency tables, could not be completed. Accordingly, specificity and sensitivity could not be calculated. An alternative statistical analysis was based on pooled event rates of positive diagnosis. A more detailed description is available in section 3.7.

3.2. Eligibility criteria

In each case clinical question was formulated with the aid of the PICO format (population, intervention, control, and outcome).

In the case of dental implants, the following question was formulated. Are there significant differences concerning implant failure rates and marginal bone level loss between machined and sand-blasted dental implants among healthy patients (Czumbel et al., 2019)? Studied meeting the following requirements were considered for inclusion: **P:** participants with partial edentulous or edentulous jaws, with no systemic diseases affecting the healing of the implants. **I:** treatment of tooth loss with sand-blasted endosteal dental implants. **C:** treatment of tooth loss with machined endosteal dental implants. **O:** implant failure rates (IF) and MBL (marginal bone level) changes using x-ray images to measure changes. Only RCTs, using similar implant macro design, written in English were included.

Exclusion criteria were defined as studies other than RCTs, studies applying growth factors, or bone augmentation, implants with modified neck macrostructure, participants with systemic or local diseases affecting implant healing, gray or black literature.

In the case of HA dermal fillers in the perioral region, the following questions were formulated. (1) To what extent are hyaluronic acid dermal fillers effective for lip augmentation? (2) What are the common and also the rare treatment-related adverse effects of HA application? (Czumbel et al., 2021) Studied meeting the following requirements were considered for inclusion: **P:** participants at age 18 or older with minimal, mild or moderate levels of lip fullness based on a validated lip fullness scale. **I:** the use of injectable HA in the perioral region and lips to enhance lip fullness and aesthetics of the face. **C:** baseline controlled. The values of lip fullness of each participant were recorded before and after treatment and these were compared. **Primary O:** measurement of effectiveness expressed as the rate of responders. Participants with one or more levels of improvement based on a validated lip fullness scale were defined as responders. **Secondary O:** Frequency and type of treatment-related AEs. Case series, cohort studies, and RCTs, written in English or available in English translation were considered for inclusion. Studies investigating other filling materials, treatment of other areas than the perioral region and the lips were excluded,

In the case of oral diagnostics publications satisfying the following research question was formulated. *Are saliva specimens reliable for detecting SARS-CoV-2 in COVID-19 patients confirmed by nasopharyngeal swab testing?* Eligibility criteria were as follows. **P:** records published in clinical trial registries or scientific journals, participants diagnosed with COVID-19. **Index test:** PCR diagnostics of saliva samples for detecting SARS-CoV-2. **Comparator test** (reference standard): PCR diagnostics of NPS samples for diagnosing SARS-CoV-2. Records in English language or with available English translation. Records such as recommendations, reviews, guidelines or publications before 01.01.2020 and after 25 04.2020 were considered for exclusion. Additionally, grey and black literature were also excluded. (Czumbel et al., 2020).

3.3. Information sources and search

In the topic of dental implants, the systematic search was performed in English in three different databases Cochrane Central Register of Controlled Trials (CENCTRAL) Library, Embase, Medline via Pubmed. Studies in English, published up to 20 August 2018 were considered. Additionally, the reference list of the included records and relevant articles were also used to find records for inclusion.

To find eligible records, the following search queries were used.

Cochrane CENTRAL: *"('machined':ti,ab,kw or 'turned':ti,ab,kw or 'blasted':ti,ab,kw or 'sandblasted':ti,ab,kw or 'sand-blasted':ti,ab,kw) and ('dental':ti,ab,kw or 'dentistry':ti,ab,kw) and 'implant':ti,ab,kw*" with Cochrane Library publication date to Aug 2018, in Trials

Embase: "*('machined':ti,ab,kw OR 'turned':ti,ab,kw OR 'blasted':ti,ab,kw OR 'sandblasted':ti,ab,kw OR 'sand-blasted':ti,ab,kw) AND ('dental':ti,ab,kw OR 'dentistry':ti,ab,kw) AND 'implant':ti,ab,kw AND 'controlled clinical trial'/de AND [english]/lim"*.

Medline via PubMed: *„(machined[Title/Abstract] OR turned[Title/Abstract] OR blasted[Title/Abstract] OR sandblasted[Title/Abstract] OR sand-blasted[Title/Abstract] OR sand blasted[Title/Abstract]) AND (dental[Title/Abstract] OR dentistry[Title/Abstract]) AND implant[Title/Abstract] AND (Clinical Trial[ptyp] AND ("0001/01/01"[PDAT]: "2018/08/20"[PDAT]) AND English[lang])"* (Czumbel et al., 2019)

In the topic of HA dermal fillers in the perioral region search was performed again in English, in the three aforementioned databases. Records published up to 31 December 2018 were considered. Additionally, the reference list of the included records and relevant articles were also used to find records for inclusion. "Lip" and "hyaluronic acid" expressions and their synonyms were used in the three databases.

Cochrane CENTRAL: *"('hyaluronic':ti, ab, kw OR 'hyaluronate':ti, ab, kw OR 'hyaluronan':ti, ab, kw OR 'dermal filler':ti, ab, kw OR 'injectable implant':ti, ab, kw) AND 'lip':ti, ab, kw." Limits applied: trials.*

Embase: *"('hyaluronic':ti, ab, kw OR 'hyaluronate':ti, ab, kw OR 'hyaluronan':ti, ab, kw OR 'dermal filler':ti, ab, kw OR 'injectable implant':ti, ab, kw) AND 'lip':ti, ab, kw". Limits applied: human.*

Medline via PubMed: *„hyaluronic[All Fields] OR hyaluronate[All Fields] OR ("hyaluronic acid"[MeSH Terms] OR ("hyaluronic"[All Fields] AND "acid"[All Fields]) OR "hyaluronic acid"[All Fields] OR "hyaluronan"[All Fields]) OR ("dermal fillers"[MeSH Terms] OR ("dermal"[All Fields] AND "fillers"[All Fields]) OR "dermal fillers"[All Fields] OR ("dermal"[All Fields] AND "filler"[All Fields]) OR "dermal filler"[All Fields]) OR (("injections"[MeSH Terms] OR "injections"[All Fields] OR "injectable"[All Fields]) AND implant[All Fields]) AND ("lip"[MeSH Terms] OR "lip"[All Fields]) AND "loattrfull text"[sb]". Limit applied: „human".* (Czumbel et al., 2021)

On the topic of oral diagnostics, the systematic search was performed in English on records published after 01.01.2020. For the search five different databases Cochrane (CENTRAL) Library, Embase, Medline via Pubmed, Scopus, Web of Science were used. Additionally, five clinical trial registers (ClinicalTrial.gov, EU Clinical Trials Register, NIPH Clinical Trial Search, ISRCTN Registry, ANZCTR Registry) were also searched (Czumbel et al., 2020). Studies in English, published up to 25.04.2020 were considered. Again, the reference list of the included records and relevant articles were also used to find records for inclusion. The following search queries were used in the corresponding databases: *(COVID 19 OR COVID19 OR Wuhan virus OR Wuhan coronavirus OR coronavirus OR 2019 nCoV OR 2019nCoV OR 2019-nCoV OR SARS CoV-2 OR SARS-CoV-2 OR NCP OR novel coronavirus pneumonia OR 2019 novel coronavirus OR new coronavirus) AND (saliva)* (Czumbel et al., 2020).

3.4. Study selection

In each topic, records were managed using EndNote (Clarivate Analytics, Philadelphia, US, version: X9.3.3) reference manager software. The study selection process was carried out by two authors independently. Disagreements within selection were resolved by discussion or by consulting with a third author.

3.5. Data collection process and data items

Collected data on each topic was organized into preconstructed and standardized data extraction tables.

On the topic of dental implants L.M.C. and K.B. performed data extraction independently. Authors' names, sample size, year of publication, gender distribution, average age of participants, design of the studies, implant systems used, and outcome parameters were extracted (Czumbel et al., 2019).

In the topic of HA dermal fillers in the perioral region L.M.C. and S.F. performed data extraction independently based. Authors' names, sample size, year of publication, gender distribution, average age of participants, design of the studies, type of HA utilized, site of injection, follow-up period, type of scale used and outcome parameters were extracted (Czumbel et al., 2021).

In the topic of oral diagnostics A.H. and I.M. performed data extraction independently. Authors' names, sample size, year of publication, gender distribution, average age of participants, design of the studies, methods of diagnosis used, type of PCR kit and outcome parameters were extracted (Czumbel et al., 2020).

3.6. Risk of bias assessment

Risk of Bias (RoB) assessment was performed based on guidelines published in the literature.

The guideline outlined in the Cochrane Handbook (J. P. Higgins et al., 2011) were followed to assess RoB in the case of RCTs. Included studies were evaluated according to 8 domains described in the guideline. These domains were: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, other bias. Domains and

evaluations outlined in the handbook were not needed to be modified to comply with the studies.

For cohort studies the Newcastle Ottawa Scale (NOS) was used (Deeks et al., 2003). In order to achieve a meaningful assessment of RoB with the NOS scale slight modifications were introduced to the scale. "Ascertainment of exposure" was removed from the Selection domain. As a consequence, the maximum scores in each domain changed accordingly: three stars in Selection, one star in Comparability, and three stars in the Outcome domain. In the Outcome domain, six months or longer follow-up was regarded acceptable, while a 10% drop-out was regarded adequate.

For evaluating the RoB in diagnostic accuracy studies the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool was used (Whiting et al., 2011). Assessment of bias was evaluated according to four domains. Patient selection, conduct of interpretation of the index test, evaluation of reference standard, and patient flow and timing. Applicability of the included studies was judged against the following question: *Are saliva specimens reliable for detecting SARS-CoV-2 in COVID-19 patients confirmed by nasopharyngeal swab testing?* (Czumbel et al., 2020). For assessment, we used preconstructed form available on the webpage of the University of Bristol (Bristol).

3.7. Summary measures and synthesis of results

In the topic of dental implants, weighted mean difference (WMD) with 95% confidence intervals (CIs) were calculated for continuous outcome – MBL changes. A decrease in MBL is indicated by negative values. A more pronounced decrease in MBL of sand-blasted implants compared to machined implants is indicated by negative values of WMD. Pooled Risk Ratio (RRs) with 95% CIs were calculated for the dichotomous outcome – IF rates. IF defined by Albrektsson et al. (Albrektsson et al., 1986) was applied in the present work. The statistical unit of calculations was the implant numbers. To estimate the intervention effect we used the random effect model with DerSimonian-Laird estimation.

In the topic of HA dermal fillers in the perioral region for the rate of responders, the untransformed proportions were calculated with 95% CIs. A responder is defined as a participant with at least one-grade improvement on a validated lip fullness scale compared to

its baseline value (Czumbel et al., 2021). Descriptive statistics were used to analyze the distribution and frequency of AEs. The statistical unit was taken as the number of participants. To estimate the intervention effect we used the random effect model with DerSimonian-Laird estimation.

In the topic of oral diagnostics, patient-based data was included from consecutive case series. Analyses were performed according to the recommendations of the working group of the Cochrane Collaboration. In each case series sensitivity of saliva and NPS tests were investigated in participants who were confirmed cases - diagnosed by NPS test and also clinical signs. The sensitivity of the NPS test was based on the matching NPS tests when saliva tests were also performed. Due to the fact that some of the sensitivity values are close to or equal to 1, the score confidence interval estimation (Wilson, 1927) was applied with the Freeman-Tukey double arcsine transformation (Freeman & Tukey, 1950). To estimate the pooled effect the random effect model with DerSimonian-Laird estimation was used.

All statistical analyses were performed using STATA 15.0 (Czumbel et al., 2020).

3.8. Risk of bias across studies and additional analysis

In each MA statistical heterogeneity and probability values were calculated using I-square and chi-square tests. Significant heterogeneity was indicated by $p<0.1$ (J. P. Higgins et al., 2011). In the sensitivity analysis included studies were omitted from the meta-analyses one by one, to investigate the influence of each study on the summary estimate. For analyses with more than 9 studies funnel plots were created and publication bias was checked by the visual inspection of the plots. In the topic of HA dermal fillers in the perioral region, the quality and certainty of evidence were assessed according to the GRADE approach (Cumpston et al., 2019; Schünemann H, 2015). It was performed by two authors independently (L.M.C. and S.F.)

4. Results

4.1. Results of the meta-analysis on the topic of dental implants

4.1.1 Study selection

The systematic search and selection process yielded a total of 188 records. After duplicate removal, 130 items remained. 114 records were excluded because of numerous reasons for ineligibility. Such as investigating participants with systemic disease, different objectives, other surface modifications, comparing different macro or micro designs of implants, evaluating surgical protocols. After all, 16 publications were eligible for full-text selection. Out of these records, seven RCTs were eligible for quantitative and qualitative analysis (Åstrand et al., 2004; Gotfredsen & Karlsson, 2001; Jacobs et al., 2010; Ravald et al., 2013; Steenberghe et al., 2000; Tawse-Smith et al., 2002; Vroom et al., 2009). *Figure 1* summarizes the selection process.

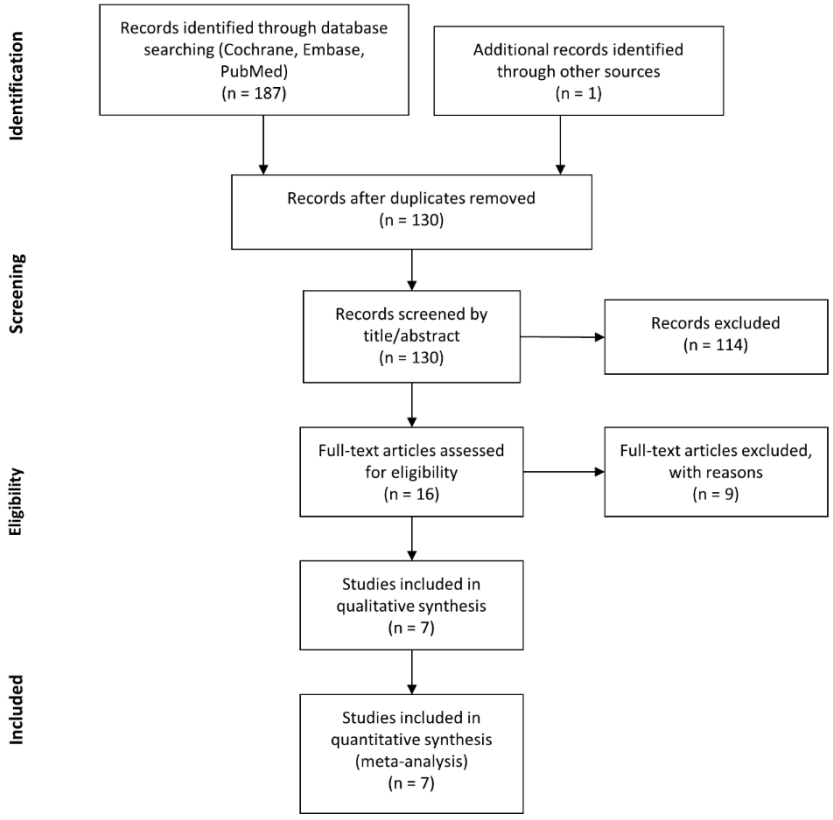

Figure 1. Visual representation of the results of the study selection process. (*Source:* (Czumbel et al., 2019)).

4.1.2. Characteristics of the included studies

All included records were randomized controlled trials (RCTs). The analysis included 722 implants (362 sand-blasted and 360 machined). The included participants in these studies were uniform. Population with excessive drug and alcohol consumption or participants with systemic diseases that might influence the process of osseointegration were excluded.

The average age of the population in the studies was between 50 and 58 years. Among the records the following implant systems were used: Astra Tech, Brånemark, Steri-Oss and Southern Implants. In the control group, each implant had a minimally rough surface (machined) and in the intervention group, each implant had a moderately rough surface (sand-blasted). None of the implants had a special collar region to enhance osseointegration. All records utilized the two-stage protocol (Branemark et al., 1986) except one study (Tawse-Smith et al., 2002). In the study of Tawse-Smith et al, (Tawse-Smith et al., 2002) although the one-stage protocol was used the implants were loaded after 3 and 6 months of healing at the lower and upper jaw, respectively. The treatment of edentulism differed across studies. Two records (Gotfredsen & Karlsson, 2001; Steenberghe et al., 2000) achieved rehabilitation with fixed partial bridges, another two (Tawse-Smith et al., 2002; Vroom et al., 2009) utilized overdentures. In another study, they used full arch bridges (Åstrand et al., 2004). The follow-up time between studies varied between 1 year (Åstrand et al., 2004; Steenberghe et al., 2000; Tawse-Smith et al., 2002; Vroom et al., 2009) and 16 years (Jacobs et al., 2010).

All records published data on MBL change calculated from x-ray measurements and implant failure rates. In the case of one record (Tawse-Smith et al., 2002), only half of the groups were included as two groups using immediate loading protocols were excluded from the analysis. In addition, the marginal bone level measurement of Ravald et al. (Ravald et al., 2013) and Astrand et al. (Åstrand et al., 2004) were not included since no implant-based statistical data could be harvested from the published results. A summary of the study characteristics is included in *Table 1 and 2*.

Table 1. Characteristics of included records. (*Source:* (Czumbel et al., 2019))

Author	**Åstrand et al. (2004)andRavald et al. (2013)***	**Gotfredsen et al. (2001)**	**Steenberghe et al. (2000) and Jacobs et al. (2010)***	**Tawse-Smith et al. (2002)**	**Vroom et al. (2009)**
Study type	block randomization separate for upper and lower jaw, with equal probability of receiving either implant type	alternating implant placement	split-mouth design	random allocation to either implant system on a one-by-one basis	alternating implant placement
Country	Sweden	4 Scandinavian countries	Belgium	New Zealand	not stated
Age	$\bar{x}$ = 61.5	$\bar{x}$ = 53	$\bar{x}$ = 59.7	55-80	$\bar{x}$ = 53
Number of participants	males: 28, females: 38	males: 25, females: 25	males: 6, females: 12	total: 48	males: 7, females: 13
Extent of teeth loss	edentulous	partially edentulous	partially edentulous	edentulous (mandible only)	edentulous (mandible only)
Sand-blasted implant (intervention)	Astra Tech implants	Astra Tech implants	Astra Tech implants	Southern Implants	Astra Tech implants
Machined implants (control)	Branemark System MK II	Astra Tech implants	Branemark System MK II	Sterioss	Astra Tech implants
Surgical protocol	two-stage technique (3 months and 6 months healing in the lower and upper jaw respectively before abutment placement	two-stage technique (3-4 months and 6-7 months healing in the lower and upper jaw respectively before abutment placement	two-stage technique (3-4 months and 6-7 months healing in the lower and upper jaw respectively before abutment placement	one-stage technique (3 months of healing before loading)	two-stage technique (3-4 months healing before abutment placement

*The publications of Ravald et al (2013) and Jacobs et al (2010) are the continuations of the studies published by Åstrand et al (2004); and Steenberghe et al (2000) respectively. (*Source:* (Czumbel et al., 2019))

Table 2. Characteristics of included records (continued). (*Source:* (Czumbel et al., 2019))

Author	**Åstrand et al. (2004) and Ravald et al. (2013)***	**Gotfredsen et al. (2001)**	**Steenberghe et al. (2000) and Jacobs et al. (2010)***	**Tawse-Smith et al. (2002)**	**Vroom et al. (2009)**
Type of prosthesis	full-arch fixed bridges	screw retained fixed partial prosthesis	screw retained fixed partial prosthesis	implant supported overdenture	implant supported overdenture
Outcome	IF, MBL change, BOP, plaque accumulation, pain, suprastructure complications	IF, MBL change, BOP, paraesthesia, periimplant inflammation, pain, suprastructure complications	IF, MBL change, sulcus bleeding index, PPD presence of plaque	IF, MBL change, sulcus bleeding index, PPD, implant stability measurement (Periotest), modified plaque index	IF, MBL change, bleeding index. PPD, presence of calculus
Follow-up time	5 and 12* years	5 years	2 and 15* years	2 years	12 years

*The publications of Ravald et al (2013) and Jacobs et al (2010) are the continuations of the studies published by Åstrand et al (2004); and Steenberghe et al (2000) respectively.

IF: implant failure; MBL: marginal bone level; BOP: bleeding on probing; PPD: probing pocket depth

(*Source:* (Czumbel et al., 2019))

4.1.3. Risk of bias in studies

The risk of bias in the included studies was assessed using the Cochrane Risk of Bias Tool. All included seven records were also included in the risk of bias assessment. However, the studies of Ravald et al. (Ravald et al., 2013) and of Åstrand et al. (Åstrand et al., 2004) and also Jacobs et al. (Jacobs et al., 2010) and Steenberghe et al. (Steenberghe et al., 2000) were evaluated two-by-two as they are the continuations of previously published work. Two records (Gotfredsen & Karlsson, 2001; Vroom et al., 2009) had a high risk of allocation concealment, as they utilized a predictable random sequence generation process. Other two publications did not clearly present the random sequence generation process used (Steenberghe et al., 2000; Tawse-Smith et al., 2002).

Each record performed blinding at radiographic evaluations, however, no blinding was possible in case of clinical inspection and evaluation of the implants. Drop-outs were reported in four records studies (Åstrand et al., 2004; Gotfredsen & Karlsson, 2001; Tawse-Smith et al., 2002; Vroom et al., 2009). In two records the reason for drop-out was not clearly stated (Tawse-Smith et al., 2002; Vroom et al., 2009). This means an unclear risk of bias.

No intext evidence of selective reporting bias was found, although there was no access to study protocols or trial registers. *Figure 2* summarizes the risk of bias assessment.

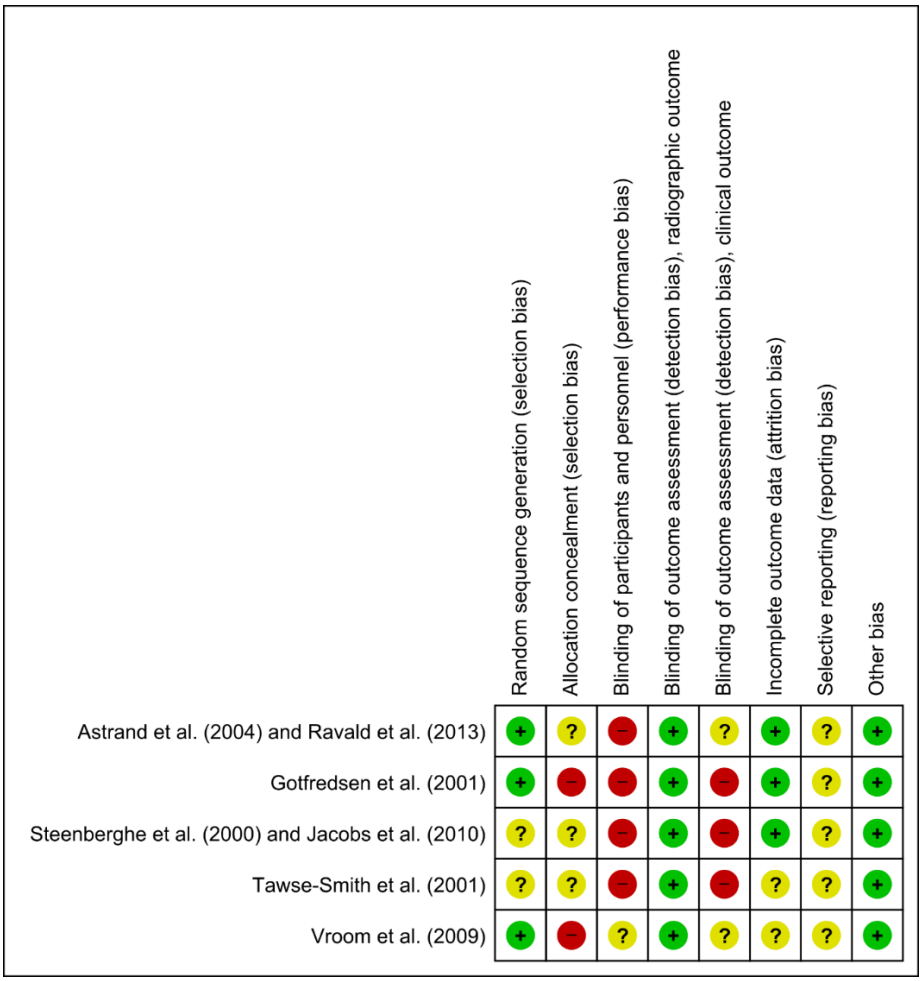

Figure 2. Detailed results of risk of bias assessment. Green represents a low risk of bias, yellow represents an unclear risk of bias, red represents a high risk of bias. (*Source:* (Czumbel et al., 2019)).

4.1.4. Results of data synthesis – IF rate

Moderately rough surface (sand-blasted) implants have significantly lower implant failure rates than smooth surface implants at 1, 2, and 5/6 years.

Data for meta-analysis of implant failure after one year follow-up were pooled from five eligible studies (Åstrand et al., 2004; Gotfredsen & Karlsson, 2001; Steenberghe et al., 2000; Tawse-Smith et al., 2002; Vroom et al., 2009). The analysis indicated that there is an 80 % lower risk of implant failure among sand-blasted implants after one year of use. RR = 0.20; 95% CI: 0.06– 0.67; statistical heterogeneity: I^2 = 0.0% p = 0.986) (*Figure 3*).

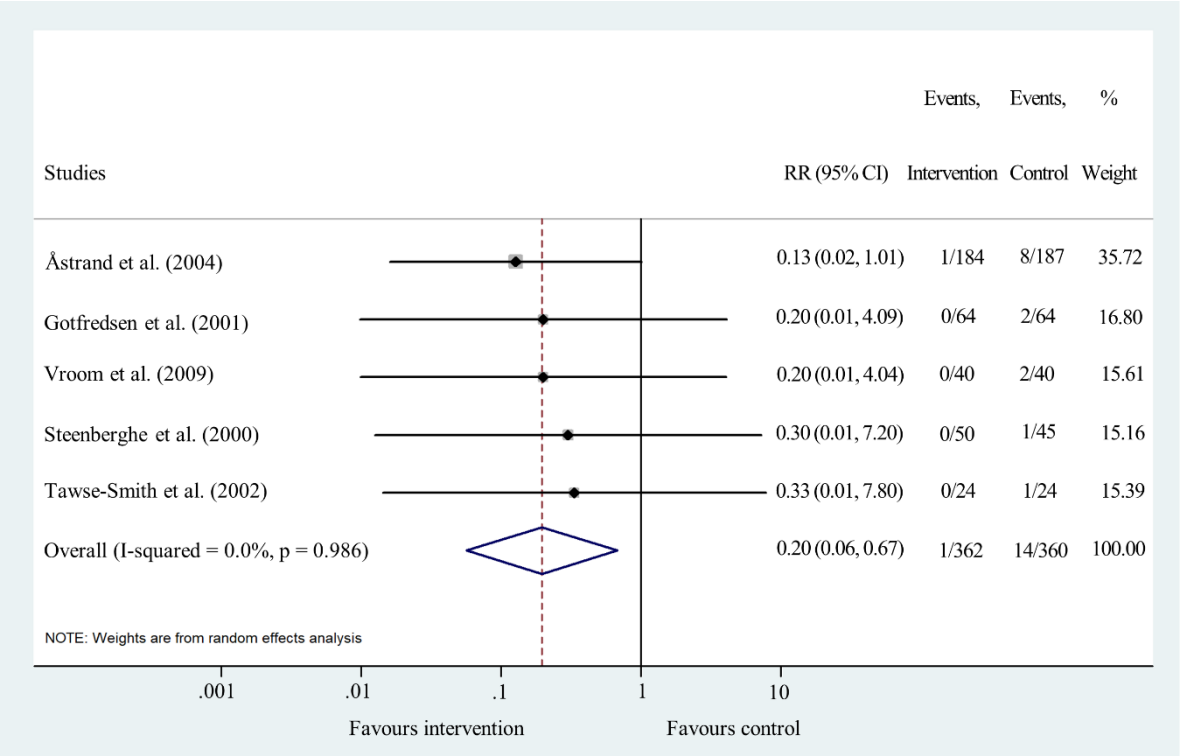

Figure 3. Forest plot of implant failure rate at one year follow-up. RR represents the risk ratio, the vertical line at 1 represents the null-effect line – no difference between the intervention and control. The red vertical dashed line indicates the pooled effect. The two sides of the rhombus represent the 95% confidence intervals. As the rhombus is not touching the null-effect line the difference is considered significant. (*Source:* (Czumbel et al., 2019)).

Analysis of pooled data (Åstrand et al., 2004; Gotfredsen & Karlsson, 2001; Steenberghe et al., 2000; Tawse-Smith et al., 2002; Vroom et al., 2009) of cumulative implant failure after two years reveals the risk of implant failure is 81% lower in case of moderately rough surface than in case of machined implants. (Risk Ratio = 0.19; 95% CI: 0.05–0.64; statistical heterogeneity: I^2 = 0.0% p = 0.977) (*Figure 4*).

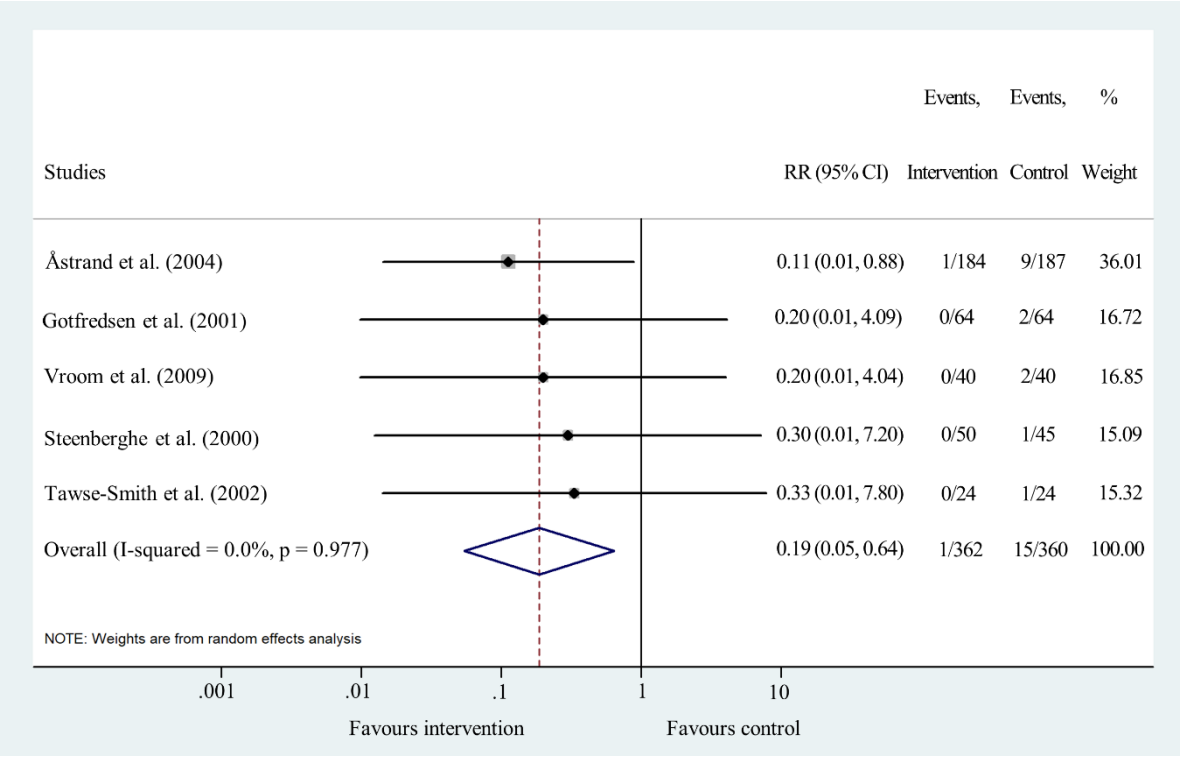

Figure 4. Forest plot of IF rate at two years follow-up. (*Source:* (Czumbel et al., 2019)).

Meta-analysis of data (Åstrand et al., 2004; Gotfredsen & Karlsson, 2001; Jacobs et al., 2010; Vroom et al., 2009) on cumulative implant failure after five or six years follow-up reveal that there is a 74 % lower risk of implant failure among moderately rough implants. (Risk Ratio = 0.26; 95% CI: 0.09–0.74; statistical heterogeneity I2 = 0.0% p = 0.968) *(Figure 5*).

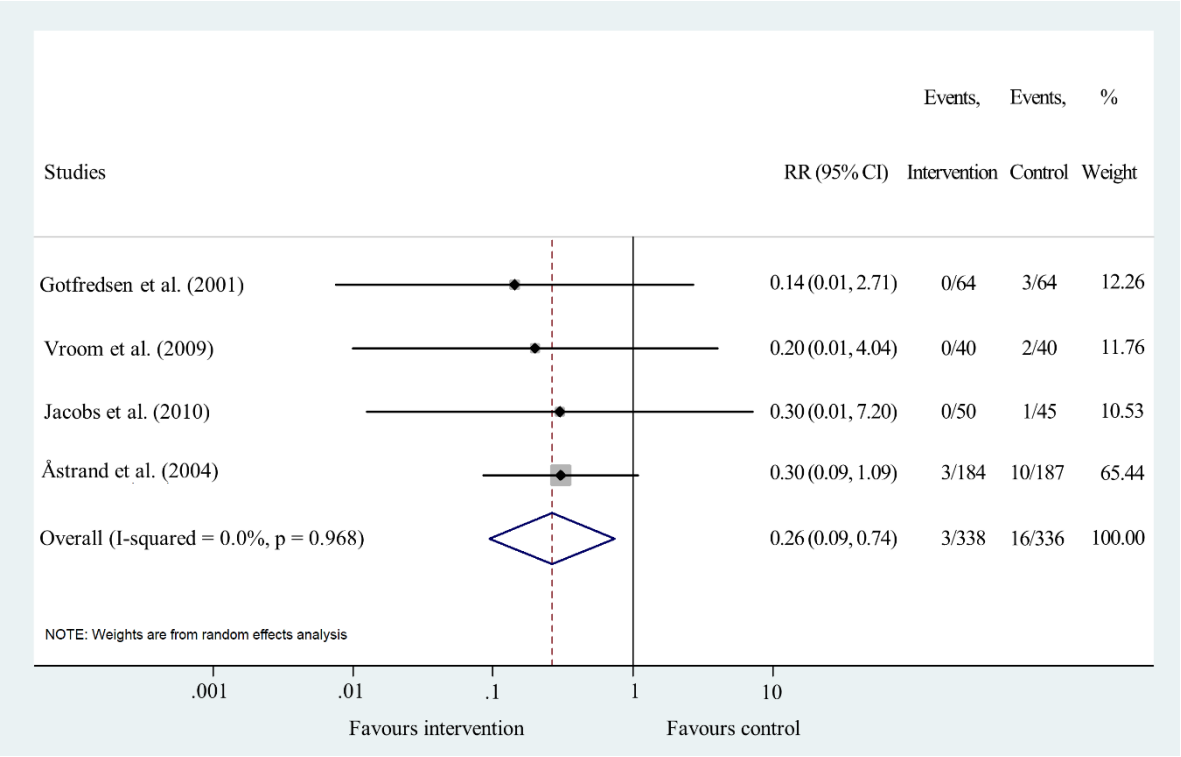

Figure 5. Forest plot of IF rate at five years follow-up. (*Source:* (Czumbel et al., 2019)).

Data on cumulative implant failure after 12 to 15 years were pooled from three studies (Jacobs et al., 2010; Ravald et al., 2013; Vroom et al., 2009). Analysis indicates no significant difference between smooth and moderately rough surfaces after 12-15 years of follow-up. Risk Ratio = 0.68; 95% CI: 0.29–1.57; statistical heterogeneity I2 = 0.0% p = 0.590) (*Figure 6*).

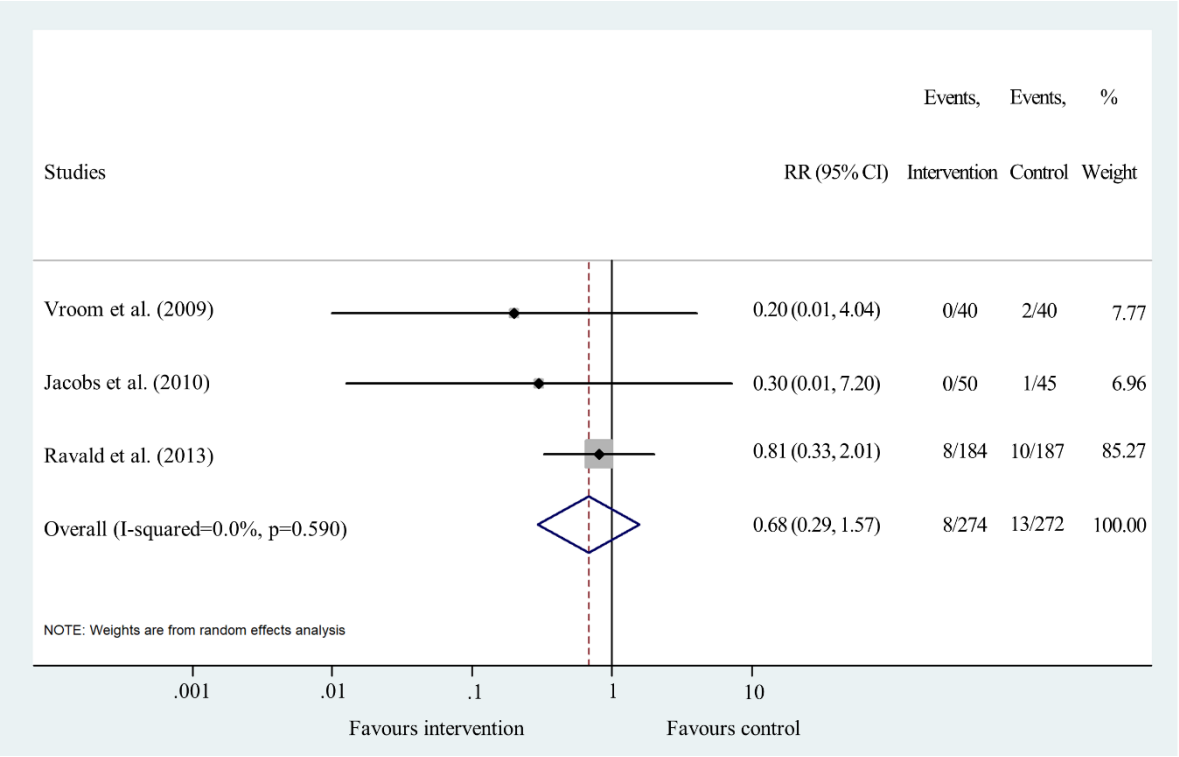

Figure 6. Forest plot of IF rate at 12/15 years of follow-up. As the rhombus is touching the null-effect line the difference is considered non-significant between intervention and control. (*Source:* (Czumbel et al., 2019)).

4.1.5. Results of data synthesis – MBL change

No significant difference in MBL loss between moderately rough (sand-blasted) and smooth (machined) surface after 5 years.

Meta-analysis of marginal bone levels was conducted on data after one and five years after the delivery of the final prosthesis. Analysis reveals no significant difference between sand-blasted and machined implant types after one year of use (Steenberghe et al., 2000;

Tawse-Smith et al., 2002; Vroom et al., 2009). Weighted mean difference = -0.10 mm; 95% CI: -0.20–0.01; statistical heterogeneity: I^2 = 0.0%, p = 0.560 (*Figure 7*). Analysis of 5-year data also indicates no significant results between moderately rough and smooth implant surface. Weighted mean difference = 0.00 mm; 95% CI: -0.13–0.14; statistical heterogeneity I^2 = 26.2%, p = 0.258 (*Figure 8*).

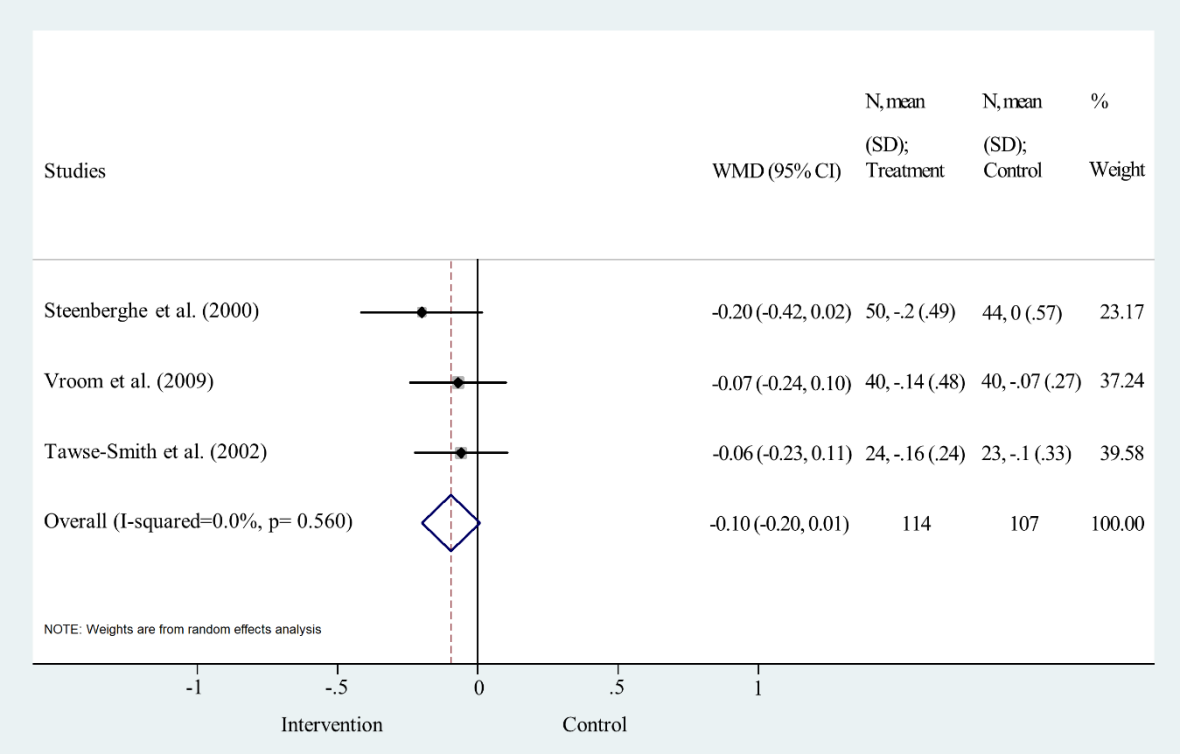

Figure 7. Forest plot of MBL loss after one year of prosthesis delivery. WMD represents the weighted mean difference in mm, the vertical line at 0 represents the null-effect line – no difference between the intervention and control. The red vertical dashed line indicates the pooled effect. The two sides of the rhombus represent the 95% confidence intervals. As the rhombus goes beyond the null effect line there is no significant difference between intervention and control. (*Source:* (Czumbel et al., 2019))

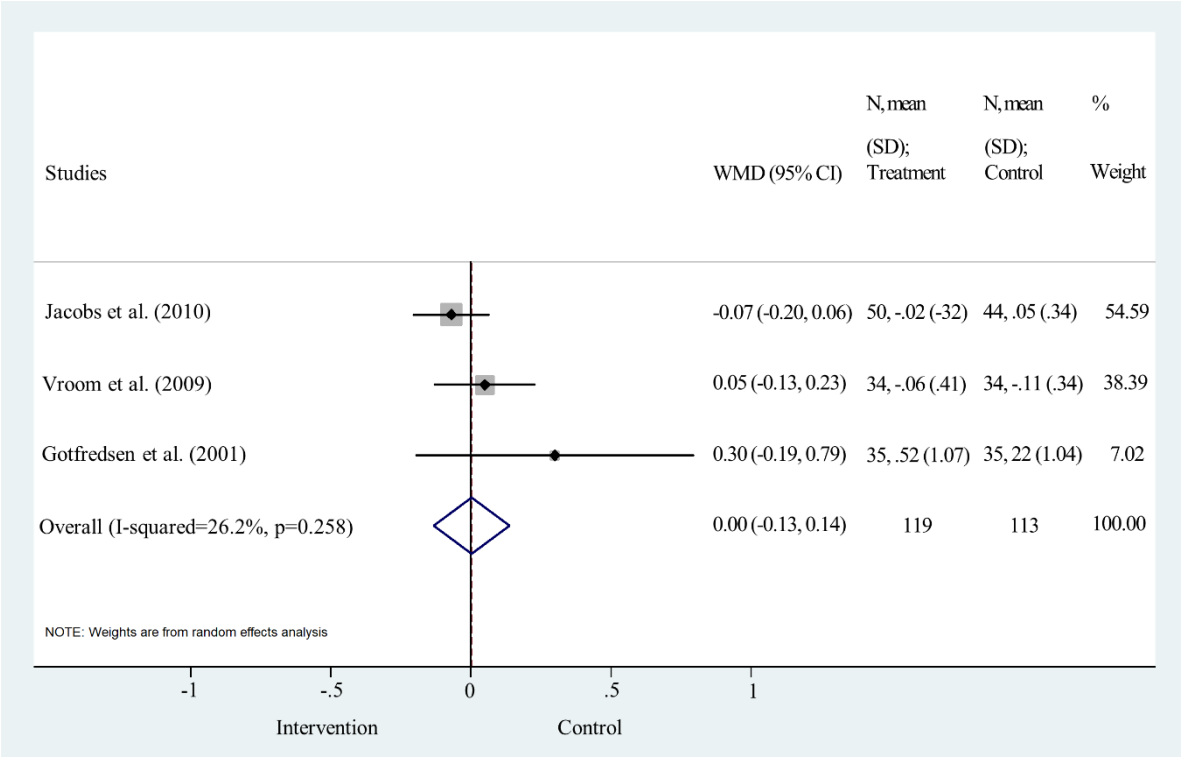

Figure 8. Forest plot of MBL loss after five years of prosthesis delivery. (*Source:* (Czumbel et al., 2019))

4.1.6 Additional analysis

Statistical heterogeneity was not important regarding the outcome of implant failure. I^2 values were 0% and the related p values were between 0.59 and 0.89 indicating no significance. (*Figures 3, 4, 5 and 6*). Statistical heterogeneity was only slightly higher in the case of marginal bone loss after 5 years I^2 (26.2%) and p (0.258), this was considered insignificant (*Figures 7 and 8*).

