UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE BIOMEDICHE

Direttore della Scuola: Prof. Andrea Fausto Piana

INDIRIZZO IN ODONTOSTOMATOLOGIA PREVENTIVA

XXVIII CICLO

Prevention, diagnosis and minimally invasive treatment of dental caries.

Direttore:

Prof. Andrea Fausto Piana

Tutor:

Prof. Guglielmo Campus

Tesi di dottorato di:

Dott. Gianfranco Lai

Anno Accademico 2014 - 2015

La presente tesi è stata prodotta nell'ambito della Scuola di Dottorato in Scienze Biomediche dell'Università degli Studi di Sassari, a.a. 2011/2012 – XXVII ciclo, con il supporto di una borsa di studio finanziata con le risorse del P.O.R. SARDEGNA F.S.E. 2007-2013 - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linea di Attività l.3.1.

ABSTRACT

Aim: Study the news overtures to prevention, diagnosis and therapy of caries disease.
Methods: We conducted one randomized controlled clinical trial (RCT), one cross-sectional study and one systematic review with meta-analysis. The RCT evaluated the efficacy of the Carisolv and CeraBur. In the cross-sectional study we evaluated the efficacy of a new device to detect caries lesions: DIAGNOcam. The systematic review of the literature was conducted to evaluate efficacy of polyols in caries prevention.
Results: We found a significantly difference in terms of time taken between control group and Carisolv ($p<0.001$). We found no difference in increment cavity size and in anti-microbial effect between techniques.
In the cross-sectional study we found a higher sensibility of the DIAGNOcam device respect to x-rays to diagnose caries in enamel ($k=0.24$); no statistically significant difference was found in dentin caries ($k=1$).
In the meta-analysis we found that xylitol gum showed a good antimicrobial effect against the mutans streptococci than control group ($p<0.01$); low increment of ΔDMFS at 2 and 3 years follow-up ($p<0.01$), and low AUC pH than sorbitol gum ($p<0.01$).
Conclusion: The clinical efficacy of Carisolv and CeraBur seems as reliable as the rotary instruments. The study on DIAGNOcam showed that this new device might be a useful tool in early caries detection. The xylitol gums showed a role in caries prevention.

CONTENTS

ORIGINAL PAPERS

This thesis is based on the following four papers, which will be referred to in the text by their Roman numerals:

I. **The use of polyols in caries prevention: a systematic review and meta-analysis.**
Lai G, Lara-Capi C, Cocco F, Cagetti MG, Campus G.
Submitted

II. **Digital imaging fiber-optic transillumination device versus radiographic and clinical examination in detection of dental caries.**
Lara-Capi C, Lingström P, Lai G, Cagetti MG, Cocco F, Simark-Mattsson C, Campus G.
Submitted

III. **Comparison of Carisolv system vs traditional rotating instruments for caries removal in the primary dentition: A systematic review and meta-analysis.**
Lai G, Lara-Capi, Cocco F, Cagetti MG, Lingström P, Almhöjd U, Campus G.
Acta Odontol Scand. 2015; 73: 569-80

IV. **Clinical randomized controlled trial of four different techniques of caries removal in primary dentition.**
Lai G, Lingström P, Sale S, Campus G
Submitted

INTRODUCTION

Dental caries is one of the most prevalent chronic diseases of people worldwide affecting the majority of individuals in all age groups during their lifetime. Dental caries forms through a complex interaction over time between acid-producing bacteria and fermentable carbohydrate, and many host factors including teeth and saliva (Selwitz RH *et al.*, 2007).

Caries prevention: use of polyols

The use of fluoridated toothpastes, other topically applied fluorides, fluoridated municipal water and pit and fissure sealants, along with dietary improvement, remain mainstays of caries management. These modalities, which are based on high-quality evidence, are the first choice for prevention and control of dental caries (Rethman MP *et al.*, 2011).
Globally, many strategies have focused on the avoidance, or at least the reduction, of sugar intake to prevent dental caries. Despite these efforts, world consumption of sugar continues to increase. The increasing demand for sugar, coupled with its potential detrimental effect on systemic health (obesity, type 2 diabetes mellitus) and oral health (dental caries), has led to increasing interest in sugar substitutes. One such class of substitutes known as “polyols” or “sugar alcohols” is non-fermentable sugars (Deshpande A *et al.*, 2008).
Non-fluoride agents may serve as adjunctive therapeutics for preventing, arresting or even reversing dental caries.
The most common polyols are sorbitol and xylitol, and they have been used extensively as sugar substitutes in chewing gum. Experts recognize that regular use of polyol-containing chewing gums could play a role in preventing caries by increasing salivary flow through mastication, reversing decreases in plaque pH and enhancing remineralisation of subsurface enamel lesions. Xylitol also may decrease the amount of dental caries as a result of its unique ability to alter microbial composition by reducing the viability and survival of virulent Streptococcus mutans (Deshpande A *et al.*, 2008; Rethman MP *et al.*, 2011).
Erythritol is a natural sugar alcohol of the tetritol type, which has been recently approved for use in the United States and throughout much of the world. Some studies have shown that erythritol has a similar effect on the risk factors of caries and seems to inhibit the growth of certain mutans streptococci strains as xylitol. Erythritol is considered to be a non-acidogenic substance (Honkala S *et al.*, 2014; Mäkinen KK, 2011).

Diagnosis

The principal methods currently used to diagnose carious lesion are visual and visual/tactile examination matched with radiographic assessments (Bader, JD 2002). Although the clinical examination was well established and universally taught, clinicians and patients did not generally recognised that this method was imperfect. While the clinical examination was mainly used to identify lesions on occlusal surface, the detection of caries in interproximal space was achieved using bitewing radiographs. The combined use of these two methods had an overall sensitivity of 50% and a specificity of 87% (Selwitz RH *et al.*, 2007). In also limitation to reveal the early stage of disease have been reported (Bader JD *et al.*, 2002). In addition, the risk related to radiographic exposure needs to be taken into consideration (Lodlow JB *et al.*, 1997).
Improvement of technology for caries detection is evident. As a complementing aid to visual examination, a Digital Imaging Fiber-Optic Transillumination Device (DIFOTI) was designed with the task to support clinicians in the identification of caries lesion in different stages of the disease (Astvaldsdòttir A *et al.*, 2012; Keem S *et al.*, 1997; Schneiderman A *et al.*, 1997). By using the specific optical properties of a carious tissue, trans-illumination of teeth with DIFOTI amplifies the change in scattering and absorption of photons and thereby, makes the lesion appear as a dark shadow (Astvaldsdòttir A *et al.*, 2012). DIFOTI was developed to facilitate in real time detection, localization and quantitative characterization of lesions (Schneiderman A *et al.*, 1997).

Therapy

The modern approach to caries treatment indicated the need to remove only dental tissue to the extent that is strictly necessary for treatment (Lozano-Chourio MA *et al.*, 2006). Modern restorative dentistry offers alternatives to the traditional tissue removal using conventional drilling instrument: the possible alternative are the chemo mechanical removal and the new type of bur.
In 1999, a product from MediTeam group called Carisolv® was marketed. This contains sodium hypochlorite and three natural amino acids: lysine, leucine and glutamic acid. When the gel of three amino acids (lysine, leucine and glutamic acid); 53mM and the gel containing 0.27M hypochlorite are mixed, amino acids bind chlorine and form chloramines whit pH of 11. This chlorination affects the secondary and/or quaternary structure of the collagen, by disrupting hydrogen bonding and causing proteolytic reaction. That does not affect healthy dentine because amino acids act as homing devices for active chlorine. The chlorine atom in hypochlorite is transferred to the amino group of each amino acid and in this way that is made less reactive and less aggressive to healthy tissue (Bohari MR *et al.*, 2012). In contrast

with conventional excavators and drills used in the traditional caries removal, in Carisolv technique carious dentine is removed using specially designed instrument, all of supposed to reduce the risk of removing intact dentine.
The first in vitro investigation on the use of Carisolv, in primary and permanent teeth was published in 1998. It was reported that Carisolv had been compared in controlled clinical trials in permanent and in primary dentition to the conventional mechanical method and the removal of caries by hand instruments (Kavvadia K *et al.*, 2004). Numerous clinical study was reported the reliability of Carisolv although this product needs significantly longer working time (Bergmann J *et al.*, 2005; Kavvadia K *et al.*, 2004; Lozano-Chourio MA *et al.*, 2006).
The most conventional method of removing caries involves the use of steel or tungsten carbide burs mounted in a low-speed contra-angle. Although very efficient in term of time spent for caries removal, the decision to stop caries removal using these burs is very subjective, and basically depends on the operator's background and clinical experience. The recently marketed CeraBur (Komer-Brasseler, Lemgo, Germany) is a self-limiting ceramic bur (alumina-based with stabilized zirconia), which according to the manufacture efficiently cut infected, soft dentin, while hardly acts on hard, sound tissue (Dammaschke T *et al.*, 2008).
Caries detector dyes based on propylene glycol were developed in order to highlight alteration in dentine collagen structure but publications have shown that clinical and laboratory results produced are open to considerable user-interpretation (Neves Ade A *et al.*, 2011).

AIMS

The goal of this thesis was to gain knowledge about difference aspects of caries management. In more details, the aims of this thesis were:

- To identify the scientific validation in literature of the role of polyols in caries prevention. (Paper I)

- To evaluate the reliability of a Digital Imaging Fiber-Optic Transillumination device (DIFOTI) for the detection of caries lesions vs. clinical or radiographic examinations. In addition, the reliability of DIFOTI method was evaluated in a group of dental professionals. (Paper II)

- To evaluate efficacy and reliability of different systems of caries removal versus the traditional method of caries removal. (Paper III and Paper IV)

MATERIAL AND METHODS

Paper I

Focused PICO Question
What is the efficacy in caries prevention, of polyols compared to the sorbitol and/or mannitol and/or maltitol or no intervention group, in terms of ΔDMFS/dmfs, salivary count of Mutans S. and plaque pH?

Eligibility Criteria
The papers included in this systematic review were randomized controlled trials (RCT) assessing the efficacy in caries prevention of chewing gums, tablets, candies and lozenges containing polyols. We selected the studies that involved both children and adults in which gums, tablets, candies or lozenges, contained xylitol, erythritol, maltitol, sorbitol or mannitol, were tested either against control group (sorbitol and/or mannitol and/or maltitol) or versus no intervention group. In addition, we have included studies where experimental agents other than polyols were tested.
We considered as primary outcome:

- Dental caries increment.
- Level of S. Mutans in the saliva.
- Plaque pH.

We excluded studies where the control group used sucrose in pellets, candies, tablets or lozenges. As well, we excluded studies where subjects had disabilities, wore orthodontics appliances or were pregnant. The studies in which the follow-up was performed under 4 weeks were excluded. For the statistical comparison of incidence of caries the minimum follow-up of 2 years was determined. For the rest of the variables no timing was settled. The length of the experimental period was classified in short-term (between 1 - 5 months), medium-term (between 6 – 11 months) and long-term (more than 12 months). When controls were performed more than one time in the short term, in the medium term or in the long term we have considered the last data performed in the same period. If the polyols were tested in different way with regard to dose and frequency of administration, we choose the data from the group in which the polyols were administer according to the guidelines (Rethman MP *et al.*, 2011). If the follow-ups were longer than the administration period of polyols, we extracted only the data of the three primary outcomes until the last control.

Considering that the dental caries increment could be reported differently in different trials, we decided to include in the meta-analysis only two types of data: decayed-filled-missing tooth (ΔDMFS – Δdmfs) or the data of decayed surface increment. Furthermore, considering that in the studies the clinical examination to determine the presence of caries could have been made according to different methods and the lesions could have been classified in different ways, we established a-priori how to designate the data: data from "combined clinical and radiological examination" were chosen over data from "separated clinical and radiological" and as a second choice we included "only clinical" data when radiological examination was not performed. Data for non-cavitated lesions combined with cavitated lesions was chosen over cavitated-only lesion; when more than one follow-up performed over 2 years was present, we included the data for each follow-up performed.
For the data of S. Mutans count in the saliva we considered for the meta-analysis only the data expressed in CFU/ml. Finally, data of the plaque pH contemplated for the meta-analysis was only from areas under the plaque pH curve for each pH cut-off value presented in the paper. The studies that satisfied the inclusion criteria but data was not serviceable, were included only in the systematic review.