4.2. Results of the meta-analysis on the topic of hyaluronic acid dermal fillers in the oral cavity

4.2.1. Study selection

The study selection process recognized 326 records in total. After rigorous selection 10 records were included in the quantitative synthesis assessing the effectiveness of lip augmentation and 32 records were included in the qualitative synthesis (*Figure 9*).

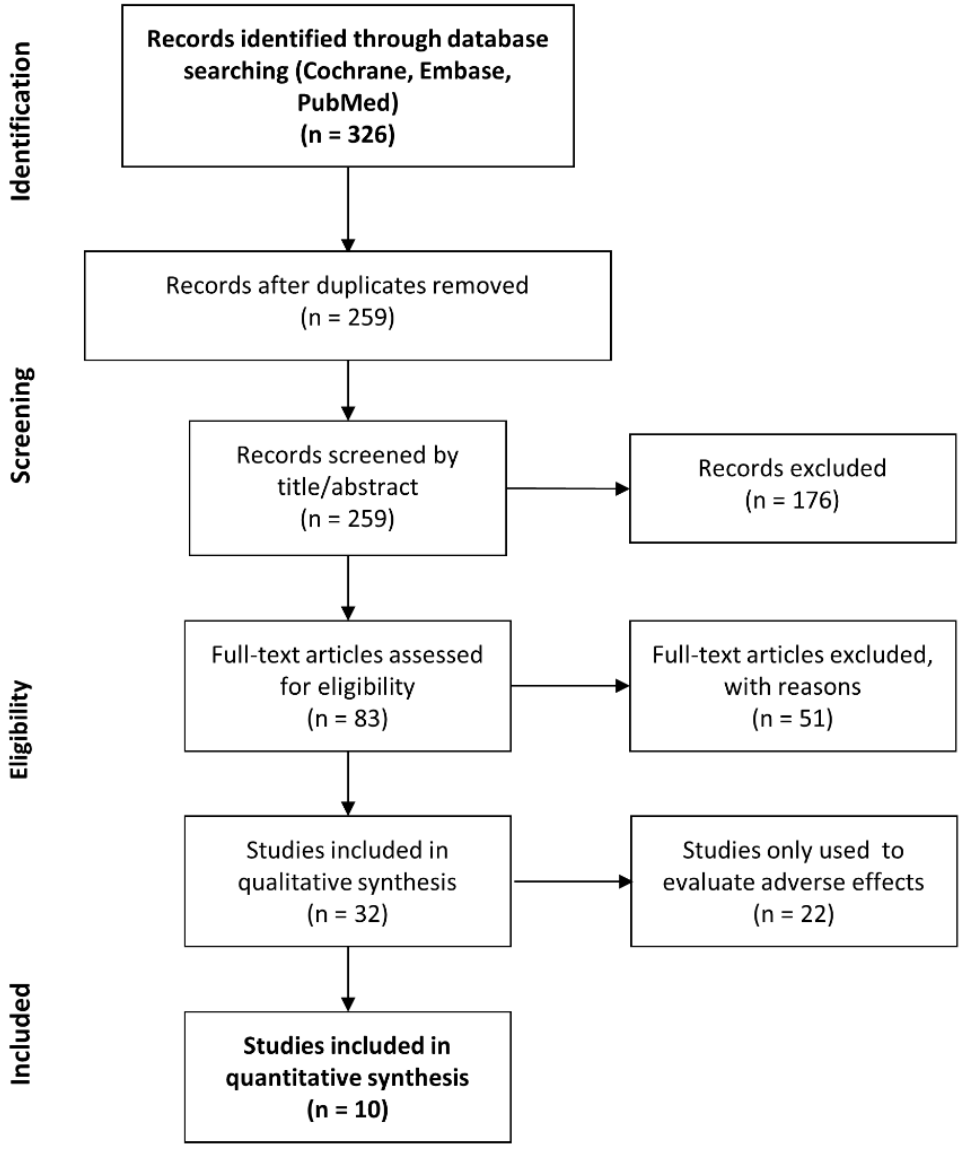

Figure 9. Study selection flow chart. (*Source:* (Czumbel et al., 2021))

4.2.2. Characteristics of included studies

To investigate the effects of HA on lip augmentation 5 RCTs (Beer et al., 2015; Dayan et al., 2015; Geronemus et al., 2017; Glogau et al., 2012; Raspaldo et al., 2015) and 5 cohort studies (Chopra et al., 2018; Eccleston & Murphy, 2012; Fagien et al., 2013; Solish & Swift, 2011; Yazdanparast et al., 2017) were included. Additionally, to assess the AEs 14 case reports (Anatelli et al., 2010; Bulam et al., 2015; Curi et al., 2015; Dougherty et al., 2011; Duhovic

& Duarte-Williamson, 2016; Edwards et al., 2006; Eversole et al., 2013; Farahani et al., 2012; Feio et al., 2013; Fernández-Aceñero Ma et al., 2003; Grippaudo et al., 2014; Leonhardt et al., 2005; Martin et al., 2018; Wolfram et al., 2006), six additional cohort studies (Artzi et al., 2016; Carruthers et al., 2005; Fischer et al., 2016; Philipp-Dormston et al., 2014; Rzany et al., 2012; Samuelson et al., 2015) and two additional RCTs (Carruthers et al., 2010; Downie et al., 2009b) were included.

A total of 1228 participants were included in the effectiveness analysis. Average subject age was between 41 and 54 years within the studies. In the included population all Fitzpatrick skin types were represented (Roberts, 2009). Participants with an allergy to injectable HA or a history of any permanent or semi-permanent aesthetic tissue augmentation were excluded. All included records used a validated lip fullness scale such as the Allergan Lip Fullness Scale (Werschler et al., 2015) or the Medicis Lip Fullness Scales (Kane et al., 2012). The sites of injection in all studies were the perioral lines and the lips. Study characteristics are summarized in *Table 3, 4, 5 and 6.*

Table 3. Summary of characteristics of studies included in the effectiveness analysis. (*Source:* (Czumbel et al., 2021))

Study	Study design	n*	Female ratio	Age: mean ± SD [median] (range),	Intervention	Control	Maximum injected volume; injection technique	Follow-up (months)	Outcome measure
Beer, K. et al. 2015	RCT, multicentre, evaluator blinded	199	97%	45.5	Restylane-L	No-treatment group	2.17 ml (mean); anterograde, retrograde linear threading, serial puncture	6	MLFS and WASULL, GAIS, TEAEs
Chopra, R. et al. 2018	Cohort, multicentre, open label, prospective	57	93%	46.5, (23 – 72)	Restylane-L	Baseline-controlled	1 – 3 ml (range); submucosa, retrograde, anterograde linear, fanning	3	GAIS, MLFS, TEAEs
Dayan, S. et al. 2015	RCT, multicentre, evaluator blinded	208	95.8%	[49], (20 – 79)	Juvéderm Ultra XC (HYC-24L)	No-treatment group	4.8 ml (max); linear threading, serial puncture, fanning, crosshatching	12	ALFS, POL, OCS, ISRs, AEs
Eccleston, D. et al. 2012	Cohort, multicentre, open label, prospective	59	100%	50, (21 – 74)	Juvéderm Volbella	Baseline-controlled	1.3 ml (median); retrograde, tunnelling, cross hatching	12	ALFS, AEs
Fagien, S. et al. 2013	Cohort, multicentre, evaluator blinded, prospective	50	96%	[47], (24 – 68)	Juvéderm Ultra	Baseline-controlled	2.2 ml (median), 2.3 ml (max); retrograde, anterograde, tunnelling, serial puncture	12	ALFS, OCS, POL, CTR, AEs
Geronemus, G., R. et al. 2017	RCT, multicentre, evaluator blinded	224	96.9%	[54], (22 – 78)	Juvéderm Volbella XC (VYC-15L)	Restylane-L	2.5 ml (median); subdermal, intradermal, tunnelling, puncture	12	ALFS, POLSS, POLM, OCS, GAIS, AEs
Glogau., R., G. et al. 2012	RCT multicentre, evaluator blinded	135	99%	47.6 ± 10.6, [50.0], (18.0 – 65.0)	Restylane	No-treatment group	1.5 ml (max), 0.3 – 2.5 ml (range); linear injection technique, serial puncture	6	MLFS, GAIS, AEs
Raspaldo, H. et al. 2015	RCT multicentre, evaluator blinded	268	97.1%	[48], (18 – 76)	Juvéderm Volbella (with Lidocaine)	Restylane-L	1.97 – 1.86 ml (mean); intradermal, subdermal, tunnelling	12	ALFS, POL, OCS, AEs, ISRs,
Solish, N. et al. 2011	Cohort, multicentre, evaluator blinded, prospective	18	86%	41.1 ± 11.4, [40], (26 – 65)	Restylane	Baseline-controlled	1.5 ml (max); anterograde, vertical, deposition formation	3	MLFS, GAIS, AEs
Yazdanparast, T. et al. 2017	Cohort, single centre, open label, prospective	10	100%	(28 – 45)	Hyamax Kiss	Baseline-controlled	1 ml (max); retrograde	6	MLFS, IGA, VAS, AEs

* Number of participants included in the MA analysis (Exclusion due to study groups using different filling material or other anatomical sites.)

Abbreviations: AEs: Adverse events, ALFS: Allergan Lip Fullness Scale, CTR: common treatment-site responses, GAIS: Global Aesthetic Improvement Scale, IGA: Investigator's Global Assessment, ISRs: Injection site responses, MLFS: Medicis Lip Fullness Scale, OCS: Oral Commissure Severity Scale, POL/POLSS: Allegran Perioral Severity Scale, POLM: Allergan Perioral Lines at Maximal Contraction scale, SP: Standardized photography, TEAE: treatment-emergent adverse events, VAS: Visual Analogue Scale, WASULL: Wrinkle Assessment Scale of Upper Lip Lines.

Table 4. Summary of characteristics of cohort studies included only in the adverse effect analysis. (*Source:* (Czumbel et al., 2021))

Study	Study design	n*	Female ratio	Age: mean ± SD [median] (range),	Intervention	Control	Statistics	Follow-up (weeks)	Outcome
Artzi, O. et al. 2016	Cohort, multicenter, retrospective	3†	90%	49.6, (28 – 70)	Juvéderm Volbella (Allergan)	No control group	Spearman correlation	96	Immediate and delayed AEs
Carruthers, J. et al. 2005	Cohort, single centre, open label	15	100%	[40.50], (33 – 60)	Restylane	No control group	Descriptive statistics	24	SP, AEs
Carruthers, A. et al. 2010	Randomized, parallel-group, multicentre, clinical trial	23	100%	48.4 ± 5.5	Juvéderm Ultra, Juvéderm Ultra Plus	OnabotulinumtoxinA, OnabotulinumtoxinA plus hyaluronic acid	Kruskal-Wallis test, Wilcoxon rank sum test	24	GAIS, CIS, AEs
Downie, J. et al. 2009	Randomized, parallel-group, double blinded, single-centre, clinical trial	23	100%	(25 – 55)	Perlane	Various collagen fillers	Kruskal Wallis Rank Sum test	48	2D and 3D facial image analysis, AEs
Fischer, T., C. et al. 2016	Cohort, multicenter, retrospective	146	98.6%	44.7 ± 14.6	CPM-HAL1 and CPM-HAL2 (Belotero Balance Lidocaine	No control group	Descriptive statistics	16	Merz scale, GAIS, VAS, AEs
Philipp-Dormston, W., G. et al. 2014	Cohort, multicenter, open label, prospective	60	88.7%	39.7 (21 – 75)	Juvéderm Volbella	No control group	Descriptive statistics	4	4-grade scale for subject and injector satisfaction, AEs
Rzany, B. et al. 2012	Cohort, multicenter, open label, prospective	76	94.8%	54.5 ± 8.2	Emervel	No control group	Descriptive statistics	24	GAIS; LRS, LFGS, satisfaction questionnaires, AEs
Samuelson, U. et al. 2015	Cohort, multicenter, evaluator blinded, prospective	29	100%	36, (19 – 59)	Restylane Lip Volume	Baseline-controlled	Proportion with 95% CI	36	GAIS, MLFS, AEs

* Number of participants included in the MA analysis (Exclusion due to study groups using different filling material or other anatomical sites.
† Study population number is 400 (mean age: 49.6, range: 28 – 70), however only 3 patients received lip augmentation with HA filler.

Abbreviations: AEs: Adverse events, CIS: Cosmetic Improvement Scale; GAIS: Global Aesthetic Improvement Scale, ISRs: Injection site responses, LFGS: Lip Fullness Grading Scale, LRS: Lemperle Rating Scale, MLFS: Medicis Lip Fullnes Scale, RCT: Randomized controlled trial, SP: Standardized photography, VAS: Visual Analogue Scale,

Table 5. Summary of characteristics of HA dermal fillers included in the meta-analysis.

(Source: (Czumbel et al., 2021))

Product name	Concentration	Composition	Studies	Source of information
Belotero Intense Lidocaine	25 mg/ml	Cross-linked	Fischer, T. et al. 2016	(Kühne et al., 2016)
Emervel (range of products)	20 mg/ml	Cross-linked to various degree	Rzany, B. et al. 2012	(Rzany et al., 2012)
Hyamax Kiss	22 mg/ml	500 µm particle size, cross-linked	Yazdanparast, T. et al. 2017	(Yazdanparast et al., 2017)
Juvéderm Ultra	24 mg/mil (0.3% Lidocaine)	Cross-linked (6%)	Fagien, S. et al. 2013, Carruthers, J. et al. 2010	(Fagien et al., 2013); (Carruthers et al., 2005)
Juvéderm Ultra XC (HYC-24L)	24 mg/mil (0.3% Lidocaine)	Cross-linked	Dayan, S. et al. 2015; Bulam, H. et al. 2015; Bulam, H. et al. 2015	(Dayan et al., 2015)
Juvéderm Volbella without Lidocaine	15mg/ml	Not available	Eccleston, D. et al. 2012; Artzi, O. et al. 2016	(Eccleston & Murphy, 2012)
Juvéderm Volbella with Lidocaine	15mg/ml HA (0.3% Lidocaine)	Cross-linked	Raspaldo, H. et al. 2015; Philipp-Dormston, W. G. et al. 2014	(Allergan, 2016a; Raspaldo et al., 2015)
Juvéderm Volbella XC (VYC-15L)	15 mg/ml HA (0.3% Lidocaine)	Cross-linked, low- and high-molecular-weight HA	Geronemus, R. G. et al. 2017	(Allergan, 2016b)
Perlane	20 mg/ml	Cross-linked, 1000 µm particle size	Downie, J. et al. 2009(WebMD, 2013)	(Downie et al., 2009a)
Restylane (without lidocaine)	Not available	SGP, 300 µm particle size, cross-linked	Glogau., R., G. et al. 2012 ; Solish, N. et al. 2011; Carruthers, J. et al. 2005; Fernández-Aceñero Ma, J., et al.2003; Anatelli, F. et al. 2010; Curi, M. M. et al. 2015; Dougherty, A. L. et al. 2011; Leonhardt, J. M. et al. 2005; Wolfram, D. et al 2006; Farahani, S. S. et al. 2012; Edwards, P. C. et al. 2006	(Glogau et al., 2012; Solish & Swift, 2011)
Restylane-L	20mg/ml HA (0.3% Lidocaine)	SGP, cross-linked	Raspaldo, H. et al. 2015; Geronemus, R. G. et al. 2017; Beer, K. et al. 2015; Chopra, R. et al. 2018;	(Geronemus et al., 2017; Medicis, 2012; Raspaldo et al., 2015); (Beer et al., 2015; Chopra et al., 2018)
Restylane Lip Volume	20mg/ml HA (0.3% Lidocaine)	Cross-linked	Samuelson, U. et al. 2015	(Samuelson et al., 2015)
HA not further specified	N/A	N/A	Duhovic, C. et al. 2016; Eversole, R. et al. 2013; Feio, P. S. et al. 2013; Grippaudo, F. R. et al 2014; Martin, L. et al. 2018	N/A

Abbreviations: N/A: not applicable, HA: hyaluronic acid.

Table 6. Study characteristics of case reports included in adverse reaction analysis. (*Source:* (Czumbel et al., 2021))

Study	Number of participants	Female ratio (%)	Age or mean age ± SD	Treatment	Description of symptoms	Diagnosis	Time after HA treatment
Anatelli, F. et al. 2010	1	100	80	Restylane	3 mm pearly translucent papule in the left upper cutaneous lip	Biopsy, amorphous basophilic deposit of HA	8 weeks or more (not clearly stated)
Bulam, H. et al. 2015	1	100	27	Juvederm Ultra Plus XC	Angioedema-type acute hypersensitivity reaction	Clinical signs, angioedema	Within minutes
Curi, M. M. et al. 2015	2	100	65	Restylane	2 nodular lesions on the right upper lip mucosa	Biopsy, granulomatous foreign body reaction related to the HA	12 years
			58	HA (unknown)	Sudden symmetric bilateral swelling on the parotid masseteric region and also on the buccal mucosa after chemotherapy	Clinical examination	4 years
Dougherty, A. L. et al. 2011	1	100	Unknown	Restylane	Angioedema-type swelling on both lips	Clinical signs, angioedema, herpes reactivation	Within 12 hours
Duhovic, C. et al. 2016	1	100	58	HA (unknown)	Indurated nodules above the upper lip bilaterally	Biopsy, multinucleated giant cells granulomatosis	4 years
Edwards, P. C. et al. 2006	1	100	74	Restylane	Firm submucosal nodule of the lower lip	Biopsy, multiple cystlike vacuolated areas with histiocytes and foamy macrophages, consistent with a foreign body reaction	6 months
Eversole, R. et al. 2013	2	100	45	HA (unknown)	Granular yellow lesion	Biopsy, granulomatous foreign body reaction	Within 5 years
			51	HA (unknown)	White nodules mandibular sulcus		
Farahani, S. S. et al. 2012	3	100	56 (mean)	Restylane	Painless discrete nodule on the labial mucosa	Biopsy	Within 2 years
Feio, P. S. et al. 2013	2	100	51	HA (unknown)	Hardness in the lower lip mucosa	Biopsy, presence of numerous giant cells around translucent particles, foreign body reaction	6 months
			30		Fibrous nodule on the left upper lip mucosa, which had quickly enlarged, then stabilised	No surgical intervention	7 years
Fernández-Aceñero Ma, J. et al. 2003	1	100	48	Restylane	Several discrete nodules in the upper lip	Biopsy, ranulomatous foreign body reaction with multinucleated cells around a blue amorphous material	2 years
Grippaudo, F. R. et al. 2014	1	100	28	HA (unknown)	"angry red nodules"	High Frequency Ultrasound (HFUS) examination	1 year
Leonhardt, J. M. et al. 2005	1	100	52	Restylane	Angioedema-type swelling on the upper lips	Clinical signs, angioedema	Within hours
Martin, L. et al. 2018	2	100	24	HA (unknown)	Nodules in the lip area	Biopsy, Foreign body reaction to exogenous material	Within years
			43				
Wolfram, D. et al. 2006	1	100	53	Restylane	Erythematous indurations of both nasolabial folds	Dense granulomatous infiltrate with multinucleated giant cells as well as sharply delineated extracellular foreign bodies	2 years

Abbreviations: HA: hyaluronic acid.

4.2.3. Risk of bias in studies

The risk of bias was assessed according to the Risk of Bias Tool (Cumpston et al., 2019; J. P. T. Higgins et al., 2011). All included RCTs used means of random sequence generation. Yet, in the case of Dayan et al. (Dayan et al., 2015) and Carruthers and coworkers (Carruthers et al., 2010) the methods of allocation concealment were not described clearly. Blinding of personnel giving the injection could not be carried out, however, blinded evaluators were used to assess the outcome. In the case of a study (Carruthers et al., 2010) the 23% of drop-outs indicate a high risk of attrition bias. Additionally, in another study (Dayan et al., 2015) as a result of ambiguous reporting on follow-up, attrition bias was marked as unclear.

The Newcastle Ottawa Scale (Deeks et al., 2003) was used to assess the bias in the observational studies. These studies had no control groups. They utilized baseline control, meaning that in the treated group the so-called "rate of responders" was compared to the baseline values. On average the bias assessment resulted in 5.5±1.3 (mean±SEM) stars on the modified seven-point scale. A summary of the risk of bias assessment is available in *Table 7* and *Figure 10*.

Table 7. Tabulated results of risk of bias assessment of included cohort studies. (*Source:* (Czumbel et al., 2021))

Study	Selection	Comparability	Outcome	Total Score	Included in effectiveness analysis
Chopra, R. et al. 2018	✸✸✸	✸	✸✸	6	Yes
Eccleston, D. et al. 2012	✸✸✸	✸	✸✸	6	Yes
Fagien, S. et al. 2013	✸✸✸	✸	✸✸✸	7	Yes
Solish, N. et al 2011	✸✸✸	✸	✸✸	6	Yes
Yazdanparast, T. et al. 2017	✸✸✸	✸	✸✸✸	7	Yes
Artzi, O. et al. 2016	✸✸✸	-	✸	4	No
Carruthers, J. et al 2005	✸✸✸	-	✸✸	5	No
Fischer, T. et al. 2016	✸✸✸	-	✸	4	No
Philipp-Dormston, W. G. et al. 2014	✸✸✸	-	-	3	No
Rzany, B. et al. 2012	✸✸✸	-	✸✸✸	6	No
Samuelson, U. et al. 2015	✸✸✸	✸	✸✸	6	No

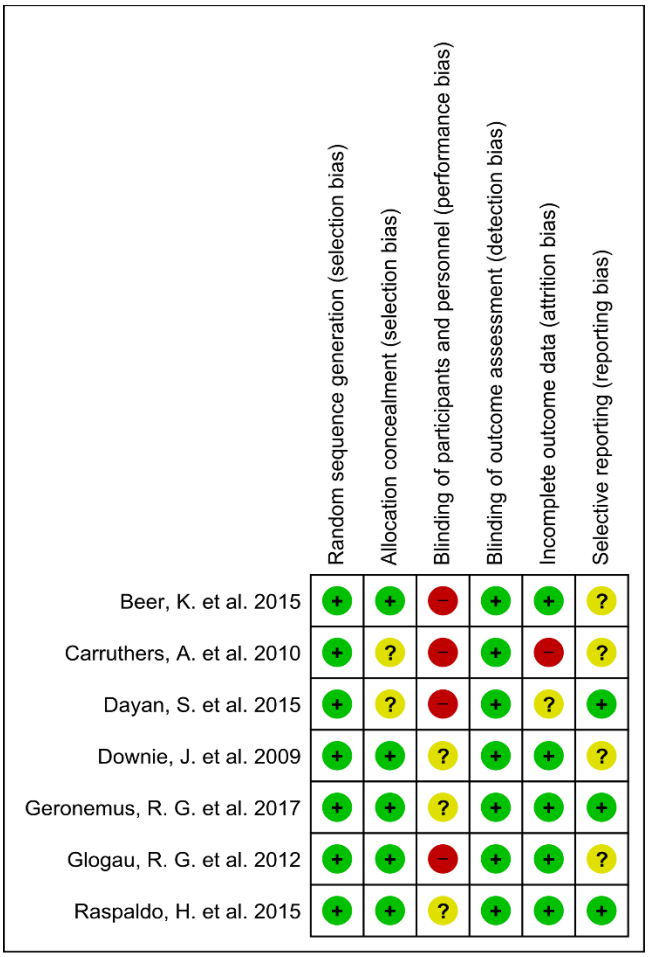

Figure 10. Riks of bias summary. (*Source:* (Czumbel et al., 2021))

4.2.4. Results of data synthesis – Effectiveness of HA

Three months after HA injection the rate of responders was calculated based on eight records (the percentage of participants with at least one-grade improvement on the MLF or ALF scales after HA injection (Czumbel et al., 2021)) (Chopra et al., 2018; Dayan et al., 2015; Eccleston & Murphy, 2012; Fagien et al., 2013; Geronemus et al., 2017; Raspaldo et al., 2015; Solish & Swift, 2011; Yazdanparast et al., 2017). Synthesized data showed that 71% of treated participants were responders, meaning that 71 out of 100 experienced a considerable, one grade or greater increase in lip fullness 3 months after the initial treatment (ES=0.71, 95% CI: 0.55—0.87; I^2 = 97.91%, p = 0.00) (*Figure 11*).

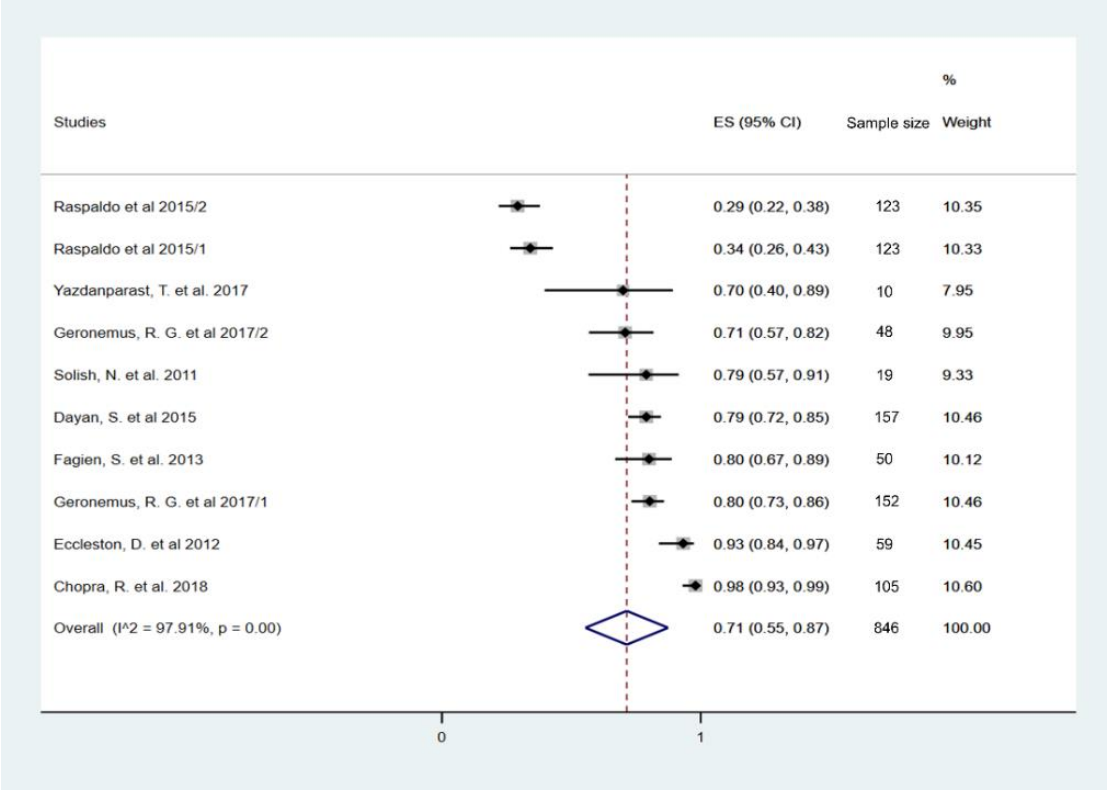

Figure 11. Forest plot visualizing the results of pooled data 3 months after treatment. (*Source:* (Czumbel et al., 2021))

Six months after treatment, the overall rate of responders was synthesized from five studies (Dayan et al., 2015; Eccleston & Murphy, 2012; Fagien et al., 2013; Geronemus et al., 2017; Yazdanparast et al., 2017). The statistical analysis indicated that 74% of participants receiving one dose of HA injection maintained their lip fullness (ES=0.74, 95% CI: 0.66—0.82; I^2 = 66.88%, p = 0.02) (*Figure 12*).

Figure 12. Forest plot visualizing the pooled data 6 months after treatment. (*Source:* (Czumbel et al., 2021))

Lip fullness data of 12 months of follow-up was available in four studies (Dayan et al., 2015; Eccleston & Murphy, 2012; Fagien et al., 2013; Geronemus et al., 2017). Pooled data revealed that the rate of responders was 46% even after one year of a single HA injection (ES=0.46, 95% CI: 0.28—0.65; I^2 = 93.21%, p = 0.00) (*Figure 13*).

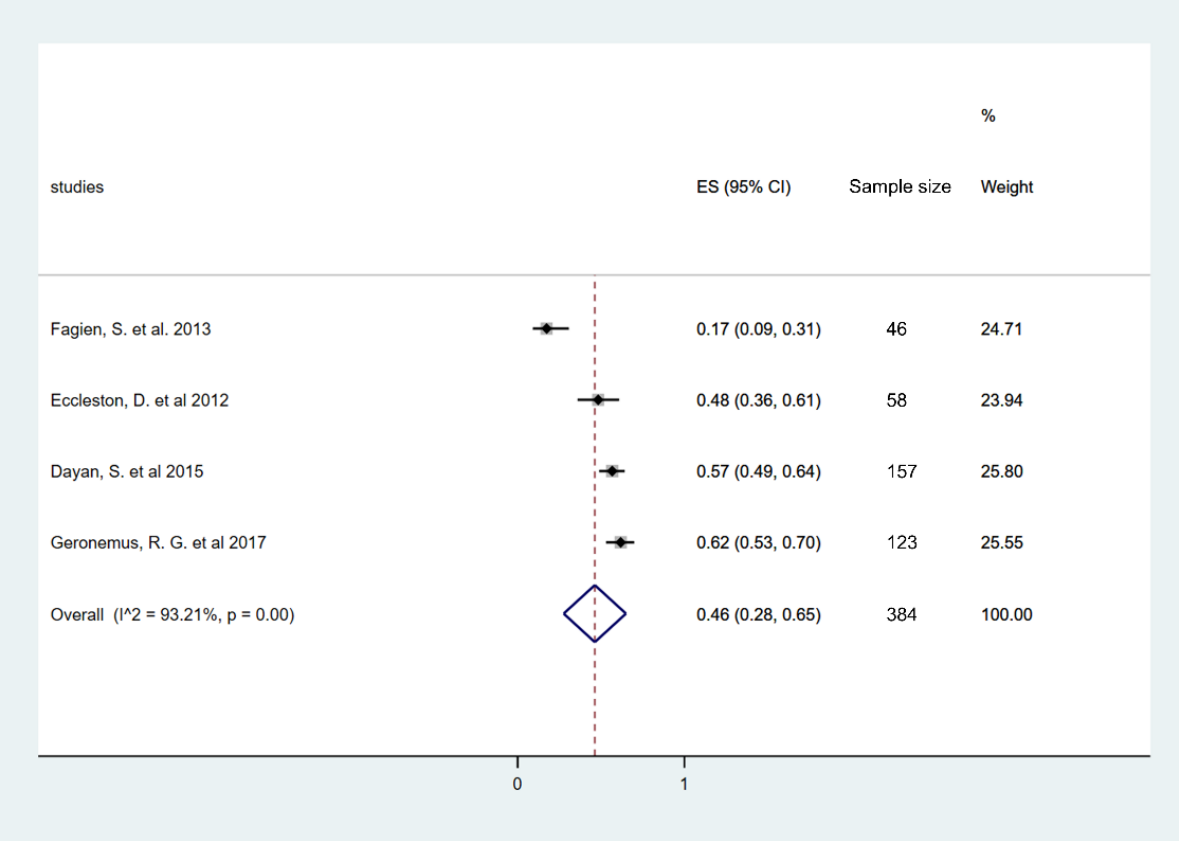

Figure 13. Forest plot Visualizing the pooled data 12 months after treatment. (*Source:* (Czumbel et al., 2021))

4.2.5. Adverse effects related to hyaluronic acid injection

This analysis includes studies reporting on AEs related to HA injection. Data were included from eligible studies overall including a population of 1488 participants (Anatelli et al., 2010; Artzi et al., 2016; Beer et al., 2015; Bulam et al., 2015; Carruthers et al., 2010; Carruthers et al., 2005; Chopra et al., 2018; Curi et al., 2015; Dayan et al., 2015; Dougherty et al., 2011; Downie et al., 2009a; Duhovic & Duarte-Williamson, 2016; Eccleston & Murphy, 2012;

Edwards et al., 2006; Eversole et al., 2013; Fagien et al., 2013; Farahani et al., 2012; Feio et al., 2013; Fernández-Aceñero Ma et al., 2003; Fischer et al., 2016; Geronemus et al., 2017; Grippaudo et al., 2014; Leonhardt et al., 2005; Martin et al., 2018; Philipp-Dormston et al., 2014; Raspaldo et al., 2015; Rzany et al., 2012; Samuelson et al., 2015; Solish & Swift, 2011; Wolfram et al., 2006; Yazdanparast et al., 2017).

The analysis showed that the five most frequent AEs were tenderness (n = 1320, 88.7 %), injection site swelling (n = 1105, 74.3 %), contusion (n = 725, 48.7 %), injection site mass (n = 406, 27.3 %) and injection site pain (n = 293, 19.7 %). More severe AEs were rare. Among them worth mentioning herpes labialis (n = 9, 0.6 %) and granulomatous foreign body reaction (n = 9, 0.6 %). Life-threatening angioedema was reported in four cases (0.3 %) (*Table 8*)**.**

Table 8. Adverse effects reported in the included studies. (*Source:* (Czumbel et al., 2021)).

Adverse effect	N (total = 1487)	%		Adverse effect	N (total = 1487)	%
Tenderness	1320	88.7		A tumorlike nodule	4	0.3
Injection site swelling	1105	74.3		Angioedema	4	0.3
Contusion	725	48.7		Dry lip	3	0.2
Injection site mass	406	27.3		Anesthesia	1	0.1
Injection site pain	293	19.7		Canker sore	1	0.1
Erythema	108	7.3		Induration	1	0.1
Tyndall effect and discolouration	84	5.7		Inflammatory nodules	1	0.1
Hematoma	27	1.8		Injection site cyst	1	0.1
Lip disorder	12	0.8		Hemorrhage	1	0.1
Granulomatous foreign body reaction	9	0.6		Papule	1	0.1
Paresthesia	9	0.6		Presyncope	1	0.1
Herpes labialis	9	0.6				

4.2.6. Additional analysis

According to the funnel plot (*Figure 14*), the asymmetrical distribution of the studies suggests the presence of a small study effect. – Meaning that small studies are present in higher numbers, increasing publication bias.

GRADE assessment of the level of the evidence (*Table 9*) indicated a low level of evidence for the effectiveness of HA treatment and AEs as well. This could be explained by the

confounding factors indicated by the statistical heterogeneity. Also the existing inconsistency, low level of study designs and wide range of CIs. Overall, implications for practice should be drawn carefully taking into consideration the limitations of the analyses.

Table 9. GRADE analysis indicating the level of certainty. (*Source:* (Czumbel et al., 2021)).

Outcome	**Study design (№ of studies)**	**Initial level of evidence**	**Evidence components**	**Upgrade/ downgrade of evidence**	**Comment**	**Final level of evidence**
Rate of responders	RCT (5) Cohort studies (5)	Low	**Risk of bias**	Considerable	Control groups are not used in each study	⊕◯◯◯ Very Low
			Inconsistency	Serious	Large I^2 value	
			Indirectness	Not serious	-	
			Imprecision	Serious	Wide range of CIs	
			Other considerations	Publication bias suspected	Small study effect	
Adverse effects	RCT (6) Cohort studies (11) Case reports (14)	Low	**Risk of bias**	Considerable	Control groups are not used in each study	⊕◯◯◯ Very Low
			Inconsistency	Serious	Lack of consistent reporting of adverse effects	
			Indirectness	Not serious	-	
			Imprecision	Not serious	-	
			Other considerations	Upgrade by one point	Large effect	

Abbreviations: RCT: randomized controlled trial

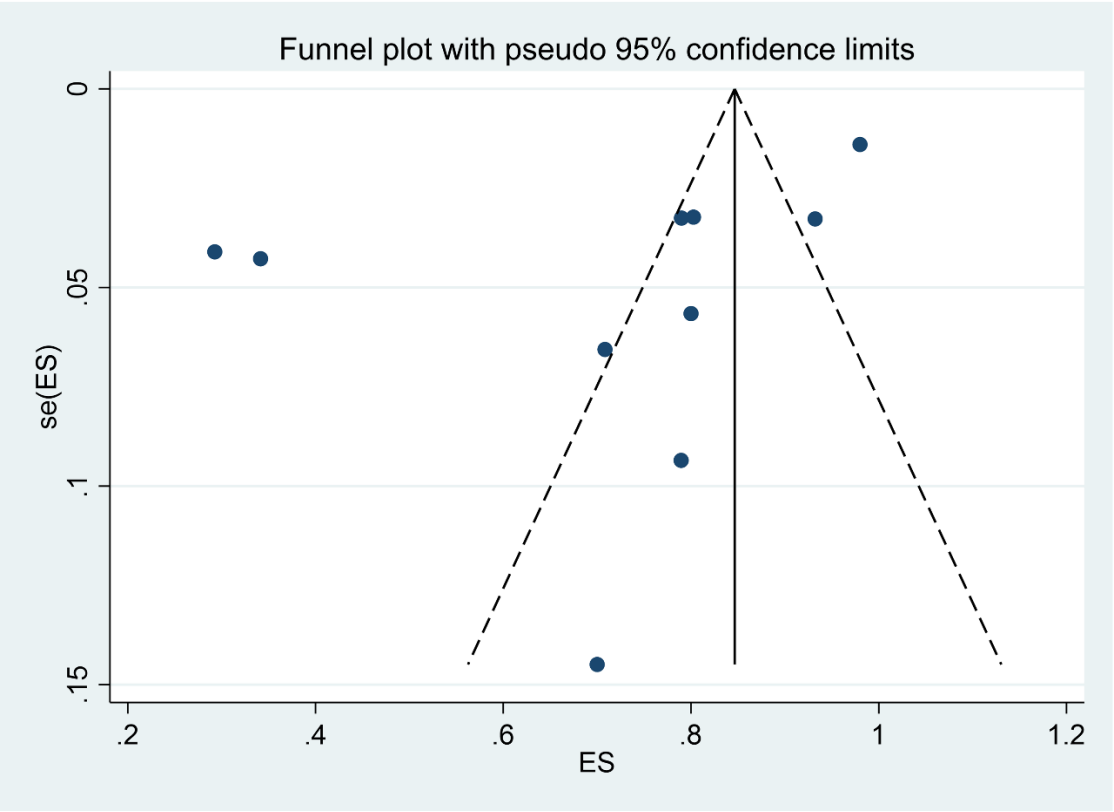

Figure 14. Funnel plot analysis of the records included in the 3 months results. The x-axis represents the effect estimate – the result of the studies. The y-axis represents the standard error of the effect estimate – studies with high power are plotted towards the top, studies with lower power are plotted towards the bottom. The dots are representing the studies included in the analysis. The dots scattered outside the "funnel" indicate heterogeneity. (*Source:* (Czumbel et al., 2021)).

4.3. Results of the meta-analysis on the topic of oral diagnostics

4.3.1. Study selection

In our analysis, we initially identified 19 records in trial registry databases, 102 records through published article databases and three additional records were found by screening the reference list of relevant articles. After duplicate removal 96 records remained for appraisal. Out of these 5 were included in quantitative synthesis (Figure 15).