Data Analysis
The outcomes considered in the studies were: the dental caries increment (continuous and dichotomous), salivary S. Mutans count (continuous) and plaque pH (continuous). When the raw data was not present in the text or tables, single authors were contacted to obtain such information. If the authors did not answer the petition, we extracted the information from the graphs. The data comparison of the primary outcome was done separately for the gums, lozenges, tablets and candies. The comparison of DMFS and dmfs index was done separately and if that was not possible we used to comparison the number of new surface or teeth decayed. Within each vector (gum, lozenges, tablets and candies) and for each primary outcome we compared separately data between control group and/or no intervention and experimental polyols group. To compare dichotomous data, a calculation of the Odd Ratio (OR) along with 95% Confidence Intervals (CIs) was used, whereas, for continuous data, the Mean Difference (MD) with the 95% Confidence Intervals (CIs) was calculated. Also, for each comparison the Z-test was used. A Fixed-effect model was applied to reassess all data extracted from the included studies. We compared the data of salivary count of S. Mutans and plaque pH at baseline in the short-medium- and at long term. For the dental caries increment we have compared data only at follow-ups. Data of gums, lozenges, tablets and candies were compared separately. Analysis was performed using Review Manager 5.3 software provided by the Cochrane Collaboration (The Cochrane Collaboration, 2012).
For the identification of studies to be included or considered for the review we developed two search strategies: one was used in two electronic databases

(PUBMED and EMBASE) (*tab. 1*) and the other was used in SCOPUS (*tab. 2*). We did not place any restriction on language or date of publication when searching the electronic database.

Search Strategy
We searched the following electronic databases:

• MEDLINE via PUBMED (to March 2015)
• EMBASE (to March 2015)
• SCOPUS (to March 2015)

A comparison of the different searches was carried out to exclude the repeated studies. Then, two authors, tasked with to evaluating the eligibility of the papers, examined independently all abstracts and titles of the studies found. If the information contained in abstract or in the title was no enough to determinate if the studies met inclusion criteria, the full paper was obtained. All studies that appeared to meet inclusion criteria were obtained in the full text format. The two authors assessed the papers independently to establish whether the studies met the inclusion criteria. Disagreements were resolved by discussion.

#1 randomized clinical trial [pt]
#2 dental caries AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR plaque pH OR streptococcus mutans OR lactobacillus) [tiab]
#3 dmft AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols) [tiab]
#4 lactobacillus AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
5# streptococcus mutans AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
6# plaque pH AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
#7 #2 OR #3 OR #4 OR #5 OR #6

Tab. 1 Search strategy used in PubMed via MedLine and Embase database

#1 randomized clinical trial [tiab]
#2 dental caries AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR plaque pH OR streptococcus mutans OR lactobacillus) [tiab]
#3 dmft AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols) [tiab]
#4 lactobacillus AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
5# streptococcus mutans AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
6# plaque pH AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
#7 #1 OR #2 OR #3 OR #4 OR #5 OR #6

Tab. 2 Search strategy used in SCOPUS database

Paper II

The study was approved by the Ethical Committee at the University of Sassari (authorization number 389/2013) and it was conducted over 6 weeks from June 9th to July 15th 2014.

Study design

The study was designed in two different parts: the first one was on the comparison among three detection methods (DIFOTI, bitewing radiographs and clinical examination), the second one was on the reliability among dental professionals using DIFOTI imagines derived by the first part.

Comparison among three detection methods

Detection methods

The new KaVo DIAGNOcam 2170 is a camera system that uses the tooth's structure to verify occlusal, approximal and secondary caries lesions when the tooth is trans-illuminated. A digital video camera records the image and displays it on a computer screen.

For the radiographic examination, Planmeca intraoral radiographic equipment (Planmeca, Helsinki, Finland) and Kodak UltraSpeed DF42 films, with settings of 70 kV and 7 mA and an exposure time of 0.25 s, were used for bitewing radiographs.

The radiographs were manually developed via conventional standard conditions and standard processing times, and examined according to O'Mullane criteria (O'Mullane DM *et al.*, 1997).
Clinical examination was performed under standard conditions. Subjects were seated in a dental unit and teeth were examined using a plan mirror (Hahnenkratt, Königsbach, Germany) and the WHO CPITN ballpoint probe (Asa-Dental, Milan, Italy) under optimal light.

Calibration of the examiners
Calibration exercises for all the three methods (visual clinical caries diagnostic system (ICDAS), DIAGNOcam unit and radiographic examination) were carried out by two dentists before the start of the study. One of the authors (GCampus) acted as benchmark, training and calibrating the two examiners. The calibration process was divided for each diagnostic method in four steps:

- lectures regarding the disease, the method (*i.e.* ICDAS, DIAGNOcam, x-ray), for eight hours;
- first examination, no discussion was allowed between the examiners and the dental advisors as to the interpretation of the criteria during the calibration sessions;
- re-valuation by the examiners after 72 hours for the clinical examination and after one week for the DIAGNOcam and x-ray;
- evaluation of the agreement or disagreement and statistical analysis.

Fifty volunteers were clinically examined for caries lesions presence in a dental chair using the ICDAS criteria and re-examined after 72 hours. Intra- and inter-examiner reliability was calculated through per cent agreement and Cohen's Kappa statistics. Good inter-examiner reliability was found with no significant difference from benchmark values ($p=0.15$) and a low mean square of error (0.47). The Pearson's correlation coefficient between the two examiners was high ($r = 0.83$, $p < 0.01$, $R^2 = 0.71$). Intra-examiner reliability was also high, Cohen's $K=0.88$.
Forty extracted human teeth (10 premolars and 30 molars), in total 80 approximal and 40 occlusal surfaces, were selected for the calibration of the DIFOTI device and the radiographic examination. The teeth were selected from a pool of extracted teeth from the Department of Oral Surgery of the University of Sassari. After extraction, the teeth were immediately collected in vials containing distilled water first, and then were carefully cleaned of soft tissues and calculus, and frozen at -20° until used. Selection criteria match the line of the first evaluation. Evaluations were carried out at one-week interval; Kappa values for inter- and intra-examiner agreement were high for both methods (0.79 for DIFOTI and 0.83 for x-ray). The Pearson's correlation coefficient for the two examiners was high ($r = 0.84$, $p < 0.01$, $R^2 = 0.74$).

The clinical examiner did not have the opportunity to look at DIAGNOcam (CAMo/a) or BW images for the entire period.

Sample

The study population consisted of students of the School of Medicine of the University of Sassari, Italy. To be suitable for enrolment, subjects had to meet these inclusion criteria: no missing teeth, no secondary caries and no fillings in premolars or molars. The exclusion criteria were subjects wearing fixed orthodontic appliances and subjects unable to be exposed to x-rays for medical/specific reasons. All students (n=1145) attending the School of Medicine were invited to participate via email/leaflet where the aim of the study was described in detail. A total of 678 students accepted and were examined (59.2% acceptance rate) and 52 subjects (19-23 years, mean age 21.2±1.2) fulfilled the inclusion/exclusion criteria.

Power analysis (G*Power 3 software) was performed to establish the number of subjects needed to evaluate the estimated difference in caries diagnosis using DIFOTI and/or clinical evaluation and x-ray. Data (Virajsilp V *et al.*, 2005) related to the reliability of a two diagnostic methods were used to calculate the sample size, even if data used were on primary teeth. The standardized effect was set at 0.39 with a sample size of 48 subjects and an upper 95% one- sided confidence limit of 0.52. All subjects (n=52) that fulfilled the inclusion/exclusion criteria were enrolled. Each subject was codified with a number in order to protect his/her identity. The flow chart of the study is displayed in figure 1.

The DIFOTI device was used to assess caries lesions in occlusal surfaces (CAMo) and in approximal surfaces (CAMa). As well, a clinical examination of the occlusal surfaces (CE) and a radiographic examination (BW) for approximal surfaces were performed.

Each tooth were cleaned for 30 seconds with a prophylaxis paste (Clinpro™ Prophy Paste: 3M ESPE Dental Products, USA) and then rinsed by a water spray for 10 seconds. The clinical examination was performed under standardized conditions describe above after drying teeth for 5 seconds. The students were examined and analysed during the same day by both examiners, first attending the clinical and radiographic examination and afterwards they were asked to go to another room where the DIFOTI device was installed with a computer in a dental chair. The International Caries Detection and Assessment System (ICDAS) was recorded for both enamel and dentinal lesions (International Caries Detection and Assessment System Coordinating Committee, 2005; Ismail AI *et al.*, 2007; Honkala E *et al.*, 2011). The radiographs were taken using an 8-inchround cone that was placed in contact with the ring of the film-holding system (RINN XCP, Dentsply, York), which in turn was placed in contact with the patient's cheek during exposure. Not perfectly clear or overlapping images were taken a second time. Then the DIFOTI device was used according to the manufacturer's instructions, placing the mouthpiece

over the occlusal surfaces. The image appeared in real time on the computer monitor, and the examiner saved it in the electronic patient record.
The ICDAS scores were performed on the occlusal surface. The DIAGNOcam was used for the detection of occlusal and approximal caries at enamel or dentine. When a defined approximal shadow in the enamel was present, it was scored as 1 and when reaching into the dentine it was scored as 2. Due to the impossibility to measure the lesion vertically all dark occlusal areas were scored as 1.
Radiographs were examined according to O'Mullane criteria (O'Mullane DM *et al.*, 1997) and mesial and distal surfaces were assessed.

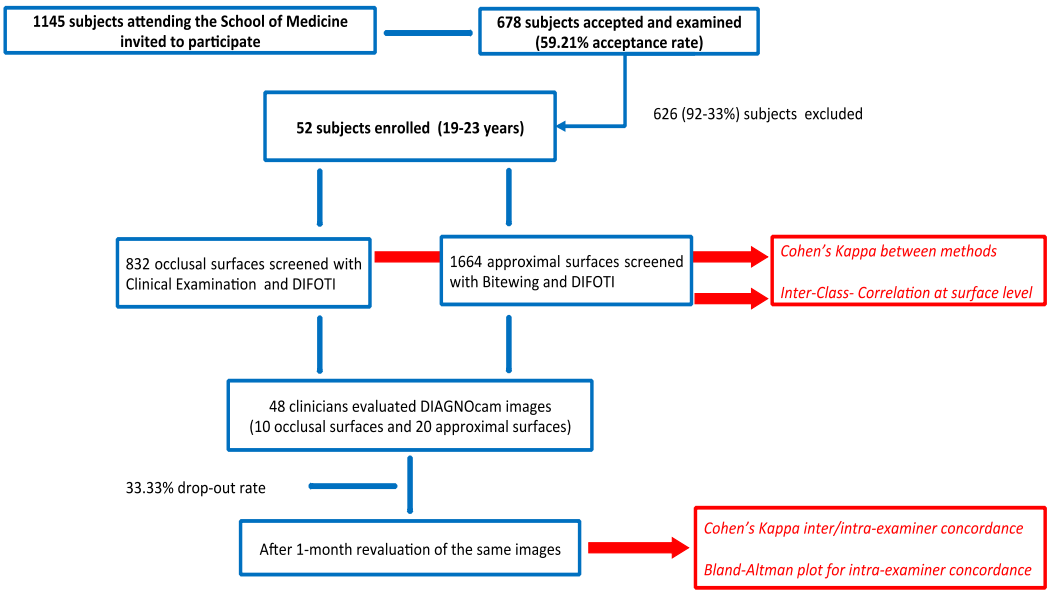


Fig. 1 Flowchart of experimental design to collect subject to test DIAGNOcam device

Reliability among dental professionals using DIFOTI

Forty-eight Italian dental professionals with no experience of the DIFOTI device were asked to participate in the second part of the study. The professional experience was at least 7 years. The day of the study they underwent at 60-minute training session describing the DIFOTI technology and the DIAGNOcam by one of the authors (CLC). Immediately after the training session, each participant had to diagnose ten teeth imagines randomly obtained from the first part of the study, analysing 10 occlusal, 10 mesial and 10 distal surfaces. Participants were asked to fill in a form containing two possible answers (1 - presence of caries, 2 - absence of caries) (EVA1). One month later, participants were contacted via email and were asked to revaluate the same images with the same criteria (EVA2). These results were compared with their previous answers.

Statistical Analysis

All data were analysed using STATA 13. For all analysis a p-value<0.05 was considered statistically significant. The general grade of accordance between the different detection methods was evaluated using the Cohen's Kappa (Cohen J *et al.*, 1960), while the reproducibility for the two methods for each surfaces (occlusal or approximal) was assessed using Intra-Class Correlation coefficients (ICC). ICC values equal to 0 represent agreement equivalent to that expected by chance, while 1 represents full agreement.