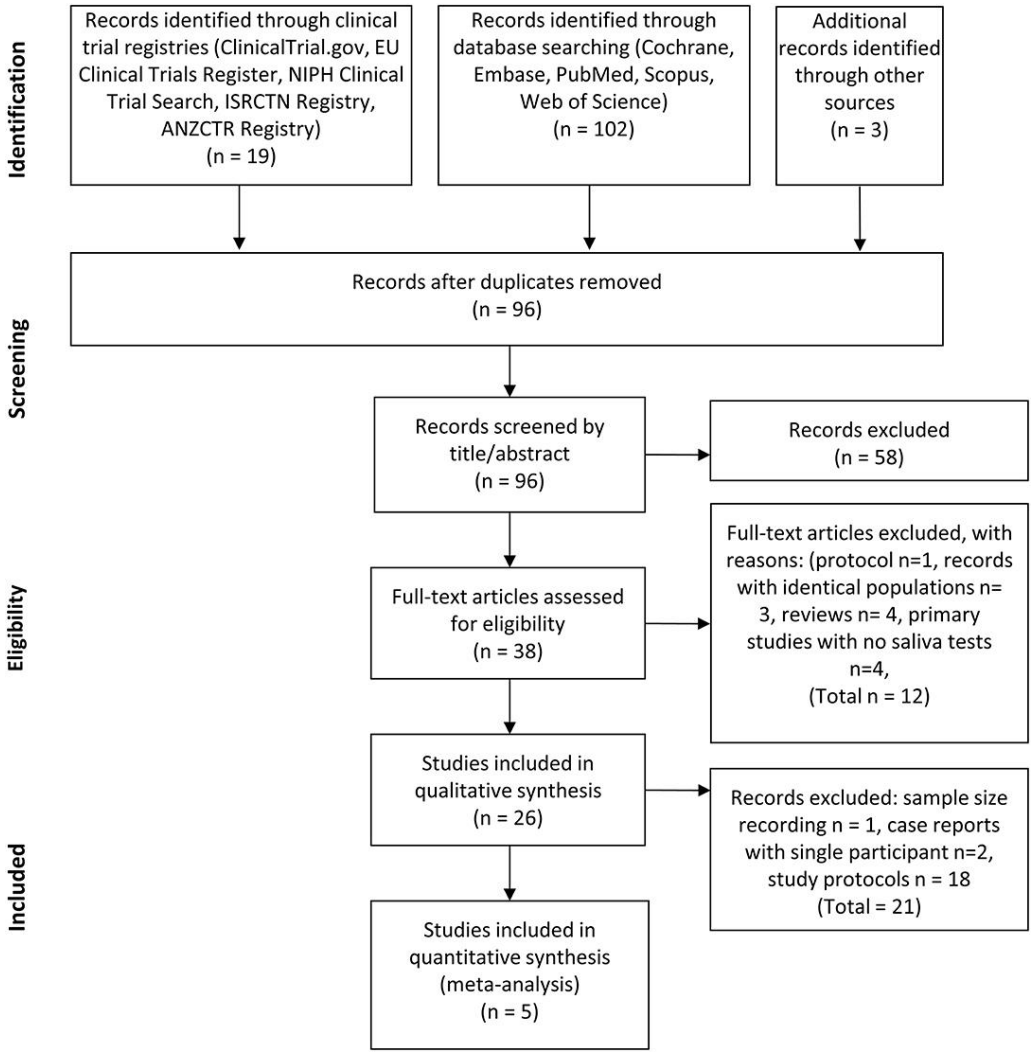

Figure 15. Results of study selection (*Source:* (Czumbel et al., 2020)).

4.3.2. Characteristics of included studies

The five studies included in the quantitative analysis were consecutive case series with 123 participants. (Azzi et al., 2020; Bae et al., 2020; Fang et al., 2020; To et al., 2020; Williams et al., 2020). Another consecutive case series (Wyllie et al., 2020) were also included in the qualitative synthesis. However, it cannot be included in the quantitative work as no clear patient-wise data was reported for comparison with the rest of the studies. The diagnosis of COVID-19 was confirmed in all included cases. The included studies did not declare any

further exclusion criteria for eligibility regarding patient selection. *Table 10* contains the summary of study characteristics.

Table 10. Tabulates summary of characteristics of eligible studies. (*Source*: (Czumbel et al., 2020).)

First author and year	Country	Study type	Population		Diagnoses of COVID-19	PCR kit	Reference standard	Index test	Outcome parameters
			n (m/f)	Age					
Azzi et al. (2020)	Italy	Consecutive case series	25 (17/8)	61 (mean) (39-85)	Viral RNA detection with PCR from NPS	Luna Universal qPCR Master Mix	NPS	Saliva	Number of positive and negative index tests
Bae et al. (2020)	South Korea	Consecutive case series	4 (2/2)	61.5 (35-82)	Viral RNA detection with PCR from NPS And clinical signs of pneumonia	NA	NPS	Saliva	Number of positive and negative index tests
Fang et al. (2020)	China	Consecutive case series	32 (16/16)	41 (34-54)	Viral RNA detection with PCR from NPS	NA	NPS	Saliva	Number of positive and negative index tests
To et al. (2020)	Hong Kong, China	Consecutive case series	23 (13/10)	62 (37-75)	Viral RNA detection with PCR from NPS	QuantiNova Probe RT-PCR Kit	NPS	Saliva	Number of positive and negative index tests
Williams et al. (2020)	Australia	Consecutive case series	39 (not published)	Not published	Viral RNA detection with PCR from NPS	Coronavirus Typing (835 well) assay	NPS	Saliva	Number of positive and negative index tests
Not included in quantitative synthesis:									
Deng and Hu (2020)	China	Case report	1 (0/1)	39	Viral RNA detection with PCR from NPS And clinical signs of pneumonia	NA	NPS	Saliva	Number of positive and negative reference tests and index tests
Han et al. (2020)	South Korea	Case report	1 (0/1)	Neonate (27 day-old)	Viral RNA detection with PCR from NPS	PowerChek TM 2019-nCoV Real-time PCR Kit	NPS	Saliva	Number of positive and negative reference tests and index tests
Wyllie et al. (2020)	USA	Consecutive case series	29 (16/13)	59 (mean) (23-91)	Viral RNA detection with PCR from NPS	The US CDC real-time RT-PCR primer/probe sets	NPS	Saliva	Number of positive and negative reference tests and index tests

NPS - Nasopharyngeal swab; NA – Not available

4.3.3. Risk of bias assessment in the studies

Quadas-2 tool was used to assess the risk of bias in the included six case series (Azzi et al., 2020; Bae et al., 2020; Fang et al., 2020; To et al., 2020; Williams et al., 2020; Wyllie et al., 2020). In the case of four records (Azzi et al., 2020; Bae et al., 2020; Fang et al., 2020; To et al., 2020) bias analysis revealed a high risk of bias regarding the index test as results of saliva tests were assessed with prior knowledge of the results of the nasopharyngeal tests. In all studies, due to the lack of sufficient information on the time passed between sample collection for the reference standard and index test flow and timing was marked as high risk or unclear risk of bias. Overall, the risk of bias analyses indicated a moderate level of bias in the studies. A summary of the risk of bias assessment is available in *Table 11*.

Table 11. Summary of risk-of-bias and applicability concerns in included studies. (*Source:* (Czumbel et al., 2020)).

STUDY	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Azzi et al. (2020)	✓	✗	✓	✗	✓	✓	✓
Bae et al. (2020)	✓	✗	✓	?	✓	?	?
Fang et al. (2020)	✓	✗	✓	?	✓	?	✓
To et al. (2020)	✓	✗	?	?	✓	✓	✓
Williams et al. (2020)	?	?	?	✗	✓	✓	?
Not included in the quantitative analysis:							
Wyllie et al. (2020)	✓	?	?	?	✓	✓	✓

✓ = Low Risk ✗ = High Risk ? = Unclear Risk

4.3.4. Results of data synthesis - sensitivity

Among the included studies the sensitivity of saliva tests ranged between 78% (Fang et al., 2020) to 100% (Azzi et al., 2020) among the SARS-CoV-2 infected participants.

Pooled event rates (negative and positive saliva test results) indicate a sensitivity of 91% (CI 80-99%) among COVID-19 patients (*Figure 16A*). Pooled event rates of NPS tests taken at the same time as saliva specimens show that the sensitivity of NPS test was 98% (CI 89-100%) (*Figure 16B*). As the confidence intervals of the two tests overlap, it suggests that the percentage of positive tests from the NPS tests and saliva tests are not very different. However, to strengthen our observation larger clinical studies are needed.

Evidence exists that in some cases NPS tests sometimes give negative results while the saliva test is positive (Azzi et al., 2020; Deng et al., 2020). Wyllie and coworkers (Wyllie et al., 2020) in a sample-based study out of 38 participants in eight (21%) detected the virus based on saliva specimens, while the virus was not detected in the corresponding NPS samples. Whereas only in three instances (8%) happened that NPS was positive but saliva tests gave a negative result.

Specificity was assessed in two studies (Williams et al., 2020; Wyllie et al., 2020). In the study of Williams et al. (Williams et al., 2020) SARS-CoV-2 was detected in 2% (CI 0.1-11.5%) of PCR-negative patients (n=50) (test was taken by nasal swab). The study of Wyllie and coworkers (Wyllie et al., 2020) included 98 asymptomatic healthcare personnel in their analysis. Saliva and NPS tests were taken in parallel. All tests turned out to be negative for NPS and two were positive for saliva.

4.3.5. Additional analysis

The moderate level of statistical heterogeneity for the proportion of positive saliva tests (I^2=60.96%) and the proportion of positive NPS tests I^2 = 46.56% indicates the presence of co-founding factors that might influence the results of the data synthesis. Due to the fact that the influence of confounding factors could not be well explored as a lack of sufficient data, results should be interpreted within the context of the analysis.

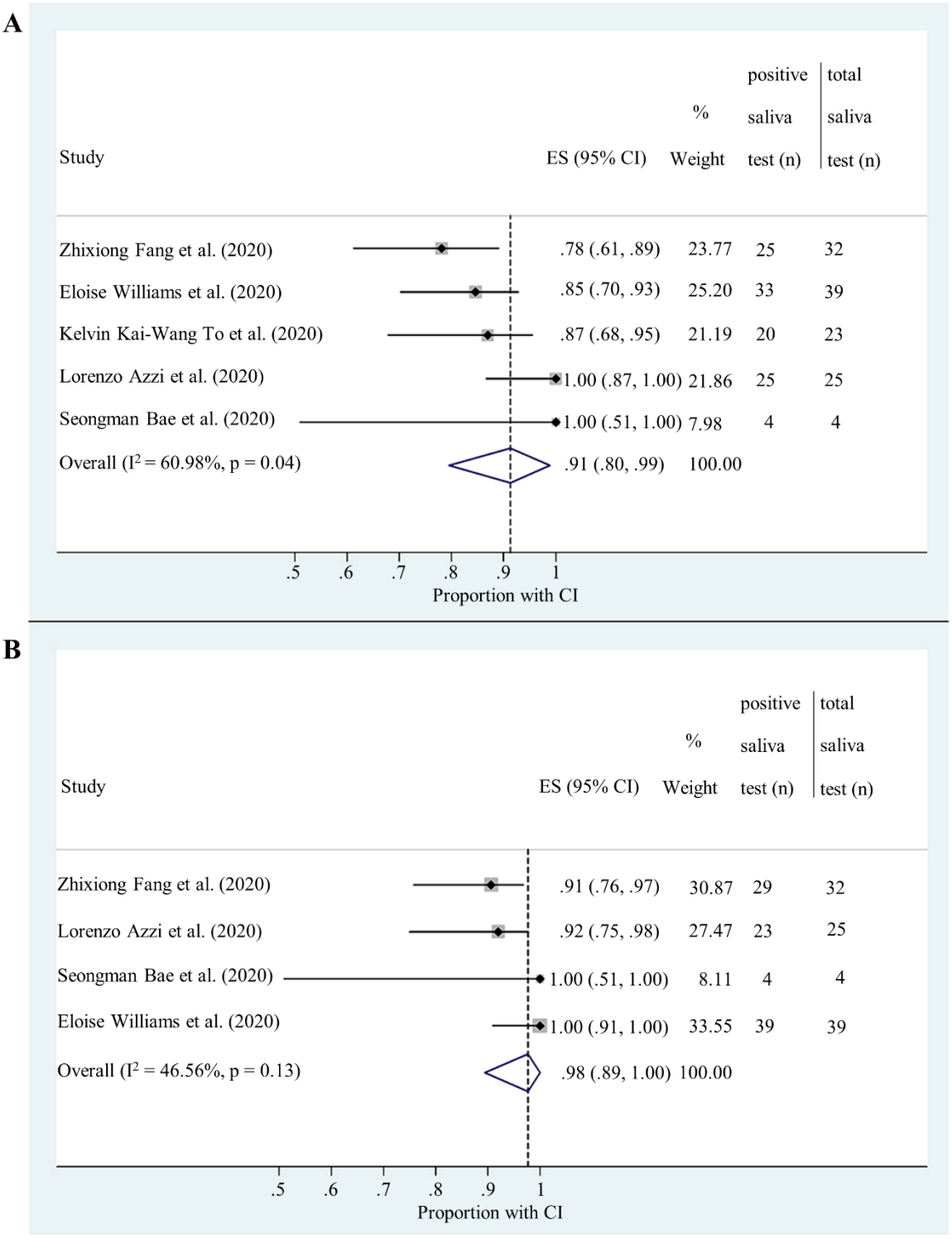

Figure 16. Forest plot visualizing the detection sensitivity. Part A: proportion of positive saliva tests included five studies. Part B: proportion of positive NPS tests included four studies. (*Source:* (Czumbel et al., 2020))

5. Discussion

For decades the amount of available scientific literature has been exponentially increasing, meaning that it is becoming extremely difficult to find the relevant information within the published data. Moreover, clinical trials contain bias, in certain cases they include an insufficient number of participants, in other cases contradictory conclusions emerge. These conditions decrease the level of certainty of the findings, leaving researchers and clinicians with uncertainty. The present scientific work utilized the methodology of meta-analysis in three specific dental areas, such as the comparison of healing potential machined and sand-blasted dental implants, the performance of HA dermal fillers in the perioral region and the use of saliva as a specimen for diagnosing COVID-19. In these areas, this present work used the methodology of MA for the first time.

The scientific analyses in the present work integrated the available data from eligible original studies and appraised them systematically and critically to synthesize new findings with a higher level of evidence. This was achieved by increasing power and precision as more participants were included in the analyses overall. Furthermore, controversies among individual studies were investigated. Moreover, bias in studies was assessed using appropriate assessment tools developed by research groups.

Although several studies have been conducted to find the optimal surface type for promoting osseointegration, the final conclusion is still missing. Several novel materials and surface modifications are being invented to outperform the existing ones. However, the evidence is weak, coming from non-standardized sources and contradictory (Borges et al., 2020; Bornstein et al., 2007; Degidi et al., 2012; Rocci et al., 2008; Roehling et al., 2019; Wennerberg et al., 2018). The aim of this present work was to perform a systematic review and a re-analysis of the already existing evidence in the form of a meta-analysis regarding smooth and moderately rough (sand-blasted) implant surfaces. We aimed to decrease bias, increase precision and certainty, overall to obtain the highest level of evidence in this research field.

In the meta-analysis to obtain the highest level of evidence only RCTs were included. Retrospective trials and uncontrolled trials were excluded. Implant performance was evaluated by comparing cumulative implant failure rates and measuring marginal bone loss.

After rigorous selection seven randomized clinical trials, 202 participants and 360 machined and 362 sand-blasted implants were included in this complex approach.

The Meta-analysis demonstrated that implant failure rate significantly differed between moderately rough and smooth implants. Contrary, none of the included randomized controlled trials could alone detect significant differences between sand-blasted and machined implants. The majority of implant failures were registered during the first follow-up year. A possible explanation for this phenomenon could be that sand-blasted implants are rougher thus enhancing bone formation in the implant itself (Andrukhov et al., 2016). Other research groups publish similar findings on different moderately rough surfaces (Chrcanovic et al., 2016). The findings of the present meta-analysis also highlight that after one year of insertion, there is no significant difference between machined and sand-blasted implants, once osseointegration has occurred. In addition, another study also confirmed that in the long term both, sand-blasted and machined implants preserved a satisfactory level of osseointegration (Iezzi et al., 2012).

Regarding marginal bone loss, the present meta-analysis revealed no statistically significant difference between machined and sand-blasted implants. Contrary, a previous meta-analysis and a review found that moderately rough implants might induce more bone loss than smooth implants (Doornewaard et al., 2017). The difference between the present and the previous meta-analysis could be explained by the different populations included, and also the higher statistical heterogeneity in the work of Doornewaard and coworkers (Doornewaard et al., 2017). However, all included studies recorded marginal bone losses with high standard deviations. This hinders accurate statistical comparison; thus, results must be interpreted judiciously.

On the other hand, literature data indicate some general tendencies. Åstrand et al. found that most of the bone was lost between implant placement and prosthesis delivery. Additionally, this was less in the case of sand-blasted implants (Åstrand et al., 2004).

However, due to the lack of sufficient data, no meta-analysis could be performed on MBL change between implant placement and prosthesis delivery. Moreover, according to Figure 7, the mean bone change was in the range of 0 and -0.10 mm for machined; 0.14 and -0.20 mm for sand-blasted implants in the first year in the included studies (Åstrand et al.,

2004; Tawse-Smith et al., 2002). In reality, the difference is very small with negligible clinical significance. Based on the findings of Ravald and coworkers, in the time range of five years to 12-15 years follow-up the annual mean bone loss gradually decreased. The annual mean bone level change was -0.02 for machined and -0.04 mm for sand-blasted implants (Ravald et al., 2013).

On the other hand, evidence exists that bone gain might also happen around implants. Åstrand and coinvestigators found a more than 0.6 mm increase in marginal bone level around four sand-blasted and two machined implants over a period of five years (Åstrand et al., 2004). Another study also observed some increase in marginal bone level, regardless of surface varieties. Vroom et al. argued that this results from enhanced corticalization (Vroom et al., 2009).

The different measuring methods of the two outcomes (IF rates and MBL changes), might explain the different trends observed in IF rates and MBL changes regarding the machined and sand-blasted implant types.

Annual measurements of MBL changes may only detect changes in bone level if the dental implant is still integrated into the bone with a slow or no rate of bone resorption. When bone resorption occurs at a fast rate that all the bone surrounding the implant resorbs within a year, implants will be marked as failed and will be excluded from the measurements of MBL changes.

Nevertheless, several studies show that in terms of the rate of initial bone formation machined implants are inferior to implants with rough surface (Bruyn et al., 2017; Piattelli et al., 1996; Velasco-Ortega et al., 2016). The aforementioned findings can be an explanation for why a smaller number of implants with rough surface have failed compared to implants with smooth surface after the first year of implantation.

Ultimately, when it comes to choosing implant types rapid healing stays a key characteristic of rough implants as healing time is a crucial factor in modern implantology and faster healing is prioritized (Vandeweghe et al., 2016; Vervaeke et al., 2016).

In our analysis patients with no history of periodontitis were included. In contrast, studies conducted on a population with a history of periodontitis show different trends. A review article by Quiryen and coinvestigators reports that patients with a history of

aggressive periodontitis have two times more bone loss than subjects with healthy periodontium (Quirynen et al., 2007). These findings are also supported by a recent RCT conducted on patients with severe periodontitis (Raes et al., 2018). Several articles found that surface roughness has a great effect on biofilm accumulation (SCHMIDLIN et al., 2013; Shrestha et al., 2013; Stavropoulos et al., 2021; Teughels et al., 2006). In the cases where the development of periimplantitis is at high risk, machined implants could be the best treatment option. Since machined surfaces have a favourable impact on biofilm formation and in cases where bacterial colonization already had occurred decontamination could be carried out with higher efficiency than in cases of rough implants (Dank et al., 2019; Lin et al., 2013). A review by Quirynen et al. found that there is no difference in implant failure between patients with healthy periodontium and patients with a history of periodontitis if machined implants were used and supportive periodontal therapy was given. (Quirynen et al., 2007).

The present work has a clear implication for clinicians. It was hypothesized that, regarding MBL loss and IF rates, there are significant differences between machined and sand-blasted implant types. Our study found that sandblasting significantly decreases IF rates, however, MBL changes are not significantly affected by sand-blasting. Thus, it is recommended to use moderately rough, sand-blasted implants over machined ones in the case of patients with no systemic diseases. Sand-blasted implants support osseointegration with fewer complications.

To improve research in the field the protocols must be further improved to allow the collection of standardized data for further analysis. Consistent and standardized reporting on several clinical outcomes is needed, for example implant success, bleeding on probing and pocket probing depth.

HA is an often-used dermal filler for lip augmentation among non-surgical techniques (Chung et al., 2020; Cohen et al., 2013). Numerous primary studies investigated the effect of HA fillers, however, their relatively small number of participants involved in the studies makes it difficult to draw strong conclusions. Thus this present work aimed to conduct a meta-analysis of the available records to increase certainty and level of evidence regarding the effectiveness and the AEs related to HA lip augmentation.

Our investigation made an important observation that lip fullness had a substantial decrease over a 12-month period as only 46% (CI: 28% – 65%) of participants remained a responder. The loss of volume in augmented lips could be attributed to the natural biodegradation of HA (Salwowska et al., 2016). Creating cross-links between the HA molecules can slow down the biodegradation process (Ali et al., 2007), however, the direct relation between the speed of degradation and so the long-term effectiveness and the degree of cross-linking is unclear.

Regarding AEs case report revealed additional AEs not reported by RCTs and other prospective studies. Our review of the literature revealed that the most common AEs were injection-related. Such as bruising, injection site swelling, injection site mass, tenderness, and injection site pain. Similar AEs were found in other anatomical areas using HA fillers for augmentation (Chung et al., 2020; Cohen et al., 2013). However, other immune system-related AEs were also noted, such as granulomatous foreign body reaction, activation of herpes virus, and angioedema.

Contamination in HA fillers may be a possible reason for immune system-related AEs. Although HA itself is a non-toxic, non-allergic molecule (Liu et al., 2011), in industry HA products are produced from different xenogeneic sources and procedures (San Miguel Moragas et al., 2015; Schuurmans et al., 2021; Selyanin et al., 2015). Although product are being purified from xenogenic materials, nucleic acid fragments, residual proteins can still exists in the final products. (Boeriu et al., 2013; Schuurmans et al., 2021; Selyanin et al., 2015). Another systematic review studied the occurrence of delayed inflammatory reactions associated with HA filler injection (Chung et al., 2020). Although they have found a relatively low estimated incidence, they recommend the use of skin tests before injections to avoid AEs related to the sensitivity of the product.

From the above, it is evident that in practice HA dermal fillers last for 6 months in more than 70% of the included population. Additionally, it was found that severe AEs such as angioedema may happen in rare cases related to HA injections. As only published events could be considered for statistical analysis the number of AEs actually might be higher. Hence, it is advised to perform an allergy test before injection. For research, the present work

implies that studies with longer follow-ups are needed. Additionally, publishing AEs should be harmonized for meaningful analysis.

There are several FDA-approved saliva-based assays to test COVID-19 infection. However, the studies investigating the effectiveness of saliva-based tests use a relatively small sample size. Thus yielding results with high uncertainty and low precision, with weak statistical power. Our meta-analysis is the first to evaluate the existing evidence of a saliva-based approach with the methodology of meta-analysis.

The present work based on pooled event rates of COVID-19 patients revealed that sensitivity for NPS and saliva tests and were 98% (CI 89-100%) and 91% (CI 80-99%) respectively. The overlap of confidence intervals suggests that there is not much of a difference between the two tests. Although NPS tests tend to be more sensitive no significant difference was found between the two tests. In the future thoroughly designed and performed studies are needed to establish the relative diagnostic specificities and accuracies of NPS and saliva tests.

Among the included studies only two records have investigated the specificity of the saliva (index) test. In one study only one out of 50 healthy participants were detected as positive based on the saliva test (Williams et al., 2020). In the other study, only two out of 98 participants were detected positive based on the index test and they were negative according to the reference standard (Wyllie et al., 2020). These results may indicate the true difference in specificity of the saliva and NPS tests.

Although the concept of utilizing saliva specimens to detect viral infections and various systemic conditions is by now well-proven (Corstjens et al., 2012; Dawes & Wong, 2019; Kaczor-Urbanowicz et al., 2017; Keremi et al., 2017; Niedrig et al., 2018), in everyday practice application of saliva-based testing is still rare due to scarcity of standardized, well-established protocols.

Three conditions must be improved for optimal saliva-based testing (Bhattarai et al., 2018).

A) Selection and optimization of saliva collecting methods through systematic comparison of the available approaches.

B) Resolution to the issue regarding collection, transportation and storing of saliva specimens. C) Optimalization of the RNA assay method for use with saliva specimens.

Particularly, focusing on the internal control, which cannot be human DNA as it is poorly presented in saliva (Bae et al., 2020; Fang et al., 2020; To et al., 2020; Williams et al., 2020; Wyllie et al., 2020). The standardization of all these three specifications must be carried out to obtain sensitive and reliable saliva tests.

For practice, the present work implies that saliva-based tests with appropriate collection methods and carefully adjusted qPCR protocol can be successfully used to screen masses. For researchers, the present work implies that more healthy participants should be included in the studies to estimate the false positive rate with higher certainty.

Limitations

A limitation of the present work is the relatively small number of RCTs available and the small sample sizes in the topic. The limited amount of published data makes it difficult to conduct thorough analyses and comprehensively investigate the grounds of certain trends. Insufficient reported data hinders sub-group analysis and thorough investigation of co-founding factors affecting the results.

Another limitation is the heterogeneity regarding the methodology used in the articles. Although, a huge effort was put into the selection of homogeneous studies this was only possible to a limited extent.

Another limitation was the nonuniform and inconsistent reporting of outcomes. In the case of the topic of dental implants, each study published data on clinical outcomes such as BoP, however, studies used different reporting schemes hindering meaningful comparison of data. In the topic of oral diagnostics, many of the studies did not report false-positive data.

Another limitation of the present work is that in the topic of dental implants, the participants were selected according to prespecified criteria. Hence patients with certain health conditions might have an increased risk of complications, not revealed in the studies. It is also worth noting that the present analyses could only include published events, unpublished events could not be taken into account. Hence AEs associated with interventions might be underestimated.

6. Conclusion

The present PhD work applied the methodology of meta-analysis in three extremely important, controversial and unsettled dental topics, dental implant surface modifications, application of hyaluronic acid dermal fillers in the perioral region and oral diagnostics for COVID-19 identification. Analyses were performed to obtain a high level of evidence and to outline possible directions for further research in these specified fields of dentistry. As a result, the following conclusions were drawn.

1. The data revealed that there is an important and significant difference between moderately rough (sand-blasted) and smooth (machined) implant surfaces in terms of implant failure rate after five years of follow-up. Sand-blasted implants performed significantly better. On the other hand, no significant difference was found in marginal bone level loss between the two implant surfaces.
2. From the findings it can be also concluded that there is a need for comprehensive protocols to perform standardized clinical trials in the field of oral implant dentistry. Consistent reporting on several clinical outcomes is needed. Such as pocket probing depth, bleeding on probing and implant success.
3, Additionally, we found evidence that hyaluronic acid dermal filler injections are greatly efficient for at least up to six months. The present work also provided evidence that the lip volume was still significantly increased after a year in almost half of the participants.
4. Moreover, our analysis revealed that most of the AE related to HA injection were moderate or mild, however, due to the lack of a longer follow-up period possible delayed reaction could not be revealed. Longer follow-up periods are required to establish the long-term effect of HA dermal filler injection.
5. Furthermore the present work provided evidence that saliva-based tests are promising candidates to replace nasopharyngeal swab tests for diagnosing COVID-19.
6. Enhanced and standardized saliva assays may offer a safe collection and reliable diagnosis of SARS-CoV-2 virus in the future. However further validation and standardization

are still needed for saliva-based tests before they become the standards of clinical practice.

Overall, in the three studies high level of scientific evidence was provided by systematically finding and selecting all eligible articles, and then synthetizing them by meta-analyses, also applying an extremely rigorous quality control.

7. Summary

The present work utilized the methodology of meta-analysis to overcome the limitations of individual studies on three topics related to the oral cavity. As conducting a meta-analysis the data of individual studies are synthesized to produce evidence with higher certainty and precision, increase statistical power to confirm the significance of small differences with higher confidence, to identify trends and confounding factors not addressed by individual studies. Furthermore, to assess bias in findings available in the literature. Overall, to increase the strength of conclusions drawn from the findings to provide meaningful information for clinicians, researchers and decision-makers. The topics of research are I) the efficiency of osteointegration of two implant types with different surface treatments, II) the efficiency and adverse effects related to hyaluronic acid injectable fillers, III) estimating the diagnostic sensitivity of saliva-based detection of SARS-CoV-2 virus.

In all the cases, protocols were written prior to conducting the research. Systemic search was conducted using preconstructed search queries. Search was performed in three independent databases Cochrane CENTRAL, Embase and MEDLINE (via PubMed). Data synthesis and statistical estimations were performed according to the nature of the data sets.

The present work found that I) there is a 74% (RR = 0.26 95% CI:0.09–0.74) lower risk of implant failure in the group of sand-blasted implants compared to machined after five years of use. II) In the case of hyaluronic acid injection we found that the rate of responders (the percentage – in relation to baseline values of those who experienced a maintained lip fullness after 6 months) was 74% (ES=0.74, 95% CI:0.66─0.82). III) In the case of covid diagnosis we found 91% (CI 80-99%) sensitivity for index tests (saliva) and 98% (CI 89-100%) sensitivity for reference standard (nasopharyngeal swab).

The present study reveals that sand-blasted implants are superior over machined implants regarding implant failure rates, but not marginal bone level changes. Additionally, HA fillers maintain their effect in a six-month period. Moreover, results indicate that nasopharyngeal tests do not perform significantly better than saliva tests. Furthermore, meta-analyses performed on the three topics come with limitations, thus results should be evaluated in context and measures should be taken to improve the quality of future clinical trials.

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9. Bibliography of the candidate's publications

List of Candidate's publications connected to the dissertation

Czumbel, László Márk; Kerémi, Beáta; Gede, Noémi; Mikó, Alexandra; Tóth, Barbara; Csupor, Dezső; Szabó, Andrea; Farkasdi, Sándor; Gerber, Gábor ; Balaskó, Márta et al. *Sandblasting reduces dental implant failure rate but not marginal bone level loss: A systematic review and meta-analysis* PLOS ONE 14 : 5 Paper: e0216428, 19 p. (2019)

Citations: 12;

Quartile: 2022 – Q2;

IF: 2019 – 2,740; 2020 – 3,240; 2021 – 3,752; 2022 – 3,700 (Source: Clarivate, Journal Citation Reports)

Czumbel, Laszlo Mark; Kiss, Szabolcs; Farkas, Nelli; Mandel, Ivan; Hegyi, Anita; Nagy, Akos; Lohinai, Zsolt; Szakacs, Zsolt; Hegyi, Peter; Steward, Martin C. et al. *Saliva as a Candidate for COVID-19 Diagnostic Testing: A Meta-Analysis* FRONTIERS IN MEDICINE 7 Paper: 465 , 10 p. (2020)

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Quartile: 2022 – Q2;

IF: 2020 – 5,903; 2021 – 5,058; 2022 – 3,900 (Source: Clarivate, Journal Citation Reports)

Czumbel, László Márk; Farkasdi, Sándor; Gede, Noémi; Mikó, Alexandra; Csupor, Dezső ; Lukács, Anita; Gaál, Valéria; Kiss, Szabolcs; Hegyi, Péter; Varga, Gábor *Hyaluronic Acid Is an Effective Dermal Filler for Lip Augmentation : A Meta-Analysis* FRONTIERS IN SURGERY 8 Paper: 681028 , 16 p. (2021)

Citations: 12;

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List of Candidate's publications not related to the dissertation

Czumbel, LM ; Farkasdi, S ; Pató, L ; Varga, G *Mintavételező szerkezet trepán fúróhoz* 5240 , NSZO: A61B 17/16 , Ügyszám: U2000032/21 Magyar szabadalom (Oltalmi formák)
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Czumbel, LM ; Farkasdi, S ; Pató, L ; Varga, G *Szerkezet trepán fúró fúrási mélységének pontos beállítására* 5239 , NSZO: A61B 17/16 , Ügyszám: U2000031/22 Magyar szabadalom (Oltalmi formák)
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Csupor, Dezső ; Viczián, Réka ; Lantos, Tamás ; Kiss, Tivadar ; Hegyi, Péter ; Tenk, Judit ; Czumbel, László Márk ; Thanyaporn, Sang-Ngoen ; Gyöngyi, Zoltán ; Varga, Gábor et al. *The combination of hawthorn extract and camphor significantly increases blood pressure: A meta-analysis and systematic review* PHYTOMEDICINE: INTERNATIONAL JOURNAL OF PHYTOTHERAPY AND PHYTOPHARMACOLOGY 63 Paper: 152984 , 9 p. (2019)
Citations: 7.
IF: 2019 – 4,268; 2020 – 5,340; 2021 – 6,656; 2022 – 7,900

Ruksakiet, Kasidid ; Hanák, Lilla ; Farkas, Nelli ; Hegyi, Péter ; Sadaeng, Wuttapon; Czumbel, László Márk ; Sang-Ngoen, Thanyaporn ; Garami, András ; Mikó, Alexandra ; Varga, Gábor et al. *Antimicrobial efficacy of chlorhexidine and sodium hypochlorite in root canal disinfection: A systematic review and meta-analysis of randomized controlled trials*JO URNAL OF ENDODONTICS 46 : 8 pp. 1032-1041.e7. , 10 p. (2020)
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Candidate's publications related to the dissertation

PLOS ONE

RESEARCH ARTICLE

Sandblasting reduces dental implant failure rate but not marginal bone level loss: A systematic review and meta-analysis

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Abstract

Introduction

Sandblasting is one of the oldest implant surface modifications to enhance osseointegration. Regarding its superiority over machined surface controversies still exist. Our objective was to compare implant failures (IF) and marginal bone level (MBL) changes between sand-blasted and machined dental implants by a meta-analysis utilizing the available data. The PROSPERO registration number of the meta-analysis is CRD42018084190.

Methods

The systematic search was performed in Cochrane, Embase and Pubmed. Inclusion criteria included participants with neither systemic diseases, nor excessive alcohol consumption, nor heavy smoking. We calculated pooled Risk Ratio (RRs) with confidence intervals of 95% (CIs) for dichotomous outcomes (implant failure) and weighted mean difference (WMD) CIs of 95% for continuous outcomes (marginal bone level change). We applied the random effect model with DerSimonian-Laird estimation. I^2 and chi^2 tests were used to quantify statistical heterogeneity and gain probability-values, respectively.

Results

Literature search revealed 130 records without duplicates. Out of these, seven studies met the inclusion criteria and all were included in data synthesis, involving 362 sand-blasted and 360 machined implants. The results indicate that there is an 80% (RR = 0.2 95% CI:0.06–0.67; I^2 = 0.0% p = 0.986) lower among sandblasted compared to machined implants after one year of use and 74% (RR = 0.26 95% CI:0.09–0.74; I^2 = 0.0% p = 0.968) five years of use, respectively. In contrast, there is no significant difference in MBL (WMD:-0.10mm, 95%

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CI:-0.20, 0.01; p>0.05; I^2 = 0.0%, p = 0.560 and WMD:-0.01mm, 95% CI:-0.12, 0.09; p>0.05; I^2 = 26.2%, p = 0.258) between the two implant surfaces after one and five years of use.

Conclusions

This meta-analysis reveals that sandblasting is superior over machined surface in implant failure but not in marginal bone level in healthy subjects. It also points out the need for further randomized clinical trials with large sample size for objective determination of the clinical benefits of certain implant surface modifications.

Introduction

Since machined titanium dental implants were first used [1], enormous effort has been put into research to enhance osseointegration and increase the life span of implants. Many parameters have been identified that influence the period of healing time and bone stability [2–4].

It has been suggested that surface roughness is one of the several key factors influencing the degree of biological integration and success rates of inserted implants [5–7]. As a result of extensive investigation, several surface modifications have emerged. These include sandblasting, acid-etching, anodization, plasma-spraying, coating with different bioactive surfaces and the combination of these [5, 8]. Generally, implant surface roughness is modified by these processes. For roughness classification, four categories exist: smooth ($S_a < 0.5$ μm), minimally rough (S_a = 0.5–1 μm), moderately rough (1 μm < S_a < 2 μm) and rough implant surfaces (S_a > 2 μm) [9].

Sandblasting was one of the first modifications invented, resulting in moderately rough or rough surfaces, and it is still used by many implant manufacturers [4, 7]. During the blasting process, ceramic particles such as titanium oxide, aluminum oxide or silica [10] are blustered onto the implant surface at high velocity [11]. The size of sand particles and their speed when they reach the implant surface are the key parameters influencing surface roughness [8, 12]. The size of the particles usually varies between 25–250 μm [8, 13]. As a result, the surface becomes irregular with depressions and pits, and roughness (S_a) is between 1.2–2.2 μm [9, 14]. In contrast, machined surfaces are smoother, having only shallow grooves on the surface [8]. The roughness of a machined surface is usually between 0.5–1 μm [9].

Several *in vitro* studies have demonstrated the positive effects of sandblasted surfaces on osseointegration [7, 15, 16]. However, some preclinical and clinical investigations and reviews indicated that moderately rough surfaces may not perform better. These studies suggest that a rougher surface may modify the properties of biofilm formation and, therefore, bacteria could attach to the surface more easily [9, 16, 17]. Hence, the marginal bone around rough implants may be less stable [18] and more vulnerable to peri-implantitis [19, 20].

Although the attention and utilization shifted from machined to sandblasted surface, the scientific reason behind is not well-founded. In other words, for clinical practice, no clear and strong evidence exists to support the use of sandblasted implants over machined ones.

The RCTs investigating the effect of sandblasted implants applied relatively small sample sizes providing weak evidence. Conducting meta-analysis could overcome the weaknesses of the individual RCTs by increasing sample size and the validity of the statistical analysis Several review papers have been published on this topic[9, 20–22], which are, however, either not based on meta-analyses (because the authors, due to the great heterogeneity of the included

studies, did not perform any) or even if they are, the meta-analyses performed combine all kinds of moderately rough surfaces. As an outstanding example, the most recent systematic review pooled together extremely heterogeneous studies, in which there were great differences in the study design. Thus, in addition to the results of RCTs, also those of uncontrolled trials and retrospective studies were combined in a single statistical analysis [20], thereby representing a very high level of bias. To our knowledge, no meta-analysis was performed involving exclusively RCTs, comparing the effect exerted on osseointegration by sandblasted implants with that exerted on it by machined implants. We assumed that identifying all relevant publications and conducting a meta-analysis might overcome the weaknesses of small sample size and increase the value of evidence in the topic.

The objective of the present meta-analysis and systematic review was to test the hypothesis that there are significant differences in implant failure rates and marginal bone level changes between sand-blasted and machined dental implants.

Materials and methods

Protocol and registration

This meta-analysis follows the PRISMA guideline [23]. The PRISMA checklist summarizing the content of this review is available in the supporting information (S1 Appendix).

The meta-analysis has been registered in Prospero (International Prospective Register of Systematic Reviews) database, 07/02/2018, registration number: CRD42018084190 (S2 Appendix).

Eligibility criteria

The PICO (patient characteristics, type of intervention, control and outcome) format was applied to the following clinical question: are there significant differences concerning implant failure rates and marginal bone level loss between machined and sandblasted dental implants among healthy patients?

For analysis, we considered records published in scientific journals compiling with our selected PICO. Patient characteristics: edentulous or partially edentulous participants who do not have any systemic diseases that would affect the osseointegration of implants. Type of intervention: treating tooth loss with endosteal dental implants, having undergone sandblasting surface modification. Control: treating tooth loss with endosteal dental implants, with machined surface (no surface modification). Outcome: the number of implants survived at each check-up, and changes in marginal bone level around the implants, which are measured using radiographic images.

Inclusion and exclusion criteria. Publications meeting the following eligibility criteria were included: 1) randomized controlled trials; 2) intervention: sandblasted implants; 3) control group: machined implants; 4) healthy participants; 5) similar implant designs. Records written in English or available in English translations. Exclusion criteria: 1) any publication type other than randomized controlled trials; 2) application of growth factors; 3) bone augmentation; 4) surface modification only on the implant neck; 5) participants with systemic or local conditions affecting osseointegration; 6) gray or black literature.

Information sources

A systematic search in English language limited to randomized controlled clinical trials was performed in three different major electronic databases (Cochrane Central Library, Embase and PubMed) with records published up to 20 August 2018. Besides electronic databases, an

extensive hand search in the reference list of relevant articles and included records were also performed to find eligible records.

Search

The following research string, was used in the Cochrane database: *"('machined':ti,ab,kw or 'turned':ti,ab,kw or 'blasted':ti,ab,kw or 'sandblasted':ti,ab,kw or 'sand-blasted':ti,ab,kw) and ('dental':ti,ab,kw or 'dentistry':ti,ab,kw) and 'implant':ti,ab,kw*" with Cochrane Library publication date to Aug 2018, in Trials.

The following search string was used for finding records in Embase: "*('machined':ti,ab,kw OR 'turned':ti,ab,kw OR 'blasted':ti,ab,kw OR 'sandblasted':ti,ab,kw OR 'sand-blasted':ti,ab,kw) AND ('dental':ti,ab,kw OR 'dentistry':ti,ab,kw) AND 'implant':ti,ab,kw AND 'controlled clinical trial'/de AND [english]/lim"*.

The following string was used to search on PubMed: *„(machined[Title/Abstract] OR turned [Title/Abstract] OR blasted[Title/Abstract] OR sandblasted[Title/Abstract] OR sand-blasted [Title/Abstract] OR sand blasted[Title/Abstract]) AND (dental[Title/Abstract] OR dentistry [Title/Abstract]) AND implant[Title/Abstract] AND (Clinical Trial[ptyp] AND ("0001/01/01"[PDAT]: "2018/08/20"[PDAT]) AND English[lang])"*

Besides electronic databases, the reference lists of relevant articles were also searched.

Study selection

EndNote reference manger was used to organize and manage records. After removing duplicates, the remaining records were screened for suitability by two authors (L.M.Cz. and B.K.) based on the titles and abstracts of the published original papers. The eligibility of full texts of the remaining records was assessed by two reviewers independently (L.M.Cz. and B.K.). Disagreement between reviewers was resolved by discussion or, if it was necessary, by consulting with a third reviewer (G.V.).

Data collection process and data items

Data extraction was performed by two authors independently (L.M.C. and K.B.) using a preconstructed standardized data extraction form. The following information was extracted: first author's name, year of publication, sample size, population type (type of edentulism), average age of participants, gender distribution, design of the studies, implant system for intervention and control, outcome (implant failure rate, marginal bone loss), conclusion of each study. In case of disagreement, a third author (G.V.) was also involved.

Risk of bias assessment

Quality and bias of the studies were evaluated according to the Cochrane Handbook [24], which is a broadly used guideline to assess randomized controlled trials. Studies were evaluated according to 8 domains. 1) Random sequence generation evaluates the strength of the method used for randomization. 2) Allocation concealment appraises the potential bias during allocation of the participants. 3) Blinding of participants and personnel assesses whether the patients and investigators were appropriately blinded to the treatment type. 4) Blinding of outcome assessment, radiographic outcome evaluates whether the personnel assessing x-ray images have been blinded. 5) Blinding of outcome assessment, clinical outcome appraises whether the clinical investigators evaluating the clinical outcome have been blinded. 6) Incomplete outcome data evaluate the risk of attrition bias due to withdrawals, loss of participants during follow ups and other missing data. 7) Selective reporting assesses whether all pre-

determined outcomes have been measured and reported. 8) Other bias evaluates any other type of bias not falling into the previous 7 domains [24].

Summary measures and synthesis of results

Pooled Risk Ratio (RRs) with 95% confidence intervals (CIs) and weighted mean difference (WMD) with 95% CIs were calculated for dichotomous outcomes (IF) and for continuous outcomes (MBL change expressed in mm) respectively. Negative values in MBL change indicate a decrease in marginal bone level. Negative values of weighted mean differences indicate a greater decrease in MBL in sand-blasted implants compared to machined ones. Criteria for implant failure were defined according to Albrektsson et al. [25] Implant number was chosen as statistical unit. We only considered results credible if raw data for meta-analysis could be drawn from at least three records. We applied the random effect model with DerSimonian-Laird estimation. I^2 and chi-square tests were used to quantify statistical heterogeneity and gain probability-values, respectively; $p<0.1$ indicated a significant heterogeneity. [24] All statistical analyses were performed using STATA 15.0.

Risk of bias across studies and additional analyses

Sensitivity analysis was performed by omitting studies (one by one) from the analyses and recalculating them in order to investigate the impact of the individual studies on the summary estimate. To check for publication bias, a visual inspection of funnel plots was performed.

Results

Study selection

During the study selection process, a total of 188 records were identified, including one record found in the reference list of related articles. After removing duplicates, 130 items remained. During the screening process, 114 records were excluded due to reasons such as other surface modification (n = 38) or different objectives (n = 76), investigating populations with systemic disease, evaluating surgical protocols, or comparing different macro designs of implants. For full-text evaluation 16 records were searched. Out of these publications nine records were excluded. The reasons for exclusion are explained below. Seven studies were eligible for qualitative and quantitative analysis [19, 26–31] (Fig 1).

Study characteristics

Description of excluded studies. Out of the nine excluded records, three records were not eligible because of evaluating other surface modifications than the ones investigated in this meta-analysis [32–34]. Two studies reported on previous results of ongoing studies that have been republished in updated records [35, 36]. Two other records investigated different populations (periodontitis-susceptible) [37, 38]. One record was not RCT [39] and one other paper did not describe the surface modification used [40].

Description of the included studies. All involved studies were randomized controlled trials. A total of 722 implants (362 sandblasted and 360 machined) were included in the data synthesis. The populations represented in these studies were uniform, patients with alcohol and drug consumption or other medication abuse were excluded. Exclusion criteria also included bruxism, uncontrolled diabetes mellitus or any other significant medical condition that would affect the process of osseointegration. The mean age of participants in the studies varied between 50 and 58 years. In the five studies four different implant systems (Astra Tech, Brånemark, Steri-Oss and Southern Implants) were used. All implants in the control group had

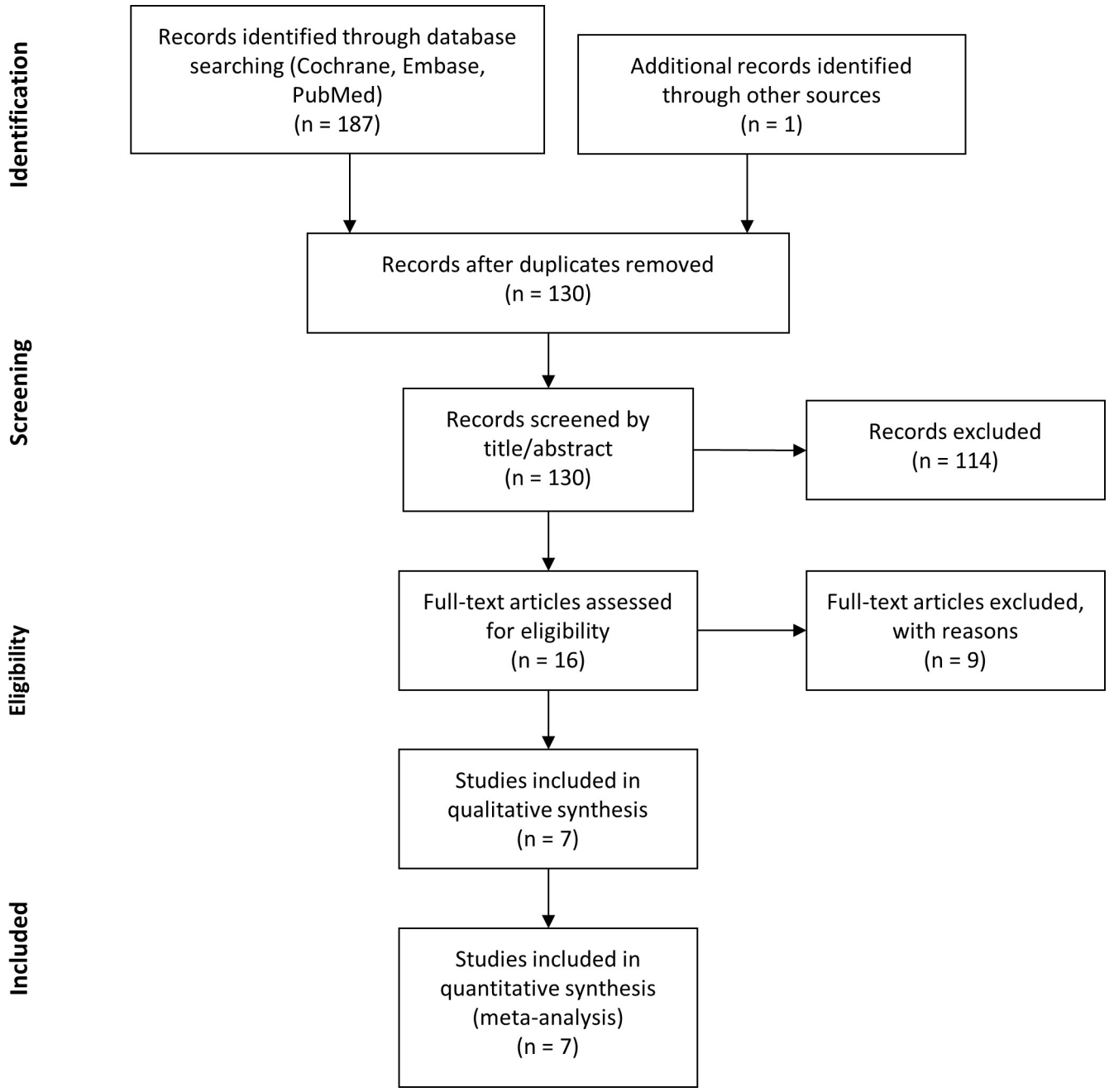

Fig 1. PRISMA flow diagram of study selection process.

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minimally rough surface and all implants in the intervention group had moderately rough surface [9].

All study groups except one [26] followed the two-stage protocol [41]. However, even in the study using one-stage protocol, implants were only loaded 3 months following healing at the lower jaw, and 6 months of healing at the upper jaw. Out of the five studies two [19, 26] treated edentulism with overdentures, another two [27, 30] used fixed partial bridges. One study [42] achieved rehabilitation with full arch bridges. The shortest follow-up was 2 years long, and the

Table 1. Summary of study characteristics.

Author	Åstrand et al. (2004)andRavald et al. (2013)*	Gotfredsen et al. (2001)	Steenberghe et al. (2000) and Jacobs et al. (2010)*	Tawse-Smith et al. (2002)	Vroom et al. (2009)
Study type	block randomization separate for upper and lower jaw, with equal probability of receiving either implant type	alternating implant placement	split-mouth design	random allocation to either implant system on a one-by-one basis	alternating implant placement
Country	Sweden	4 Scandinavian countries	Belgium	New Zealand	not stated
Age	$\bar{x}$ = 61.5	$\bar{x}$ = 53	$\bar{x}$ = 59.7	55–80	$\bar{x}$ = 53
Number of participants	males: 28, females: 38	males: 25, females: 25	males: 6, females: 12	total: 48	males: 7, females: 13
Extent of teeth loss	edentulous	partially edentulous	partially edentulous	edentulous (mandible only)	edentulous (mandible only)
Sand-blasted implant (intervention)	Astra Tech implants	Astra Tech implants	Astra Tech implants	Southern Implants	Astra Tech implants
Machined implants (control)	Branemark System MK II	Astra Tech implants	Branemark System MK II	Sterioss	Astra Tech implants
Surgical protocol	two-stage technique (3 months and 6 months healing in the lower and upper jaw respectively before abutment placement	two-stage technique (3–4 months and 6–7 months healing in the lower and upper jaw respectively before abutment placement	two-stage technique (3–4 months and 6–7 months healing in the lower and upper jaw respectively before abutment placement	one-stage technique (3 months of healing before loading)	two-stage technique (3–4 months healing before abutment placement

*The publications of Ravald et al (2013) and Jacobs et al (2010) are the continuations of the studies published by Åstrand et al (2004); and Steenberghe et al (2000) respectively.

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longest lasted for 16 years [29]. Each study provided information on implant failure rate and marginal bone level change calculated from blinded radiographic measurements. In one study [26], only two out of four groups were included, two groups using immediate loading protocol were not included in the analysis. Additionally, MBL measurements of Astrand et al. [31] and Ravald et al. [28]were excluded since the reported patient-based data could not be converted to implant-based data to match the statistics of other studies. A detailed description of these studies is shown in Tables 1 and 2.

Table 2. Summary of study characteristics.

Author	Åstrand et al. (2004) and Ravald et al. (2013)*	Gotfredsen et al. (2001)	Steenberghe et al. (2000) and Jacobs et al. (2010)*	Tawse-Smith et al. (2002)	Vroom et al. (2009)
Type of prosthesis	full-arch fixed bridges	screw retained fixed partial prosthesis	screw retained fixed partial prosthesis	implant supported overdenture	implant supported overdenture
Outcome	IF, MBL change, BOP, plaque accumulation, pain, suprastructure complications	IF, MBL change, BOP, paraesthesia, periimplant inflammation, pain, suprastructure complications	IF, MBL change, sulcus bleeding index, PPD presence of plaque	IF, MBL change, sulcus bleeding index, PPD, implant stability measurement (Periotest), modified plaque index	IF, MBL change, bleeding index. PPD, presence of calculus
Follow-up time	5 and 12*years	5 years	2 and 15*years	2 years	12 years

*The publications of Ravald et al (2013) and Jacobs et al (2010) are the continuations of the studies published by Åstrand et al (2004); and Steenberghe et al (2000) respectively.

IF: implant failure

MBL: marginal bone level

BOP: bleeding on probing

PPD: probing pocket depth

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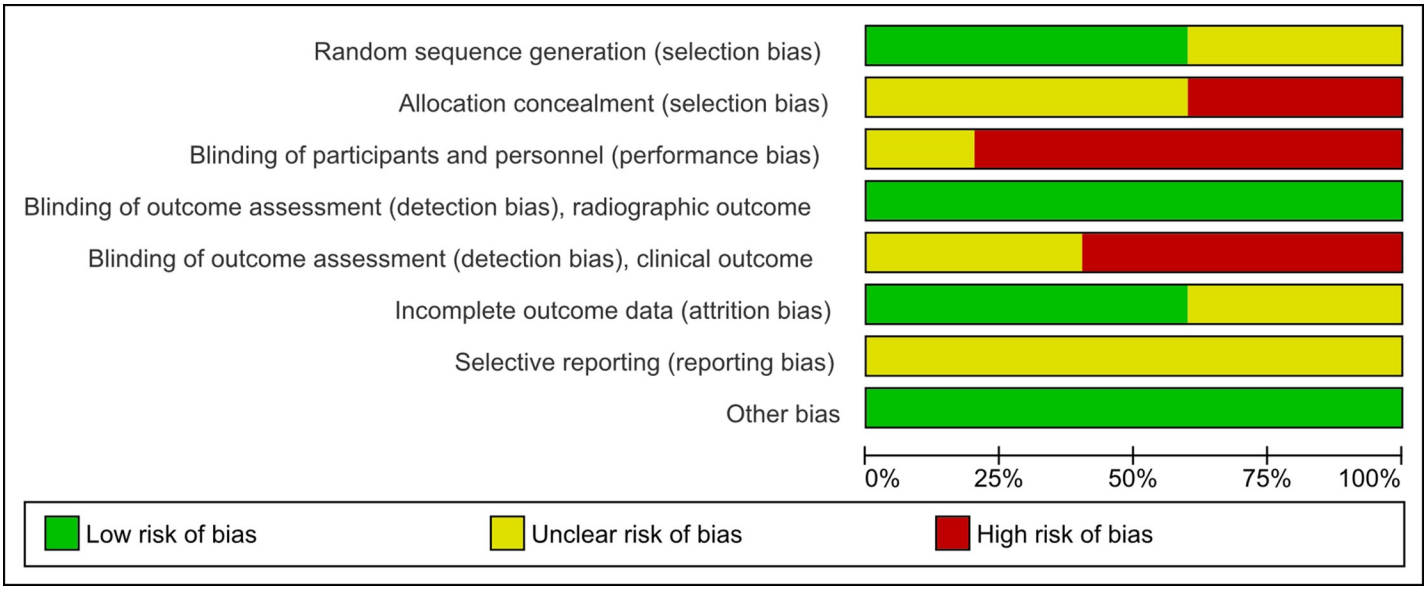

Fig 2. Risk of bias graph. Percentage of each risk of bias item across included studies.

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Risk of bias within studies

Bias in the studies was assessed according to the Cochrane Risk of Bias Tool. All seven included studies were included in the risk of bias assessment, however two pairs of studies [28, 31] and [27, 29] were evaluated together because the study of Ravald et al [28] and Jacobs et al. [29] are the continuation of previous studies of Åstrand and coworkers [31] and those of Steenberghe and coinvestigators [27], respectively.

Two studies [26, 27] had unclear random sequence generation, and other two [19, 30] had a high risk of allocation concealment, due to the predictable sequence generation process used. All studies performed blinding during the evaluation of x-ray images. However, due to its nature, no blinding could be carried out evaluating the implants clinically. Dropouts were identified in four studies [19, 26, 30, 31], two of these with unclear risk of bias.[19, 26]. Access was not gained to study protocols or trial registers, however, no intext evidence of selective reporting was found. Fig 2, S1 Table and S3 Appendix contain the summary of the risk of bias assessment.

Results of individual studies and synthesis of results

Sandblasted implants are better than machined implants concerning implant failure at 1, 2 and 5–6 years. Data for implant failure analysis after one year were pooled from five studies [19, 26, 27, 30, 31]. The results show that there is an 80% lower risk for sand-blasted implants to fail compared to machined implants after one year of use (RR = 0.20 95% CI: 0.06–0.67; I^2 = 0.0% p = 0.986) (Fig 3 and S1 File).

Data for cumulative implant failure after two years could be pooled from five studies [19, 26, 27, 30, 31]. The meta-analysis revealed that the risk of sand-blasted implant failure is 81% lower than that of machined implants (RR = 0.19 95% CI: 0.05–0.64; I^2 = 0.0% p = 0.977) (Fig 4 and S1 File).

Data for analyzing the effect of sandblasting on implant failure after five or six years' follow-up were pooled from four studies [19, 29–31]. The results indicate that there is a 74% lower risk of sandblasted implants to fail (RR = 0.26 95% CI: 0.09–0.74; I^2 = 0.0% p = 0.968) (Fig 5 and S1 File).

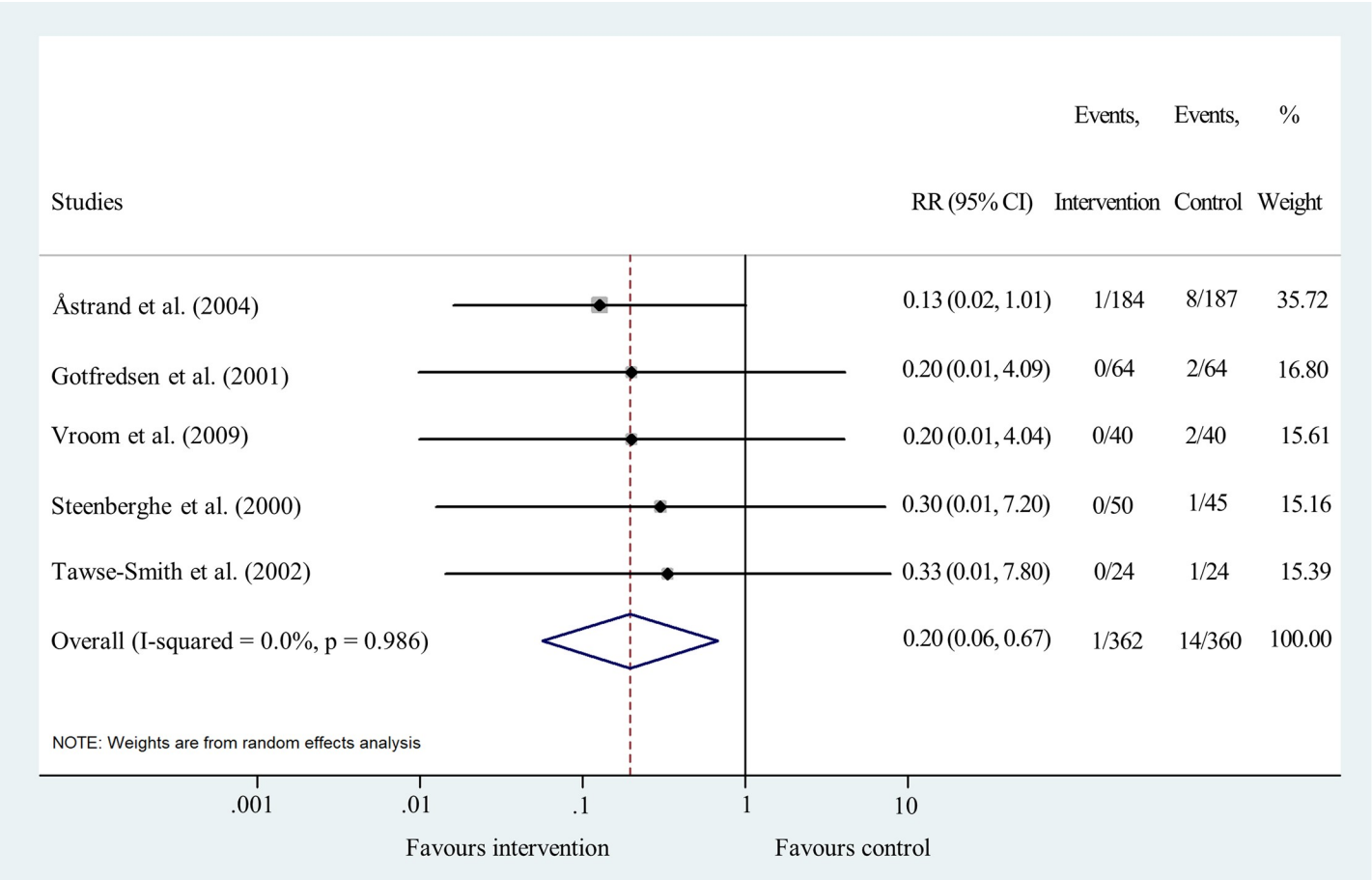

Fig 3. Forest plot analysis of implant failure rate after one year.

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Results for cumulative IF after 12–15 years were synthesized from 3 studies [19, 28, 29]. Results show that there is no significant difference between the two treatment types (RR = 0.68 95% CI: 0.29–1.57; I^2 = 0.0% p = 0.590) (Fig 6 and S1 File).

No detectable difference in MBL between sand-blasted and machined implants after 5 years of follow up

MBL change was analyzed one and five years after the delivery of the final prosthesis One-year data were pooled from three studies [19, 26, 27]. No significant difference was found between the two surface treatments, (weighted mean difference = -0.10, 95% CI: -0.20–0.01; p>0.05; I^2 = 0.0%, p = 0.560) (Fig 7 and S1 File). Data for 5-year analysis were pooled from three studies [19, 29, 30]. The statistical analysis clearly shows that the difference is not significant between the two implant surface types, the line of null effect falls within the range of the confidence interval (weighted mean difference = 0.00, 95% CI: -0.13–0.14; p>0.05; I^2 = 26.2%, p = 0.258) (Fig 8 and S1 File).

Risk of bias across studies and additional analysis

Funnel plot analyses indicated a moderate level of publication bias (S4 Appendix). Statistical heterogeneity was not important in the results of IF at all time points. I^2 values were 0.0% and

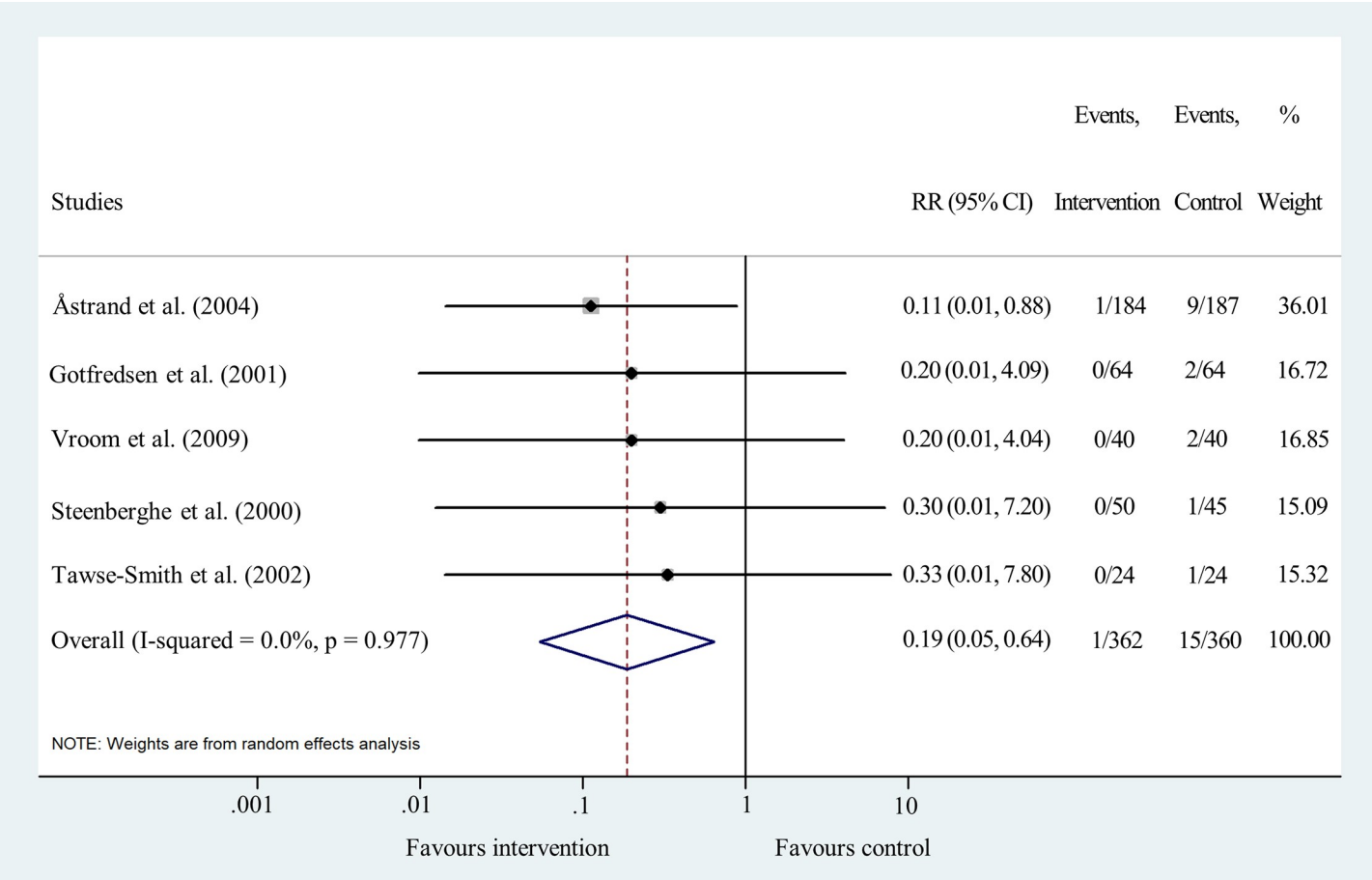

Fig 4. Forest plot analysis of cumulative implant failure rate after two years.

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p values varied between 0.590 and 0.986 (Figs 3, 4, 5 and 6). Heterogeneity was also negligible in the results of MBL change at 1 year (I^2 = 0.0%, p = 0.560) (Fig 7). I^2 (26.2%) and p (0.258) values indicated a slightly higher level of statistical heterogeneity for the results of MBL change at 5 years (Fig 8), however, this was still considered insignificant. [24].

Sensitivity analysis showed that the removal of the study of Astrand et al. [31] decreases the significance of the results of pooled risk ratio analysis at one, two and five/six years following implantation. This is most likely due to the large sample size of that study compared to the other RCTs.

Discussion

Summary of evidence

As a result of extensive investigations conducted in the past decades, several methods for implant surface modifications have emerged and numerous studies claimed superiority for one or other roughened surfaces. [43–45] However, no evidence supports a single decisive hypothesis. Therefore, the contradictory conclusions of the literature require further studies and careful re-analysis. As our objective stated, we re-evaluated the performance of sand-blasted implant surface over machined ones. To obtain the highest level of evidence, a meta-analysis was conducted including only RCTs available on the topic but excluding uncontrolled

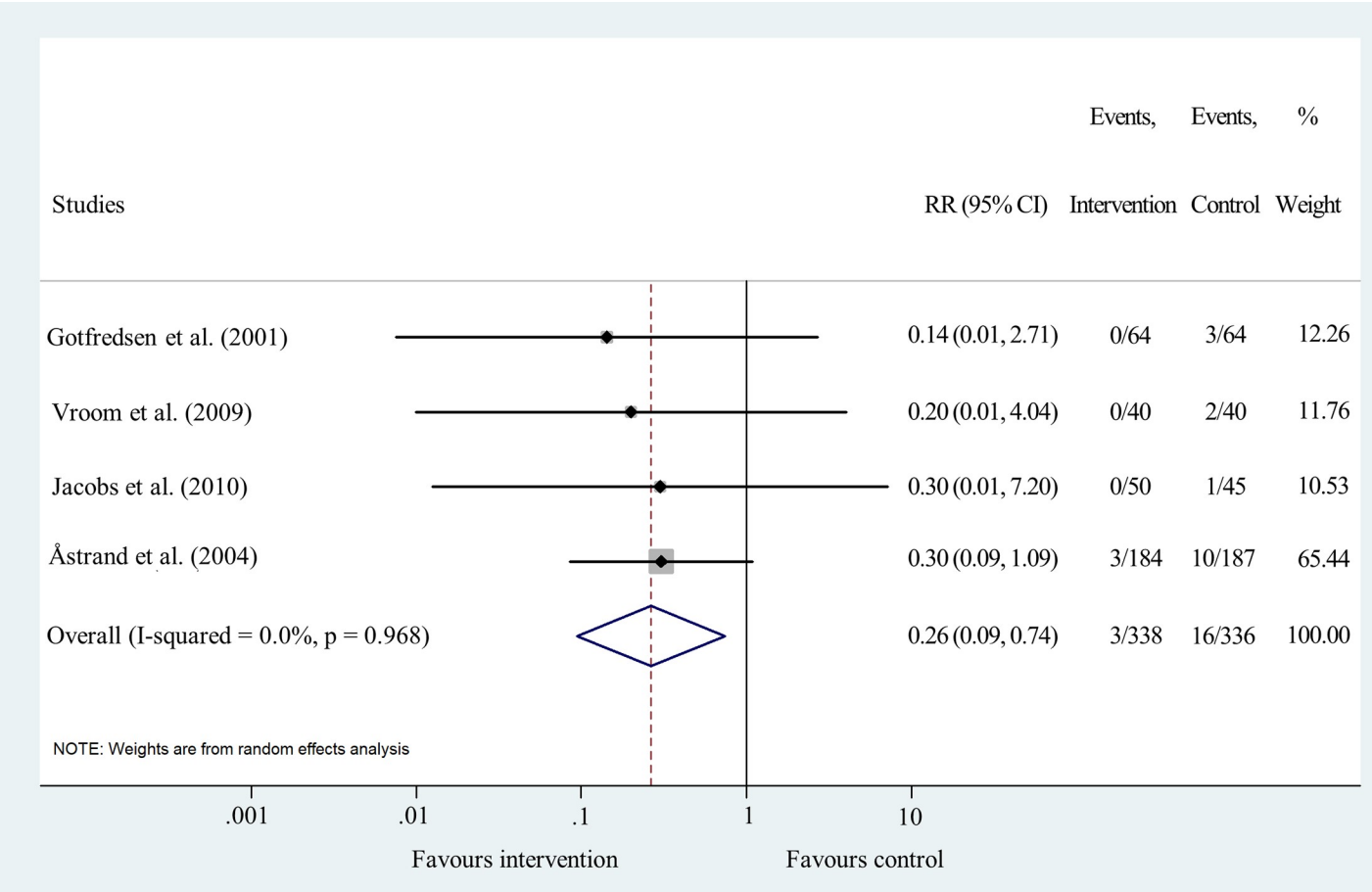

Fig 5. Forest plot analysis of cumulative implant failure rate after 5/6 years.

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trials and retrospective studies. Implant stability was evaluated by measuring MBL changes and comparing cumulative implant failure rates. After appropriate selection, seven RCTs, 202 patients, having 362 sand-blasted and 360 machined implants could be included in our complex approach.

Our meta-analysis revealed that implant failure rates were significantly different between machined implants and sand-blasted ones. In contrast, the results of the individual RCTs could not reveal a significant difference between the two types of surfaces. This gained difference reflects the increased number of samples and the high power of statistical methods of meta-analysis. Most implant failures happened during the first year after implantation. The reason for this could be that the surface modification of sandblasted implants creates a rougher surface which enhances the processes of bone formation on the implant itself. [46] Independent researchers published similar observations on other moderately rough surfaces, too. [47] Our results also show that after one year, when osseointegration has already taken place, the difference between the two surfaces diminish and the significance of the difference between implant failures disappear. Additionally, a histological study, using small sample size, also confirmed that, in the long term, both implant types maintained a decent level of osseointegration. It was found that after 5 years the bone-implant contact level was 92.7% for machined and 81.2% for sand-blasted implants. [48].

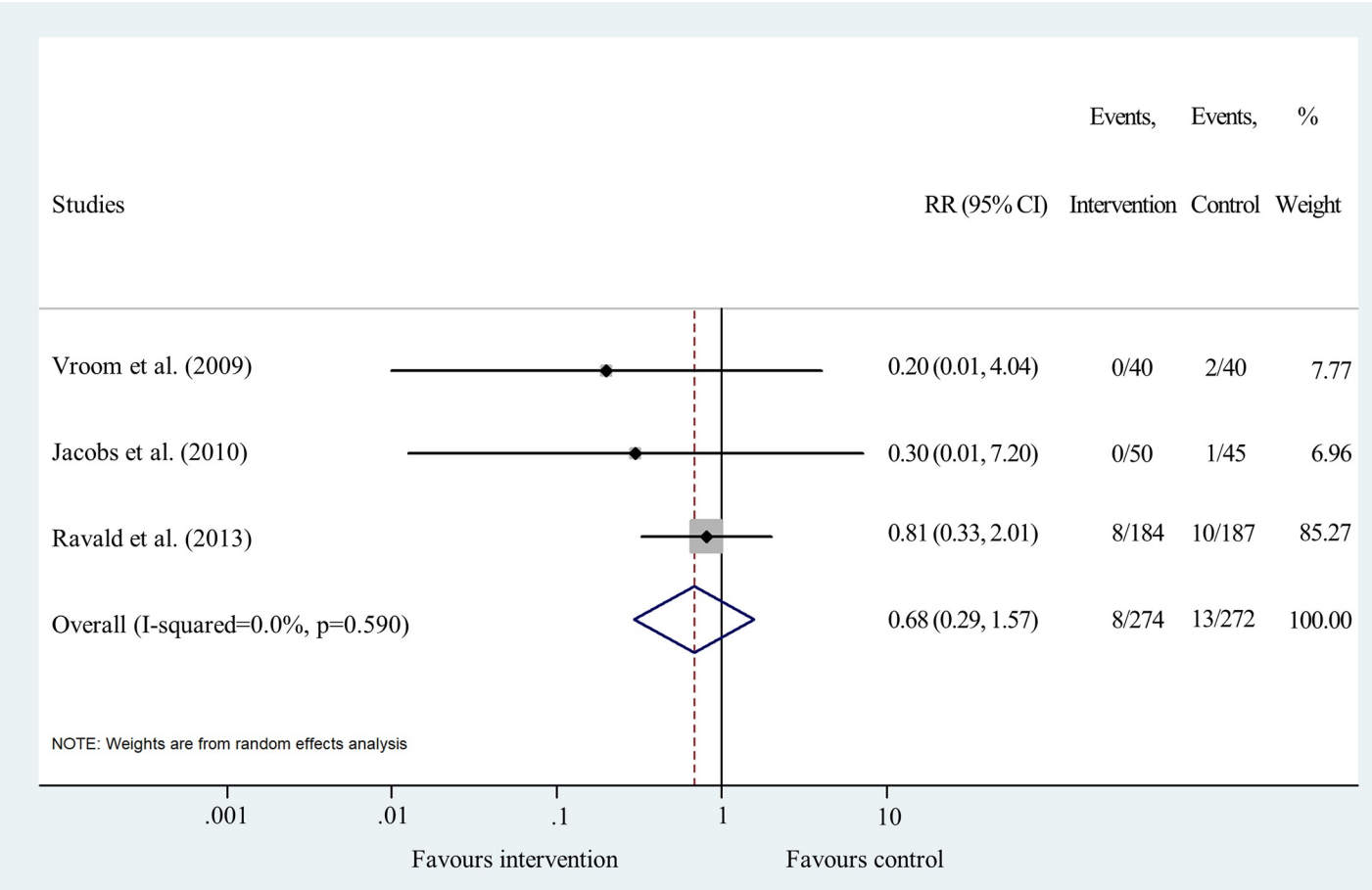

Fig 6. Forest plot analysis of cumulative implant failure rate after 12/15 years.

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In the case of MBL change, our meta-analysis demonstrated no significant difference between the two implant types. The results of the individual RCTs included in our meta-analysis did not reveal any significant difference either. In contrast, a recent meta-analysis and also a novel review comparing machined implants to surface-modified implants concluded that rough implants may cause more bone loss [9, 20]. The discrepancy between the meta-analysis reported by Doornewaard and coworkers [9] and our results could arise from the fact that we only examined sandblasted implants and excluded all other surface modifications in order to decrease heterogeneity. Additionally, they might have included patients with periodontitis, which was an exclusion factor in our case. Moreover, Wennerberg et al. [20] included not only RCTs, but also uncontrolled trials and retrospective studies in their analysis. Thus, they used a different statistical approach yielding high statistical heterogeneity, which might fundamentally influence the outcome [20]. Nevertheless, in all included studies, MBL measurements resulted in high standard deviations, which hinder any accurate statistical comparison. Therefore, the results have to be interpreted carefully.

However, some general trends are indicated by literature data. Åstrand and coworkers argued that the greatest loss in marginal bone occurred between implant placement and prosthesis connection [31]. This change was found to be greater in machined than in sand-blasted implants [31]. Unfortunately, no published data on MBL change between implant placement and prosthesis connection are available for meta-analysis. Furthermore, based on data shown

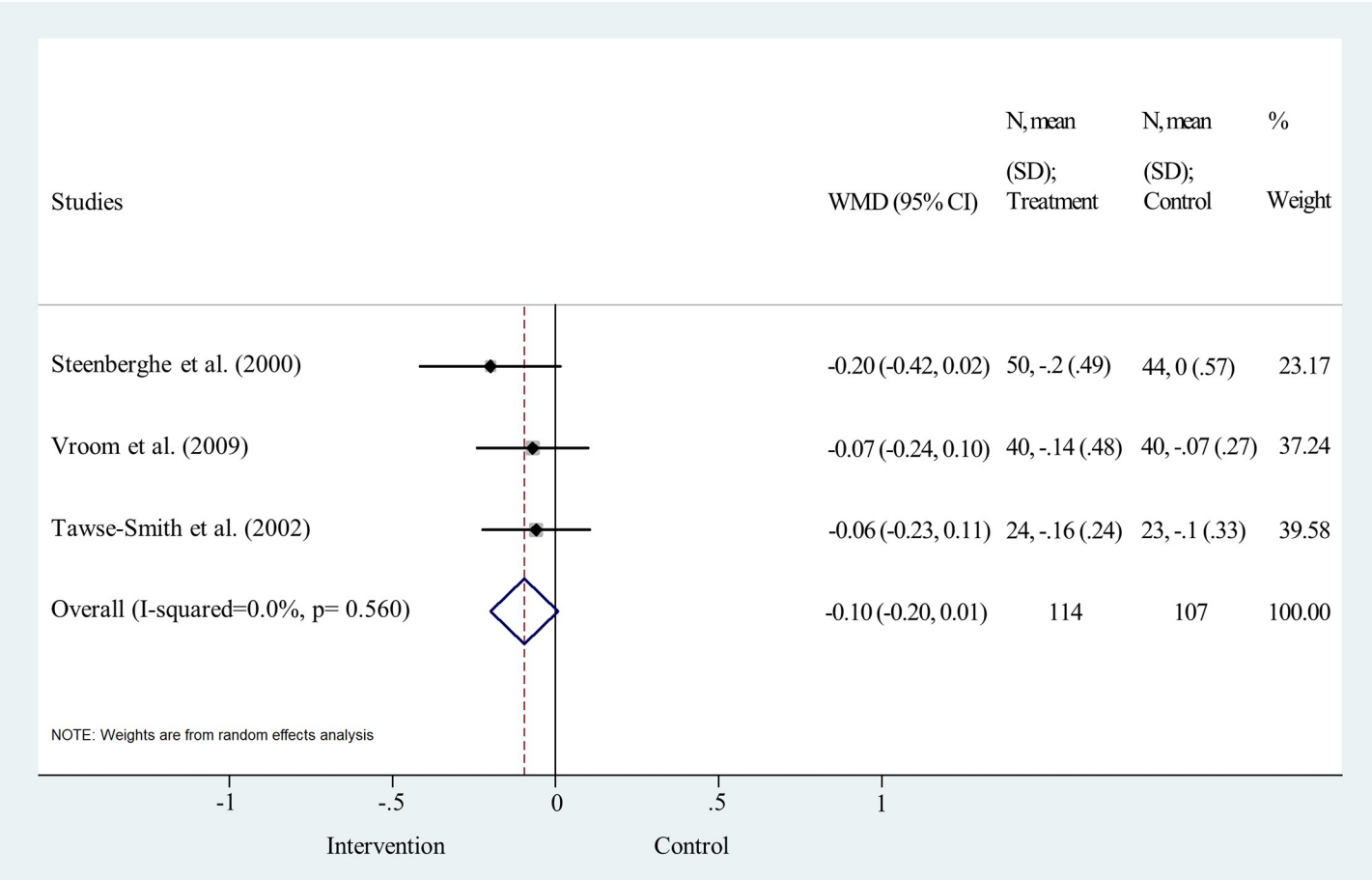

Fig 7. Forest plot analysis of marginal bone level change after one year.

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in Fig 7, the mean bone change within the RCTs was between 0 and -0.10 mm for machined, -0.14 and -0.2 mm for sand-blasted implants from baseline to the first year, which is, in fact, very small [26, 31]. According to Ravald and coinvestigators, the mean annual bone loss decreased gradually after five years. The annual mean bone attachment change was -0.02 and -0.04 mm for machined and sand-blasted implants, respectively, between five years and the end of the 12–15 years' follow-up period [28]. In addition, there is evidence that bone gain can also occur around implants. The RCT of Åstrand et al. reported more than 0.6 mm increase in MBL around 4 sandblasted and 2 machined implants over five years [31]. Vroom and coworkers also noted an increase in MBL around some implants with not much difference between the two surface types. The authors of this study argue that bone gain is a result of increased bone corticalization [19].

The different trends in MBL change and IF concerning the two implant types can be explained by the differences between the two measuring methods. MBL measurements can only detect bone changes when the implant is still stable at the annual checkup. If bone resorption takes place so quickly that all the bone is resorbed within a year, the implants will be labeled to have failed and excluded from the MBL measurements, hence they no longer influence MBL changes. Indeed, numerous studies show that the initial bone formation takes place at a faster rate around rough surface implants than around machined ones [7, 49, 50]. This may also explain why fewer sand-blasted implants failed compared to machined ones in the

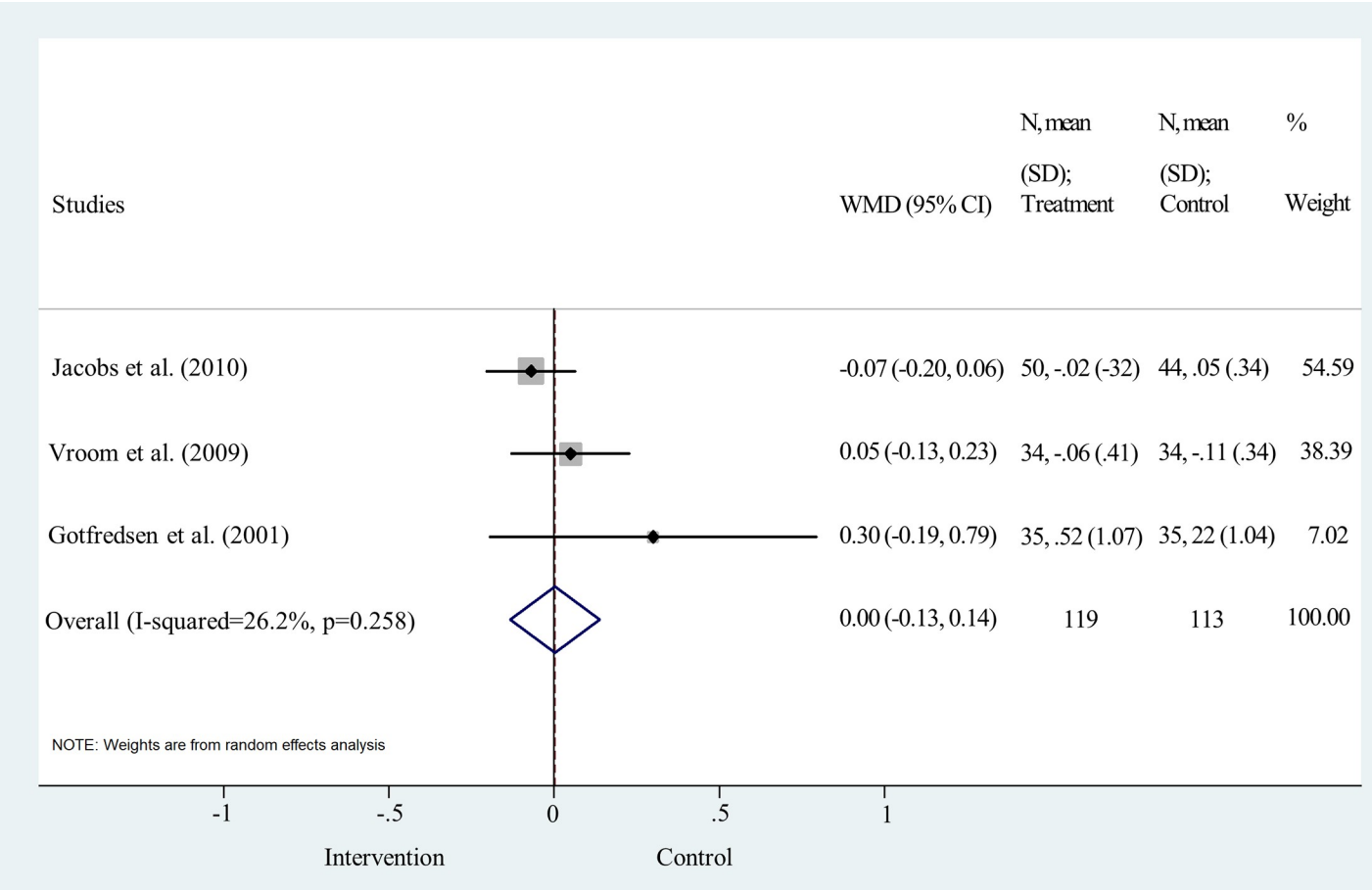

Fig 8. Forest plot analysis of marginal bone level change after 5 years.

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first year after implantation. In addition, fast healing remains a key attribute of rough implants since healing time is a key element in modern implantology and implants with faster healing are prioritized [51, 52].