The inter-examiner DIFOTI reliability among dental professionals compared to the results derived from DIAGNOcam analysis was evaluated categorizing the kappa value of each professional respect to DIAGNOcam following the criteria described by Landis and Koch (Landis JR *et al.*, 1997), who characterized values < 0 as indicating no concordance and 0-0.20 as slight, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as almost perfect concordance. The method by Bland and Altman (Bland JM *et al.*, 1986) was used to display the variability of the two examinations (EVA1 and EVA2) by each examiner and the plot of EVA1 respect to the DIAGNOcam results, the plot of EVA2 respect to DIAGNOcam and the comparison between EVA1 and EVA2. This method allows to investigate the existence of any systematic difference between the measurements and to identify possible outliers.

Paper III

The systematic review was performed following the guidelines of the Transparent Reporting of Systematic Reviews and Meta-Analyses (Moher D *et al.*, 2009).

Focused PICO question

In primary dentition, what is the efficacy of Carisolv in caries removal rate (clinically appreciated) compared to the traditional drill technique, the clinical efficiency (treatment time) and patient's comfort (need of anaesthesia)?

Eligibility criteria

The studies included in the present review are Clinical Trials, Randomized Clinical Trials and Controlled Trials assessing the efficacy on the primary dentition of Carisolv compared to traditional mechanical caries removal (control) with drilling instruments. Only studies where total caries removal in each group was completed using Carisolv systems or rotary instruments used without any time limit were considered eligible. The studies including other experimental groups in addition to Carisolv and drilling were also included in this review. Studies assessing the complete caries removal different from clinical criteria (i.e. using a sharp probe) were excluded.

For the identification of studies to evaluate for this review, a unique search strategy to be applied for each database research was developed. The following key words were used: “Carisolv” and “Chemo mechanical Caries Removal”. No Mesh term match was found. The terms were searched following the Boolean term ‘OR’ for a total of three inquiries.

Database research:
. MEDLINE via PUBMED (from 1948 to December 2014);
. Web of Science (from 1948 to December 2014); and
. SCOPUS (from 1969 to December 2014).

A comparison of the different searches was carried out to delete the repeated studies. Then, two authors (GL and CLC), on charge to evaluate the eligibility of the studies, examined independently all abstracts of the selected papers. If an abstract didn’t supply enough information to determine if the paper met the inclusion criteria, the full report was obtained. All studies, which appeared to meet the inclusion criteria, were obtained in the full text format. The two authors assessed the papers independently, to establish whether or not the studies met the inclusion criteria. Disagreements were resolved by discussion. If not possible, other authors were consulted.

Data analysis
The outcomes considered in the studies were: the caries removal rate clinically appreciated (binary yes/ no), the time required to complete the tissue removal (continuous) and the pain threshold during the procedure, assessed through the need for local anaesthesia by patients (binary yes/no). When raw data was not available in the text, tables or graphs, single authors were contacted to obtain such information. To com- pare dichotomous data, a calculation of the Odd Ratio (OR) along with 95% Confidence Intervals (CIs) was used, whereas, for continuous data, the Mean Difference (MD) with 99% Confidence Intervals (CIs) was calculated. Also, for each comparison the Z-test was used. A random-effect model was applied to reassess all data extracted from the included studies.
Analysis was performed using Review Manager 5.3 software provided by the Cochrane Collaboration (The Cochrane Collaboration, 2012).

Paper IV

This prospective, randomized and controlled clinical trial was performed at the Faculty of Medicine and Surgery, Sassari University between March 2013 and December 2014.

Experimental Design

Before starting the study, there was a preparation period. The training lasted 4 weeks. The operator (GL), who performed the clinical procedures of the study, reached a good clinical in vivo agreement with the benchmark operator (GC) about what constitutes a cavity with complete and incomplete caries removal.

The steps of the study were the following: a preliminary examination, informed consent, randomization of samples, recording of cavity characteristic, collect dentine sample, caries removal, cavity inspection, collect dentine sample and final restoration. The same operator performed both caries treatment and cavity examination.

Inclusion Criteria

Between all the patients who appeared for a regular dental examination, who met the inclusion criteria was invited to enter in the study. To be selected children had to have at least 1 caries lesion interesting either occlusal, interproximal or in cervical surface of first and second primary molars prone to exfoliation. The lesion considered in this study was between a D1 and D3 stage evaluated by radiographic examination. Teeth with pathological processes of dental tissue other than caries, or pulpal disease or with adjacent soft tissue lesion were excluded. The children with systemic disease were excluded.

Clinical Procedure

Each tooth treated was distributed among the five groups by computer randomization. The five treatments groups with a total of 50 teeth are (*fig. 2*):

A. Cavity preparation with traditional technique (control group)
B. Cavity preparation with CeraBur
C. Cavity preparation with Carisolv and hand instruments dedicate
D. Cavity preparation with Carisolv and CeraBur
E. Cavity preparation with Carisolv and CeraBur. Cavity inspection with caries detector

In all groups was registered information about cavity size. This procedure was performed before and after excavation. Three different measurement were made using a periodontal probe:

1. The outer diameter in buccal – lingual and in mesial – distal sense
2. The depth of the lesion (when possible before excavation)

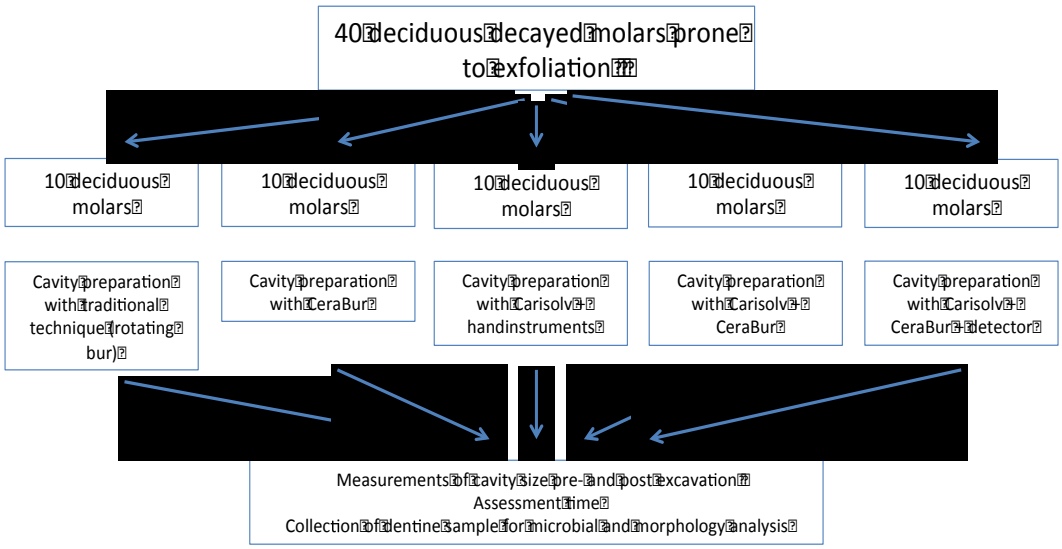


Fig. 2 Summary of experimental design to collect primary teeth to test different method of caries removal

To calculate the volume of cavity size before and after excavation we used pyramid as geometric model that could simulate the caries lesion shape. From cavity data we calculated the volume of geometric model that simulated the extension of caries lesions and clean cavity. Difference between post and pre-operative size was used to estimate the increment of cavity size.

Treatment time related to the caries excavation was measured. The clock was started when the first step of excavation or opening of the cavity began and stopped when the caries excavation and cavity preparation was completed. Time was measured in seconds.

In group A (control group) the carious lesions were treated using drills with two types of bur: Komet 880 314 012 to remove enamel and Komet H1SE 204 014 to eliminate tissue decay. In group B the enamel was removed using diamond bur (Komet 880 314 012) and ceramic bur was used to remove dentine decay. In group C Carisolv was used to remove caries: the gel was applied on dentine infected and after 30 seconds the softened tissue was removed using dedicated hand tools. This procedure was repeated until complete caries removal. When necessary enamel was removed using diamonds bur (Komet 880 314 012). In group D caries was removed using Carisolv gel. Finally ceramic bur (Komet Cerabur K1SM 2014 014) was used to finish the walls and the floor of the cavity. The procedure to remove tissue decay was the same in the D and E groups. In group E a caries detector was used for cavity inspection. In groups A, B, C and D the completion of caries removal was judged by standard clinical criteria, i.e. the probe did not stick in the remaining dentine. In the group E the complete caries removal was evaluated with caries detector. The data of

complete caries removal was registered. After the cavity check the teeth were restored with ionomer glass cement.

Microbiological Analysis

After drying and isolation with cotton rolls, dentine was sampled from the cavity before and at the end of the cavity preparation using sterile excavator. Each sample was placed in an Eppendorf tube containing 150 µl of sterile TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6). Then 100 µl of 0.5 M NaOH was added to the dentine sample and the bacterial suspension was stored at -20°C pending further processing (Gellen LSS *et al.*, 2007).

The analysis of bacterial species was performed using the checkerboard DNA-DNA hybridisation method (Wall-Manning GM *et al.*, 2002). Whole genomic probes were prepared from the 15 bacterial strains known to be related to caries as shown in (*tab. 1*). An evaluation of the bacterial count in the samples was performed by comparing the obtained signals with the ones generated by the pooled standard samples containing a count of 10^6 and 10^5 of each bacterial species, respectively. The signals were coded on a scale from 0 to 5 as follows: 0 = no signal; 1 = a signal density weaker than that of the low standard ($<10^5$ bacteria); 2 = a signal density equal to that of the low standard ($=10^5$ bacteria); 3 = a signal density higher than that of the low standard but lower than that of the high standard ($>10^5$ but $<10^6$ bacteria); 4 = a signal density equal to that of the high standard ($=10^6$ bacteria) and 5 = a signal density higher than that of the high standard ($>10^6$ bacteria).

Bacterial nomenclature	Strain description & source
Mutans streptococci	
Streptococcus mutans	ATCC-25175
Streptococcus sobrinus	CCUG-27507
Non-muntans streptococci	
Streptococcus sanguinis	ATCC-10556D-5
Streptococcus salivarius	ATCC-9759D-5
Streptococcus gordonii	ATCC-35105D-5
Streptococcus mitis	ATCC-49456D-5
Lactobacilli	
Lactobacillus casei	ATCC-334D-5
Lactobacillus fermentum	OMGS-3182
Lactobacillus salivarius	CCUG-55845
Actinomycens	
Actinomycens odontolyticus	NCTC-9935
Actinomucens oris	ATCC-12104D-5
Veillonella Parvula	ATCC-10709D-5
Rothia Dentocariosa	CCUG-17835
Bifidobacterium Dentium	OMGS-1956
Parvimonas Migra	ATCC-33270

Tab. 1 Bacterial strain used for preparation of DNA probes.

Statistical Analysis

Statistical difference in time taken and cavity size increment were performed using ANOVA analysis, adjusting statistical significance for the multiple comparisons (Bonferroni correction). For the analysis of microbiological data Shapiro-Francia normality test was used to assess the normality distribution of collect variables. Statistical difference in score of bacterial count was performed using the Kruskall-Wallis analysis of post-operative samples. In case of difference between groups comparisons were performed (Bonferroni correction). Statistical differences between pre- and post-operative in bacterial count, in each group and for each bacterial species, were calculated performing Mann-Whitney U test.

Statistical analysis was carried out using STATA®14.

RESULTS

Paper I

A total of 518 studies published from 1975 to 2015 were found. Forty-eight papers were analysed and 25 met the eligibility criteria. Three papers (Mäkinen KK *et al.*, 1996; Seki M *et al.*, 2011; Lenkkeri AM *et al.*, 2012) were not included in the meta-analysis but only in the systematic review because primary outcome data was missing (*fig. 3*).

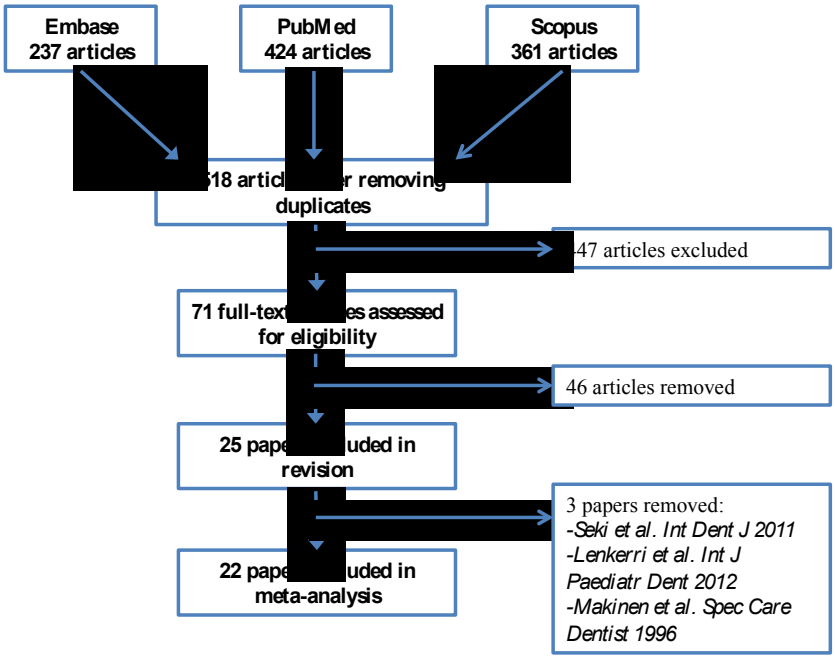


Fig. 3 Flowchart of search strategy

The trials admitted in the review involved a total of 5464 patients. From the selected studies, three were conducted in the US (Hildebrandt GH *et al.*, 2000; Milgrom P *et al.*, 2006; Ly KA *et al.*, 2006), one in Hungary (Szöke J *et al.*, 2001), one in England (Simons D *et al.*, 2002), four in Estonia (Honkala S *et al.*, 2014; Mäkinen KK *et al.*, 2005; Alanen P *et al.*, 2000; Runnel R *et al.*, 2013), three in Sweden (Oscarson P *et al.*, 2006; Stecksen-Blicks C *et al.*, 2008; Holgerson PL *et al.*, 2006), one in Puerto Rico (Beiswanger BB *et al.*, 1998), one in China (Peng B *et al.*, 2004), one in Lithuania (Machiulskiene V *et al.*, 2001), one in Colombia (Martinez-Pabòn MC *et al.*, 2014), three in Italy (Campus G *et al.*, 2009; Campus G *et al.*, 2011; Campus G *et al.*, 2013) and one in Germany (Splieth CH *et al.*, 2009). One of the studies

included in the meta-analysis (Spieth CH *et al.*, 2009) did not specify the randomization method of the sample. All the other studies had a randomization clinical trial with parallel arms design. Five studies (Honkala S *et al.*, 2014; Mäkinen KK *et al.*, 2005; Alanen P *et al.*, 2000; Peng B *et al.*, 2004; Machiulskiene V *et al.*, 2001) used a cluster-randomized design. Eleven studies (Milgrom P, 2006; Honkala S *et al.*, 2014; Mäkinen KK *et al.*, 2005; Stecksen-Blicks C *et al.*, 2008; Martinez-Pabon MC *et al.*, 2014; Runnel R *et al.*, 2013; Holgerson PL *et al.*, 2006; Campus G *et al.*, 2013; Campus G *et al.*, 2011; Campus G *et al.*, 2009, Splieth CH *et al.*, 2009) were double blind and one single blind (Oscarson P *et al.*, 2006). Seven studies were performed in adults (Hildebrandt GH *et al.*, 2000; Milgrom P *et al.*, 2006; Simons D *et al.*, 2002; Martinez-Pabon MC *et al.*, 2014; Ly KA *et al.*, 2006; Campus G *et al.*, 2011; Splieth *et al.*, 2009), eleven in patients with mixed dentition, aged between 6 and 13 years old, (Szöke J *et al.*, 2001; Honkala S *et al.*, 2014; Beiswanger BB *et al.*; 1998; Runnel R *et al.*, 2013; Holgerson PL *et al.*, 2007; Campus G *et al.*, 2013; Campus G *et al.*, 2009; Stecksen-Blicks C *et al.*, 2007; Machiulskiene V *et al.*, 2001; Alanen P *et al.*, 2000; Peng B *et al.*, 2004) and one in children with deciduous dentition (Oscarson P *et al.*, 2006).

Out of fifteen studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011; Campus G *et al.*, 2013; Holgerson PL *et al.*, 2007; Ly KA *et al.*, 2006; Simons D *et al.*, 2002; Martinez-Pabon MC *et al.*, 2014; Machiulskiene V *et al.*, 2001; Peng B *et al.*, 2004; Beiswanger BB *et al.*, 1998; Szöke J *et al.*, 2001; Milgrom P *et al.*, 2006; Hildebrandt GH *et al.*, 2000) where gums were used, thirteen tested for xylitol; eight used gums with sorbitol and/or mannitol or maltitol as control; five did not give gums the control group. In two studies (Szöke J *et al.*, 2001; Beiswanger BB *et al.*, 1998) was tested a combination of sorbitol and mannitol was tested versus a control group with no gum. In one study (Hildebrandt GH *et al.*, 1998) as control was present both sorbitol gum and no intervention group. From two studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011), which tested gums, we could extract two types of data: salivary count of S. Mutans and plaque pH. From seven studies (Campus G *et al.*, 2013; Holgerson PL *et al.*, Ly KA *et al.*, 2006; Simons D *et al.*, 2002; Martinez-Pabon MC *et al.*, 2014; Milgrom P *et al.*, 2006, Hildebrandt GH *et al.*, 2000) we extracted data of S. Mutans count whereas from four studies (Szöke J *et al.*, 2001; Beiswager BB *et al.*, 1998; Machiulskiene V *et al.*, 2001; Peng B *et al.*, 2004) we gained data of dental caries increment. From study of Ly et al. the data of salivary count of S. Mutans at baseline was no present in the text. We contacted the authors to obtain that data but received no reply. The follow-up data were deduced from the graph in the papers. We also extracted data of salivary count of S. Mutans from the graphs in the paper text from Simons et al., 2006 study.

Two studies included in the meta-analysis used lozenges (Splieth CH *et al.*, 2009; Stecksen-Blicks C *et al.*, 2007) and tested xylitol. In the study of Splieth et al., was used as control group lozenges with sorbitol while in Stecksen-Blicks et al. paper in

the control group was not administrated any lozenges. From Splieth et al. study we extracted data of plaque pH while from Stecksen-Blicks et al. paper we gained data of ΔDMFS.
Two studies included in the meta-analysis used tablets (Mäkinen KK *et al.*, 2005; Oscarson P *et al.*, 2006) as vector. The paper of Oscarson et al. was tested xylitol while in the study of Mäkinen et al. the polyols tested were erythritol, xylitol and sorbitol. Both studies in the control group did not administer tablets. From one study (Mäkinen KK *et al.*, 2005) was gained data of salivary count of S. Mutans was gained while from the other study (Oscarson P *et al.*, 2006) we extracted data of Δdmfs.
In two studies included in the meta-analysis were used candies were used (Runnel R *et al.*, 2013; Honkala S *et al.*, 2014) as vector. Both studies were tested erythritol and xylitol while the control group were used candies with sorbitol. From one studies (Honkala S *et al.*, 2014) we extracted data of number of decayed surface while in the other we gained data of salivary count of S. Mutans. From the study of Honkala S et al., the data of decayed surface affecting both the primary teeth and permanent teeth.
In the study of Alanen P et al., were used both candies and gum. In the study xylitol was tested while in the control group the subject did not received gum or candy. From this study we extracted data of ΔDMFS.

Xylitol gum versus sorbitol gum - MS count
For this comparison we considered seven studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011, Campus G *et al.*, 2013; Hildebrandt GH *et al.*, 200; Holgerson PL *et al.*, 2007; Milgrom P *et al.*, 2006; Ly KA *et al.*, 2006). At baseline and in the short term we found data in six studies, in the medium term we extracted data from two studies and in the long term only one study presented data of MS (Mutans S.) salivary count. At baseline (*fig. 4*) we found no difference in MS salivary count (mean difference (MD) 0.01, 95% confidence interval (CI) -0.04 to 0.06, P value = 0.78). In the short term (*fig. 4*) the MS salivary count is significantly lower in the Xylitol group (MD -0.20, 95% CI -0.28 to -0.12, P value = 0.002). In the medium term (*fig. 4*) we found no difference between gum with xylitol and gum with sorbitol in terms of reduction of MS salivary count (MD -0.16, 95% CI -0.32 to 0.01, P value = 0.26. In the long term (*fig. 4*) from the analysis of Campus et al., 2013 study, we found that the MS salivary count is smaller in the xylitol gum group than in the control group with sorbitol gum (MD -0.70, 95% CI -1.31 to -0.09, P =0.02).

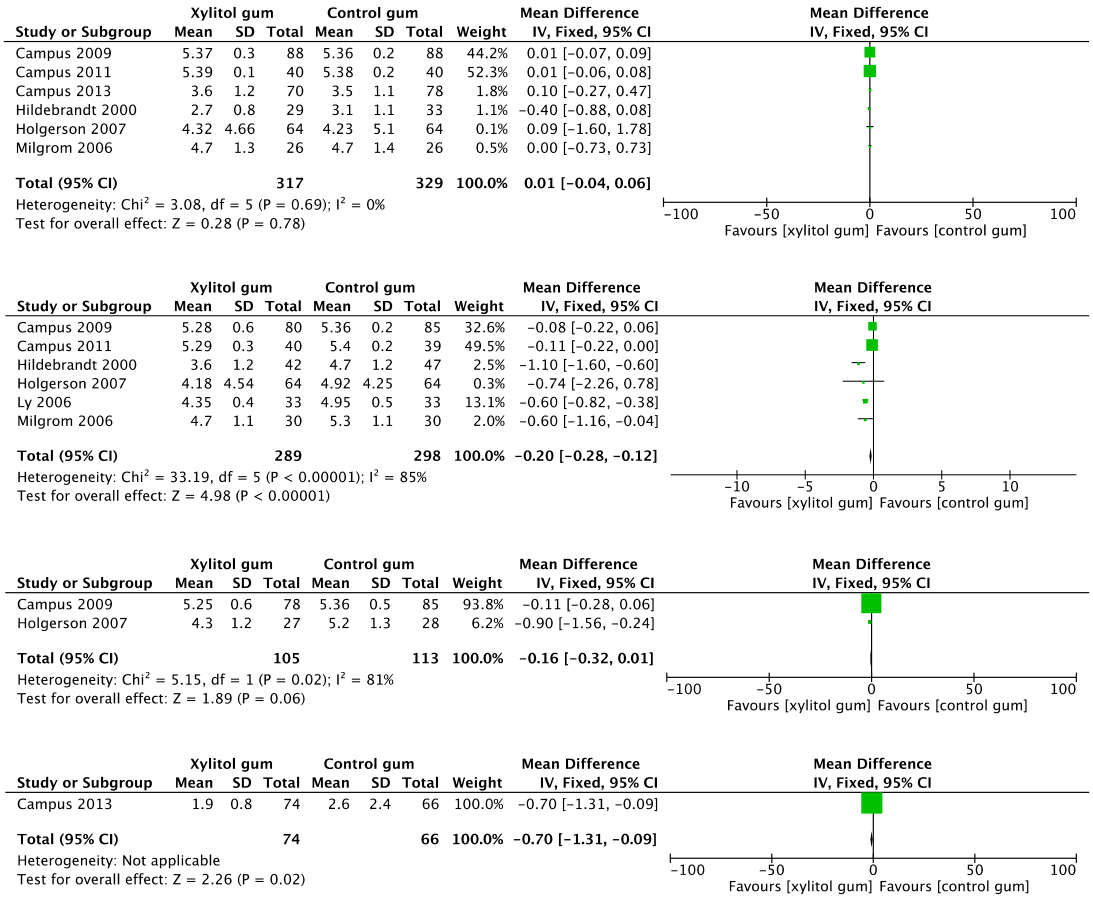


Fig. 4 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of xylitol gum vs. sorbitol gum at short, medium and long term

Xylitol gum versus no gum – MS count

For this comparison we had included three studies (Hildebrandt GH *et al.*, 2000; Martinez-Pabon MC *et al.*, 2014; Simons D *et al.*, 2002). At baseline and in the short term we found data in all studies whereas for the comparison in the medium and long terms only one study (Simons D *et al.*, 2002) was used to extract MS salivary count. At baseline (*fig. 5*) we found no difference in MS salivary count (MD -0.17; 95% CI -0.58 to 0.24, P value = 0.42). In the short term (*fig. 5*) MS salivary count was significantly lower in the Xylitol group (MD -0.70, 95% CI -1.14 to -0.25, P = 0.002). In the medium and long term (*fig. 5*) no difference was found in MS salivary count. In the medium term P-value = 0.08 and in the long term P-value = 0.85.