The present study has a clear message for clinicians. We hypothesized that, concerning implant failure rates and marginal bone level loss, there are significant differences between sandblasted and machined dental implants. Our meta-analysis provided evidence that sandblasting, indeed, significantly lowers implant failure rates although does not significantly affect marginal bone level changes. Thus, we recommend the use of sandblasted, moderately rough implants for patients with no systemic diseases as such implants support the osseointegration process with fewer complications than machined implants.

Limitations

A major limitation of the present paper is the relatively small number of randomized controlled trials available regarding this topic. Despite the large number of records found by the systematic search, only seven could be included. The limited number of reported data makes it impossible to perform sub-group analyses and to thoroughly investigate the causes behind certain trends. Another issue that hinders in-depth analysis is the inhomogeneous reporting of outcome parameters. Some studies report the data separately for the lower and upper jaws, whereas others only report combined data. All studies reported one or two clinical parameters

such as bleeding on probing. However, the use of different reporting schemes made comparison impossible for bleeding on probing tests, among others. Another limitation of the present work is that its conclusions apply only to healthy populations. There are several confounding factors which might create unfavorable conditions for moderately rough implants, such as patients with severe periodontitis [53]. However, these conditions were excluded from our analysis. Uncontrolled or unknown confounding factors not evenly affecting intervention and control groups may also contribute to differences in the outcomes. Finally, limitations of this meta-analysis include the heterogeneity of the implants used. Although implants with identical macro designs would be preferred, this was not really possible.

In conclusion, within the limitations of this meta-analysis, the results reveal that sandblasting is superior over machined surface concerning implant failure. On the other hand, no significant difference was found regarding marginal bone level changes between the two implant types. Our in-depth analysis of the literature also highlights that results are highly sensitive to heterogeneity and study design, which may lead to contradictory conclusions. In the future, consistent reporting on more clinical outcomes such as bleeding on probing, pocket probing depth and implant success rates are needed. Evaluation could be more meaningful if implant success is evaluated by RCTs rather than case-based implant failure studies. Therefore, a comprehensive protocol should be compiled to guide clinicians conducting valuable RCTs evaluating implant performance in order to decrease heterogeneity of papers and to increase clinical applicability.

Supporting information

S1 Appendix. PRISMA checklist.
(PDF)

S2 Appendix. PROSPERO registration.
(PDF)

S3 Appendix. Risk of bias summary: Review authors' judgements about each risk of bias item for each included study.
(TIFF)

S4 Appendix. Publication bias (funnel plots).
(PDF)

S1 Table. Detailed evaluation of risk of bias of individual studies.
(PDF)

S1 File. Data included in meta-analysis.
(XLSX)

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S1 Appendix. PRISMA checklist.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7-8
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8-9
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8-9
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9-10
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9-10

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	10
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	10
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10-11
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	11-14
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	15
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	15-17
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	15-17
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	17
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	17
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	18-21
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	21
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	21-22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	22

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Systematic review

1. * Review title.

Give the working title of the review, for example the one used for obtaining funding. Ideally the title should state succinctly the interventions or exposures being reviewed and the associated health or social problems. Where appropriate, the title should use the PI(E)COS structure to contain information on the Participants, Intervention (or Exposure) and Comparison groups, the Outcomes to be measured and Study designs to be included.

Survival Rates and Marginal Bone Level Changes of Sand-Blasted versus Machined Dental Implants: Meta-Analysis

2. Original language title.

For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.

3. * Anticipated or actual start date.

Give the date when the systematic review commenced, or is expected to commence.

25/09/2017

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

14/01/2019

5. * Stage of review at time of this submission.

Indicate the stage of progress of the review by ticking the relevant Started and Completed boxes. Additional information may be added in the free text box provided.
Please note: Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. Should evidence of incorrect status and/or completion date being supplied at the time of submission come to light, the content of the PROSPERO record will be removed leaving only the title and named contact details and a statement that inaccuracies in the stage of the review date had been identified.
This field should be updated when any amendments are made to a published record and on completion and publication of the review.

The review has not yet started: No

Review stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	No
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Provide any other relevant information about the stage of the review here (e.g. Funded proposal, protocol not yet finalised).

6. * Named contact.

The named contact acts as the guarantor for the accuracy of the information presented in the register record.

László Márk Czumbel

Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence:

Dr Czumbel

7. * Named contact email.

Give the electronic mail address of the named contact.

czumbel.laszlo@dent.semmelweis-univ.hu

8. Named contact address

Give the full postal address for the named contact.

9. Named contact phone number.

Give the telephone number for the named contact, including international dialling code.

10. * Organisational affiliation of the review.

Full title of the organisational affiliations for this review and website address if available. This field may be completed as 'None' if the review is not affiliated to any organisation.

Department of Oral Biology, Semmelweis University, Budapest
Institute for Translational Medicine, Medical School, University of Pécs

Organisation web address:

11. Review team members and their organisational affiliations.

Give the title, first name, last name and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong.

12. * Funding sources/sponsors.

Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Include any unique identification numbers assigned to the review by the individuals or bodies listed.

EFOP GRANT NUMBER: EFOP-3.6.2.-16-2017-00006

13. * Conflicts of interest.

List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.

None

14. Collaborators.

Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members.

15. * Review question.

State the question(s) to be addressed by the review, clearly and precisely. Review questions may be specific or broad. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS where relevant.

Is there a significant difference in implant survival and marginal bone loss between sand-blasted and machined dental implants?

16. * Searches.

Give details of the sources to be searched, search dates (from and to), and any restrictions (e.g. language or publication period). The full search strategy is not required, but may be supplied as a link or attachment.

We will perform our search in three electronic databases: Cochrane Library, EMBASE and PubMed. In addition, reference list of eligible studies and review articles will be also searched.
The search query for EMBASE is: (('machined':ti,ab,kw OR 'turned':ti,ab,kw OR 'blasted':ti,ab,kw OR 'sandblasted':ti,ab,kw OR 'sand-blasted':ti,ab,kw) AND 'dental':ti,ab,kw OR 'dentistry':ti,ab,kw) AND 'implant':ti,ab,kw AND [randomized controlled trial]/lim. Similar search terms are used for the other two databases too. Eligibility for inclusion will be decided following the inclusion/exclusion criteria.

17. URL to search strategy.

Give a link to the search strategy or an example of a search strategy for a specific database if available (including the keywords that will be used in the search strategies).

Alternatively, upload your search strategy to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

Do not make this file publicly available until the review is complete

18. * Condition or domain being studied.

Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.

Treatment of teeth loss with dental implants.

19. * Participants/population.

Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.

Inclusion criteria: 1) randomized controlled trials; 2) control group: machined implants; 3)intervention: sand-blasted implants; 4) healthy participants, 5) similar implant design. Exclusion criteria: 1) using growth factors; 2) bone augmentation; 3) surface modification only on the implant neck; 4) participants with systemic condition affecting osseointegration.

20. * Intervention(s), exposure(s).

Give full and clear descriptions or definitions of the nature of the interventions or the exposures to be reviewed.

Treating teeth loss with endosteal dental implants, undergoing sand-blasting surface modification.

21. * Comparator(s)/control.

Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g. another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.

Treating teeth loss with endosteal dental implants, with no surface modification (machined surface).

22. * Types of study to be included.

Give details of the types of study (study designs) eligible for inclusion in the review. If there are no restrictions on the types of study design eligible for inclusion, or certain study types are excluded, this should be stated. The preferred format includes details of both inclusion and exclusion criteria.

Only randomized controlled trials will be included in the meta-analysis.

23. Context.

Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.

24. * Primary outcome(s).

Give the pre-specified primary (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.

Primary outcomes are the number of survived implants at check-ups, and changes in marginal bone level around the implants, which are measured using radiographic images.

Timing and effect measures

25. * Secondary outcome(s).

List the pre-specified secondary (additional) outcomes of the review, with a similar level of detail to that required for primary outcomes. Where there are no secondary outcomes please state ‘None’ or ‘Not applicable’ as appropriate to the review

None

Timing and effect measures

26. Data extraction (selection and coding).

Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.

27. * Risk of bias (quality) assessment.

State whether and how risk of bias will be assessed (including the number of researchers involved and how discrepancies will be resolved), how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.

Risk of bias will be assessed according to the Cochrane Handbook, Cochrane Risk of Bias Tool. Included records will be assessed by two review authors independently. Differences between the two reviews will be discussed until agreement is reached. If it is necessary a third review author will be involved. Records which show evidence of no randomization, will be excluded from data synthesis.

28. * Strategy for data synthesis.

Give the planned general approach to synthesis, e.g. whether aggregate or individual participant data will be used and whether a quantitative or narrative (descriptive) synthesis is planned. It is acceptable to state that a quantitative synthesis will be used if the included studies are sufficiently homogenous.

In our meta-analysis we will focus on analytical approaches besides descriptive synthesis of the findings. Where appropriate, for continuous data mean difference and for dichotomous data relative risk (RR) values (with 95% confidence interval) will be calculated. In addition, contribution weight of studies and statistical heterogeneity will be also calculated. For statistical significance p 0.05 will be used. Statistical calculations will be performed with STATA software (StataCorp LLC, USA)

29. * Analysis of subgroups or subsets.

Give details of any plans for the separate presentation, exploration or analysis of different types of participants (e.g. by age, disease status, ethnicity, socioeconomic status, presence or absence or co-morbidities); different types of intervention (e.g. drug dose, presence or absence of particular components of intervention); different settings (e.g. country, acute or primary care sector, professional or family care); or different types of study (e.g. randomised or non-randomised).

If possible, subgroup analysis will be performed to decrease potential heterogeneity.

30. * Type and method of review.

Select the type of review and the review method from the lists below. Select the health area(s) of interest for your review.

Type of review

Cost effectiveness
No

Diagnostic
No

Epidemiologic
No

Individual patient data (IPD) meta-analysis
No

Intervention
Yes

Meta-analysis
Yes

Methodology
No

Network meta-analysis
No

Pre-clinical
No

Prevention

No

Prognostic
No

Prospective meta-analysis (PMA)
No

Qualitative synthesis
Yes

Review of reviews
No

Service delivery
No

Systematic review
No

Other
No

Health area of the review

Alcohol/substance misuse/abuse
No

Blood and immune system
No

Cancer
No

Cardiovascular
No

Care of the elderly
No

Child health
No

Complementary therapies
No

Crime and justice
No

Dental
Yes

Digestive system
No

Ear, nose and throat
No

Education
No

Endocrine and metabolic disorders
No

Eye disorders
No

General interest
No

Genetics
No

Health inequalities/health equity

No

Infections and infestations
No

International development
No

Mental health and behavioural conditions
No

Musculoskeletal
No

Neurological
No

Nursing
No

Obstetrics and gynaecology
No

Oral health
Yes

Palliative care
No

Perioperative care
No

Physiotherapy
No

Pregnancy and childbirth
No

Public health (including social determinants of health)
No

Rehabilitation
No

Respiratory disorders
No

Service delivery
No

Skin disorders
No

Social care
No

Surgery
No

Tropical Medicine
No

Urological
No

Wounds, injuries and accidents
No

Violence and abuse
No

31. Language.

Select each language individually to add it to the list below, use the bin icon to remove any added in error.

English

There is not an English language summary

32. Country.

Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved.

Hungary

33. Other registration details.

Give the name of any organisation where the systematic review title or protocol is registered (such as with The Campbell Collaboration, or The Joanna Briggs Institute) together with any unique identification number assigned. (N.B. Registration details for Cochrane protocols will be automatically entered). If extracted data will be stored and made available through a repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.

34. Reference and/or URL for published protocol.

Give the citation and link for the published protocol, if there is one

Give the link to the published protocol.

Alternatively, upload your published protocol to CRD in pdf format. Please note that by doing so you are consenting to the file being made publicly accessible.

No I do not make this file publicly available until the review is complete

Please note that the information required in the PROSPERO registration form must be completed in full even if access to a protocol is given.

35. Dissemination plans.

Give brief details of plans for communicating essential messages from the review to the appropriate audiences.

Do you intend to publish the review on completion?

Yes

36. Keywords.

Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords will help users find the review in the Register (the words do not appear in the public record but are included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.

37. Details of any existing review of the same topic by the same authors.

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.

38. * Current review status.

Review status should be updated when the review is completed and when it is published.
Please provide anticipated publication date

Review_Ongoing

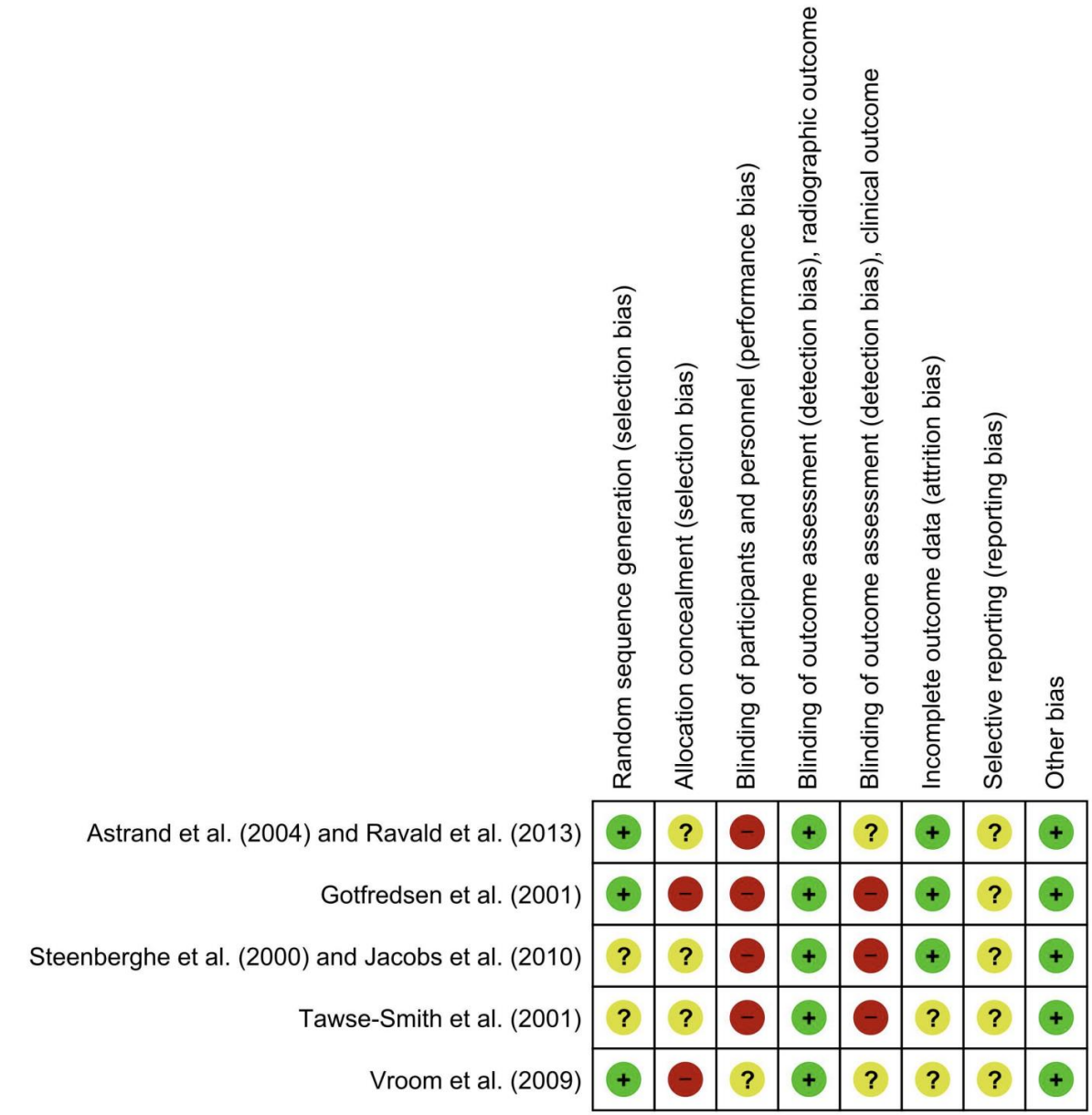
39. Any additional information.

Provide any other information the review team feel is relevant to the registration of the review.

40. Details of final report/publication(s).

This field should be left empty until details of the completed review are available.

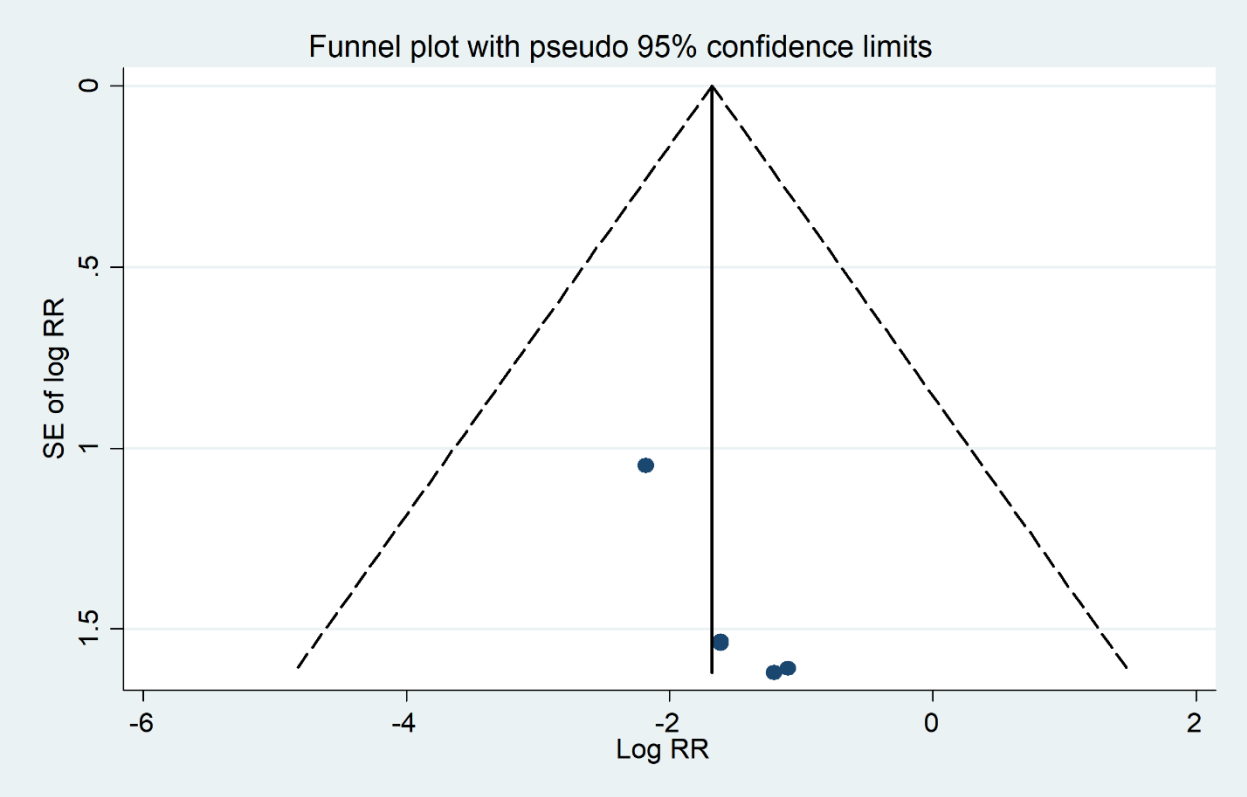
Give the link to the published review.



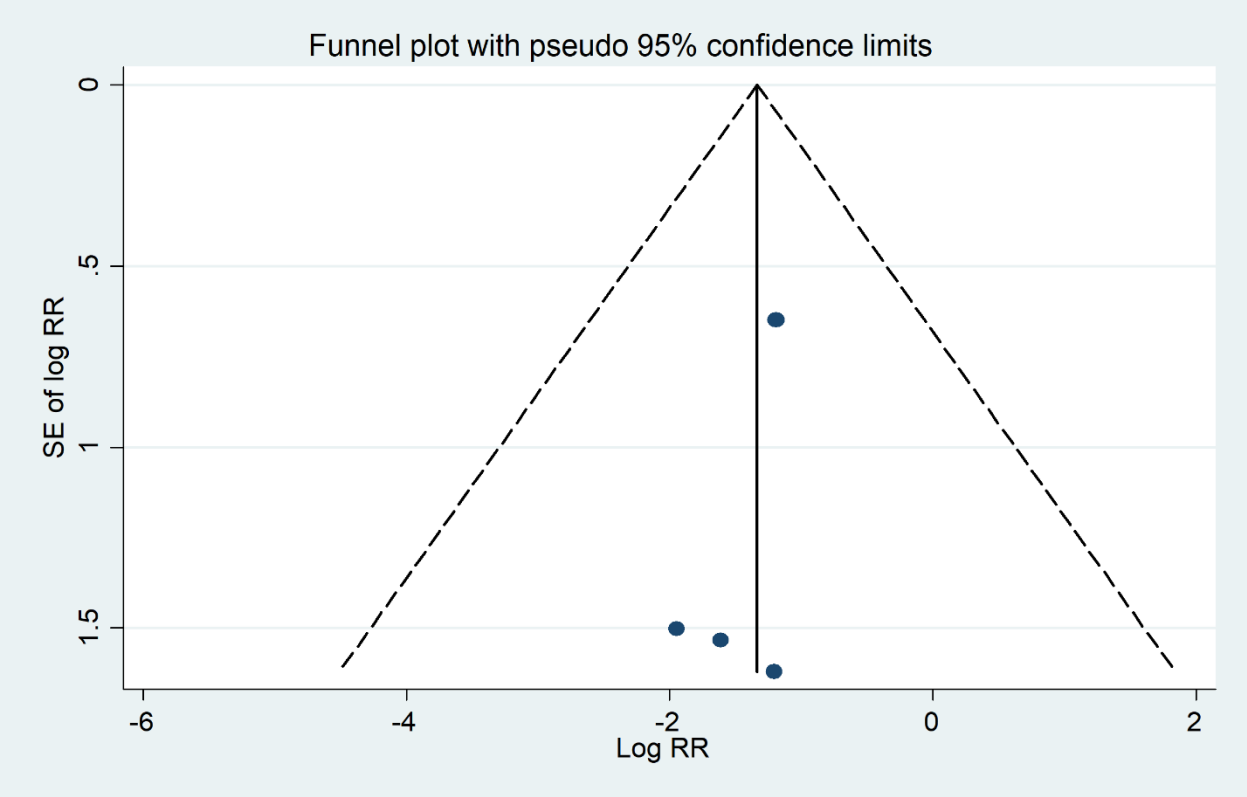
S3 Appendix. Risk of bias summary:
Review authors' judgements about each risk of bias item for each included study.

Supplementary Appendix 4. Publication bias (funnel plots).

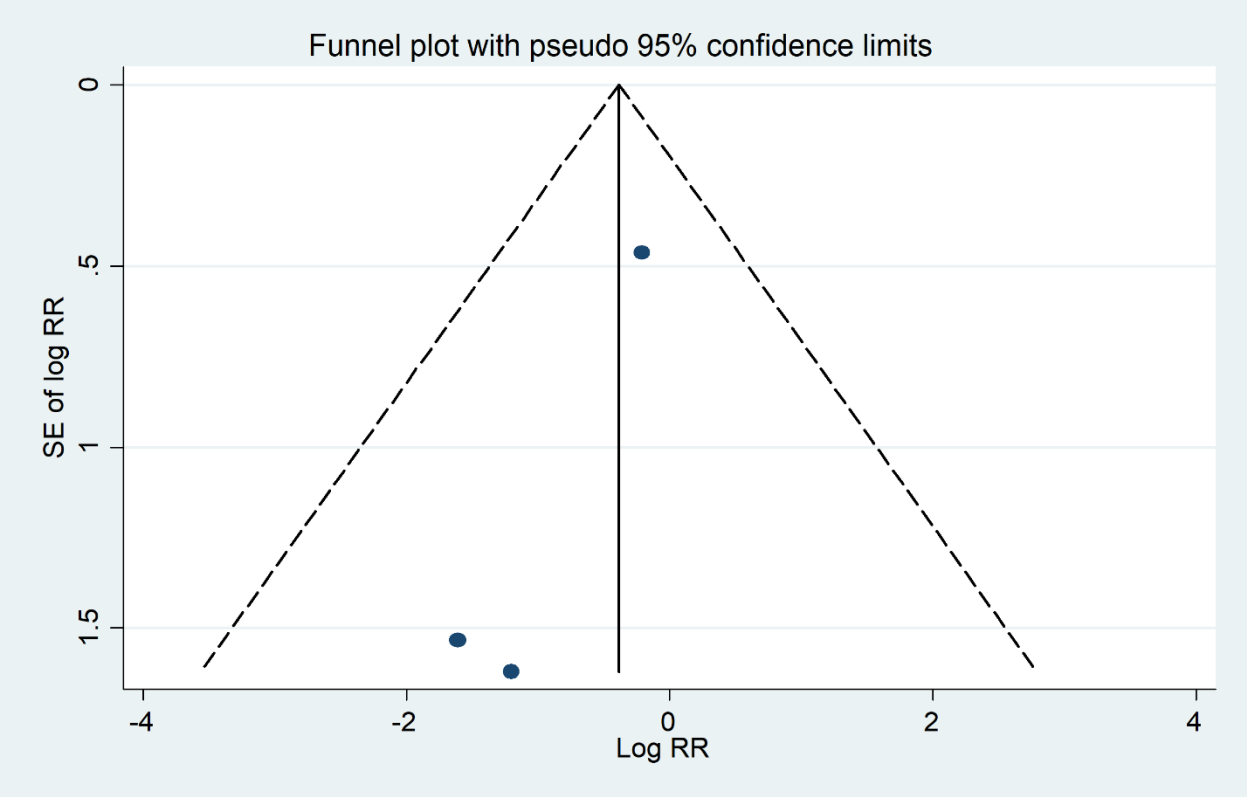
Funnel plot for pooled risk ratio analysis of implant failure rate after 1 year.



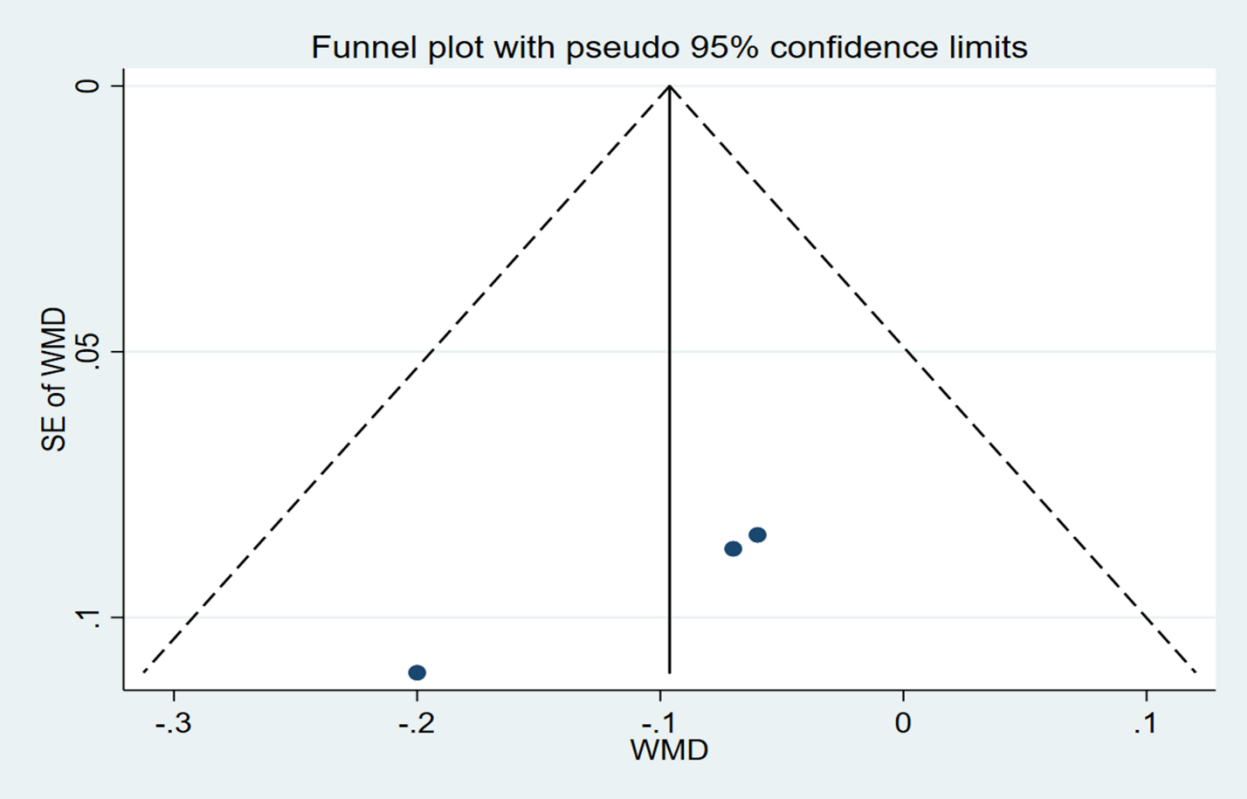
Funnel plot for pooled risk ratio analysis of implant failure rate after 2 years.



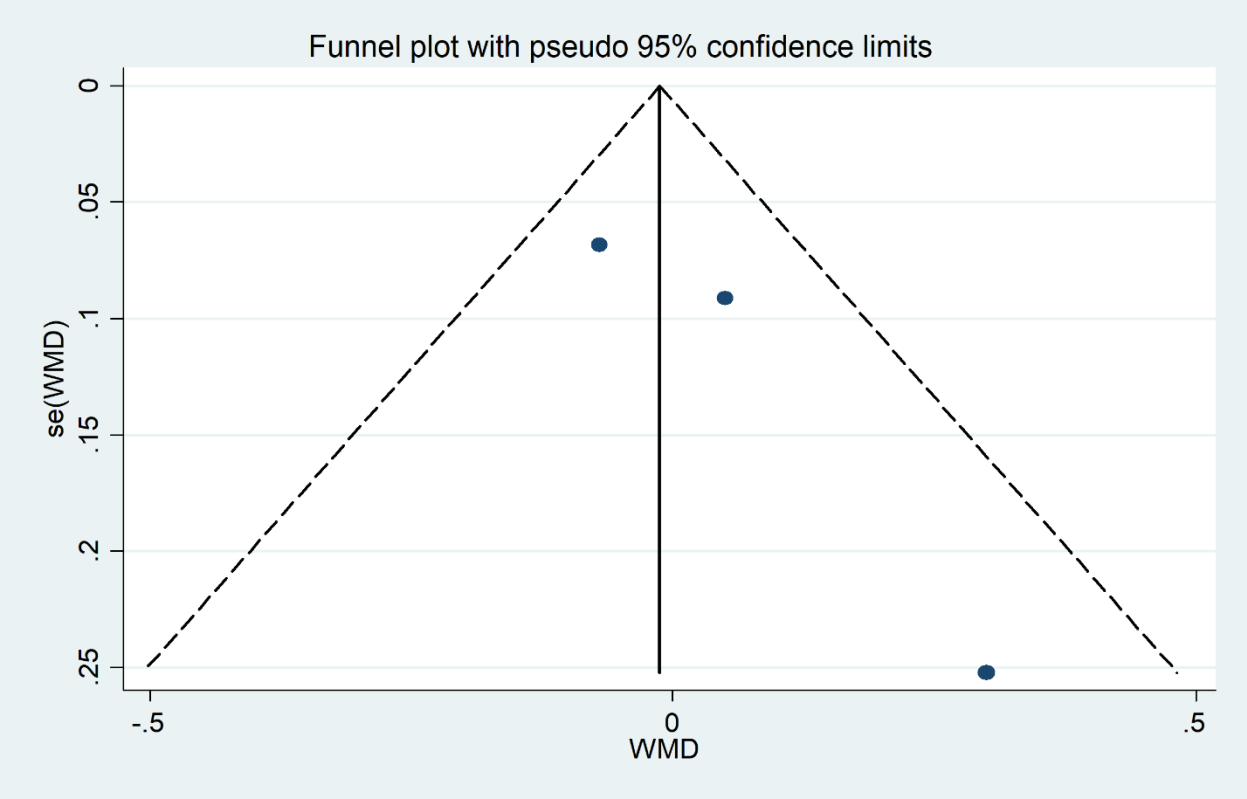
Funnel plot for pooled risk ratio analysis of implant failure rate after 5-6 years.



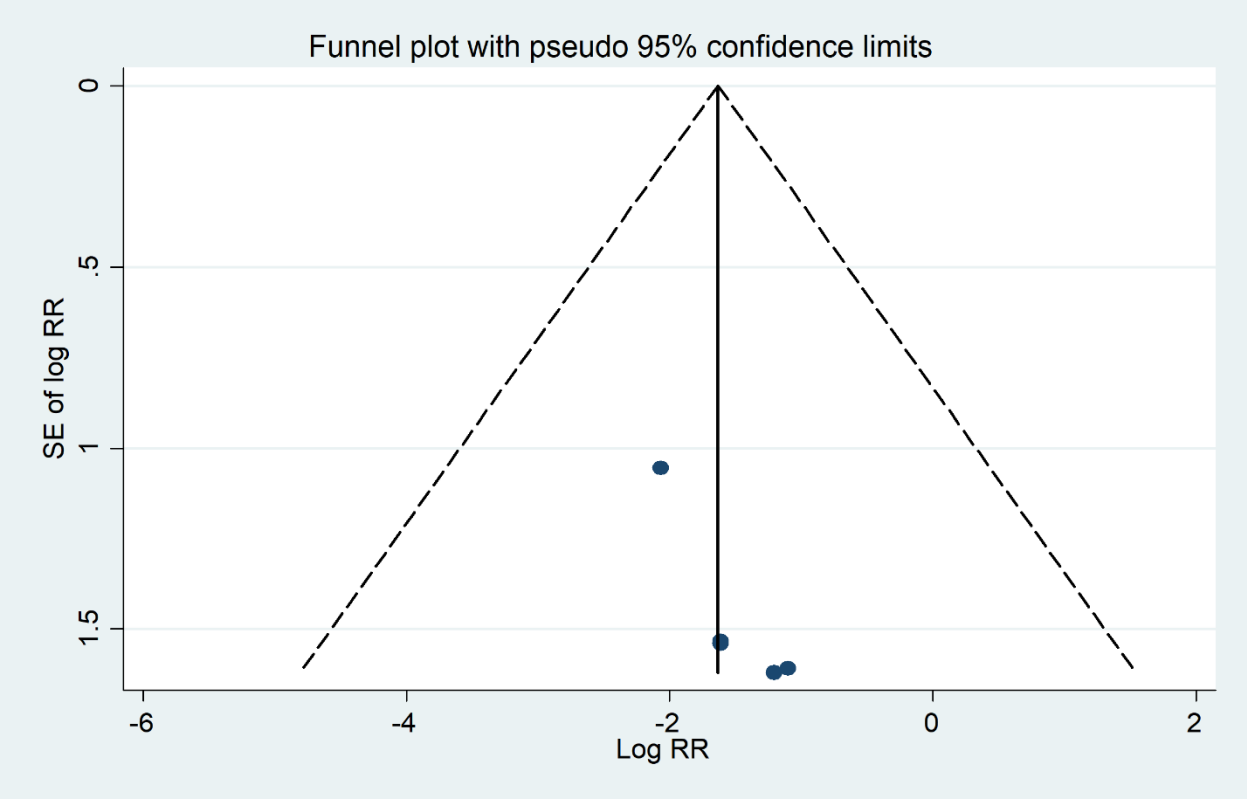
Funnel plot for pooled risk ratio analysis of implant failure rate after 12-15 years.



Funnel plot for weighted mean difference of marginal bone level change after 1 year.



Funnel plot for weighted mean difference of marginal bone level change after 5 years.



S1 Table. Detailed evaluation of risk of bias of individual studies.

First author and title: Astrand, P. 2004 *Astra Tech and Brånemark system implants: a 5-year prospective study of marginal bone reactions* (The continuation of the study was published by Ravald et al. 2013 *Long-term evaluation of Astra Tech and Brånemark implants in patients treated with full-arch bridges. Results after 12-15 years*)		
Bias	**Authors judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quote: "…patients were randomized in blocks with an equal probability of receiving Astra Tech or Brånemark system implants."
Allocation concealment (selection bias)	Unclear	Comment: Not described.
Blinding of participants and personnel (performance bias)	High risk	Comment: No blinding described, and probably no blinding occurred, due to the fact that different implant systems need different surgical protocols and special abutments for denture fixation thus study personnel must know the implant type.
Blinding of outcome assessment (detection bias) radiographic outcome	Low risk	Quote: "A specialist in oral radiology, who did not take part in the clinical treatment, performed the radiographic evaluation."
Blinding of outcome assessment (detection bias) clinical outcome	High risk	Comment: Probably no blinding due to the nature of outcome.
Incomplete outcome data (attrition bias)	Low risk	Comment: 3 patients were excluded, one lost the implants (Brånemark), the other two died (Astra). However, all patients were included in the cumulative survival analysis.
Selective reporting (reporting bias)	Unclear risk	Comment: No access to study protocol or trial registry entry, but no intext evidence of reporting bias.
Other bias	Low risk	Comment: Study appears to be free of other sources of risk.

First author and title: Gotfredsen, K. 2001 *A prospective 5-year study of fixed partial prostheses supported by implants with machined and TiO_2-blasted surface*		
Bias	**Authors judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quote: ”A stratification and a randomization of the 2 surface groups were done. The first implants were selected at random by drawing lots…”
Allocation concealment (selection bias)	High risk	Quote: “…thereafter the implants with different surface configurations were inserted alternately.”
Blinding of participants and personnel (performance bias)	High risk	Comment: No blinding described, and probably no blinding occurred, due to the fact that different implant systems need different surgical protocols and special abutments for denture fixation thus study personnel must know the implant type.
Blinding of outcome assessment (detection bias) radiological outcome	Low risk	Quote: “An experienced radiologist, not otherwise involved in the study, evaluated all the radiographs, blindly.”
Blinding of outcome assessment (detection bias) clinical outcome	High risk	Comment: No blinding described, due to the difference in characteristics of the two implant systems evaluators could differentiate between the two.
Incomplete outcome data (attrition bias)	Low risk	Comment: 10 % of participants dropped out (eight from both implant types), otherwise no missing data.
Selective reporting (reporting bias)	Unclear risk	Comment: No access to study protocol or trial registry entry, but no intext evidence of reporting bias.
Other bias	Low risk	Comment: Study appears to be free of other sources of risk.

Article's first author and title: Steenberghe, D 2000 *A prospective split-mouth comparative study of two screw-shaped self-tapping pure titanium implant systems* (The continuation of the study was published by Jacobs et al. 2010 *A split-mouth comparative study up to 16 years of two screw-shaped titanium implant systems*)		
Bias	**Authors judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Quote: "…randomized for the jaw in which both implant systems were applied."
Allocation concealment (selection bias)	Unclear risk	Comment: Way of randomization and allocation concealment are not described in the study.
Blinding of participants and personnel (performance bias)	High risk	Comment: No blinding described, and probably no blinding occurred, due to the fact that different implant systems need different surgical protocols and special abutments for denture fixation thus study personnel must know the implant type.
Blinding of outcome assessment (detection bias) radiological outcome	Low risk	Comment: Blinding of outcome assessment not described but unlikely to affect measurement of this outcome.
Blinding of outcome assessment (detection bias) clinical outcome	High risk	Comment: No blinding described, due to the difference in characteristics of the two implant systems evaluators could differentiate between the two.
Incomplete outcome data (attrition bias)	Low risk	Comment: No drop outs.
Selective reporting (reporting bias)	Unclear risk	Comment: No access to study protocol or trial registry entry, but no intext evidence of reporting bias.
Other bias	Low risk	Comment: Study appears to be free of other sources of risk.

Article's first author and title: Tawse-Smith, A. 2001 *One-stage operative procedure using two different implant systems: a prospective study on implant overdentures in the edentulous mandible*		
Bias	**Authors judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Quote: "participants were randomly selected from those requesting the placement of osseointegrated Implants…" Comment: However, the method for randomization is not described.
Allocation concealment (selection bias)	Unclear risk	Comment: Selection method not described.
Blinding of participants and personnel (performance bias)	High risk	Comment: No blinding described, and probably no blinding occurred, due to the fact that different implant systems need different surgical protocols and special abutments for denture fixation thus study personnel must know the implant type.
Blinding of outcome assessment (detection bias) radiological outcome	Low risk	Comment: Blinding of outcome assessment not described but unlikely to affect measurement of this outcome, "standardized intraoral radiographs" were used.
Blinding of outcome assessment (detection bias) clinical outcome	High risk	Comment: No blinding described, due to the difference in characteristics of the two implant systems evaluators could differentiate between the two.
Incomplete outcome data (attrition bias)	Unclear risk	Comment: Two participants dropped out, no reason was given.
Selective reporting (reporting bias)	Unclear risk	Comment: No access to study protocol or trial registry entry, but no intext evidence of reporting bias.
Other bias	Low risk	Comment: Study appears to be free of other sources of risk.

Article's first author and title: Vroom, M. G. 2009 *Effect of surface topography of screw-shaped titanium implants in humans on clinical and radiographic parameters: a 12-year prospective study*		
Bias	**Authors judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quote: "The type of implant surface texture used at this location was randomly assigned using a computer-generated randomization schedule."
Allocation concealment (selection bias)	High risk	Quote: "Thereafter, the two types of implants were placed alternatively…"
Blinding of participants and personnel (performance bias)	Unclear risk	Comment: The blinding was not described.
Blinding of outcome assessment (detection bias) radiographic outcome	Low risk	Comment: Blinding of outcome assessment not described but unlikely to affect measurement of this outcome. Quote: "Standardized intra-oral radiographs were… made… The radiographs … were analyzed using a commercially available dental X-ray software program".
Blinding of outcome assessment (detection bias) clinical outcome	Unclear risk	Comment: The blinding was not described.
Incomplete outcome data (attrition bias)	Unclear risk	Comment: At the 12 years checkup the sample size decreased by 7. Authors give no explanation.
Selective reporting (reporting bias)	Unclear risk	Comment: No access to study protocol or trial registry entry, but no intext evidence of reporting bias.
Other bias	Low risk	Comment: Study appears to be free of other sources of risk.

S1 File. Data included in meta-analysis.