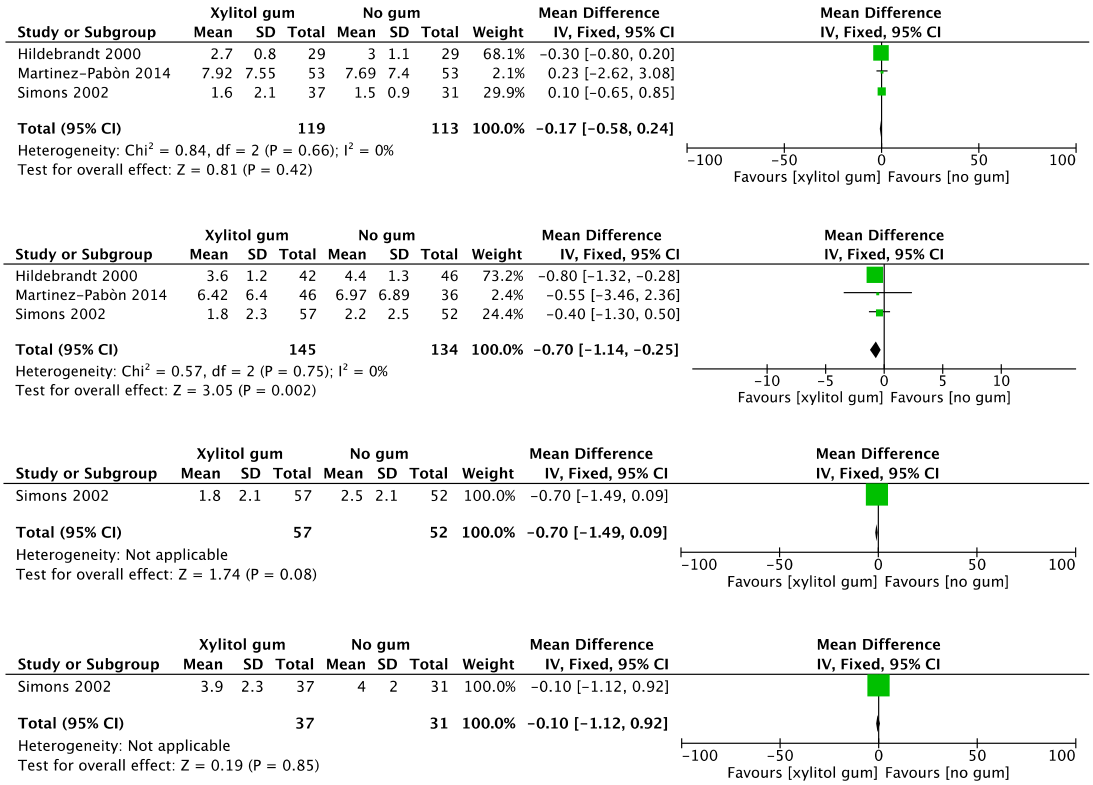
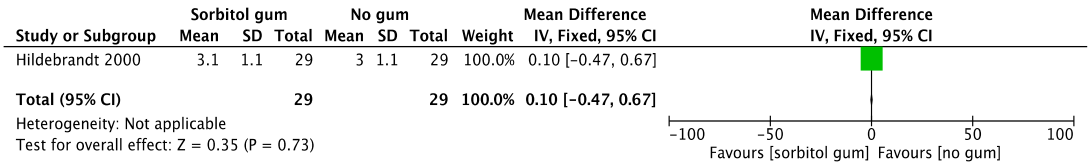


Fig. 5 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of xylitol gum vs. no intervention group at short, medium and long term.

Sorbitol gum versus no gum – MS count

For this comparison we had extracted data from one study (Hildebrandt GH *et al.*, 2000). In this study control were present at baseline and in the short term. At baseline there was no difference in terms of MS salivary count (MD 0.10, 95% CI -0.47 to 0.67, P = 0.73) (*fig. 6*). In the control in the short-term period we found no difference between sorbitol gum and control group without chewing gums (MD 0.30, 95% CI -0.21 to 0.81, P = 0.25) (*fig. 6*).



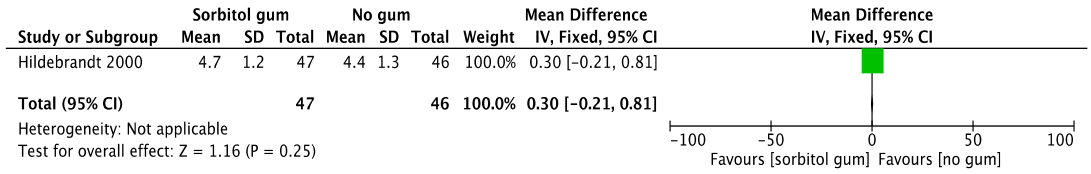


Fig. 6 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of sorbitol tablet vs. no intervention group at medium term

Xylitol tablet versus no tablet – MS count

For this comparison we had included only one study (Mäkinen KK *et al.*, 2005) and we have data at baseline and in the medium term. At baseline there was no difference in MS salivary count (MD 0.32, 95% CI -0.71 to 0.07, P = 0.10) whereas in the medium term (*fig. 7*) salivary presence of MS was significantly lower in the xylitol group than in the control group (MD -0.70, 95% CI -1-12 to -0.28, P = 0.001) (*fig. 7*).

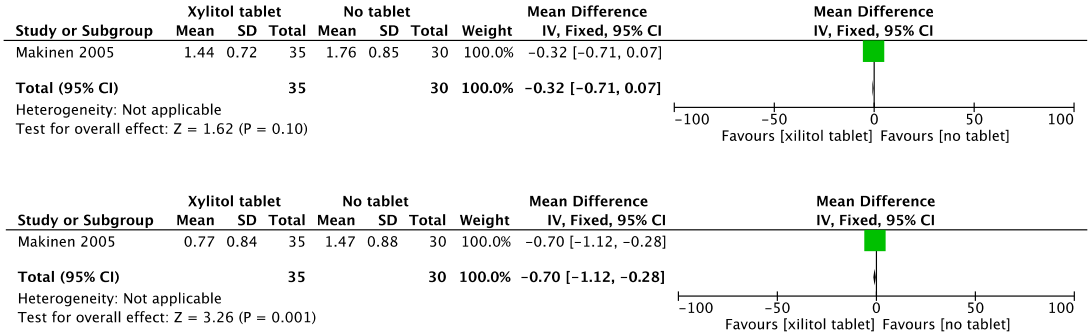


Fig. 7 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of xylitol tablet vs. no intervention group at medium term

Xylitol tablet versus control tablet – MS count

For this comparison we included only one study (Mäkinen KK *et al.*, 2005) and we had data at baseline and in the medium term. No significant difference was found at baseline in MS salivary count (MD -0.03, 95% CI -0.40 to 0.34, P = 0.87) whereas in the medium term (*fig. 8*) the MS salivary count appeared higher in xylitol group than in the control group (MD -0.61, 95% CI -1.01 to -0.21, P = 0.003) (*fig. 8*).

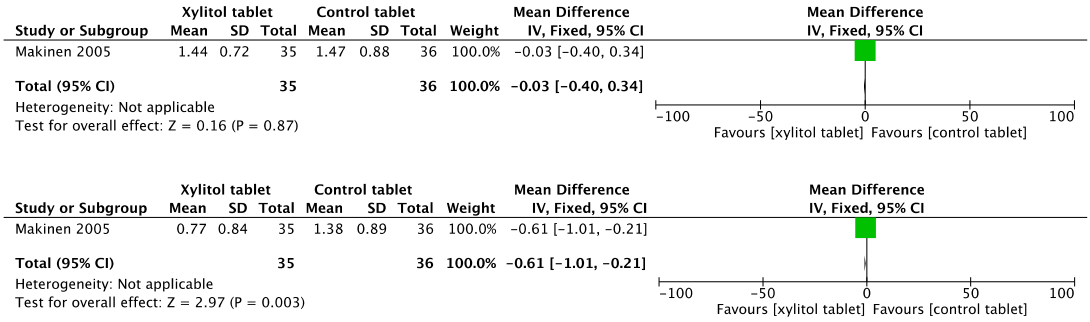


Fig. 8 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of xylitol tablet vs. sorbitol tablet at medium term

Erythritol tablet versus no tablet – MS count

For this comparison we included only one study (Mäkinen KK *et al.*, 2005) and we had data at baseline and in the medium term. At baseline (*fig. 9*) there was not difference in MS salivary count (MD -0.04, 95% CI -0.48 to -0.40, P = 0.86) whereas in the medium term (*fig. 9*) salivary presence of MS is significantly lower in xylitol group than in the control group (MD -0.85, 95% CI -1.26 to -0.44, P < 0.0001).

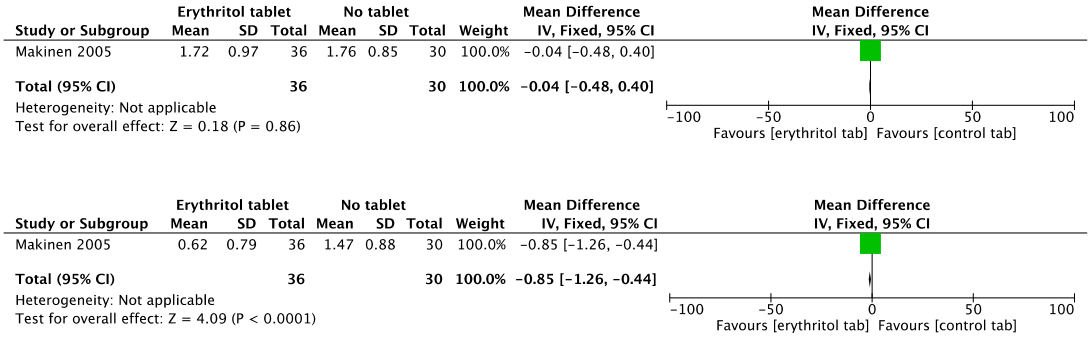


Fig. 9 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of erythritol tablet vs. no intervention group at medium term

Erythritol tablet versus control tablet – MS count

For this comparison we included only one study (Mäkinen KK *et al.*, 2005) and we have data in the baseline and in the medium term. No significant difference was found at baseline in MS salivary count (MD 0.25, 95% CI -0.18, 0.68, P = 0.25) (*fig.10*) whereas in the medium term the MS salivary count resulted higher in control group than erythritol group (MD -0.76, 95% CI -1.15 to -0.37, P = 0.0001) (*fig.10*).

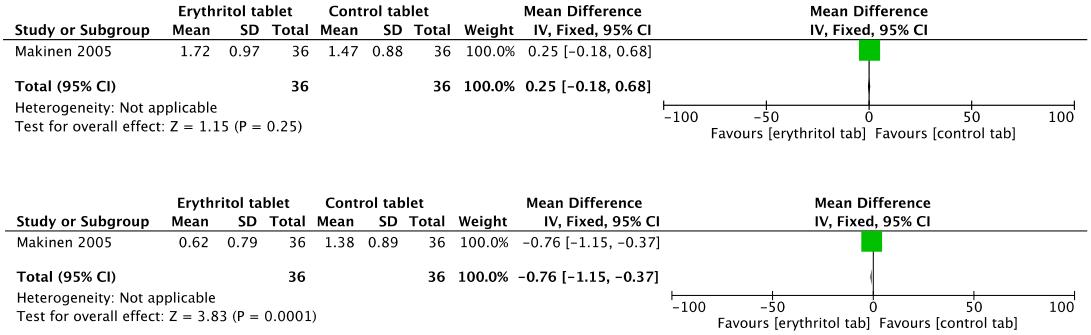


Fig. 10 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of erythritol tablet vs. no intervention group at medium term

Sorbitol tablet versus no tablet – MS count

For this comparison we included only one study (Mäkinen KK *et al.*, 2005) and we had data at baseline and in the medium term. No significant difference was found at baseline in MS salivary count (MD 0.25, 95% CI -0.18, 0.68, P = 0.25) (*fig. 11*) whereas in the medium term (tab. 1) the MS salivary count resulted higher in control group than in the erythritol group (MD -0.76, 95% CI -1.15 to -0.37, P = 0.0001) (*fig. 11*).

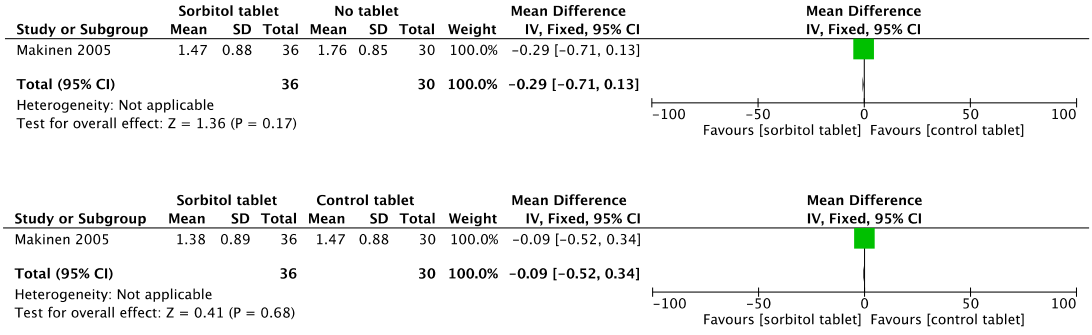


Fig. 11 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of sorbitol tablet vs. no intervention group at medium term

Xylitol candy versus control candy – MS count

For this comparison we have found one study (Runnel R *et al.*, 2013). In the paper data at baseline and at long term was presented. At baseline (*fig. 12*) the MS salivary count was significantly higher in xylitol group (MD 0.11, 95% CI 0.09 to 0.13, P < 0.00001) whereas at control in the long term we found the opposite situation: the MS salivary count was lower in the xylitol group (MD -0.18, 95% CI -0.20, -0.16, P < 0.0001) (*fig.12*).

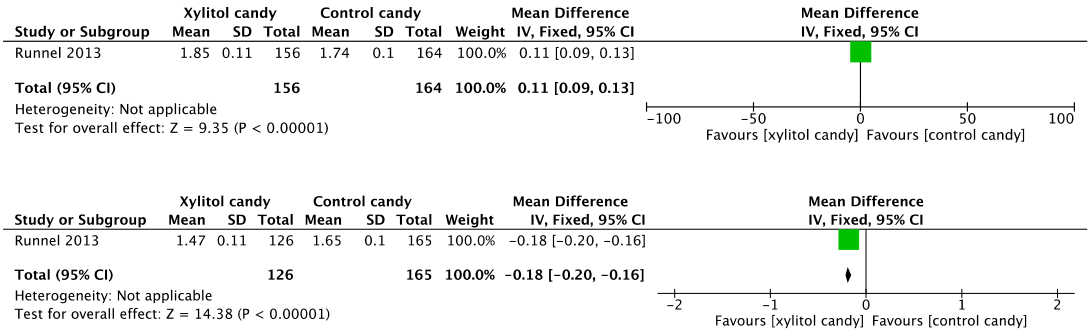


Fig. 12 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of xylitol candy vs. sorbitol candy at long term

Erythritol candy versus control candy – MS count

For this comparison we included only one study (Runnel R *et al.*, 2013) and we had data at baseline and in the long term. At baseline (*fig. 13*) there was not difference in MS salivary count (MD 0.02, 95% CI -0.00 to -0.04, P = 0.08) whereas in the medium term salivary presence of MS is significantly lower in xylitol group than in the control group (MD -0.44, 95% CI -0.46 to -0.42, P < 0.0001) (*fig. 13*).

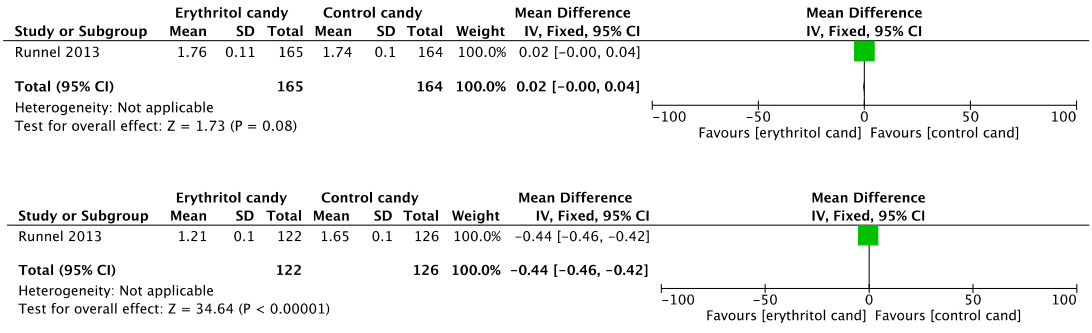


Fig. 13 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control MS salivary count increment of erythritol candy vs. sorbitol candy in the long term

Xylitol gum versus sorbitol gum – DMFS

For this comparison we had included one study (Machiulskiene V *et al.*, 2001) and we had data at 2 years and at 3 years of follow-ups. The data comparison of ΔDMFS at two years (*fig. 14*) showed, in the control group with sorbitol gum, a significantly smallest increase of decayed surface (MD 2.45, 95% CI 2.20 to 2.70, P < 0.00001. At 3 years (*fig. 14*) the increment of DMFS is higher in the control group with sorbitol gum (MD -0.90, 95% CI -1.35 to 0.45, P < 0.0001).

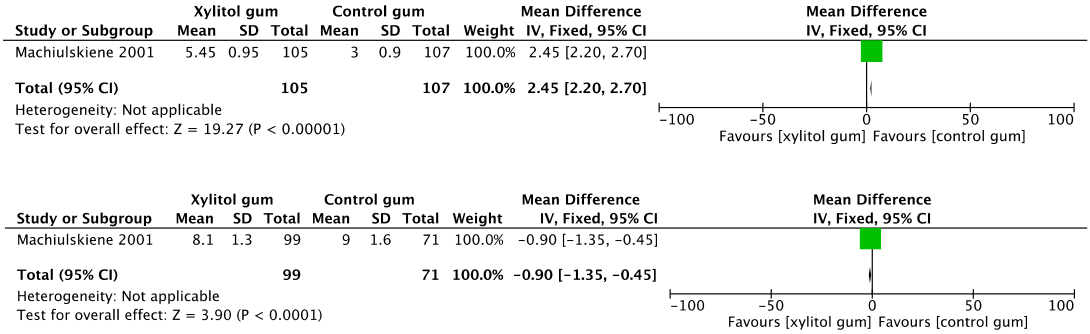


Fig. 14 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control ΔDMFS increment of xylitol gum vs. sorbitol gum at 2 and 3 years follow-up.

Xylitol gum versus no gum – DMFS

For this comparison we included three studies (Machiulskiene V *et al.*, 2001; Peng B *et al.*, 2004; Alanen P *et al.*, 2000); from one (Machiulskiene V *et al.*, 2001) we extracted data at 2 and at 3 years follow-ups whereas from studies of Peng B et al., and Alanen P et al., the follow-ups were performed respectively at 2 and 3 years. At 2 years the increase of DMFS was significantly lower in the xylitol group (MD -0.01, 95% CI -0.17 to -0.02, P = 0.01) (*fig. 15*). This trend was confirmed at 3 years of follow-ups: the ΔDMFS was smaller in the xylitol than control group (MD -0.69, 95% CI -1.08 to 0.30, P = 0.0005) (*fig. 15*).

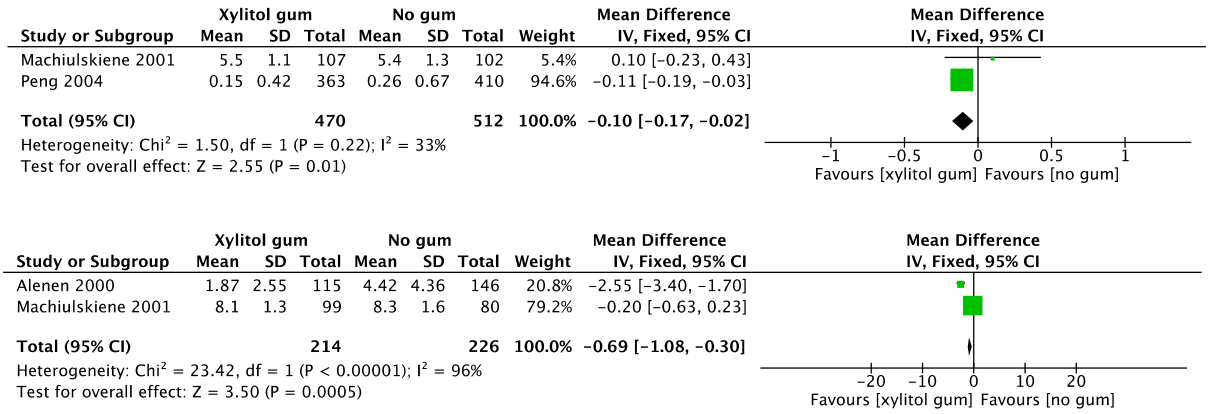


Fig. 15 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control ΔDMFS increment of xylitol gum vs. no intervention group at 2 and 3 years follow-up.

Sorbitol gum versus no gum – DMFS

For this comparison we included three studies (Machiulskiene V *et al.*, 2001; Szöke J *et al.*, 2001; Beiswanger BB *et al.*, 1998); from two studies (Machiulskiene V *et al.*, 2001, Beiswanger BB *et al.*, 1998) we extracted data at 2 and at 3 years follow-ups whereas from studies of Szöke et al. the follow-ups were performed only at 2 years. At 2 years the increase of DMFS was significantly lower in the xylitol group (MD -0.01, 95% CI -0.17 to -0.02, P = 0.01) (*fig. 16*). This trend was confirmed at 3 years follow-ups: the ΔDMFS was smaller in the xylitol than control group (MD -0.69, 95% CI -1.08 to 0.30, P = 0.0005) (*fig. 16*).

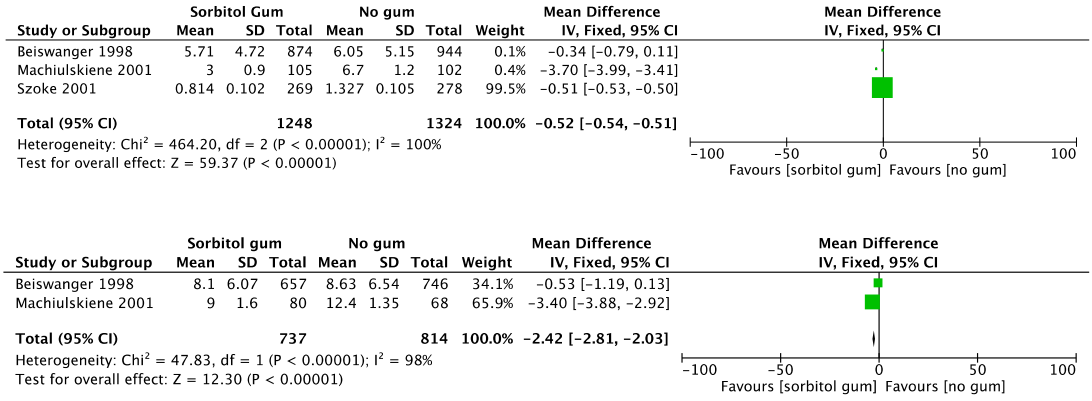


Fig. 16 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control ΔDMFS increment of sorbitol gum vs. no intervention group at 2 and 3 years follow-up.

Xylitol lozenges versus no lozenges – DMFS

For this comparison we found data in one study (Stecksen-Blick C *et al.*, 2008) and the follow-up was performed at 2 years. We found no difference in terms of ΔDMFS between xylitol lozenges and the group without lozenges (MD 1.00, 95% CI -0.42 to 2.42, P = 0.17) (*fig. 17*).

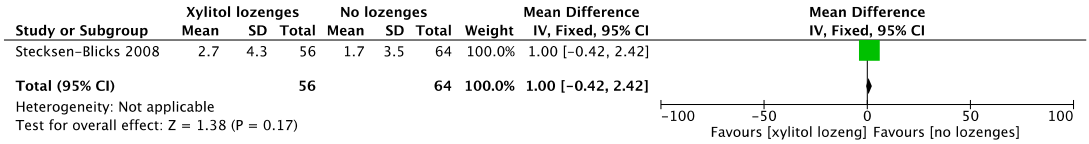


Fig. 17 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control ΔDMFS increment of xylitol lozenges vs. no intervention group at 3 years follow-up.

Xylitol candy versus no candy – DMFS

For this comparison we found data in one study (Alanen P *et al.*, 2000) and the follow-up was performed at 3 years. At 2 years the increase of DMFS was significantly lower in the xylitol group (MD -1.65, 95% CI -2.67 to -0.63, P = 0.002) (*fig. 18*).

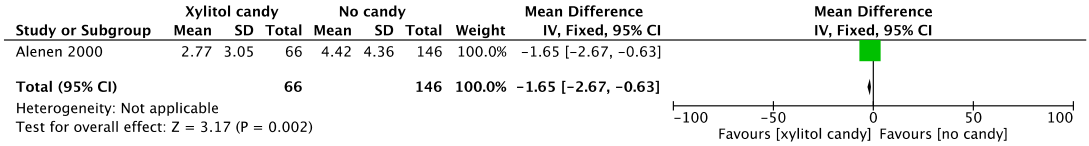


Fig. 18 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control ΔDMFS increment of xylitol candy vs. no intervention group at 3 years follow-up.

Xylitol candy versus no candy – DMFS + dmfs (number of decayed surface/total surface analysed)

For this comparison we have found data in one study (Honkala E *et al.*, 2014) in which the follow-up was performed at 3 years. We have no found difference between the xylitol group and the control group (OR 1.05, 95% CI 0.96 to 1.15, P = 0.26) (*fig. 19*).