0 - 1 years (cumulative failure)				
Studies	intervention		control	
	Failed (n)	Total (n)	Failed (n)	Total (n)
Åstrand et al. (2004)	1	184	8	187
Gotfredsen et al. (2001)	0	64	2	64
Steenberghe et al. (2000)	0	50	1	45
Tawse-Smith et al. (2002)	0	24	1	24
Vroom et al. (2009)	0	40	2	40

0 - 2 years (cumulative failure)				
Studies	intervention		control	
	Failed (n)	Total (n)	Failed (n)	Total (n)
Åstrand et al. (2004)	1	184	9	187
Gotfredsen et al. (2001)	0	64	2	64
Steenberghe et al. (2000)	0	50	1	45
Tawse-Smith et al. (2002)	0	24	1	24
Vroom et al. (2009)	0	40	2	40

0-5/6 years (cumulative failure)				
Studies	intervention		control	
	Failed (n)	Total (n)	Failed (n)	Total (n)
Åstrand et al. (2004)	3	184	10	187
Gotfredsen et al. (2001)	0	64	3	64
Jacobs et al. (2010)	0	50	1	45
Vroom et al. (2009)	0	40	2	40

0-12/15 years (cumulative failure)				
Studies	intervention		control	
	Failed (n)	Total (n)	Failed (n)	Total (n)
Ravald et al. (2013)	8	184	10	187
Jacobs et al. (2010)	0	50	1	45
Vroom et al. (2009)	0	40	2	40

from 0 to 1 year change							
Studies	Group	Sample size	Marginal bone level change				P value
			Mean	Range	SEM	SD	
Steenberghe et al. (2000)	control	44	0	-1.5 - +1.0			no sig. diff.
	intervention	50	-0.2	-1.6 - +0.6			
Tawse-Smith et al. (2002)	control	23	-0.1			0.33	no sig. diff.
	intervention	24	-0.16			0.24	
Vroom et al. (2009)	control	40	-0.07			0.27	no sig. diff.
	intervention	40	-0.14			0.48	

from 0 to 5/6 years change							
Studies	Group	Sample size	Marginal bone level change				P value
			Mean	Range	SEM	SD	
Gotfredsen et al. (2001)	control (maxilla)	10	-0.21			0.83	no sig. diff.
	control (mandible)	25	-0.22			1.13	
	intervnetion (maxilla)	10	-0.51			1.11	
	Intervention (mandible)	25	-0.52			1.07	
Vroom et al. (2009)	control	34	-0.11			0.34	no sig. dif.
	intervention	34	-0.06			0.41	
Jacobs et al. (2010)	control	44	0.05			0.34	no sig. dif.
	intervention	50	-0.02			0.32	

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Saliva as a Candidate for COVID-19 Diagnostic Testing: A Meta-Analysis

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Background: COVID-19 is a serious and potentially deadly disease. Early diagnosis of infected individuals will play an important role in stopping its further escalation. The present gold standard for sampling is the nasopharyngeal swab method. However, several recent papers suggested that saliva-based testing is a promising alternative that could simplify and accelerate COVID-19 diagnosis.

Objectives: Our aim was to conduct a meta-analysis on the reliability and consistency of SARS-CoV-2 viral RNA detection in saliva specimens.

Methods: We have reported our meta-analysis according to the Cochrane Handbook. We searched the Cochrane Library, Embase, Pubmed, Scopus, Web of Science and clinical trial registries for eligible studies published between 1 January and 25 April 2020. The number of positive tests and the total number of tests conducted were collected as raw data. The proportion of positive tests in the pooled data were calculated by score confidence-interval estimation with the Freeman–Tukey transformation. Heterogeneity was assessed using the I^2 measure and the χ^2-test.

Results: The systematic search revealed 96 records after removal of duplicates. Twenty-six records were included for qualitative analysis and 5 records for quantitative synthesis. We found 91% (CI 80–99%) sensitivity for saliva tests and 98% (CI 89–100%) sensitivity for nasopharyngeal swab (NPS) tests in previously confirmed COVID-19 patients, with moderate heterogeneity among the studies. Additionally, we identified 18 registered, ongoing clinical trials of saliva-based tests for detection of the virus.

Conclusion: Saliva tests offer a promising alternative to NPS for COVID-19 diagnosis. However, further diagnostic accuracy studies are needed to improve their specificity and sensitivity.

Keywords: coronavirus, SARS-CoV-2, COVID-19, diagnostic tests, saliva, systematic review, meta-analysis

INTRODUCTION

COVID-19, caused by the SARS-CoV-2 virus, is a serious and potentially deadly disease. Globally, as of 5 May 2020, there have been 3,489,053 confirmed cases of COVID-19 reported to WHO, including 241,559 deaths (1). Early diagnosis and isolation of infected individuals will play an vital role in stopping the further escalation of the pandemic.

At present, nasopharyngeal swabbing, followed by reverse transcription of the extracted RNA and quantitative PCR (RT-qPCR), is the gold standard for detection of SARS-CoV-2 infection (2). Specimen collection currently requires trained medical personnel (3), thus exposing staff to a high risk of infection (4). These tests are not always successful at the first attempt, and shortages of swabs and protective equipment are frequently reported (2). Additionally, mass testing requires an increased number of trained personnel at specimen acquisition sites. Consequently, the nasopharyngeal swab (NPS) collection method is causing an economic and logistic burden on healthcare systems. Additionally, nasopharyngeal swabbing causes discomfort to the patients (5) and there are several contraindications, such as coagulopathy or anticoagulant therapy, and significant nasal septum deviation (6). Clearly, there is a need for a simpler and less invasive method that also reduces the risk to healthcare personnel.

One candidate for non-invasive specimen collection is saliva. The saliva secreted by salivary glands contains water, electrolytes, mucus, and digestive and protective proteins (7–9). But whole saliva collected from the mouth is a mixture of glandular secretions, gingival crevicular fluid, serum, expectorated airway surface liquid and mucus, epithelial and immune cells from the oral mucosa and upper airways, and oral microbes and viruses (10). Despite its heterogeneous origins, this mixed fluid is used widely and successfully as a diagnostic tool to identify various oral and systemic conditions (8, 11). These already include viral infections such as dengue, West Nile, chikungunya, Ebola, Zika and Yellow Fever, and also the recently emerged coronaviruses responsible for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) (12).

Since early January 2020, several papers have been published on the possible use of saliva as a specimen for detecting SARS-CoV-2 in the diagnosis of COVID-19. Until now there has been no systematic review or meta-analysis of this topic. Our aim, therefore, was to conduct a meta-analysis, thus overcoming the limitations of the small sample sizes in individual studies, in order to estimate the diagnostic sensitivity of saliva-based detection of the virus. We also aimed to summarize the study protocols that have been registered in clinical trial registries to investigate saliva-based COVID-19 diagnosis in the future.

MATERIALS AND METHODS

Protocol and Registration

The reporting of our meta-analysis follows the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (13). The PRISMA checklist for our work is available in the supporting information (**Table S1**). We registered our meta-analysis protocol in the OSF (Open Science Framework, Center for Open Science) registries on 23 April 2020 (https://osf.io/3ajy7).

Deviation From the Registered Protocol

Studies eligible according to our inclusion criteria did not present sufficient raw data to complete 2 × 2 contingency tables. True positive, true negative, false positive and false negative values were not generally available, thus sensitivity and specificity could not be separately calculated. Instead, positive event rates were pooled for statistical analysis. Details of the analysis are described in section Summary Measures and Synthesis of Results.

Eligibility Criteria

We included records if they met the following eligibility criteria: (1) records published in scientific journals or clinical trial registries; (2) patients diagnosed with COVID-19; (3) index test: saliva specimens with PCR diagnostics for detecting SARS-CoV-2; (4) reference standard (comparator test): NPS specimens with PCR diagnostics for detecting SARS-CoV-2; (5) records written in English or available in English translation. Exclusion criteria: (1) publications with no primary results such as reviews, guidelines and recommendations; (2) publications dated before 1 January and after 25 April, 2020; (3) gray and black literature.

Search Strategy

Systematic searches for records published in English after 1 January 2020 were performed in five major literature databases (Cochrane Library, Embase, PubMed, Scopus, Web of Science) and also in five clinical trial registers (ClinicalTrial.gov, EU Clinical Trials Register, NIPH Clinical Trial Search, ISRCTN Registry, ANZCTR Registry). The last update of our systematic search was performed on 25 April 2020. Cited and citing papers of the relevant studies were screened for further eligible studies.

The following key words were applied to each database to identify eligible records: (COVID 19 OR COVID19 OR Wuhan virus OR Wuhan coronavirus OR coronavirus OR 2019 nCoV OR 2019nCoV OR 2019-nCoV OR SARS CoV-2 OR SARS-CoV-2 OR NCP OR novel coronavirus pneumonia OR 2019 novel coronavirus OR new coronavirus) AND (saliva).

Study Selection

We used EndNote X9.3.3 reference manger to organize records. After removal of duplicates, two authors (A.H. and I.M.) independently screened the records for eligibility based on the titles and abstracts. Papers included at this stage were further appraised by reading the full text. Any disagreement between reviewers was resolved by consulting a third reviewer (L.M.C.).

Data Collection

Using a preconstructed, standardized data extraction form, two authors (A.H. and I.M.) independently collected data from the included records. From primary studies the following information was extracted (**Table 1**): first author's name, year of publication, place of study, study type, population size, age, gender, method of diagnosis, type of PCR kit, and the following outcome parameters: numbers of total, positive and negative saliva tests and numbers of total, positive and negative NPS

TABLE 1 | Summary of study characteristics of included records.

References	Country	Study type	Population		Diagnoses of COVID-19	PCR kit	Reference standard	Index test	Outcome parameters
			n (m/f)	Age					
(14)	Italy	Consecutive case series	25 (17/8)	61 (mean) (39–85)	Viral RNA detection with PCR from NPS	Luna Universal qPCR Master Mix	NPS	Saliva	Number of positive and negative index tests
(15)	South Korea	Consecutive case series	4 (2/2)	61.5 (35–82)	Viral RNA detection with PCR from NPS And clinical signs of pneumonia	N/A	NPS	Saliva	Number of positive and negative index tests
(16)	China	Consecutive case series	32 (16/16)	41 (34–54)	Viral RNA detection with PCR from NPS	N/A	NPS	Saliva	Number of positive and negative index tests
(17)	Hong Kong, China	Consecutive case series	23 (13/10)	62 (37–75)	Viral RNA detection with PCR from NPS	QuantiNova Probe RT-PCR Kit	NPS	Saliva	Number of positive and negative index tests
(18)	Australia	Consecutive case series	39 (not published)	Not published	Viral RNA detection with PCR from NPS	Coronavirus Typing (835 well) assay	NPS	Saliva	Number of positive and negative index tests
Not included in quantitative synthesis:									
(19)	China	Case report	1 (0/1)	39	Viral RNA detection with PCR from NPS And clinical signs of pneumonia	N/A	NPS	Saliva	Number of positive and negative reference tests and index tests
(20)	South Korea	Case report	1 (0/1)	Neonate (27 day-old)	Viral RNA detection with PCR from NPS	PowerChek TM 2019-nCoV Real-time PCR Kit	NPS	Saliva	Number of positive and negative reference tests and index tests
(21)	USA	Consecutive case series	29 (16/13)	59 (mean) (23–91)	Viral RNA detection with PCR from NPS	The US CDC real-time RT-PCR primer/probe sets	NPS	Saliva	Number of positive and negative reference tests and index tests

NPS, Nasopharyngeal swab; N/A, Not available.

tests. From registered study protocols the following information was extracted (**Table S2**): clinical trial ID, recruiting status, study type, number of centers, study design, location, population, intervention, comparison, primary outcomes, and secondary outcomes. In cases of disagreement during extractions a third author (L.M.C.) was consulted.

Risk of Bias and Applicability Assessment

We evaluated the potential for bias, the quality of reporting and the applicability of the studies using the QUADAS-2 tool (Quality Assessment of Diagnostic Accuracy Studies 2) (22), which is a tool widely used to assess studies of diagnostic accuracy. Our appraisal consisted of evaluating the risk of bias and applicability in four domains: (1) patient selection, (2) conduct and interpretation of the index test, (3) reference standard, and (4) flow and timing. We applied the following review question to judge the applicability of the studies to our investigation: *Are saliva specimens reliable for detecting SARS-CoV-2 in COVID-19 patients confirmed by nasopharyngeal swab testing?*

We used the preconstructed form available on the QUADAS-2 web page of the University of Bristol (23).

Summary Measures and Synthesis of Results

In the synthesis of quantitative data we included patient-based data from consecutive case series. Case reports from single participants were excluded.

The sensitivities of the saliva and NPS tests were assessed in patients who had previously been confirmed to be infected, having had both a positive NPS test and well-defined clinical symptoms on admission to the hospital. Extracted data were limited to test results from subsequent occasions when both saliva and NPS samples were collected concurrently. Therefore, the sensitivity of the NPS test is based on the matching NPS tests when saliva tests were also performed.

The sensitivity of the saliva test in the patient-based pooled data was calculated using the methods recommended by the working group of the Cochrane Collaboration. Because some of the sensitivity values are close to or equal to 1, the score confidence interval estimation (24) was applied with the Freeman–Tukey double arcsine transformation (25). Because of the variability of the population sizes and methodologies in the different studies, the DerSimonian and Laird method (26)

was used, with 95% confidence intervals (CI), for a random-effects meta-analysis.

Heterogeneity was assessed using the I^2 measure and the χ^2-test, where $p < 0.1$ is taken to indicate significant heterogeneity. I^2 values of 25, 50, and 75% were identified as low, moderate and high estimates, respectively (27). Statistical analyses were carried out using STATA software version 15.0 (STATACorp, Texas, USA).

RESULTS

Study Selection

We included 20 articles for full-text evaluation of completed studies. Of these, eight were included in the qualitative synthesis, from which five were also included in the quantitative synthesis. **Figure 1** illustrates the study selection process.

Our search in the clinical trial register yielded 19 protocols, of which one was excluded due to its relating to a different topic.

Study Characteristics

Characteristics of the Studies Included

All five records included in the quantitative synthesis were consecutive case series, involving 123 patients from five distinct global locations (**Table 1**) (14–18). All of these publications included patients with confirmed diagnoses of COVID-19. No other restrictions on inclusion were stated in any of the studies.

In the qualitative synthesis we also included another consecutive case series (**Table 1**). But in their work Wyllie et al. presented 38 matching NPS and saliva samples from 29 patients without identifying the double or multiple samplings from individual patients. Therefore, their sample-wise results cannot be combined for quantitative analysis with the others which reported patient-wise data (21).

Results of Individual Studies and Synthesis of Results

Diagnostic Potential of Saliva Specimens

In the individual studies included in the quantitative synthesis, the sensitivity of the saliva test among COVID-19 infected patients ranged from 78% (16) to 100% (14).

Pooled event rates (positive and negative test results) from saliva specimens show that the sensitivity of the saliva test was 91% (CI 80–99%) among COVID-19 patients diagnosed in the recruitment period (**Figure 2A**). By definition, the nature of the initial diagnosis implies or rather assumes a 100% sensitivity for the nasal swab test in those patients at that time point. However, pooled event rates from NPS specimens taken concurrently with the saliva specimen collections, generally some time after the initial diagnosis, indicate that the sensitivity of the NPS test, based on these time-matched samples, was 98% (CI 89–100%) (**Figure 2B**). Since the two confidence intervals overlap, it appears that the proportions of positive test results from the saliva and NPS samples are not very different. However, a firm conclusion will require formal diagnostic accuracy tests based upon larger clinical studies.

We assessed our pooled results for inconsistency using the I^2-test (28). In the case of the saliva tests we found a moderate level of heterogeneity ($I^2 = 60.98\%$, $p = 0.04$) indicating the contribution of confounding factors. On the other hand, we found a low level of heterogeneity among the NPS test results ($I^2 = 46.56\%$, $p = 0.13$).

Interestingly some of the data suggest that NPS tests may occasionally be negative when the corresponding saliva test gives a positive result. Azzi et al. reported that two patients showed positive saliva tests while their NPS tests were negative (14), and a case report showed that in seven sample pairs from one individual, the NPS tests were all negative while the saliva tests were positive on each occasion (19). In a sample-based study of 38 patients, Wyllie et al. (21) detected SARS-CoV-2 in saliva but not NPS specimens from eight patients (21%), while the virus was detected in NPS but not saliva in only 3 matched samples (8%). And overall, they found significantly higher SARS-CoV-2 titers in the saliva than in the NPS specimens.

In a more detailed study, Bae et al. examined the difference in viral loads between the two sampling methods: the values were 0.06 to 3.39 $\log_{10}$ units higher in the NPS specimens than in the saliva specimens (15). One case series (18) and another case report on a 27-day-old neonate (20) also found that there were higher viral loads in the NPS specimens.

Only two studies assessed the specificity of the saliva tests (18, 21). In one, a subset of saliva specimens from 50 patients with PCR-negative nasal swabs was tested. SARS-CoV-2 was detected in 2% (CI 0.1–11.5%) of these saliva samples (18). The other study tested 98 asymptomatic healthcare workers with parallel NPS and saliva tests. NPS tests turned out to be negative for all participants, while saliva tests were positive for two (21).

Risk of Bias Within Studies

We assessed the risk of bias in the six included case series (14–18, 21) according to the QUADAS-2 tool. Five of the six (14–17, 21) had low risk of selection bias. On the other hand four studies (14–17) had high risk of bias in the index test due to the fact that the saliva tests results were interpreted with prior knowledge of the results of the reference standard. Flow and timing were high or unclear in all studies, since there was no exact information regarding the time passed between specimen collections for the two tests. Applicability had low concerns in index test in four studies (14, 17, 18, 21) and unclear in two studies (15, 16). A summary of the risk-of-bias analysis and applicability concerns is available in **Tables S3**, **S4**. Altogether, our risk-of-bias analyses demonstrated a moderate bias level in both the individual and the overall aspects of the studies.

Ongoing Registered Clinical Trials on Saliva Diagnostics for COVID-19

We also systematically searched five clinical trial registers (EU Register, ISRCTN, ANZCTR, JPRN, ClinicalTrials.gov) for clinical trial protocols that are planned to evaluate saliva specimens for COVID-19 diagnosis. By using the same keywords as for the studies already completed, we found 18 registered clinical trials on planned or ongoing clinical studies. All of them appeared in the ClinicalTrials.gov registry (**Table S2**). Among these, 13 are non-interventional, focusing primarily

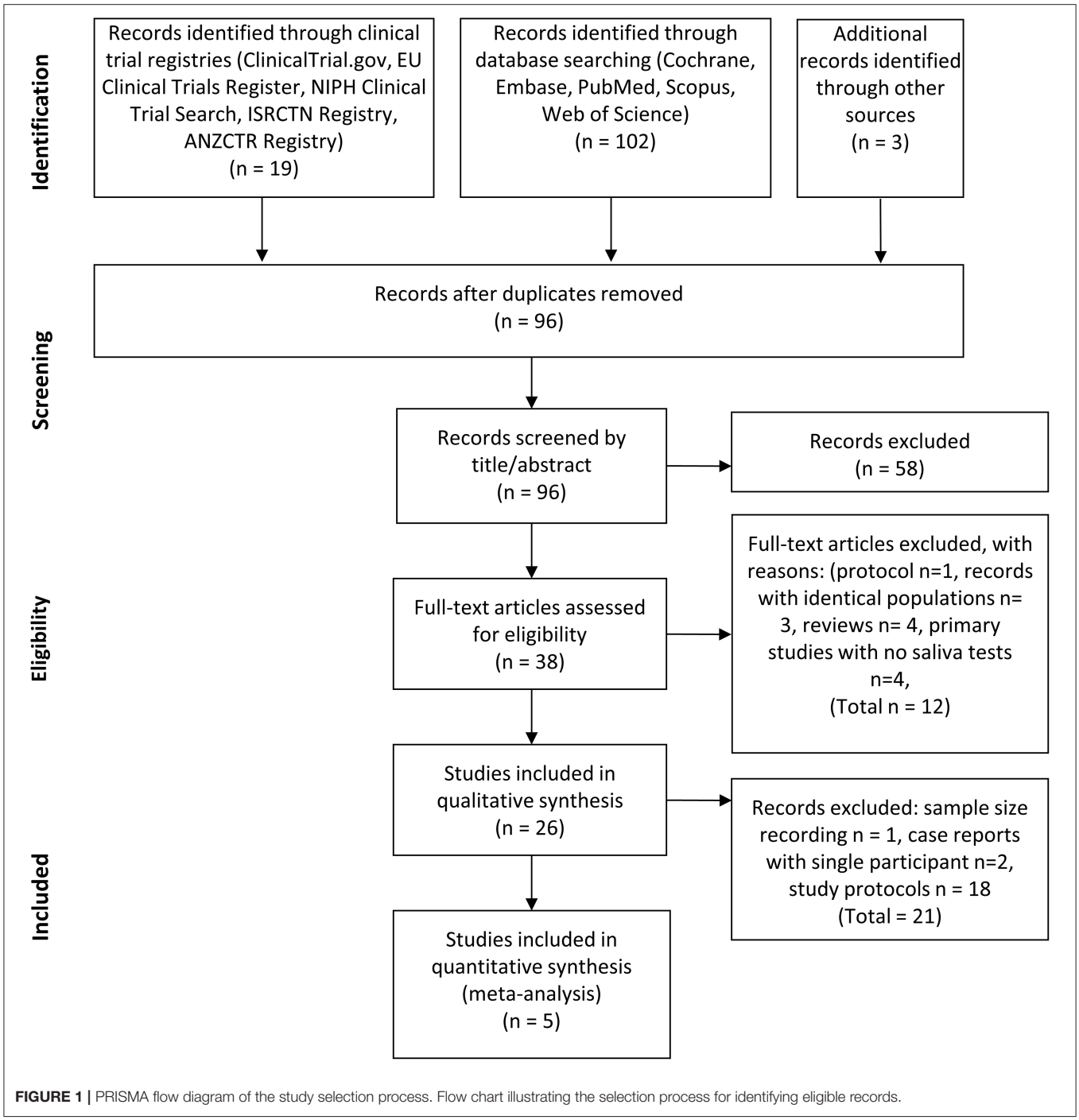

FIGURE 1 | PRISMA flow diagram of the study selection process. Flow chart illustrating the selection process for identifying eligible records.

on the diagnostic and prognostic value of various specimens collected from patients, including NPS, saliva and blood, in detecting and following the progression of COVID-19 disease. The other five, interventional studies are examining the effectiveness of several potentially beneficial compounds, including azithromycin, lopinavir/ritonavir, beta-cyclodextrin, citrox 3 and peginterferon lambda, on the outcomes of SARS-CoV-2 infection. In these studies, besides NPS specimen collections, saliva tests are also planned. Unfortunately, in the trial protocols very little information is available about the optimization and validation of the saliva collection protocols, the transportation and storage of the saliva samples, the viral RNA assay methods to be used for the saliva samples, and the choice of appropriate internal controls, which is important given the scarcity of human DNA in saliva samples.

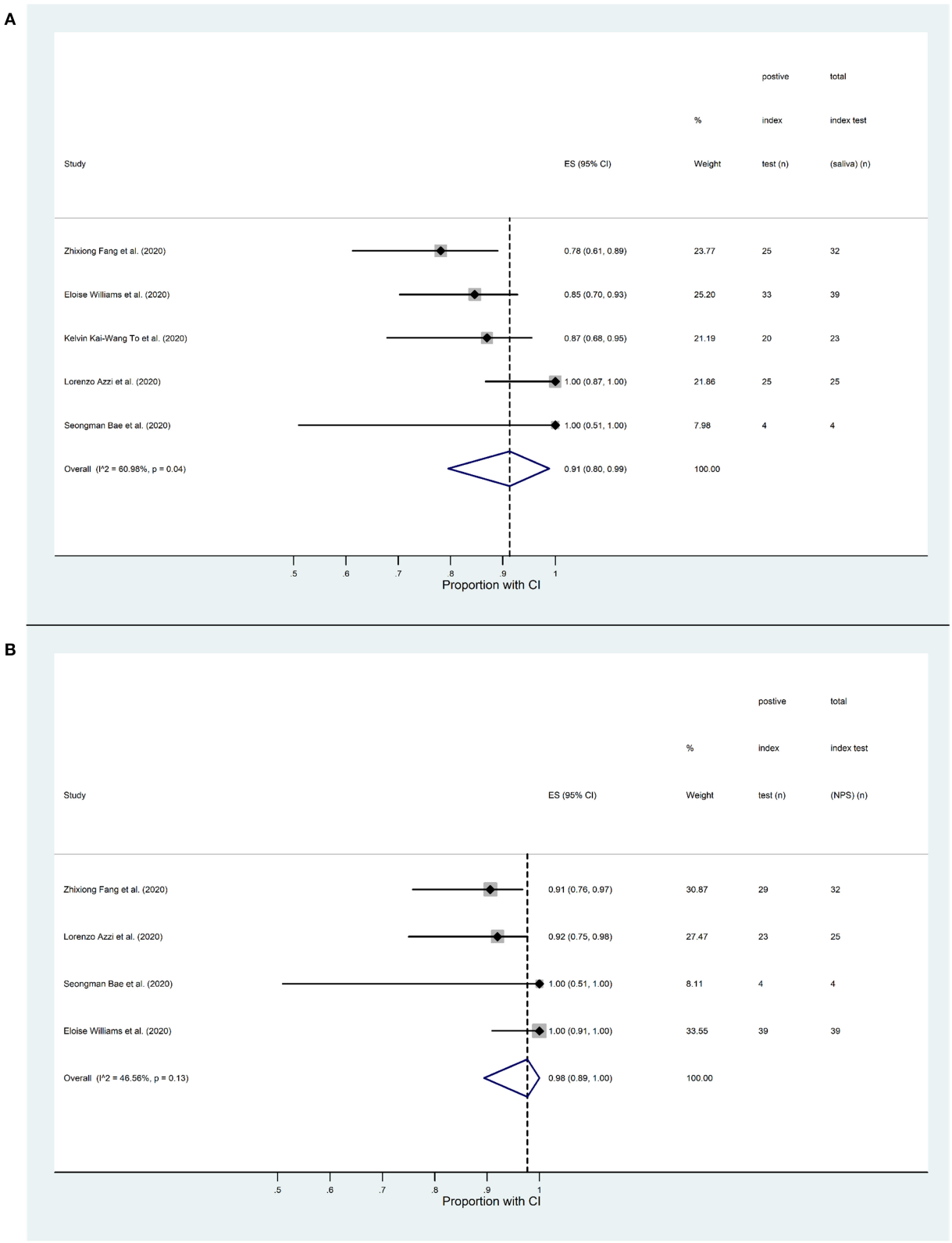

FIGURE 2 | Forest plot analysis of SARS-CoV-2 detection sensitivity based on RT-qPCR analysis of saliva and nasopharyngeal swab (NPS) specimens from COVID-19 patients. **(A)** Proportion of positive saliva tests in the five studies included in the quantitative analysis, ranging from 0.78 to 1. The overall proportion in the pooled data is 0.91 (CI 0.80–0.99). The I^2 value (60.98%, $p = 0.04$) indicates a moderate level of statistical heterogeneity. **(B)** Proportion of positive NPS tests in the four studies included in the quantitative analysis, ranging from 0.91 to 1. The overall proportion in the pooled data is 0.98 (CI 0.89-1). The I^2 value (46.56%, $p = 0.13$) indicates a low level of statistical heterogeneity.

DISCUSSION

In April 2020 the Food and Drug Administration (FDA) granted emergency use authorization (EUA) to Rutgers' RUCDR Infinite Biologics and its collaborators for a new specimen collection approach that utilizes saliva as the primary test biomaterial for the SARS-CoV-2 coronavirus, the first such approval granted by the federal agency (https://www.fda.gov/media/136877/download). This new saliva-based diagnostic collection method, which RUCDR has developed in partnership with Spectrum Solutions and Accurate Diagnostic Labs (ADL), claims to allow an easier and therefore broader screening of the population compared with the current method using nose and throat swabs. Another accelerated EUA for the "Curative-Korva SARS-Cov-2 Assay," which was specifically designed for use with oral fluid samples, was also approved to permit the testing of oral fluids, i.e., saliva (https://www.fda.gov/media/137088/download). Nasopharyngeal swabs, oropharyngeal swabs and nasal swabs can also be used with the Curative-Korva SARS-CoV-2 Assay, but their performance with this assay has not yet been assessed (https://www.fda.gov/media/137088/download). These two saliva-based, FDA-approved assays are now in use to test for COVID-19 infection, in spite of the fact that no independent, scientific analysis has yet established their effectiveness. Our present work is the first integrative meta-analysis study to review the existing multi-study evidence for validity of the saliva-based approach.

The use of saliva as a diagnostic tool for various systemic conditions is nothing new. Considerable research effort has been made in the past to seek biomarkers in saliva, since its collection is non-invasive and easy. As a result, emerging evidence indicates that whole saliva can be used to identify various oral and systemic conditions [for reviews see (8, 11, 29)]. Importantly, the concept of using saliva to detect viral infections is now well-established (12, 30).

Among RNA viruses, salivary diagnostic tests for Zika are well-established (31, 32) and a number of salivary-based detection methods have been reported for Ebola virus detection (12). The presence of considerable quantities of viral RNA in the saliva of 17 SARS-infected patients has also been shown unequivocally (33). But most studies lack any direct comparison of the sensitivity and specificity of NPS- and saliva-based assays. The one important exception is a study which compared saliva and NPS specimens for the detection of respiratory viruses by multiplex RT-PCR (4). This study, which included results from 236 patients with 11 different viral respiratory infections, including coronaviruses, revealed no significant difference in the sensitivity and specificity of saliva- and NPS-based tests (4). Taken together, although saliva-based diagnostics are supported by a considerable amount of evidence, routine applications are still rare because of the lack of well-standardized protocols.

The source of SARS-CoV-2 in saliva is unknown at present but it could come from multiple locations. One obvious source is debris from the nasopharyngeal epithelium which drains into the oral cavity (17). Secondly, SARS-CoV-2 may actually infect the salivary glands and the virus is then secreted into the saliva from the glands. No information is available on this. But it is of note that during the infection of rhesus macaques by the SARS coronavirus, epithelial cells lining salivary gland ducts are an early target of the virus (34). One consequence of this is the production of SARS-specific secretory immunoglobulin A into the saliva (35). Thirdly, SARS-CoV-2 from blood plasma may access the mouth via the crevicular fluid, an exudate derived from periodontal tissues (36). Fourthly, infected oral mucosal endothelial cells, which show overexpression of ACE2 during SARS-CoV-2 infection, may also contribute to the viral load in saliva (37). Finally, salivary cells may endocytose viruses and virus-containing exosomes from the circulation at their basolateral surface and release them into the salivary lumen by exocytosis. Such mechanisms have been revealed for other macromolecular constituents of the blood, such as DNA and RNA (8). Any or all of these five possible sources may contribute to the appearance of SARS-CoV-2 in the saliva of COVID-19 patients. Given also that the main sites of viral infection (nasal, oral, pharyngeal or respiratory tract) may differ between individuals, it is quite possible that in some patients the virus is more readily detected in the saliva and in others it is more readily detected in an NPS specimen. Such differences might also be related to genomic variations between patients (38). Consequently discrepancies between NPS and saliva test results, rather than indicating a deficiency in one or other test, may be an expected outcome, and it may have implications in terms of assessing asymptomatic carriers (39, 40). Either way, our present level of understanding paves the way for more intensive studies of these important issues, extending well-beyond the design of better diagnostics for SARS-CoV-2 infection (6, 38).

In the present meta-analysis we found that the test sensitivities for SARS-CoV-2 were 91% (CI 80–99%) and 98% (CI 89–100%) for saliva and for NPS samples, respectively, based the pooled event rates among COVID-19 patients. Clearly the two confidence intervals overlap, suggesting that the outcomes of the saliva tests and NPS tests are not very different. There appears to be a slight tendency for NPS tests to be more sensitive but this is not statistically significant. On the other hand, one study reported the opposite tendency with the virus detectable in the saliva but not the NPS sample on a significant number of occasions (21). Although NPS-based SARS-CoV-2 virus detection is currently regarded as the gold standard (2, 41, 42), carefully performed future studies need to be carried out to determine the relative diagnostic accuracies and specificities of the saliva and NPS tests.

At present only two studies have considered the specificity of the saliva tests. In one of those tests only one saliva sample was found to be positive among 50 apparently healthy individuals who were PCR-negative for the NPS test (18). In the other work two individuals were detected positive in saliva tests on 98 participants who were negative for NPS test (21). These results may reflect a real difference in the specificities of the NPS and saliva tests, or they may simply be a consequence of occasional false negatives in the NPS tests.

For optimal saliva-based testing at least three conditions have to be improved by standardization and validation (43). (1) A specific saliva collection method should be selected and optimized after systematically comparing the various methods currently used for collecting whole saliva in other clinical

and scientific contexts. (2) The optimal solution for collecting, transporting and storing saliva samples should be found. (3) The RNA assay method, either RT-qPCR, loop-mediated isothermal amplification (LAMP) or another protocol, should also be optimized for saliva, using an appropriate internal control; this cannot be human DNA which is overwhelming in NPS but not in saliva samples (15–18, 21). In order to obtain a reliable and sensitive saliva test, all of these conditions must be standardized.

Not surprisingly the studies included in our analysis used different sampling methods to collect saliva. This may have had a significant effect on the sensitivity of the saliva test. Azzi et al. used a simple drooling technique to collect saliva and they resuspended the collected specimens in 2 ml of PBS (14). In contrast, To et al. collected saliva specimens that also contained fluid from the posterior oropharynx obtained by coughing up and clearing the throat (17). Another study (18) asked patients to pool saliva in their mouth prior to collection, and to spit 1–2 ml into a collection pot. The act of pooling saliva in the mouth may have stimulated additional saliva secretion, which could have diluted the viral load in the specimen. In this case no transport medium was added to the specimens but, after transportation to the laboratories, liquid Amies medium was added. Wyllie et al. used a self-collection technique: patients were asked to spit repeatedly into a sterile urine cup until one third was full (21). This too could have diluted the sample with additional virus-free saliva. The remaining two studies did not describe the collection method at all (15, 16). Additionally, two of the studies specified that specimens were collected in early morning to avoid anomalies introduced by eating, drinking and tooth brushing (17, 21). The rest of the studies did not specify the time of collection or mention any other confounding factors that may have affected the sample. Taken together, the sample collection protocols of the included studies are quite diverse. But it is promising that even without validated, standardized collection protocols, the studies reviewed here yielded very similar results.

Other factors, such as the type of transport medium, the temperature during transportation, and the time passed between specimen collection and RNA extraction, may also affect the outcome of the tests (43). Unfortunately, there is insufficient information in these few studies to draw any conclusions about the possible effects of these confounding factors on the accuracy of saliva testing for COVID-19 diagnosis (15–18, 21). But again, although the five studies used different RNA isolation methods, and different PCR primers and conditions, it is encouraging to note that the virus could in all cases be detected in saliva samples with a consistently high level of sensitivity.

It is likely that a simple drooling technique, with no specific target volume and no extra stimulation of saliva secretion, will provide the greatest sensitivity if the viral RNA in whole saliva derives mainly from sources other than the secretions of the salivary glands. Drooling is a well-established saliva collection method that is generally recommended for analytical purposes (44). Due to its simplicity, it does not require trained personnel, it can be self-administered, and it can be done at home if necessary. Even in the clinic, the drooling method is safer than nasopharyngeal or oropharyngeal swabbing, with no need for infected swabs to be carried through the air from the patient to the container. The fact that nasopharyngeal swab sampling sometimes has to be repeated in overt COVID-19 patients before a positive result is obtained suggests that the reliability of that sampling method is lower than might be expected from saliva sampling. Moreover, this saliva collecting technique also avoids the mixing of fluids from different anatomical regions such as the oropharynx (14).

In the present meta-analysis the overall sensitivity of the saliva (index) test is assessed by comparison with the NPS (reference standard) test using patient-based pooled data. This simple comparison does not allow us to address any of the more complex questions that arise from the widely varying presentation of different COVID-19 patients. For example, are there significant differences in the sensitivities of the two sampling methods according to the primary location of the infected cells? Are there higher viral loads in the saliva, and is there therefore a higher saliva test sensitivity, in COVID-19 patients who only present with a loss of taste sensation or who are asymptomatic? Are saliva tests more or less sensitive than NPS tests in patients whose infection is mainly localized to the respiratory tract? Correlation studies comparing saliva and NPS viral loads in patients categorized by the nature and severity of their symptoms should be very informative. Time series data on the relative viral loads in the saliva and NPS specimens may be useful in predicting the progression of the disease and in guiding treatment. But, as discussed above, these studies will require careful optimization and standardization, particularly of the saliva collection protocol.

The need for reliable, non-invasive and easy-to-perform tests for COVID-19 has focused special attention on saliva in the last few months. Between 1 January and 25 April 2020, 18 clinical trials involving saliva specimens have started according to the ClinicalTrials.gov registry (**Table S2**). Among these, 13 are non-interventional, focusing on the diagnostic value of various specimens including saliva, and five interventional studies also planned to use saliva as a diagnostic tool, but with a primary focus on evaluating potential treatments for SARS-CoV-2 infections. Unfortunately, these registered clinical trials vary considerably in the amount of information presented about the proposed testing methodology. Neither the non-interventional nor the interventional protocols have clear descriptions of the collection, transportation and storage of saliva samples, and the optimization of the viral RNA assay for saliva specimens. Only a few of them emphasize the necessity for determining the sensitivity and specificity of the saliva-based test. But hopefully, during the course of execution, such studies will yield high quality, reliable data that can be used to address some of the important biological and methodological questions that we have discussed here.

LIMITATIONS

A limitation of the present work is the relatively small number of studies and small sample sizes available regarding this topic. Despite the large number of records found in the systematic search of the literature, only 6 studies could be included. Although intensive research is in progress regarding COVID-19, there are still only a handful articles fulfilling our eligibility criteria. The limited amount of reported data makes it difficult to perform comprehensive analyses and to thoroughly investigate

the causes behind certain trends in the results. Another issue that hinders in-depth analysis is the lack of methodological homogeneity, and the inadequate reporting of methods and outcome parameters. A significant limitation is the lack of false-positive data, based on an independent reference, that would be required for 2 × 2 contingency tables to allow estimation of the test specificities. Thus, the more rigorous statistical methodologies specially developed for meta-analysis of diagnostic test accuracy could not be used in this work.

All studies except two (18, 21) investigated the reliability of the saliva test only among confirmed COVID-19 infected participants, with no healthy individuals or asymptomatic COVID-19 patients recruited for comparison. Additionally, there are several other confounding factors that might have affected the detectability of viral RNA in the saliva, such as the timing and method of sample collection, the choice of transport medium, storage and transport temperatures, the time passed between specimen collection and RNA isolation, and the extraction and PCR kits used for isolation, amplification and detection. None of these factors could be properly addressed in our analysis owing to the lack of information in the reported studies.

CONCLUSION

In the present meta-analysis we provide evidence that saliva tests are a promising alternative to nasopharyngeal swab tests for COVID-19 diagnosis. Optimized and validated saliva assays offer the possibility of reliable self-collection of samples for COVID-19 testing in the future. However, there are many open questions to be answered before the precise specificity and sensitivity of the saliva-based tests can be determined and appropriate standardized procedures introduced into clinical practice.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. This data can be found here: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

AUTHOR CONTRIBUTIONS

LC, SK, ÁN, ZL, ZS, PH, MS, and GV devised the project, the main conceptual ideas and planned the research. LC, SK, NF, ZS, and GV worked out the methodology. IM, AH, and LC performed the data collection, literature search, study selection, and data extraction. LC, IM, and AH also organized and maintained research data for analysis. NF performed analytic calculations and applied statistical models for synthetizing data. ZS and SK also aided the research by interpretation of raw and synthetized data. NF visualized synthetized data into forest plots. LC and GV worked on summarizing results into figures and tables. SK and GV were responsible for managing and coordinating the research activity. PH and GV took leadership responsibility for the research activity, provided resources, and acquired financial support for the research project. ZS, PH, MS, and GV validated reproducibility of the results. LC, NF, IM, AH, and GV wrote the manuscript with input from all authors. SK, ÁN, ZL, ZS, PH, MS, and GV extensively reviewed the work and further edited the manuscript. All authors contributed to the article and approved the submitted version.

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An earlier draft of this manuscript has been released as a pre-print at medRxiv.org (45).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00465/full#supplementary-material

Table S1 | PRISMA checklist.

Table S2 | Characteristics of clinical trials including saliva as a diagnostic tool for COVID-19, registered on ClinicalTrials.gov.

Table S3 | Summary of risk-of-bias and applicability concerns in included studies.