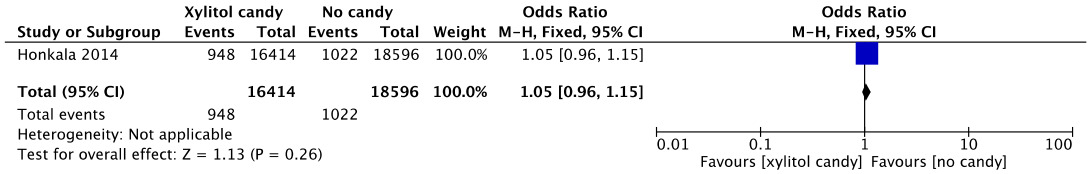


Fig. 19 Forest plot of comparison: Individual and overall Odd Ratio in the comparison of efficacy to control surface decayed of xylitol candy vs. no intervention group at 3 years follow-up.

Erythritol candy versus no candy – DMFS + dmfs (number of decayed surface/total surface analysed)

For this comparison we have found data in one study (Honkala E *et al.*, 2014) in which the follow-up was performed at 3 years. At 3 years the decayed surface in the control group was significantly higher than in the erythritol group (OR 0.83, 95% CI 0.75 to 0.91, P < 0.0001) (*fig. 20*).

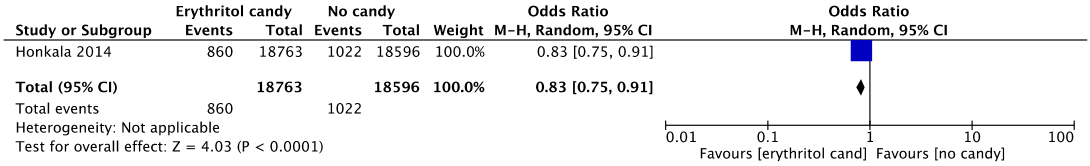


Fig. 20 Forest plot of comparison: Individual and overall Odd Ratio in the comparison of efficacy to reduce surface decayed of erythritol candy vs. no intervention group at 3 years follow-up.

Xylitol tablet versus no tablet – dmfs

For this comparison we found data in one study (Oscarson P *et al.*, 2006) and the follow-up was performed at 2 years. At 2 years we found no difference between the xylitol group and the control group (MD -0.42, 95% CI -1.12 to 0.28, P = 0.24) (*fig. 21*).

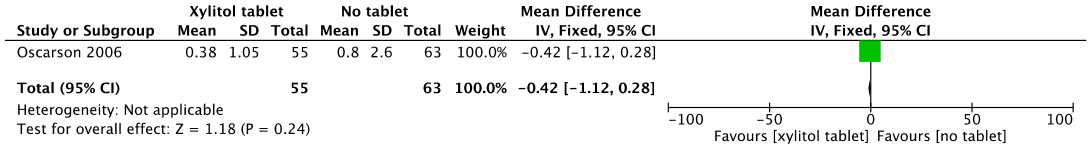


Fig. 21 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control Δdmfs increment of xylitol tablet vs. no intervention group at 2 years follow-up.

Xylitol gum versus sorbitol gum – $AUC_{5.7}$ of plaque pH

For this comparison we had included two studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011). In both studies we found data at baseline and in the short term whereas

only one study performed (Campus G *et al.*, 2009) the control in the medium term. At baseline (*fig. 22*) we found that the $AUC_{5.7}$ of plaque was significantly bigger in the xylitol group than in the control group (MD 0.98; 95% CI -0.82 to 1.14, P < 0.00001). In the short terms (*fig. 22*) the $AUC_{5.7}$ of plaque pH was smaller in the xylitol group than in the control group (MD -1.28, 95% CI -1.43 to -1.13, P < 0.00001). Also in the medium terms (*fig. 22*) we have found the $AUC_{5.7}$ in xylitol group was smaller than in the control group (MD -2.50, 95% CI -2.64 to -2.36, P<0.00001).

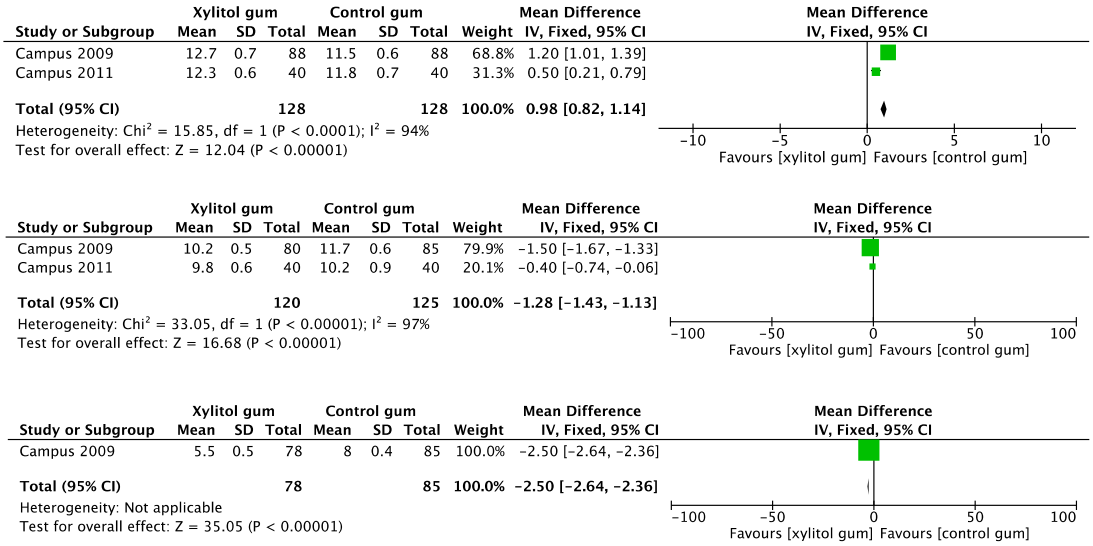


Fig. 22 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control plaque pH in $AUC_{5.7}$ of xylitol gum vs. sorbitol gum at baseline and in the short and medium term follow-ups.

Xylitol gum versus sorbitol gum – $AUC_{6.2}$ of plaque pH

For this comparison we included two studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011). In both studies we found data at baseline and in the short term whereas only one study performed (Campus G *et al.*, 2009) the control in the medium term. At baseline (*fig. 23*) comparing the data of two studies we found that the $AUC_{6.2}$ of plaque pH was significantly greater in the xylitol group (MD 1.28; 95% CI 1.09 to 1.46, P < 0.00001). In the short terms (*fig. 23*) the $AUC_{6.2}$ of plaque pH was smaller in the xylitol group than in the control group (MD -6.22, 95% CI -6.37 to -6.07, P < 0.00001). Also in the medium terms (*fig. 23*) we found the $AUC_{6.2}$ of plaque pH in the xylitol group was smaller than in the control group (MD -5.50, 95% CI -5.67 to -5.33, P<0.00001).

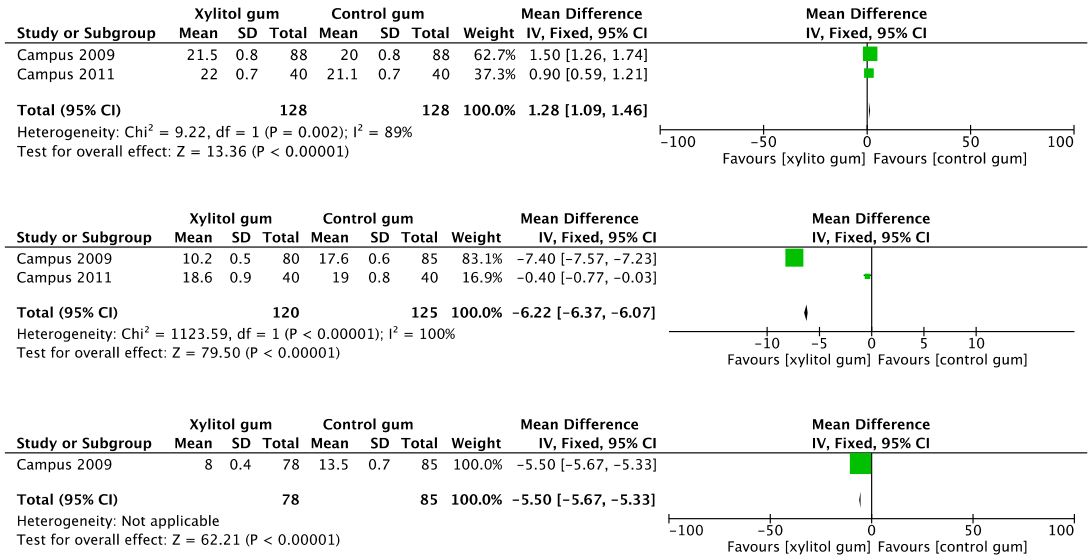


Fig. 24 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control plaque pH in $AUC_{6.2}$ of xylitol gum vs. sorbitol gum at baseline and in the short and medium term follow-ups.

Xylitol lozenges versus sorbitol lozenges – AUC7.0 of plaque pH

For this comparison we included one study (Splieth CH *et al.*, 2009) in which the controls were performed at baseline and in the short term. At baseline (*fig. 25)* comparing the data we found that $AUC_{7.0}$ of plaque pH was greater in the control group than in the experimental group with xylitol lozenges (MD -25.80, 95% -44.30 to -7.30). At control in the short term we found no difference between the two groups (MD -4.60, 95% CI -13.04 to 3.84, P =0.29) (*fig. 25*).

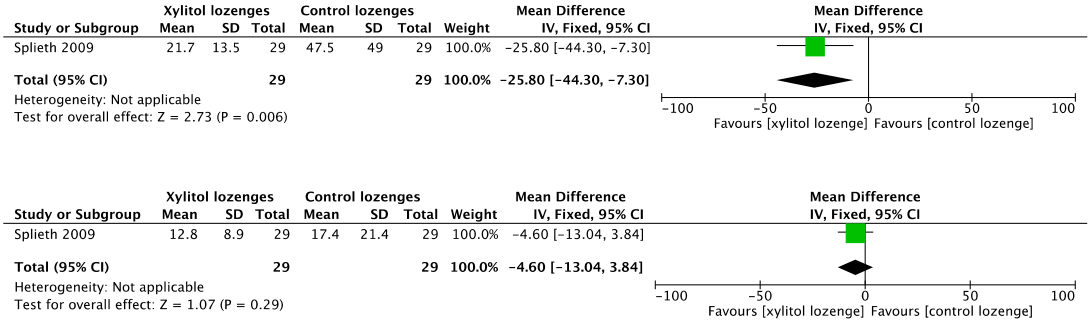


Fig. 25 Forest plot of comparison: Individual and overall Mean Difference in the comparison of efficacy to control plaque pH in AUC7.0 of xylitol lozenges vs. sorbitol gum at baseline and in the medium term follow-up.

Paper II

Comparison among the three detection methods

A total of 2496 surfaces (832 mesial, occlusal and distal, respectively) were analysed. The occlusal surfaces were analysed using DIAGNOcam (CAMo) and Clinical Examination (CE), while the approximal surfaces were analysed with DIAGNOcam (CAMa) and Bite-Wing radiographs (BW). The total number of occlusal caries lesions detected was similar, 149 using CAMo and 152 with CE with a Cohen's Kappa of 0.99. The ICC for the occlusal, mesial and distal surfaces of each tooth is reported in *fig. 26*. The mean ICC for the occlusal surface was 0.93 with a lowest value for maxillary right second molar (ICC=0.78) while a perfect agreement (ICC=1) was observed for several premolars. Approximal caries identified using CAMa were 83 and 70 using BW (Cohen's Kappa of 0.91). CAMa and BW identified the same number (31) of caries in dentine. The Cohen's Kappa was 0.24 for enamel lesions with a low agreement, while a complete concordance (Kappa=1) was observed for dentinal lesions (*tab. 3*). The mean ICC for approximal surfaces was 0.97 for the distal and 0.95 for the mesial surfaces (*tab. 4*). Regarding enamel lesions, 17 lesions in molars were detected with CAMa, while 16 with the BW method (Cohen's kappa=0.97); 35 lesions were detected in premolars with CAMa respect to 23 with BW (Cohen's kappa=0.21). Twenty-nine decayed mesial surfaces were registered with CAMa respect to 23 with BW (Cohen's kappa=0.39). For the distal surfaces, 23 lesions were recorded with CAMa and 16 with BW (Cohen's kappa=0.34). A complete concordance was observed for dentinal lesions between the two methods.