Table S4 | Detailed summary of risk of bias and applicability across studies.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Table S1. PRISMA checklist.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4-5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7-8
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	-
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	11-13
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	-
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	13-19
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	19
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	20
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	20

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Table S2. Characteristics of clinical trials including saliva as a diagnostic tool for COVID-19, registered on ClinicalTrials.gov

ID	NCT04332107	NCT04321174	NCT04352959	NCT04354259	NCT04276688
Recruiting Status	Recruiting	Recruiting	Recruiting	Recruiting	Completed
Study type	interventional	interventional	interventional	interventional	interventional
Number of Centers and Study Design	Single center, interventional, randomized (RCT), parallel assignment, quadruple masking, phase 3	Multi-locations, interventional, randomized (RCT), parallel assignment, single masking (outcomes assessor), phase 3	Multi-locations, interventional, randomized (RCT), parallel assignment, triple masking	Single center, interventional, randomized (RCT), parallel assignment, none masking, phase 2	Single center, interventional, randomized (RCT), parallel assignment, none masked, Phase 2
Location	the USA	Canada	France	Canada	China
Population	Subjects with positive SARS-CoV-2 test results received within the previous three days, but not hospitalized (n=2271)	1) High risk close contact with a confirmed COVID-19 case during their symptomatic period, 2) Successfully contacted by the study team within 24 hours of study team notification of the relevant index COVID-19 case (n=1220)	Patients with clinical diagnosis of Covid-19 infection (n=178)	1) For ambulatory cohort: patients confirmed COVID-19 infection by PCR within 5 days of symptom onset discharged to home isolation, 2) For hospitalized cohort: SARS-CoV-2 RNA-positive on nasopharyngeal swab / respiratory specimen within 5 days of symptom onset admitted to hospital for management of COVID-19 (n=140)	Subjects include patients hospitalized for confirmed 2019-n-CoV infection, temperature ≥38°C with another symptoms upon admission (n=127)
Intervention	Single oral 1g dose of Azythromycin	Lopinavir/Ritonavir 400/100 mg twice daily for 14 days	Mouthrinse with bêta-cyclodextrin and citrox 3 daily mouthrinses for 7 days	Single dose of peginterferon lambda 180µg sc at baseline for ambulatory cohort and peginterferon lambda 180µg sc at baseline and a second dose on day 7 for hospitalized cohort	Lopinavir/Ritonavir 400/100 mg twice daily for 14 days, Ribavirin 400 mg twice daily for 14 days and IFN-beta-1B 0.25 mg sc injection alternate day for 3 day / Nasopharyngeal swab, saliva, urine, stool and blood sampling
Comparison	placebo	no intervention	Placebo: mouth rinse without antiviral	No specific therapy for ambulatory cohort and the best supportive care for hospitalized cohort	Lopinavir/Ritonavir 400/100 mg twice daily for 14 days
Primary Outcomes	All-cause hospitalization or emergency room stay of >24 hours	The primary outcome is microbiologically confirmed COVID-19 infection, ie. detection of viral RNA in a respiratory specimen (mid-turbinate	Change from baseline amount of SARS-CoV-2 in salivary samples at 7 days	1) The proportion of participants with negative SARS-CoV-2 RNA on nasopharyngeal swab, Nasopharyngeal swab, saliva and blood sampling. 2) Rate of combined treatment-emergent	Time to negative nasopharyngeal swab

		swab, nasopharyngeal swab, sputum specimen, saliva specimen, oral swab, endotracheal aspirate, bronchoalveolar lavage specimen) by day 14 of the study.		and treatment-related severe adverse events	
Secondary outcome	viral load by self-collected nasal swab, Viral load by self-collected saliva swab	-	Change from Baseline amount of SARS-CoV-2 virus in nasal samples at 7 days	-	Time to negative saliva 2019-n-CoV RT-PCR

Table S2. Continued

ID	NCT04360811	NCT04354610	NCT04361604	NCT04325919	NCT04351646
Recruiting Status	Recruiting	Recruiting	Not yet recruiting	Recruiting	Recruiting
Study type	non-interventional	non-interventional	non-interventional	non-interventional	non-interventional
Number of Centers and Study Design	Single center, observational, non-randomized (NRCT), parallel assignment, none masked	Multi-locations, observational, single group assignment, none masked	Single center, observational, cohort, prospective	Observational	Single center, observational, case-control, prospective
Location	France	France	France	China	UK
Population	1) Unexposed group: COVID 19 negative pregnant woman, 2) Exposed group: COVID 19 positive (symptomatic and asymptomatic) pregnant woman (n=3600)	Patients hospitalized for critical form of Covid-19 infection within 3 days (n=57)	1) Patients co infected HIV and SRAS-CoV2 (n=250), 2) Patients infected HIV without COVID-19 (n=20)	Patients with laboratory-confirmed COVID-19, (n=170) Patients hospitalized for pneumonia tested negative for COVID-19 are controls	1) SARS-CoV-2 negative inpatients, 2) SARS-CoV-2 positive inpatients, 3) SARS-CoV-2 suspected or confirmed SARS-CoV-2 positive cases amongst health care professionals and lab staff (n=500)
Intervention	N/A	N/A	N/A	N/A	N/A
Comparison	N/A	N/A	N/A	N/A	N/A
Primary Outcomes	Exposure to SARS-CoV-2 will be measured the day of delivery by RT-PCR on maternal saliva and by serology on maternal blood	1) Worsening of renal function by at least KDIGO grade 1 during hospitalization for Covid-19 infection, 2) Troponin greater than 99th percentile during hospitalization for Covid-19 infection	Describe the course of COVID-19 disease in patients infected with HIV, biological sampling (blood, saliva, rectal swab (stool swab), urine, nasopharyngeal swab, conjonctival swab, semen	Patients' treatment and management during hospitalization. Serial viral load changes during hospitalization. Collection of blood, stool, rectal swab, urine, saliva, nasopharyngeal aspirate/flocked swab, sputum/tracheal aspirate	Antibody titres to SARS-CoV-2 at specified days post baseline samples (Nasopharyngeal swab, blood and saliva sampling)
Secondary outcome	Description of the number of positive COVID-19 RT-PCRs in the conception products: amniotic fluid, frozen placenta fragment, frozen fetal tissue, cord blood or frozen cord fragment	Blood samples, **saliva** collect ion, and urine collection to carry out biomarker assays and for the constitution of a biological collection.	-	-	-

Table S2. Continued

ID	NCT04337424	NCT04357977	NCT04356586	NCT04355533	NCT04362150
Recruiting Status	Recruiting	Recruiting	Enrolling by invitation	Recruiting	Recruiting
Study type	non-interventional	non-interventional	non-interventional	non-interventional	non-interventional
Number of Centers and Study Design	Single center, observational, case-control, prospective	Multi-locations, observational, cross-sectional	Single center, observational, cohort, prospective	Single center, observational, non-randomized (NRCT), single group assignment, none masked	Single center, observational, cohort, prospective
Location	France	USA	Belgium	France	USA
Population	1) Patients diagnosed positive, 2) Healthcare staff presumed negative for SARS-CoV-2 (n=180)	Patients and study staff at the testing site who have been flagged for COVID-19 testing or who are being treated for COVID-19 (n=300)	Healthcare workers with mild symptoms for Covid-19 (n=300)	Children hospitalized since at most 4 days and their parents (n=1920)	Individuals with positive test for COVID-19 who have recovered from acute infection (wide spectrum of age, race, gender and disease severity) (n=800)
Intervention	N/A	N/A	N/A	N/A	N/A
Comparison	N/A	N/A	N/A	N/A	N/A
Primary Outcomes	Comparison of LAMP test with reference RT-PCR on viral detection (Saliva and nasopharyngeal swab sampling)	RBA-2 saliva monitoring device development. Nasopharyngeal swab and saliva sample. The comparison of the results obtained from the current testing methods will be used to calibrate machine learning algorithms of the RBA-2	1) Percentage of serological positive healthcare workers, 2) Percentage of healthcare workers with positive saliva swabs	Seroconversion against SARS-CoV2 in children, Nasopharyngeal, rectal swabs, saliva and blood sampling	Demographic data on participants and Proportion of participants previously hospitalized. Whole blood, peripheral blood mononuclear cells, plasma, serum and saliva.
Secondary outcome	-	-	-	-	-

Table S2. Continued

ID	NCT04357327	NCT04336215	NCT04348240
Recruiting Status	Recruiting	Recruiting	Recruiting
Study type	non-interventional	non-interventional	non-interventional
Number of Centers and Study Design	Single center, non-randomized (RCT), parallel assignment, single masking	Multi-locations, observational, cohort, prospective	Single center, observational, cohort, prospective
Location	Italy	USA	USA
Population	1) Patients with symptoms associated with COVID-19, 2) Asymptomatic patients with low risk phenotype (n=100)	1) Healthcare workers (n=500), 2) Non-healthcare workers: faculty staff and students, who do not have patient contact (n=250), 3) Multigenerational household members, who test positive and negative for SARS-CoV-2 (n=540)	1) Asymptomatic high-risk subjects with known history of close personal contact with a COVID-19 positive person not tested (SARS-CoV2 status unknown), 2) Asymptomatic or mildly symptomatic subjects who are COVID-19 positive, 3) COVID-19 positive individuals retesting negative (n=60)
Intervention	N/A	N/A	N/A
Comparison	N/A	N/A	N/A
Primary Outcomes	1) Sensibility after 10 minutes for salivary test and after 6 hours for the nasopharyngeal swab, 2) Specificity after 10 minutes for salivary test and after 6 hours for the nasopharyngeal swab	1) Prevalence, 2) Incidence, Nasopharyngeal swab, saliva and blood sampling	Determination of SARS-CoV-2 viral load and infectivity in saliva that may contribute to asymptomatic transmission. Collection of nasal and oral secretions and droplets produced by participants while they speak
Secondary outcome	-	-	-

Table S3. Summary of risk-of-bias and applicability concerns in included studies.

STUDY	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Azzi et al. (2020)	✓	✗	✓	✗	✓	✓	✓
Bae et al. (2020)	✓	✗	✓	?	✓	?	?
Fang et al. (2020)	✓	✗	✓	?	✓	?	✓
To et al. (2020)	✓	✗	?	?	✓	✓	✓
Williams et al. (2020)	?	?	?	✗	✓	✓	?
Not included in the quantitative analysis:							
Wyllie et al. (2020)	✓	?	?	?	✓	✓	✓

✓ = Low Risk ✗ = High Risk ? = Unclear Risk

Table S4. Detailed summary of risk of bias and applicability across studies.

Risk of bias	**Yes**	**No**	**Unclear**
DOMAIN 1: PATIENT SELECTION			
Was a consecutive or random sample of patients enrolled?	6	0	0
Was a case-control design avoided?		N/A	
Did the study avoid inappropriate exclusions?	5	0	1
Could the selection of patients have introduced bias?	**5**	**0**	**1**
DOMAIN 2: INDEX TEST(S)			
Were the index test results interpreted without knowledge of the results of the reference standard?	0	4	2
If a threshold was used, was it pre-specified?	0	0	6
Could the conduct or interpretation of the index test have introduced bias?	**0**	**4**	**2**
DOMAIN 3: REFERENCE STANDARD			
Is the reference standard likely to correctly classify the target condition?	4	0	2
Were the reference standard results interpreted without knowledge of the results of the index test?	3	0	3
Could the reference standard, its conduct, or its interpretation have introduced bias?	**3**	**0**	**3**
DOMAIN 4: FLOW AND TIMING			
Did all patients receive a reference standard?	6	0	0
Did patients receive the same reference standard?	6	0	0
Were all patients included in the analysis?	5	1	0
Could the patient flow have introduced bias?	**0**	**2**	**4**
Applicability concerns	**Low**	**High**	**Unclear**
DOMAIN 1: PATIENT SELECTION			
Is there concern that the included patients do not match the review question?	**6**	**0**	**0**
DOMAIN 2: INDEX TEST(S)			
Is there concern that the index test, its conduct, or interpretation differ from the review question?	**4**	**0**	**2**
DOMAIN 3: REFERENCE STANDARD			
Is there concern that the target condition as defined by the reference standard does not match the review question?	**4**	**0**	**2**

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Hyaluronic Acid Is an Effective Dermal Filler for Lip Augmentation: A Meta-Analysis

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Introduction: The lips and the mouth play an indispensable role in vocalization, mastication and face aesthetics. Various noxious factors may alter and destruct the original structure, and appearance of the lips and the anatomical area surrounding the mouth. The application of hyaluronic acid (HA) may serve as a safe method for lip regeneration. Although a number of studies exist for HA effectiveness and safety, its beneficial effect is not well-established.

Aim: The present meta-analysis and systematic review was performed to investigate the effectiveness of HA on lip augmentation. We also investigated the types and nature of adverse effects (AEs) of HA application.

Methods: We reported our meta-analysis in accordance with the PRISMA Statement. PROSPERO protocol registration: CRD42018102899. We performed the systematic literature search in CENTRAL, Embase, and MEDLINE. Randomized controlled trials, cohort studies, case series and case reports were included. The untransformed proportion (random-effects, DerSimonian-Laird method) of responder rate to HA injection was calculated. For treatment related AEs descriptive statistics were used.

Results: The systematic literature search yielded 32 eligible records for descriptive statistics and 10 records for quantitative synthesis. The results indicated that the overall estimate of responders (percentage of subjects with increased lip fullness by one point or higher) was 91% (ES = 0.91, 95% CI:0.85−0.96) 2 months after injection. The rate of responders was 74% (ES = 0.74, 95% CI:0.66−0.82) and 46% (ES = 0.46, 95% CI:0.28−0.65) after 6 and 12 months, respectively. We included 1,496 participants for estimating the event rates of AEs. The most frequent treatment-related AEs were tenderness (88.8%), injection site swelling (74.3%) and bruising (39.5%). Rare AEs included foreign body granulomas (0.6%), herpes labialis (0.6%) and angioedema (0.3%).

Conclusion: Our meta-analysis revealed that lip augmentation with injectable HA is an efficient method for increasing lip fullness for at least up to 6 months after augmentation. Moreover, we found that most AEs of HA treatment were mild or moderate, but a small number of serious adverse effects were also found. In conclusion, further well-designed RCTs are still needed to make the presently available evidence stronger.

Keywords: hyaluronic acid, dermal filler, lip augmentation, effectiveness, adverse effects

INTRODUCTION

The lips and the mouth have a crucial functional importance in vocalization and mastication. Additionally, they also play an important role in the aesthetics of the face (1, 2). Particularly, lip fullness is a key factor associated with attractiveness, beauty and youth (2, 3). A number of noxious and hereditary factors contribute to the deterioration of the perioral tissues with age (2, 4–7). Consequently, volume loss of the lips may occur with other signs of aging, such as the appearance of perioral lines, marionette lines and flattening of the cupid bow (2). There are several surgical and non-surgical reconstructive procedures aiming to restore oral competence, anatomical structures and to provide appealing aesthetic outcomes and in order to be more attractive (6, 8).

Theoretically, there is a wide range of possible reconstructive methods that can be applied to rebuild damaged tissues such as tissue engineering using stem cells (9–11), gene therapy (12, 13) and artificial biocompatible scaffolds (14, 15), but their use has been not well-established in routine clinical settings. Among the non-surgical regenerative and reconstructive procedures, hyaluronic acid (HA)-based dermal filling is one of the most frequently used treatments (16, 17). Its advantages over other filling materials include its natural occurrence, which provides non-immunogenic properties (18). It also exerts an antioxidant effect (19, 20), and anti-inflammatory activity (18, 21). Additionally, HA highly supports tissue regeneration and wound-healing by providing a suitable structure for cell ingrowth (22, 23). Due to its multiple advantageous properties, HA is also broadly used in other areas of tissue regeneration, such as orthopedics to treat osteoarthritis and rheumatoid arthritis (24, 25). Moreover, it is utilized in ophthalmology, dermatology (26), as well as in certain dental procedures (27–29).

The initial production of HA from animal sources was shifted to bacterial production. In this process, various genetically modified bacteria such as *B. subtilis* and Group A and C *Streptococci* are used to produce HA, which is then extracted and chemically further modified to create cross-links between HA polymers (30, 31). This advancement in production greatly contributed to its recent success with decreased manufacturing costs, increased purity of the products, and decreased immune reactions (32).

Since the approval of the first non-animal based HA in 2004 (8) several clinical trials aimed to reveal its true potentials. HA is believed to be an excellent candidate for soft tissue augmentation to restore lip fullness, cosmetic asymmetries and to deal with rhytids due to the loss of elasticity of connective tissue (7). However, clinical studies investigating effectiveness were conducted with small sample sizes and with short follow-up periods. Therefore, conclusions rely on weak evidence, including high levels of uncertainty.

No meta-analysis has been conducted to determine the effectiveness of HA for lip augmentation and to confirm its long-term aesthetic results. Thus, the main objective of the present meta-analysis and systematic review was to increase the power and precision of the estimated HA effect on lip augmentation. Secondarily, we investigated the number and nature of adverse effects (AEs) of HA published in the literature.

MATERIALS AND METHODS

Protocol and Registration

This meta-analysis was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement (33) using similar approaches that we have recently reported (34–36). The PRISMA checklist summarizing the content of this review is enclosed in the supporting information (**Supplementary Table 1**). The meta-analysis was registered in PROSPERO (International Prospective Register of Systematic Reviews), 10/12/2018, Registration Number: CRD42018102899. There were no deviations from the study protocol.

Eligibility Criteria

The PICO (patient characteristics, type of intervention, control, and outcome) format was applied to investigate the following clinical questions: (1) To what extent are hyaluronic acid dermal fillers effective for lip augmentation? (2) What are the common and also the rare treatment-related adverse effects of HA application?

For analysis, we considered records published in scientific journals meeting the requirements of our selected PICO. Patient characteristics: subjects above 18 years having a minimal, mild or moderate score on a validated lip fullness scale. Type of intervention: injecting hyaluronic acid dermal filler into the lips and perioral area to increase lip fullness and enhance aesthetic appearance. Control: base-line control—baseline values of lip fullness recorded before treatment. Lip fullness values recorded after treatment were compared to baseline values. The effectiveness was evaluated as the rate of responders. A responder was defined as a participant with at least one grade improvement on a validated lip fullness scale. Outcome, primary: effectiveness measurement, i.e., the number of responders at each check-up; secondary: number and type of AEs related to treatment.

Inclusion and Exclusion Criteria

Publications which met the following eligibility criteria were included: (1) randomized controlled trials, cohort studies or case series and case reports; (2) intervention: hyaluronic acid used for lip augmentation; (3) healthy adult participants; (4) records written in English or available in English translation; (5) site of injection: lips; (6) use of validated scale to measure outcome. Exclusion criteria were: (1) filling material other than hyaluronic acid; (2) site of injection other than lips and perioral area; (3) Previous facial surgery, permanent facial implants or any facial cosmetic procedure in the last 24 months.

Information Sources and Search

A systematic search limited to English language records was performed in three different major electronic databases [Cochrane Central Register of Controlled Trials (CENTRAL), Embase and MEDLINE (*via* PubMed)] on 31 December, 2018. Besides electronic databases, an extensive hand search in the reference list of relevant articles and included records were also performed to find eligible records. Gray and black literature was not considered for this meta-analysis. "Hyaluronic acid" and "lip" search terms and their synonyms were used in each database adapted to their specific search engines. **Supplementary Table 2** contains the detailed search quey.

Study Selection

The EndNote (Clarivate Analytics, Philadelphia, US, version: X9.3.3) reference manager was used to organize and manage records. After removing duplicates, the remaining records were screened for suitability by two authors (L.M.C. and S.F.), in duplicate, based on the titles and abstracts of the published original papers. The eligibility of full texts of the remaining records was assessed by the same two review authors independently. Disagreements between reviewers were resolved by discussion or, if it was necessary, by consulting a third review author (G.V.).

Data Collection Process and Data Items

Data extraction was performed by two authors independently (L.M.C. and S.F.) using a preconstructed standardized data extraction form. The following information was extracted: first author's name, year of publication, sample size, age and gender distribution, study design, type of HA used, site of injection, follow-up period, type of validated scales used for evaluation, outcome (rate of responders, number and type of AEs). In case of disagreement, a third author (G.V.) was also involved.

Risk of Bias Assessment

Quality and risk of bias of the RCTs were evaluated by two authors (L.M.C. and S.F.) independently. Assessment was based on the recommendation of the Cochrane Collaboration, the Cochrane Risk of Bias assessment tool (37, 38). In case of disagreement a third author was involved (G.V.). Studies were evaluated according the domains specified in the Cochrane Handbook for Systematic Reviews of Interventions (38).

Cohort studies were evaluated based on the Newcastle Ottawa Scale (NOS) for Cohort Studies (39). We slightly modified the original NOS scale. We removed "Ascertainment of exposure" subdomain from Selection domain. Thus, in the Selection domain three sub domains remained: "Representativeness of the exposed cohort," "Selection of the non-exposed cohort," and "Demonstration that outcome of interest was not present at start of study." Scores for these subdomains were given according to the original NOS scale (39). Hence the maximum score was three, one and three stars for Selection, Comparability and Outcome domains, respectively. In the outcome 6 months or more of follow-up was considered acceptable. Drop-out below 10% was considered adequate. **Supplementary Table 3** summarizes the modified NOS.

Summary Measures and Synthesis of Results

Untransformed proportions with 95% confidence intervals (CIs) were calculated for the rate of responders. A responder is defined as a participant with at least one grade improvement on a validated lip fullness scale compared to its baseline value. For analyzing AEs we used descriptive statistics, summing the sample sizes of included studies and the incidence of each AEs described in any of the included publications. The number of participants was chosen as statistical unit.

We only considered results credible if raw data for meta-analysis could be drawn from at least three records. We applied the random effect model with DerSimonian-Laird method. I^2 and chi-square tests were used to quantify statistical heterogeneity and gain probability-values, respectively; $p < 0.1$ indicated significant heterogeneity (38). All statistical analyses were performed using STATA 15.0.

Publication Bias

We constructed funnel plots and performed visual inspection of their results to check for publication bias.

Certainty of Evidence Pont

The GRADE approach was followed to evaluate the quality and certainty of evidence (37, 40). Assessment was performed independently by two review authors (L.M.C and S.F.).

RESULTS

Study Selection

During the study selection process, we identified a total of 326 records. After removing duplicates, 259 items remained. During the screening process, 176 records were excluded due to various reasons such as filler material other than HA ($n = 30$) or different injection sites ($n = 7$), focusing on novel methods of injection ($n = 24$), investigating the effect of hyaluronic acid in special implications outside the scope of this meta-analysis ($n = 64$), review articles ($n = 17$) or miscellaneous ($n = 34$). Afterwards, 83 full text records were searched. Out of these publications, 32 were included in the qualitative synthesis and 10 in the quantitative synthesis assessing the effectiveness of lip augmentation (**Figure 1**).

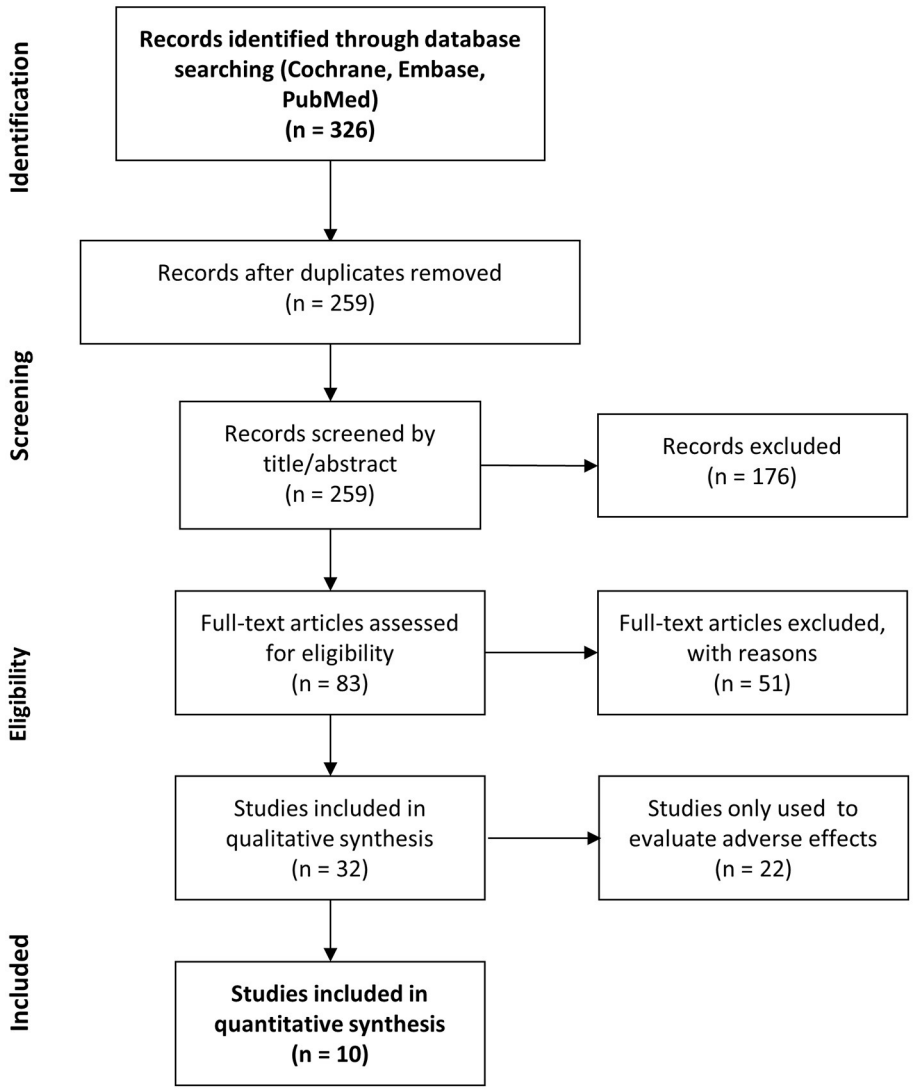

FIGURE 1 | PRISMA flow chart. Summarizing the study selection process.

Study Characteristics

Description of the Included Studies

We included 5 RCTs (41–45) and 5 cohort studies (46–50) to analyze the effects of HA on lip augmentation. Two additional RCTs (51, 52), six additional cohort studies (53–58) and 14 case reports (59–72) were included for assessing AEs.

In the effectiveness analysis, a total of 1,228 participants were included. Subjects aged 18 or older desiring lip augmentation, had lip fullness of minimal, mild or moderate on a validated lip fullness scale were included. In the study population all Fitzpatrick skin types have been represented. Exclusion criteria included a history of allergy to injectable HA, history to any semi-permanent or permanent tissue augmentation or aesthetic surgery or any temporary dermal filler treatments in the last 24 months in the facial region. Subjects with scarce or significant abnormalities of the lips were also excluded. The mean age of subjects in the studies varied between 41 and 54 years. Altogether 4 different injectable HA products were used, Juvéderm and Restylane were the two most commonly applied ones. Follow-up periods varied between 12 and 48 weeks. All included records utilized a validated lip fullness scale such as the Medicis Lip Fullness Scales (73) or the Allergan Lip Fullness Scale (74).

AEs were collected and assessed from a total of 32 records including 1,488 participants and more than 12 different HA products. In all includes studies the lips and perioral lines were the site of injection. A tabulated summary of the characteristics of the included studies and HA products is provided in **Tables 1–3** and **Supplementary Table 4**.

Description of Excluded Studies

During full-text analysis, we excluded 51 records. Seven RCTs, nine cohort studies, 11 case reports and nine review articles.

TABLE 1 | Study characteristics of records included in effectiveness analysis.

References	Study design	*n**	Female ratio	Age: mean ± SD (median) (range)	Intervention	Control	Maximum injected volume; injection technique	Follow-up (months)	Outcome measure
Beer et al. (41)	RCT, multicentre, evaluator blinded	199	97%	45.5	Restylane-L	No-treatment group	2.17 ml (mean); anterograde, retrograde linear threading, serial puncture	6	MLFS and WASULL, GAIS, TEAEs
Chopra et al. (46)	Cohort, multicentre, open label, prospective	57	93%	46.5, (23–72)	Restylane-L	Baseline-controlled	1–3 ml (range); submucosa, retrograde, anterograde linear, fanning	3	GAIS, MLFS, TEAEs
Dayan et al. (42)	RCT, multicentre, evaluator blinded	208	95.8%	(49), (20–79)	Juvéderm Ultra XC (HYC-24L)	No-treatment group	4.8 ml (max); linear threading, serial puncture, fanning, crosshatching	12	ALFS, POL, OCS, ISRs, AEs
Eccleston et al. (47)	Cohort, multicentre, open label, prospective	59	100%	50, (21–74)	Juvéderm Volbella	Baseline-controlled	1.3 ml (median); retrograde, tunneling, crosshatching	12	ALFS, AEs
Fagien et al. (48)	Cohort, multicentre, evaluator blinded, prospective	50	96%	(47), (24–68)	Juvéderm Ultra	Baseline-controlled	2.2 ml (median), 2.3 ml (max); retrograde, anterograde, tunneling, serial puncture	12	ALFS, OCS, POL, CTR, AEs
Geronemus, et al. (43)	RCT, multicentre, evaluator blinded	224	96.9%	(54), (22–78)	Juvéderm Volbella XC (VYC-15L)	Restylane-L	2.5 ml (median); subdermal, intradermal, tunneling, puncture	12	ALFS, POLSS, POLM, OCS, GAIS, AEs
Glogau et al. (44)	RCT multicentre, evaluator blinded	135	99%	47.6 ± 10.6, (50.0), (18.0–65.0)	Restylane	No-treatment group	1.5 ml (max), 0.3–2.5 ml (range); linear injection technique, serial puncture	6	MLFS, GAIS, AEs
Raspaldo et al. (45)	RCT multicentre, evaluator blinded	268	97.1%	(48), (18–76)	Juvéderm Volbella (with Lidocaine)	Restylane-L	1.97–1.86 ml (mean); intradermal, subdermal, tunneling	12	ALFS, POL, OCS, AEs, ISRs,
Solish and Swift (49)	Cohort, multicentre, evaluator blinded, prospective	18	86%	41.1 ± 11.4, (40), (26–65)	Restylane	Baseline-controlled	1.5 ml (max); anterograde, vertical, deposition formation	3	MLFS, GAIS, AEs
Yazdanparast et al. (50)	Cohort, single center, open label, prospective	10	100%	(28–45)	Hyamax Kiss	Baseline-controlled	1 ml (max); retrograde	6	MLFS, IGA, VAS, AEs

**Number of participants included in the MA analysis (Exclusion due to study groups using different filling material or other anatomical sites.).*

AEs, Adverse events; ALFS, Allergan Lip Fullness Scale; CTR, common treatment-site responses; GAIS, Global Aesthetic Improvement Scale; IGA, Investigator's Global Assessment; ISRs, Injection site responses; MLFS, Medicis Lip Fullness Scale; OCS, Oral Commissure Severity Scale; POL/POLSS, Allegran Perioral Severity Scale; POLM, Allergan Perioral Lines at Maximal Contraction scale; SP, Standardized photography; TEAE, treatment-emergent adverse events; VAS, Visual Analog Scale; WASULL, Wrinkle Assessment Scale of Upper Lip Lines.

TABLE 2 | Study characteristics of RCTs and cohort studies only included in adverse effect analysis.

Study	Study design	*n**	Female ratio	Age: mean ± SD (median) (range)	Intervention	Control	Statistics	Follow-up (weeks)	Outcome
Artzi et al. (53)	Cohort, multicenter, retrospective	3[†]	90%	49.6, (28–70)	Juvéderm Volbella (Allergan)	No control group	Spearman correlation	96	Immediate and delayed AEs
Carruthers et al. (54)	Cohort, single center, open label	15	100%	(40.50), (33–60)	Restylane	No control group	Descriptive statistics	24	SP, AEs
Carruthers et al. (52)	Randomized, parallel-group, multicentre, clinical trial	23	100%	48.4 ± 5.5	Juvéderm Ultra, Juvéderm Ultra Plus	OnabotulinumtoxinA, OnabotulinumtoxinA plus hyaluronic acid	Kruskal-Wallis test, Wilcoxon rank sum test	24	GAIS, CIS, AEs
Downie et al. (51)	Randomized, parallel-group, double blinded, single-center, clinical trial	23	100%	(25–55)	Perlane	Various collagen fillers	Kruskal Wallis Rank Sum test	48	2D and 3D facial image analysis, AEs
Fischer et al. (55)	Cohort, multicenter, retrospective	146	98.6%	44.7 ± 14.6	CPM-HAL1 and CPM-HAL2 (Belotero Balance Lidocaine)	No control group	Descriptive statistics	16	Merz scale, GAIS, VAS, AEs
Philipp-Dormston et al. (56)	Cohort, multicenter, open label, prospective	60	88.7%	39.7 (21–75)	Juvéderm Volbella	No control group	Descriptive statistics	4	4-grade scale for subject and injector satisfaction, AEs
Rzany et al. (57)	Cohort, multicenter, open label, prospective	76	94.8%	54.5 ± 8.2	Emervel	No control group	Descriptive statistics	24	GAIS: LRS, LFGS, satisfaction questionnaires, AEs
Samuelson et al. (58)	Cohort, multicenter, evaluator blinded, prospective	29	100%	36, (19–59)	Restylane Lip Volume	Baseline-controlled	Proportion with 95% CI	36	GAIS, MLFS, AEs

**Number of participants included in the MA analysis (Exclusion due to study groups using different filling material or other anatomical sites.*

[†]Study population number is 400 (mean age: 49.6, range: 28–70), however only 3 patients received lip augmentation with HA filler.

AEs, Adverse events; CIS, Cosmetic Improvement Scale; GAIS, Global Aesthetic Improvement Scale; ISRs, Injection site responses; LFGS, Lip Fullness Grading Scale; LRS, Lemperle Rating Scale; MLFS, Medicis Lip Fullnes Scale; RCT, Randomized controlled trial; SP, Standardized photography; VAS, Visual Analog Scale.

TABLE 3 | Characteristics of hyaluronic acid dermal fillers assessed in the analysis.

Product name	Concentration	Composition	References	Source of information
Belotero intense lidocaine	25 mg/ml	Cross-linked	Fischer et al. (55)	(75)
Emervel (range of products)	20 mg/ml	Cross-linked to various degree	Rzany et al. (57)	(57)
Hyamax Kiss	22 mg/ml	500 μm particle size, cross-linked	Yazdanparast et al. (50)	(50)
Juvéderm Ultra	24 mg/mil (0.3% Lidocaine)	Cross-linked (6%)	Fagien et al. (48); Carruthers et al. (52)	(48, 54)
Juvéderm Ultra XC (HYC-24L)	24 mg/mil (0.3% Lidocaine)	Cross-linked	Dayan et al. (42); Bulam et al. (60)	(42)
Juvéderm Volbella without Lidocaine	15 mg/ml	Not available	Eccleston et al. (47); Artzi et al. (53)	(47)
Juvéderm Volbella with Lidocaine	15 mg/ml HA (0.3% Lidocaine)	Cross-linked	Raspaldo et al. (45); Philipp-Dormston et al. (56)	(45, 76)
Juvéderm Volbella XC (VYC-15L)	15 mg/ml HA (0.3% Lidocaine)	Cross-linked, low- and high-molecular-weight HA	Geronemus et al. (43)	(77)
Perlane	20 mg/ml	Cross-linked, 1,000 μm particle size	Downie et al. (51)	(51, 78)
Restylane (without lidocaine)	Not available	SGP, 300 μm particle size, cross-linked	Glogau et al. (44); Solish and Swift (49); Carruthers et al. (54); Fernández-Aceñero Ma et al. (68); Anatelli et al. (59); Curi et al. (61); Dougherty et al. (62); Leonhardt et al. (70); Wolfram et al. (72); Farahani et al. (66); Edwards et al. (64)	(44, 49)
Restylane-L	20 mg/ml HA (0.3% Lidocaine)	SGP, cross-linked	Raspaldo et al. (45); Geronemus et al. (43); Beer et al. (41); Chopra et al. (46)	(41, 43, 45, 46, 79)
Restylane lip volume	20 mg/ml HA (0.3% Lidocaine)	Cross-linked	Samuelson et al. (58)	(58)
HA not further specified	N/A	N/A	Duhovic and Duarte-Williamson (63); Eversole et al. (65); Feio et al. (67); Grippaudo et al. (69); Martin et al. (71)	N/A

N/A, not applicable; HA, hyaluronic acid.

Three records had non-English texts. Additionally, in 11 cases we found no full text to the records and 1 record was a non-interventional study. Out of the 32 articles included in the analysis of AEs, we excluded 22 publications from the effectiveness analysis. Several excluded articles did not report sufficient information on effectiveness, while the others used incomparable scales to measure the effectiveness of lip augmentation.

Risk of Bias Within Studies

All RCTs applied means of random sequence generation. However, in the case of Carruthers et al. (52) and Dayan et al. (42) the methods used for allocation concealment were not clearly described. Due to the nature of the intervention, none of the studies applied blinding of personnel. On the other hand, the outcome assessment was performed by blinded evaluators in all studies. In the case of one study (42) attrition bias was unclear due to ambiguous reporting on lost to follow-ups. In another study (52) we found a high risk of attrition bias due to the 23% of dropouts. The level of reporting bias was low in all studies except three. Study protocols for Beer and coinvestigators (41), Carruthers et al. (52) and Glogau et al. (44) were not found. However, no intext evidence of reporting bias was found. **Figure 2**, **Supplementary Figure 1**, and **Supplementary Table 5** contain the summary of the risk of bias assessment of the RCTs.

Bias in the observational studies was assessed based on the Newcastle Ottawa Scale (39). Observational studies did not have control groups. Instead, they measured the rate of responders only within the treatment group (baseline controlled). The average bias assessment score of the studies was 5.5 ± 1.3 stars on the modified seven-point scale. All 11 publications, earned three stars for selection (46–50, 53–58). Five studies received no starts for comparability (53–57). In three studies (54, 56, 58) the outcome assessments were only self-reports. One study (56) was considered to have inadequately short follow-ups for valuable results, while three studies (53, 55, 56) did not give any explanation for drop-outs. **Supplementary Tables 6, 7** show the summary of the risk of bias assessment of observational studies.

Results of Individual Studies and Their Synthesis

Hyaluronic Acid Treatment Effectively Increases Lip Fullness

Two months after HA injection the overall pooled rate of responders, i.e., the percentage of participants with at least one grade improvement on a validated lip fullness

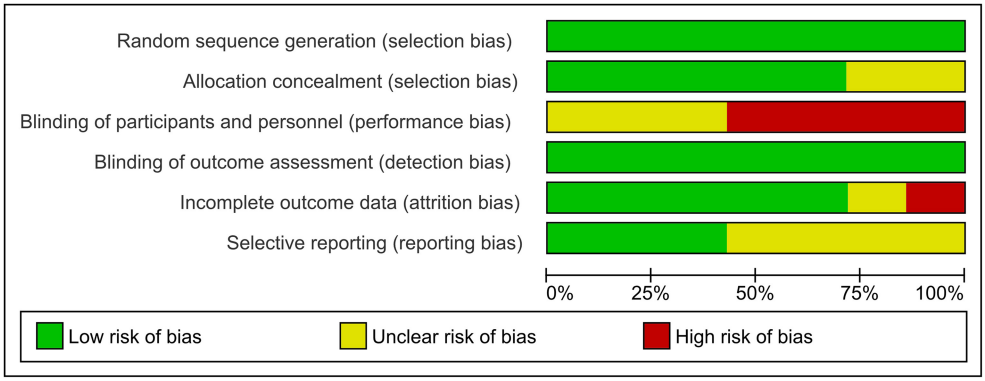

FIGURE 2 | Risk of bias graph. Representing the portion of bias in each domain.

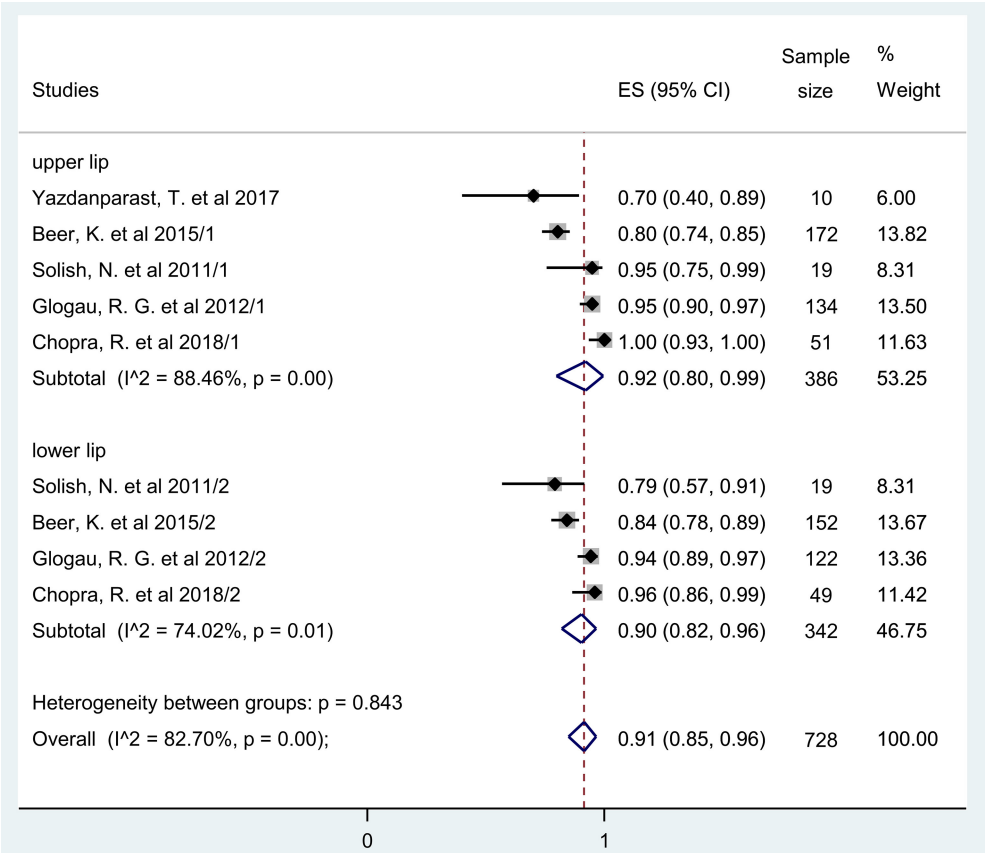

FIGURE 3 | Estimate of rate of responders at 2 months after treatment for the upper and lower lips. Overall, 92% (95% CI: 80–99%) and 90% (95% CI: 0.82–96%) of included participants had at least one grade improvement on a validated lip fullness scale regarding their upper and lower lips, respectively, after 2 months of initial treatment.

scale [Medicis Lip Fullnes Scale (MLF) or Allergan Lip Fullness Scale (ALFS)] was 91% (95% CI: 0.85−0.96) (untransformed proportion, random-effects DerSimonian-Laird method). I^2-values indicating statistical heterogeneity was 82.7% (p = 0.0). Data were pooled from 5 studies (41, 44, 46, 49, 50) (**Figure 3**).

When the rate of responders for volume increase in the upper and lower lips were compared, only a very minor, 2% difference was observed between them 2 months after HA application (41, 44, 46, 49, 50). Upper lips: ES = 0.92, 95% CI: 0.88−0.99; I^2 = 88.46%, p = 0.00 and lower lips: ES = 0.90, 95% CI: 0.82−0.96; I^2 = 74.02%, p = 0.01 (**Figure 3**).

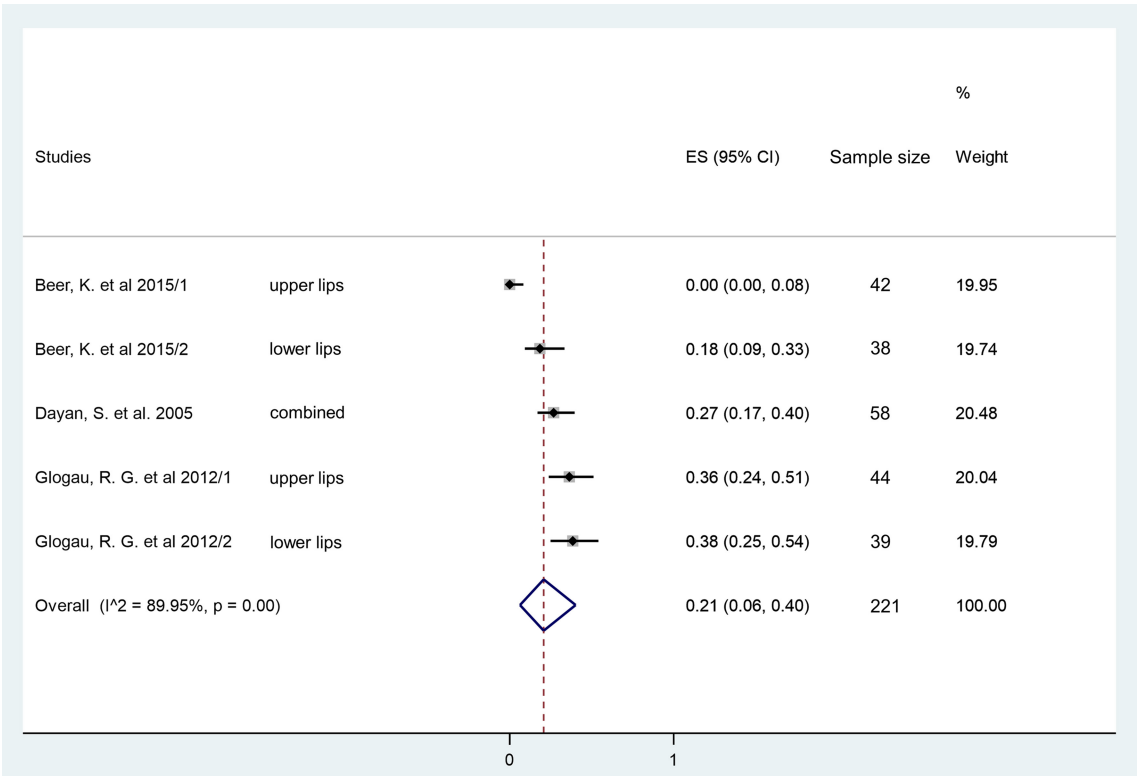

FIGURE 4 | Estimate of overall rate of responders at 2 months in the no treatment group. Overall, 21% (95% CI: 6–40%) of included participants had at least one grade improvement on a validated lip fullness scale after 2 months in the no treatment group.

An additional analysis was also performed using data of the three available studies (41, 42, 44) investigating lip fullness augmentation in non-treated controls. Even among these subjects, who received no HA injection, 21% were demonstrated to be responders 2 months after baseline assessment indicating a possible placebo effect in HA injection studies (ES = 0.21, 95% CI: 0.06−0.40; $I^2 = 89.95\%$, $p = 0.00$) (**Figure 4**).

The rate of responders to HA treatment, i.e., the percentage of participants with at least one grade improvement on the MLF or ALF scales after 3 months, was also calculated including eight studies (42, 43, 45–50). The untransformed proportion (random-effects DerSimonian-Laird method) of the pooled data showed that 71% of the HA-treated participants were responders, meaning that 71 out of 100 experienced a substantial, at least one grade increase in lip fullness 3 months after the initial treatment (ES = 0.71, 95% CI: 0.55−0.87; $I^2 = 97.91\%$, $p = 0.00$) (**Figure 5**).

Six months after the HA injection, the overall rate of responders, i.e., again the percentage of those who still have an increase of lip fullness scale by one grade or higher, were synthetized from five studies (42, 43, 47, 48, 50). This analysis revealed that 74% of those who received the one dose HA treatment maintained their increase of lip volume (ES = 0.74, 95% CI: 0.66−0.82; $I^2 = 66.88\%$, $p = 0.02$) (**Figure 6**).

The lip volume data 12 months after HA application were available only in four studies (42, 43, 47, 48). Our meta-analysis revealed that rate of responders was 46% even after 1 year of a single HA injection (ES = 0.46, 95% CI: 0.28−0.65; $I^2 = 93.21\%$, $p = 0.00$) (**Figure 7**).

Adverse Effects of Hyaluronic Acid Injection

Studies reporting the AEs related to HA injections were included in this analysis. Data were pooled from six RCTs (41–43, 45, 51, 52), 11 cohort studies (46–50, 53–58) and 14 case reports (59–72) including 1,488 participants overall.

The results revealed that the five most common AEs were tenderness ($n = 1{,}320$, 88.7%), injection site swelling ($n = 1{,}105$, 74.3%), contusion ($n = 725$, 48.7%), injection site mass ($n = 406$, 27.3%), and injection site pain ($n = 293$, 19.7%). The appearance of herpes labialis ($n = 9$, 0.6%) was identified in a few cases, while filler-associated necrosis of the lips was also found very rarely in case reports. More serious AEs such asgranulomatous foreign body reaction ($n = 9$, 0.6%), were infrequent. Life-threatening angioedema was reported only in four cases out of the 1,488 patients (0.3%) included in the studies on HA injection into the lip (**Supplementary Table 8**).

Publication Bias

Funnel plot constructed from studies with 3 months follow-up shows asymmetry of published records suggesting small-study effect (**Supplementary Figure 2**). Due to the small number

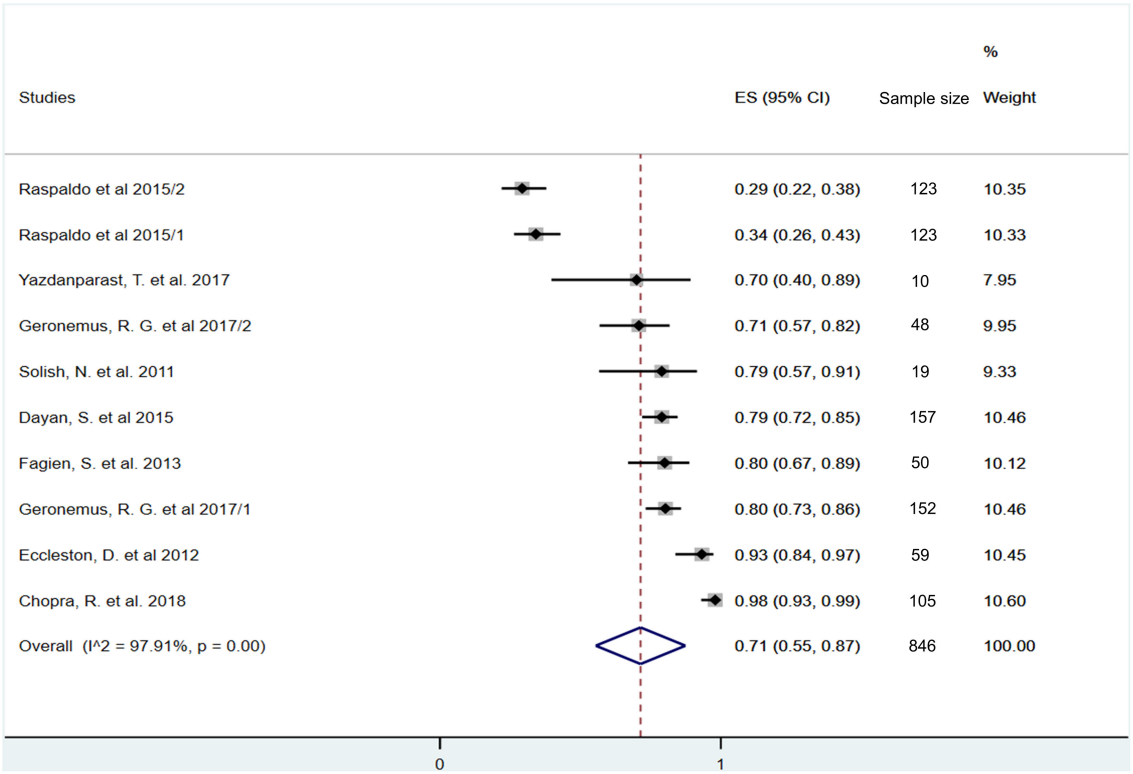

FIGURE 5 | Estimate of overall rate of responders at 3 months after treatment. Overall, 71% (95% CI: 55–87%) of included participants had at least one grade improvement on a validated lip fullness scale after 3 months of initial treatment.

of studies included, no further statistical analysis could be performed to test for small-study effect.

Certainty of Evidence

The assessement based on the GRADE approach revealed that the final level of evidence for the effectiveness of HA treatment on lip augmentation is very low. This is explained by some low level of study designs (cohort studies), the significant inconsistency due to statistical heterogeneity indicating confounding factors, imprecision indicated by wide range of confidence intervals and suspected publication bias due to small study effect.

In the case of EAs the level of evidence is also very low due to study design, the high risk of bias and the lack of consistent reporting on EAs (**Supplementary Table 9**).

DISCUSSION

Summary of Evidence

As it is the entrance of the gastrointestinal tract, the health and esthetics of lips are important for the well-being of the human body. Although HA is a frequently used dermal filler for non-surgical aesthetic treatment (8, 17), its benefits and possible AEs for lip augmentation have not been assessed quantitatively by meta-analysis. Although several primary studies existed on the matter, their relatively small sample size did not allow to draw strong conclusions. Our study is the first meta-analysis to integrate the available data from individual primary studies for the effectiveness of HA for lip augmentation after HA injections. In our analysis, we included studies which used validated scales to assess changes in lip fullness. We also included case reports to find-long term and rare events of treatment-related AEs. We found that HA injection effectively increases lip fullness up to 6 months among the majority of treated patients. Moreover, our analysis revealed that approximately half of the successfully treated participants still had a significantly increased lip fullness after 12 months. Most AEs related to the treatment were consistent across prospective studies. AEs were mostly mild or moderate, but rare severe AEs could also be observed in a very small number of cases.

Although our meta-analysis clearly showed the effectiveness of HA injection on the lip, the variability of the individual studies was also very obvious (41–45). This heterogeneity suggests that there were significant confounding factors that might influence the outcome of HA treatments. Several factors have been suggested to influence the outcome of lip augmentation, such as the injected volume, the number of touch-up treatments, the type of injection technique, the number of cross-links in HA product, and also the skin type of the patients, the experience of investigators, as well as the evaluation method (42, 44, 45, 80). In our analysis of effectiveness, more than 8 HA products using 5 different HA concentrations were included. The different papers reported several injection techniques and

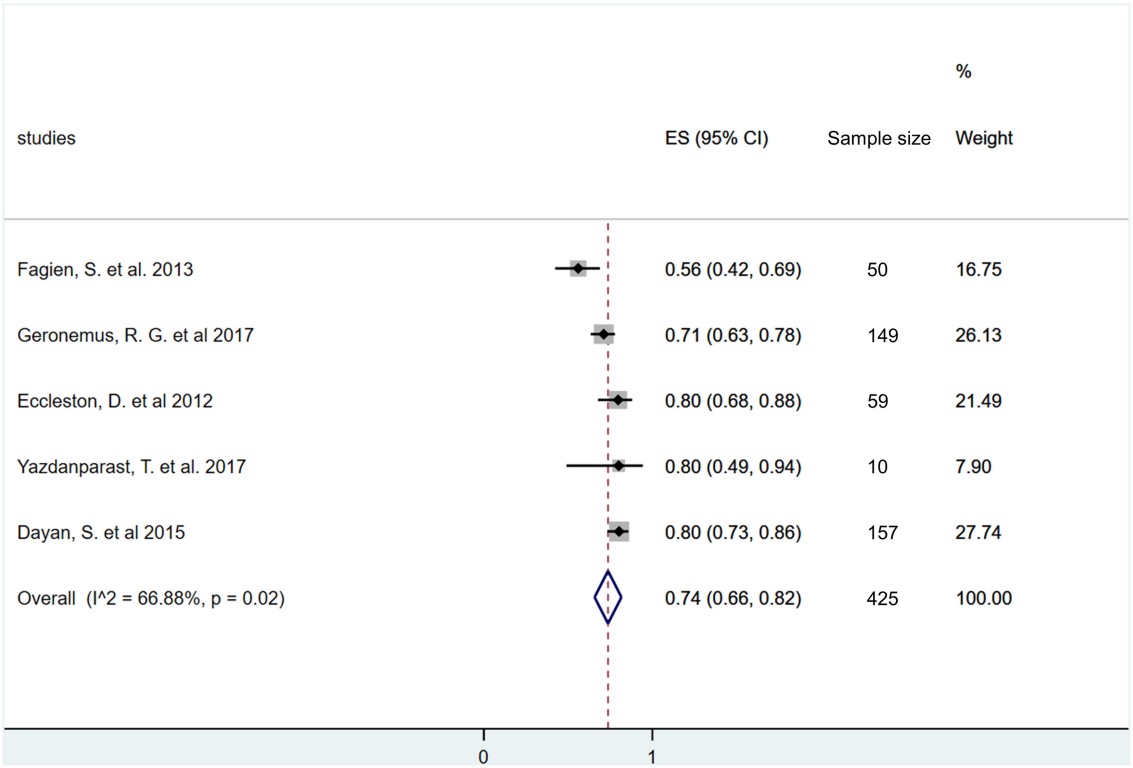

FIGURE 6 | Estimate of overall rate of responders at 6 months after treatment. Overall, 74% (95% CI: 66–82%) of included participants had at least one grade improvement on a validated lip fullness scale after 6 months of initial treatment.

various injection volumes. Due to the high variability and the low number of studies containing identical subgroups, it was not possible to perform a comprehensive statistical analysis to investigate the effects of such confounding factors. Raspaldo and coinvestigators found that live assessment yielded more precise results compared to photo analysis based on 3D images. They argued that photographs can alter shadows and smaller rhytids, thereby altering evaluation outcomes (45). On the contrary, Moragas et al. argued that the use of a validated scale is most appropriate for evaluating lip augmentation outcomes. Yet, in their review, they suggested that anthropometric measures were far from being perfect. Therefore, they did not evaluate natural appearance or changes in the shape of the lips (80).

An important observation of our analysis is the considerable decrease in lip fullness over a 12-month period as HA treatment remained effective in only about the half of the treated patients after 1 year. Although HA is regarded to be a temporary filler, its longevity on lip volume have not been investigated with statistical methods. The decreased number of augmented lips at 12 months follow-up period could be ascribed to the natural biodegradation of HA (26). Cross-linking slows down the biodegradation of HA (81), but it is unclear to what level the concentration and degree of cross-links of HA affects its long-term effectiveness.

Unfortunately, no studies are available on the effectivness of hydratization of the lips in response to hyaluronic acid treatment. HA fillers were described to increase not only the volume, but also hydratize the treated tissue when applied (82). Namely, Seok and coinvestigators observed increased skin hydration levels after HA injections into various parts of the face (83). For AEs, we found that similar event rates were reported from the included RCTs (41–45) and other prospective studies (46–50). However, case reports revealed additional AEs (59–72), which were not reported in clinical trials. Our analysis revealed that the most frequent AEs were injection-related, such as tenderness, injection site swelling, bruising, injection site mass, injection site pain. All of these AEs resolved without the need of treatment within a few weeks. Similar AE rates were found in earlier studies for lips (3, 80, 84) and for other anatomical sites as well (8, 17).

Remarkably, in one study (51), herpes labialis was found to be the most common AE (17%). The reason for this is unclear since the prevalence of this viral infection was much lower in the other included studies (0.6%). Most probably, in the work of Downie et al. the needle puncture could have triggered the reactivation of herpes virus infection (51, 85). In this context. a systematic review on HA filling of nasolabial folds found that the correct injection technique (avoiding fan-like injection) applying slow rate injection (0.3 ml/min) can minimize the risk of injection-related effects (8).

Moreover, our analysis uncovered some AEs that have been reported only in case reports, such as foreign granulomatous reactions with histology (0.6%), tumor-like nodule (0.3%). Angioedema (0.3%) was reported in one RCT (42) and in three

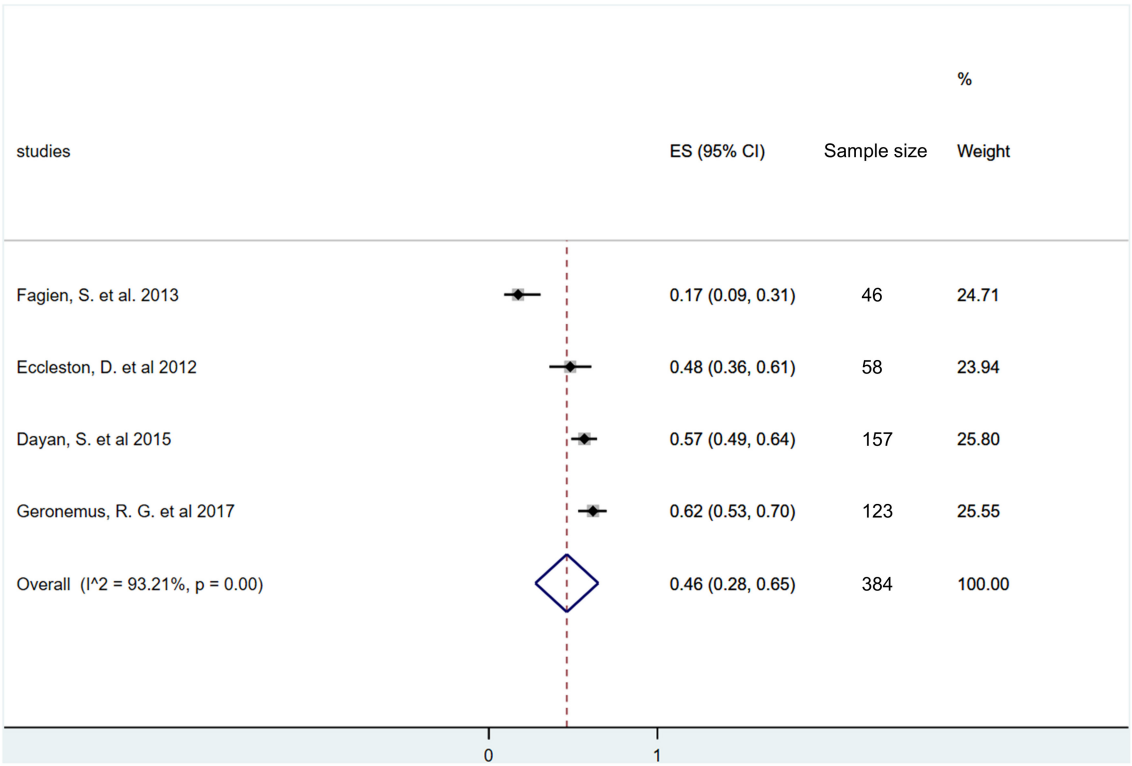

FIGURE 7 | Estimate of overall rate of responders at 12 months after treatment. Overall, 46% (95% CI: 28–65%) of included participants had at least one grade improvement on a validated lip fullness scale after 12 months of initial treatment.

case reports (60, 62, 70). Filler-associated necrosis of the lips were noted in three case reports (86–88). The available systematic reviews have not identified such AEs in similar prevalence (3, 8, 17, 80, 89). Additionally, vocalization and mastication may also be disturbed but no reports are available about this.

Impurities in HA could be a potential explanation for immune system-related AEs. HA itself is a non-allergic and a non-toxic molecule (90). However, in health industry products HA is manufactured from various xenogeneic sources. Also, there are differences between the various HA manufacturing procedures (31, 80, 91). In our meta-analysis all included studies used HA produced by bacterial transduction. HA products originating from bacterial transduction, using advanced purification technologies are thought to reduce the risk of host immune response compared to HA products from animal sources (80). Rough HA preparations may be further modified chemically to create the cross-links that extend the lifespan of the injected HA (80, 91). However, impurities, residual proteins and nucleic acid fragments leading to immune reactions may still exist after purification (31, 32, 91). It is unclear whether the few cases of angioedema and granulomas were due to impurities in the used HA products or by the possible contamination of the needle with bacteria used to puncture the skin (92–94). But a recent systematic review investigated the incidence of delayed inflammatory reactions associated with HA injection (17). That work concluded that although the estimated incidence is relatively low, preceding skin tests could be still relevant before HA injection to prevent certain types of granulomas, such as the ones caused by delayed-type hypersensitivity reaction (17).

LIMITATIONS

A major limitation of the present work is the relatively small number of RCTs found on the topic. Although our analysis revealed the importance of confounding factors, no sub-group analysis could be performed due to the limited reported data, and to uncomprehensive data-reporting. For example, the volume of HA injection and the injection technique were not given, and subdivision of the results according to skin types were not always provided. Additionally, different studies used different reporting schemes and wording for detecting AEs. Hence, due to the lack of clear definition, we had to merge certain reported AEs based on our estimation.

In several papers, the documentation of AEs was not optimal for comparison. Additionally, most of the prospective studies had a short follow-up period, up to a maximum of 1 year. Moreover, our analysis was based on reported events, unreported events could not be taken into account. This may cause underestimation of the number and type of AEs associated with lip augmentation.

CONCLUSION

In conclusion, our meta-analysis provided evidence that hyaluronic acid injections are highly efficient at least up to 6 months. Even after 1 year following HA injections, in almost half of the patients, the lip volume was still significantly increased. Additionally, we found that most of the AEs after HA treatment were mild or moderate. But the lack of longer follow-ups could not reveal possible delayed reactions. Based on our present meta-analysis, we suggest that more high quality RCTs are needed to strengthen the certainty of evidence and firmly establish the long-term effect of HA injection for lip augmentation.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LC, SF, AL, VG, SK, PH, and GV devised the project, the main conceptual ideas, and planned the research. LC, SF, NG, DC, SK, and GV worked out the methodology. LC, SF, and GV performed the data collection: literature search, study selection, and data extraction. LC, AM, and DC also organized and maintained research data for analysis. NG performed analytic calculations, applied statistical models for synthetizing data, and visualized synthetized data into forest plots. AM, DC, and SK also aided the research by interpretation of raw and synthetized data. LC and SF worked on summarizing results into figures and tables. SK and GV were responsible for managing and coordinating the research activity. PH and GV took leadership responsibility for the research activity, provided resources, and acquired financial support for the research project. SK, PH, and GV validated reproducibility of the results. LC, NG, AM, DC, and GV wrote the manuscript with input from all authors. SF, AL, VG, SK, PH, and GV extensively reviewed the work and further edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2021.681028/full#supplementary-material

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Supplementary Table 1. PRISMA checklist.

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2 – 3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4 – 5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7 – 8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	8
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8 – 9
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	9

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	9 – 10
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	10
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	10 – 12
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	12 – 13
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	13 – 16
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	13 – 16
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	16
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	16
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	17 – 21
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	21
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	21
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	22

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Supplementary Table 2. Search query.

Database	Search query
CENTRAL	*"*('hyaluronic':ti, ab, kw OR 'hyaluronate':ti, ab, kw OR 'hyaluronan':ti, ab, kw OR 'dermal filler':ti, ab, kw OR 'injectable implant':ti, ab, kw) AND 'lip':ti, ab, kw." Limits applied: trials.
Embase	"('hyaluronic':ti, ab, kw OR 'hyaluronate':ti, ab, kw OR 'hyaluronan':ti, ab, kw OR 'dermal filler':ti, ab, kw OR 'injectable implant':ti, ab, kw) AND 'lip':ti, ab, kw*"*. Limits applied: human.
MEDLINE	*„*hyaluronic[All Fields] OR hyaluronate[All Fields] OR ("hyaluronic acid"[MeSH Terms] OR ("hyaluronic"[All Fields] AND "acid"[All Fields]) OR "hyaluronic acid"[All Fields] OR "hyaluronan"[All Fields]) OR ("dermal fillers"[MeSH Terms] OR ("dermal"[All Fields] AND "fillers"[All Fields]) OR "dermal fillers"[All Fields] OR ("dermal"[All Fields] AND "filler"[All Fields]) OR "dermal filler"[All Fields]) OR (("injections"[MeSH Terms] OR "injections"[All Fields] OR "injectable"[All Fields]) AND implant[All Fields]) AND ("lip"[MeSH Terms] OR "lip"[All Fields]) AND "loattrfull text"[sb]*"*. Limit applied: „human".

Supplementary Table 3. Description of the modified Newcastle Ottawa Scale to suite included study assessment.

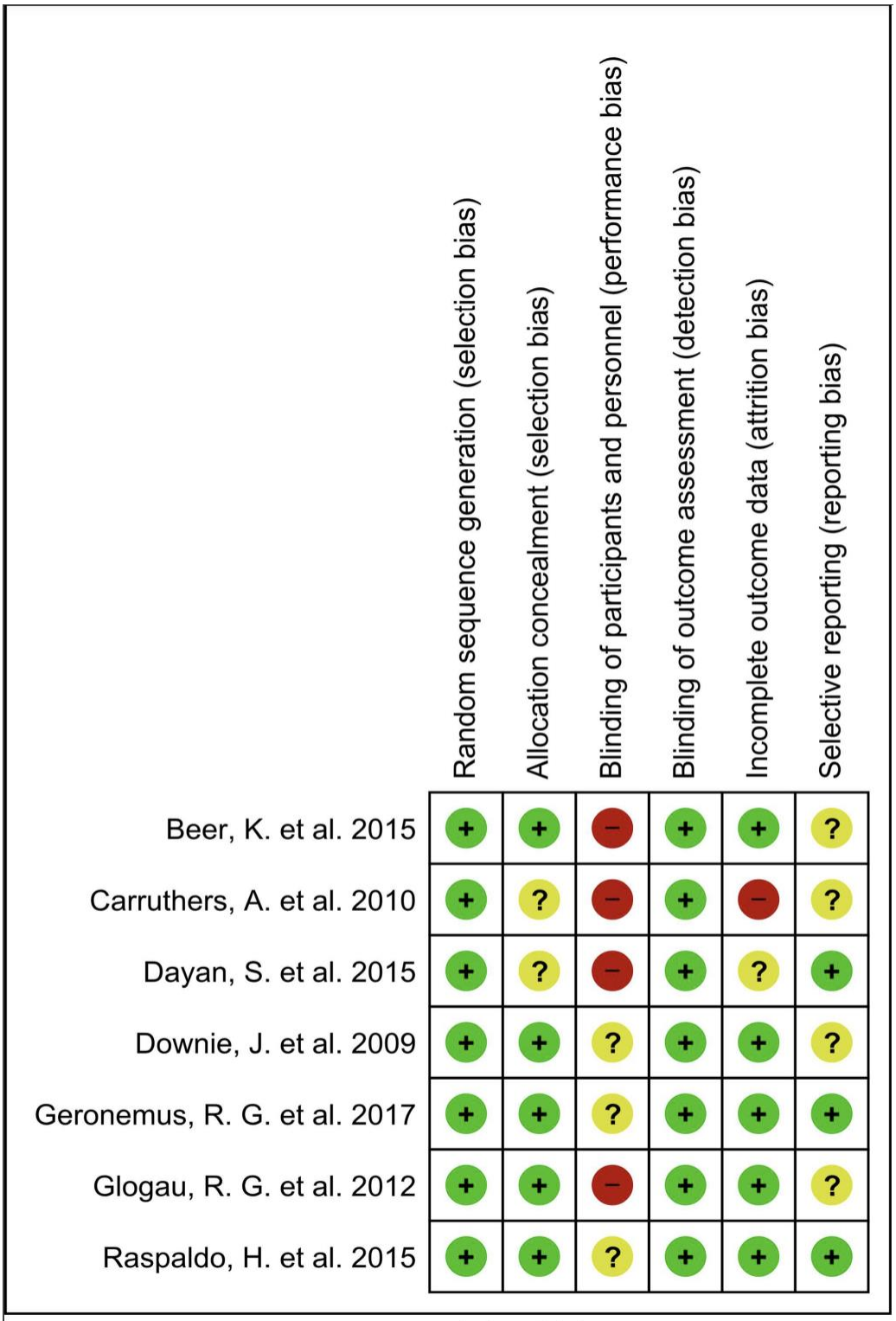
Selection	1) Representativeness of the exposed cohort a) truly representative of the average subjects for lip augmentation (describe) in the community ✸ b) somewhat representative of the average subjects for lip augmentation in the community ✸ c) selected group of users eg nurses, volunteers d) no description of the derivation of the cohort
	2) Selection of the non - exposed cohort a) drawn from the same community as the exposed cohort ✸ b) drawn from a different source c) no description of the derivation of the non exposed cohort
	4) Demonstration that outcome of interest was not present at start of study a) yes ✸ b) no
Comparability	1) Comparability of cohorts on the basis of the design or analysis a) study controls for __lip fullness_____ (select the most important factor) ✸ b) study controls for any additional factor ✸
Outcome	1) Assessment of outcome a) independent blind assessment ✸ b) record linkage – *outcome was assessed by non blinded treating investigator using a validated lip fulness scale.*✸ c) self report d) no description 2) Was follow-up long enough for outcomes to occur a) yes (6 months) ✸ b) no 3) Adequacy of follow up of cohorts a) complete follow up - all subjects accounted for ✸ b) subjects lost to follow up unlikely to introduce bias - 90 % follow up, or description provided of those lost) ✸ c) follow up rate < 90% and no description of those lost d) no statement

Supplementary Table 4. Study characteristics of case reports included in adverse reaction analysis.

Study	Number of participants	Female ratio (%)	Age or mean age ± SD	Treatment	Description of symptoms	Diagnosis	Time after HA treatment
Anatelli, F. et al. 2010 (56)	1	100	80	Restylane	3 mm pearly translucent papule in the left upper cutaneous lip	Biopsy, amorphous basophilic deposit of HA	8 weeks or more (not clearly stated)
Bulam, H. et al. 2015 (57)	1	100	27	Juvederm Ultra Plus XC	Angioedema-type acute hypersensitivity reaction	Clinical signs, angioedema	Within minutes
Curi, M. M. et al. 2015 (58)	2	100	65	Restylane	2 nodular lesions on the right upper lip mucosa	Biopsy, granulomatous foreign body reaction related to the HA	12 years
			58	HA (unknown)	Sudden symmetric bilateral swelling on the parotid masseteric region and also on the buccal mucosa after chemotherapy	Clinical examination	4 years
Dougherty, A. L. et al. 2011 (59)	1	100	Unknown	Restylane	Angioedema-type swelling on both lips	Clinical signs, angioedema, herpes reactivation	Within 12 hours
Duhovic, C. et al. 2016 (60)	1	100	58	HA (unknown)	Indurated nodules above the upper lip bilaterally	Biopsy, multinucleated giant cells granulomatosis	4 years
Edwards, P. C. et al. 2006 (61)	1	100	74	Restylane	Firm submucosal nodule of the lower lip	Biopsy, multiple cystlike vacuolated areas with histiocytes and foamy macrophages, consistent with a foreign body reaction	6 months
Eversole, R. et al. 2013 (62)	2	100	45	HA (unknown)	Granular yellow lesion	Biopsy, granulomatous foreign body reaction	Within 5 years
			51	HA (unknown)	White nodules mandibular sulcus		
Farahani, S. S. et al. 2012 (63)	3	100	56 (mean)	Restylane	Painless discrete nodule on the labial mucosa	Biopsy	Within 2 years
Feio, P. S. et al. 2013 (64)	2	100	51	HA (unknown)	Hardness in the lower lip mucosa	Biopsy, presence of numerous giant cells around translucent particles, foreign body reaction	6 months
			30		Fibrous nodule on the left upper lip mucosa, which had quickly enlarged, then stabilised	No surgical intervention	7 years
Fernández-Aceñero Ma, J. et al. 2003 (65)	1	100	48	Restylane	Several discrete nodules in the upper lip	Biopsy, ranulomatous foreign body reaction with multinucleated cells around a blue amorphous material	2 years
Grippaudo, F. R. et al. 2014 (66)	1	100	28	HA (unknown)	“angry red nodules”	High Frequency Ultrasound (HFUS) examination	1 year
Leonhardt, J. M. et al. 2005 (67)	1	100	52	Restylane	Angioedema-type swelling on the upper lips	Clinical signs, angioedema	Within hours

Martin, L. et al. 2018 (68)	2	100	24 43	HA (unknown)	Nodules in the lip area	Biopsy, Foreign body reaction to exogenous material	Within years
Wolfram, D. et al. 2006 (69)	1	100	53	Restylane	Erythematous indurations of both nasolabial folds	Dense granulomatous infiltrate with multinucleated giant cells as well as sharply delineated extracellular foreign bodies	2 years

Abbreviations: HA: hyaluronic acid.



Supplementary Figure 1. Risk of bias summary**.**
Bias in each risk of bias item for each included study.

Supplementary Table 5. Detailed risk of bias assessment of included RCTs using the Cochrane risk of bias tool.

Beer, K. et al. 2015 (38)		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Patients were randomly assigned 3:1 to receive small particle [...] HA [...] or no treatment."
Allocation concealment (selection bias)	Low risk	"centralized randomization system was used"
Blinding of participants and personnel (performance bias)	High risk	Comment: no blinding was applied on the treating investigator and participants.
Blinding of outcome assessment (detection bias)	Low risk	"evaluator-blinded study"
Incomplete outcome data (attrition bias)	Low risk	"A total of199 patients completed the study (91%). No patient discontinued because of AEs; main reasons were lost to follow-up (6%) and withdrawal of consent (3%)."
Selective reporting (reporting bias)	Unclear risk	Comment: No access to study protocol or trial registry entry, but no intext evidence of reporting bias.
Carruthers, A. et al. 2010 (49)		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"multicenter (3 site), prospective, single-blind, randomized, parallel-group study..."
Allocation concealment (selection bias)	Unclear risk	Comment: no description of method used for allocation concealment.
Blinding of participants and personnel (performance bias)	High risk	Comment: no blinding was applied on the treating investigator and participants.
Blinding of outcome assessment (detection bias)	Low risk	"an assessing investigator who was masked to the treatment that the subject received conducted effectiveness evaluations."
Incomplete outcome data (attrition bias)	High risk	Comment: 23% of participants dropped out of the study group in interest.
Selective reporting (reporting bias)	Unclear risk	Comment: No access to study protocol or trial registry entry, but no intext evidence of reporting bias.
Dayan, S. et al. 2015 (39)		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Block randomization (i.e., randomization within subgroups) was performed"

Allocation concealment (selection bias)	Unclear risk	Comment: Not mentioned in publication.
Blinding of participants and personnel (performance bias)	High risk	Comment: no blinding was applied on the treating investigator and participants.
Blinding of outcome assessment (detection bias)	Low risk	"the blinded evaluating investigator"
Incomplete outcome data (attrition bias)	Unclear risk	"One subject in the treatment group discontinued because of an AE; all other discontinuations were due to withdrawn consent, lost to follow-up, or other. One subject in the control group who subsequently received treatment discontinued because of pregnancy; all other discontinuations were due to withdrawn consent, lost to follow-up, or other." Comment: "other" is not specified.
Selective reporting (reporting bias)	Low risk	Comment: Protocol is available, and all pre-specified outcomes are reported.
Downie, J. et al. 2009 (72)		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Patients [...] were randomized by using a computerized Interactive Voice Response (IVR) system."
Allocation concealment (selection bias)	Low risk	"...patients assigned to PRI 1, PRI 2 or Perlane treatments received a similar saline skin test." & "The IVR was accessed using a push button telephone; the system provided both randomization and unblinding facility for the study."
Blinding of participants and personnel (performance bias)	Unclear risk	Comment: Participants were blinded, but personnel injecting dermal fillers were not.
Blinding of outcome assessment (detection bias)	Low risk	"Patients were assessed post-operatively [...] by an independently qualified blinded assessor."
Incomplete outcome data (attrition bias)	Low risk	"Seventy-nine patients were enrolled into the study: PRI 1: 19 patients, PRI 2: 19 patients, Perlane: 23 patients, Zyplast: 18 patients, one patient dropped out of the study."
Selective reporting (reporting bias)	Unclear risk	Comment: No evidence found.
Geronemus, R. G. et al. 2017 (40)		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"subjects were randomized 3:1 to treatment with VYC-15LorNASHA"
Allocation concealment (selection bias)	Low risk	"Subjects were blinded to treatment assignment, which was based on a central block randomization schedule and an automated interactive voice/web response system."
Blinding of participants and	Unclear risk	"Subjects were blinded to treatment" & "unblinded treating investigator"

personnel (performance bias)		
Blinding of outcome assessment (detection bias)	Low risk	"blinded evaluating investigator"
Incomplete outcome data (attrition bias)	Low risk	"One subject assigned to VYC-15L discontinued before receiving treatment."
Selective reporting (reporting bias)	Low risk	Comment: Protocol is available, and all pre-specified outcomes are reported.
Glogau, R. G. et al. 2012 (41)		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"randomly assigned 3:1 to receive SGPHA or no treatment"
Allocation concealment (selection bias)	Low risk	"centralized randomization system was used"
Blinding of participants and personnel (performance bias)	High risk	Comment: no blinding was applied on the treating investigator and participants.
Blinding of outcome assessment (detection bias)	Low risk	"evaluator-blinded study"
Incomplete outcome data (attrition bias)	Low risk	"One hundred sixteen (86%) in the SGP-HA group and 39 (87%) in the no-treatment group completed the study."
Selective reporting (reporting bias)	Unclear risk	Comment: No access to study protocol or trial registry entry, but no intext evidence of reporting bias.
Raspaldo, H. et al. 2015 (42)		
Bias	**Author's judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"subjects were randomized (1:1) to receive Juvéderm Volbella [...] or Restylane-L"
Allocation concealment (selection bias)	Low risk	"randomization and treatment assignment were managed by a centralized, automated, interactive voice and Web response system"
Blinding of participants and personnel (performance bias)	Unclear risk	"Subjects, independent central reviewers and investigational staff except for investigators and study coordinators remained blinded to treatment assignment."
Blinding of outcome assessment (detection bias)	Low risk	"Subjects, independent central reviewers and investigational staff except for investigators and study coordinators remained blinded to treatment assignment."
Incomplete outcome data (attrition bias)	Low risk	"Most subjects completed the study: 118 (84.9%) in the Juvéderm Volbella with Lidocaine group and 115 (81.0%) in the Restylane - L group."
Selective reporting (reporting bias)	Low risk	Comment: Protocol is available, and all pre-specified outcomes are reported.

Supplementary Table 6. Detailed assessment of risk of bias of cohort studies using the Newcastle-Ottawa Scale.

Study	Selection				Comparability	Outcome			Score (max 7)
	Representative-ness of the intervention group	Selection of the non-exposed cohort	Ascertainment of intervention	Demonstration that outcome of interest was not present at start of the study	Comparability	Assessment of outcome	Was follow-up long enough for outcome to occur?	Adequacy of follow up of cohorts	
Chopra, R. et al. 2018 (43)	a)	a)	N/A	a)	Baseline-controlled	b)	3 months	3 dropouts (95% completed)	6
Eccleston, D. et al. 2012 (44)	a)	a)	N/A	a)	Baseline-controlled	b)	12 months, primary effect endpoint at 3 months	1 dropout (98.3% completed)	6
Fagien, S. et al. 2013 (45)	a)	a)	N/A	a)	Baseline-controlled	b)	6 months	No dropout	7
Solish, N. et al 2011 (46)	b)	a)	N/A	a)	Baseline-controlled	a)	2 months	3 dropouts (85.7% completed)	6
Yazdanparast, T. et al. 2017 (47)	b)	a)	N/A	a)	Baseline-controlled	a)	6 months	No dropout	7
Artzi, O. et al. 2016 (50)	a)	a)	N/A	a)	No control group	d)	11 months	d)	5
Carruthers, J. et al 2005 (51)	b)	a)	N/A	a)	No control group	c)	6 months	1 drop out (94% completed)	6
Fischer, T. et al. 2016 (52)	b)	a)	N/A	a)	No control group	b)	4 months	d)	5
Philipp-Dormston, W. G. et al. 2014 (53)	b)	a)	N/A	a)	No control group	c)	1 months	d)	4
Rzany, B. et al. 2012 (54)	b)	a)	N/A	a)	No control group	b)	6 months	1 drop out (98% completed)	7
Samuelson, U. et al. 2015 (55)	b)	a)	N/A	a)	Baseline-controlled	c)	9 months	1 drop out (96% completed)	6

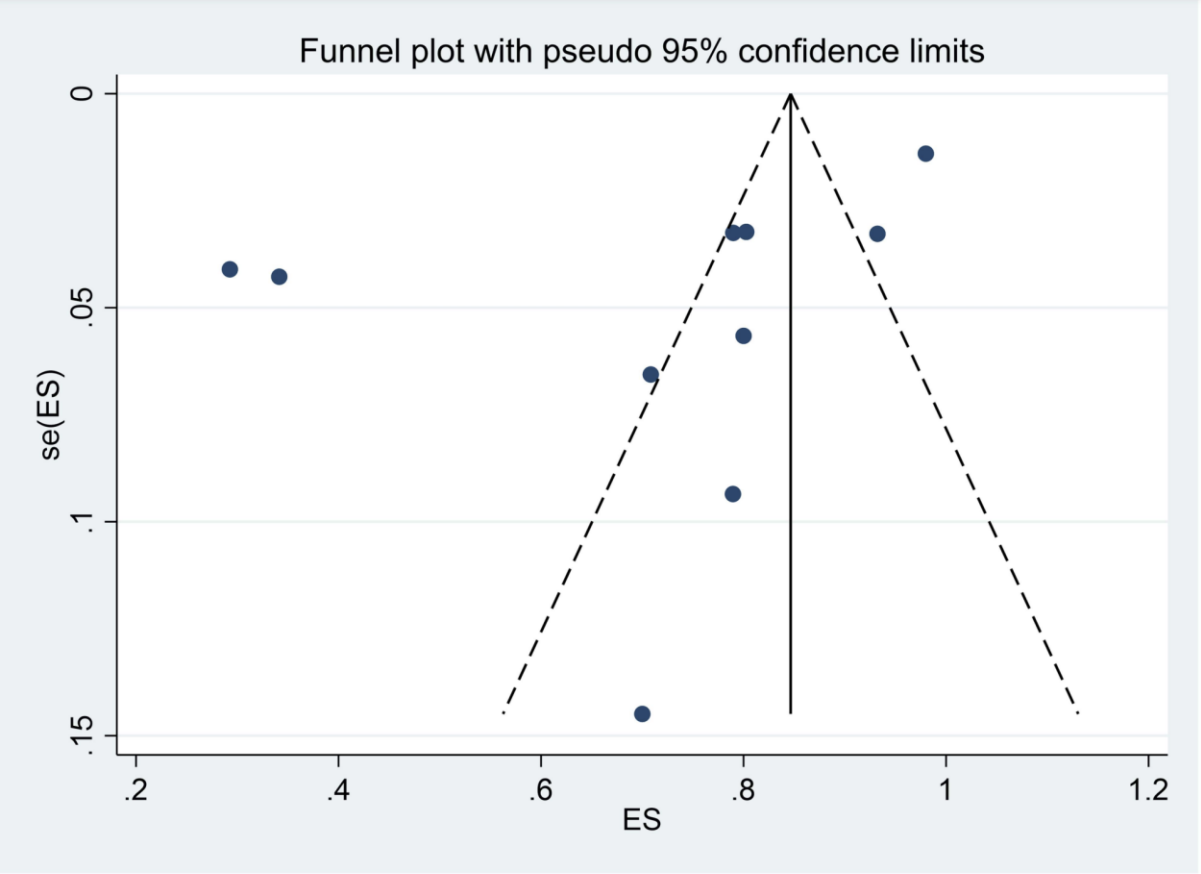
Abbreviations: N/A: not applicable.

Supplementary Table 7. Summary of the risk of bias assessment of cohort studies.

Study	Selection	Comparability	Outcome	Total Score	Included in effectiveness analysis
Chopra, R. et al. 2018 (43)	✸✸✸	✸	✸✸	6	Yes
Eccleston, D. et al. 2012 (44)	✸✸✸	✸	✸✸	6	Yes
Fagien, S. et al. 2013 (45)	✸✸✸	✸	✸✸✸	7	Yes
Solish, N. et al 2011 (46)	✸✸✸	✸	✸✸	6	Yes
Yazdanparast, T. et al. 2017 (47)	✸✸✸	✸	✸✸✸	7	Yes
Artzi, O. et al. 2016 (50)	✸✸✸	-	✸	4	No
Carruthers, J. et al 2005 (51)	✸✸✸	-	✸✸	5	No
Fischer, T. et al. 2016 (52)	✸✸✸	-	✸	4	No
Philipp-Dormston, W. G. et al. 2014 (53)	✸✸✸	-	-	3	No
Rzany, B. et al. 2012 (54)	✸✸✸	-	✸✸✸	6	No
Samuelson, U. et al. 2015 (55)	✸✸✸	✸	✸✸	6	No

Supplementary Table 8. Summary of adverse effects reported in included studies.

Adverse effect	N (total = 1487)	%		Adverse effect	N (total = 1487)	%
Tenderness	1320	88.7		A tumorlike nodule	4	0.3
Injection site swelling	1105	74.3		Angioedema	4	0.3
Contusion	725	48.7		Dry lip	3	0.2
Injection site mass	406	27.3		Anesthesia	1	0.1
Injection site pain	293	19.7		Canker sore	1	0.1
Erythema	108	7.3		Induration	1	0.1
Tyndall effect and discoloration	84	5.7		Inflammatory nodules	1	0.1
Hematoma	27	1.8		Injection site cyst	1	0.1
Lip disorder	12	0.8		Hemorrhage	1	0.1
Granulomatous foreign body reaction	9	0.6		Papule	1	0.1
Paresthesia	9	0.6		Presyncope	1	0.1
Herpes labialis	9	0.6				



Supplementary Figure 2. Funnel plot**.**
Asymmetrical distribution of studies suggests the presence of small study effect.

Supplementary Table 9. Certainty of evidence point according to GRADE approach.

Outcome	Study design [№ of studies]	Initial level of evidence	Evidence components	Upgrade/ downgrade of evidence	Comment	Final level of evidence
Rate of responders	RCT [5] (38-42) Cohort studies [5] (43-47)	Low	**Risk of bias**	Considerable	Control groups are not used in each study	⊕◯◯◯ Very Low
			Inconsistency	Serious	Large I^2 value	
			Indirectness	Not serious	-	
			Imprecision	Serious	Wide range of CIs	
			Other considerations	Publication bias suspected	Small study effect	
Adverse effects	RCT [6] (38-40, 42, 49, 72) Cohort studies [11] (43-47, 50-55) Case reports [14] (56-69)	Low	**Risk of bias**	Considerable	Control groups are not used in each study	⊕◯◯◯ Very Low
			Inconsistency	Serious	Lack of consistent reporting of adverse effects	
			Indirectness	Not serious	-	
			Imprecision	Not serious	-	
			Other considerations	Upgrade by one point	Large effect	

Abbreviations: RCT: randomized controlled trial