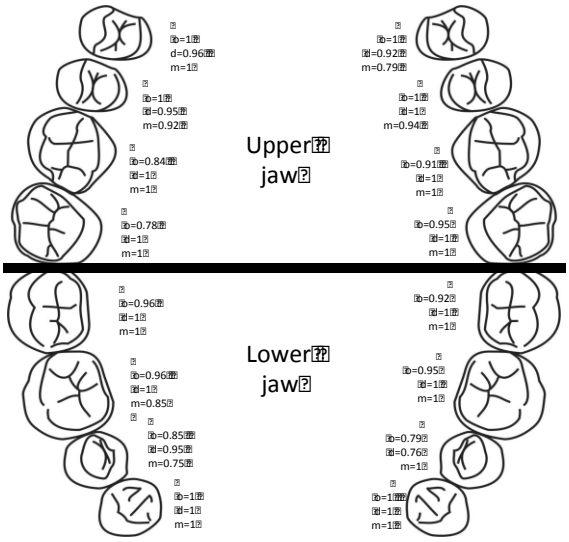


Fig. 26 Comparison among the three detection methods. Intraclass Coefficient Correlation between the DIAGNOcam and Clinical Evaluation for the occlusal surfaces (o) and between DIAGNOcam and Bite-wing for the approximal surfaces mesial (m) and distal (d) are reported

	Bitewing (BW) *n (%)*	**DIAGNOcam (CAMa)** *n (%)*	Cohen's kappa *value (SE) 95% CI*
Enamel	39 (2.3)	52 (3.1)	0.24 (0.06) 0.12-0.36
Dentine	31 (1.9)	31 (1.9)	1

Tab. 3 Comparison among the three detection methods. Caries lesions (enamel and dentinal lesions) according to the radiographic evaluation (BW) and the DIAGNOcam on approximal surfaces (CAMa). Percentage were calculated based on the total of the surfaces examined (n=1664)

Lesions for teeth/surfacs	**Enamel**			**Dentine**		
	DIAGNOcam **(CAMa)** n=52 *n (%)*	**Bitewing** **(BW)** n=39 *n (%)*	Cohen's kappa *value (SE) 95% CI*	**DIAGNOcam** **(CAMa)** n=31 *n (%)*	**Bitewing** **(BW)** n=31 *n (%)*	Cohen's kappa *value (SE) 95% CI*
Molars	17 (32.7)	16 (41.03)	0.97 (0.03) 0.91-1.00	13 (41.94)	13 (41.94)	1
Premolars	35 (67.3)	23 (58.97)	0.21 (0.08) 0.05-0.36	18 (58.06)	18 (58.06)	1
Mesial	29 (55.77)	23 (58.97)	0.39 (0.08) 0.23-0.56	11 (35.48)	11 (35.48)	1
Distal	23 (44.23)	16 (41.03)	0.34 (0.10) 0.15-0.54	20 (64.52)	20 (64.52)	1

Tab. 4 Reliability among dental professionals using the DIFOTI technique. Inter and Intra-examiner reliability categorized following the scale of the concordance degree proposed by Landis and Koch (1977) after two examinations (EVA1 and EVA2). n = 33

Reliability among dental professionals using DIFOTI

Forty-eight dental professionals participated in the first evaluation (EVA1) and thirty-two (drop out rate 33.3%) in the second evaluation (EVA2). The Cohen's Kappa of each subject regarding the reliability between the two evaluations was categorized following the scale proposed by Landis and Koch (Landis JR *et al.*, 1997) (*tab. 5*). Regarding inter-examiner reliability, in EVA1 the majority of the examiners (87.5%) had either a substantial (46.9%) or an almost perfect concordance (40.6%) compared to DIAGNOcam results, while in EVA2 a higher percentage had a substantial concordance (75.00%) and a lower percentage an almost perfect (18.8%), with a shift towards substantial concordance grade. Nineteen examiners (59.4%) showed a substantial/almost perfect agreement, while 13 examiners (40.6%) a fair/moderate agreement (*fig. 27*). The Bland-Altman plot showed a good intra-examiner (*fig. 27b*) and a higher over-rating of the number of the lesions in EVA2 (*fig. 27c*).

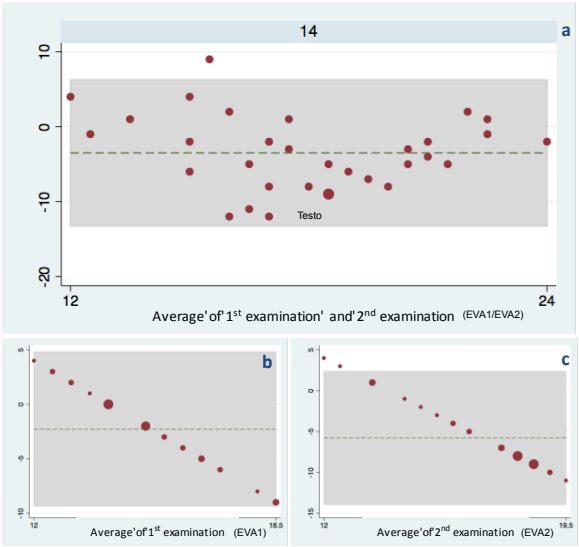


Fig. 27 Reliability among dental professionals using DIAGNOcam. Intra-examiner reliability using Bland-Altman plot of difference. Each small dot is the average value of one single examiner observations, larger dots are the sum of two or more examiners. Shaded region indicates 95% limits of agreement around the dashed line representing the mean.

	Fair concordance	Moderate concordance	Substantal concordance	Almost perfect concordance
	n (%)	*n (%)*	*n (%)*	*n (%)*
EVA 1	-	4 (12.50)	15 (46.87)	13 (40.63)
EVA 2	-	2 (6.25)	24 (75.00)	6 (18.75)
				$\chi^2=10.96$ $p<0.01$
Drop-out after EVA1 *n=16*	-	4 (25.00)	6 (37.50)	6 (37.50)
Intraexaminers reliability EVA1/EVA 2	4 (12.50)	9 (28.12)	10 (31.25)	□□□□□□□□□□

Tab. 5 Reliability among dental professionals using the DIFOTI technique. Inter and Intra-examiner reliability categorized following the scale of the concordance degree proposed by Landis and Koch (1977) after two examinations (EVA1 and EVA2). n = 33

Paper III

A total of 195 studies published from 1999–2014 were identified and assessed. Twenty-eight papers were analysed and 10 studies met the eligibility criteria (*fig. 28*).

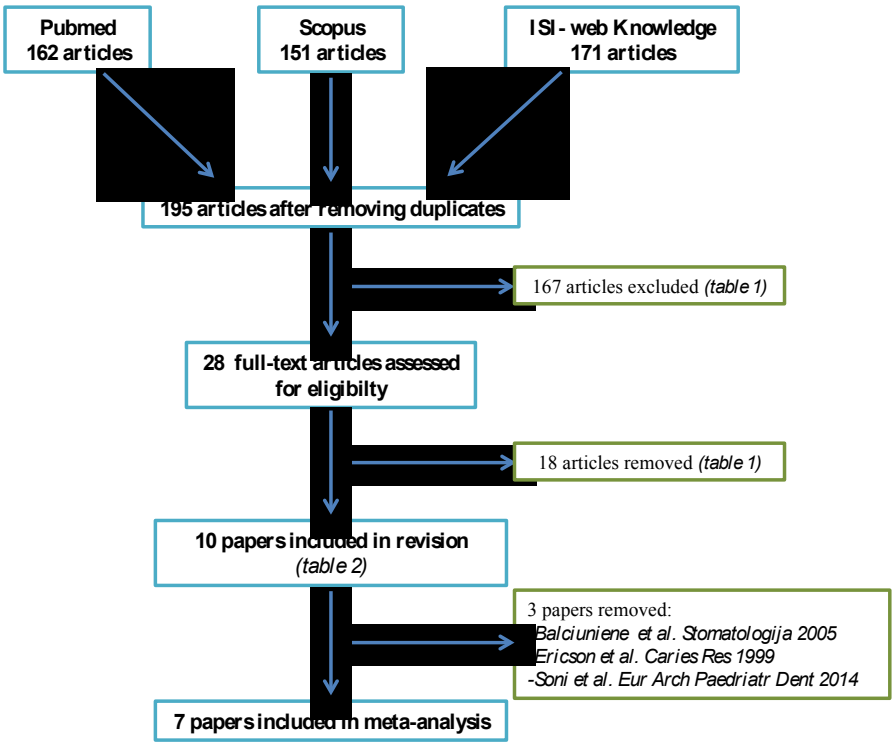


Fig. 28 Flowchart of search strategy

The trials included in the review involved a total of 348 patients and 532 treated teeth. In three studies (Ericson D *et al.*, 1999; Balciuniene I *et al.*, 2005; Soni HK *et al.*, 2014) it was not possible to extract the number of patients treated and so these studies were excluded. From the selected studies, two were conducted in India (Bohari MR *et al.*, 2012; Soni HK *et al.*, 2014), one in Venezuela (Lozano-Chourio MA *et al.*, 2006), two in Greece (Kavvadia K *et al.*, 2004; Maragakis GM *et al.*, 2001), one in Lithuania (Balciuniene I *et al.*, 2005), one in Serbia (Peric T *et al.*, 2009), one in Sweden (Ericson D *et al.*, 1999), one in the US (Peters MC *et al.*, 2006) and one in both Denmark and Portugal (Bergmann J *et al.*, 2005). Two of the papers reported data from multi-center (Sweden; Denmark and Portugal) studies.
One of the studies had a crossover design (Lozano-Chourio MA *et al.*, 2006), three were split mouth (Balciuniene I *et al.*, 2005; Bergmann J *et al.*, 2005; Maragakis GM *et al.*, 2001) and six had a parallel group design (Bohari MR *et al.*, 2012; Kavvadia K

et al., 2004; Ericson D *et al.*, 1999; Peric T *et al.*, 2009; Soni HK *et al.*, 2014; Peters MC *et al.*, 2006).

Most studies compared the Carisolv system (Schutzbank SG *et al.*, 1978) with the conventional rotary drill excavation for caries removal, but in three papers four different methods were reported (Bohari MR *et al.*, 2012; Ericson D *et al.*, 1999; Soni HK *et al.*, 2014).

In two studies no details about the operator and co-investigator were reported (Bohari MR *et al.*, 2012; Soni H *et al.*K, 2014), in another two studies there was one operator and one co-investigator (Peric T *et al.*, 2009; Balciuniene I *et al.*, 2005), while in another one there was one operator and two co-investigators (Lozano-Chourio MA *et al.*, 2006), in another one (Kavvadia K *et al.*, 2004) two operators but no co-investigator and, finally, in two studies there was only one operator (Soni HK *et al.*, 2014; Peters MC *et al.*, 2006). In one of the two multi-center studies there was one operator and one co-investigator for each centre (Ericson D *et al.*, 1999; Bergmann J *et al.*, 2005).

Six of the trials included only primary teeth (Bohari MR *et al.*, 2012; Kavvadia K *et al.*, 2004; Bergmann J *et al.*, 2005; Lozano-Chourio MA *et al.*, 2006; Maragakis GM *et al.*, 2001; Peters MC *et al.*, 2006) with participants' ages ranging from 28 months to 11 years. Four trials were carried out on permanent teeth also (Ericson D *et al.*, 1999; Peric T *et al.*, 2009; Balciuniene I *et al.*, 2005; Soni HK *et al.*, 2014) and the ages of the subjects ranged from 30 months to 85 years.

In five trials (Kavvadia K *et al.*, 2004; Ericson D *et al.*, 1999; Peric T *et al.*, 2009; Balciuniene I *et al.*, 2005; Bergmann J *et al.*, 2005) the teeth involved in the studies were molars and anterior primary teeth; in four studies (Bohari MR *et al.*, 2012; Lozano-Chourio MA *et al.*, 2006; Maragakis GM *et al.*, 2001; Peters MC *et al.*, 2006) only primary molars with occlusal caries were treated; while in one study (Soni HK *et al.*, 2014) primary molars were treated, but it was not mentioned which surfaces were treated.

Data regarding the clinical efficacy in decayed tissue removal of the Carisolv system vs. a control group were obtained from three papers (Kavvadia K *et al.*, 2004; Bergmann J *et al.*, 2005; Lozano-Chourio MA *et al.*, 2006), with a total of 264 analysed teeth. Complete caries removal was obtained in 100% (151 of 151) of the teeth using Carisolv and 99.2% (112 of 113) using the drill. When data were combined in meta-analysis, the summary OR was 0.33 (99% CI = 0.00–22.65). On the basis of the available evidence, there was no statistically significant difference in caries removal between the chemo mechanical system (Carisolv) and the rotary instruments ($z = 0.68$ $p = 0.50$) (*fig. 29*).

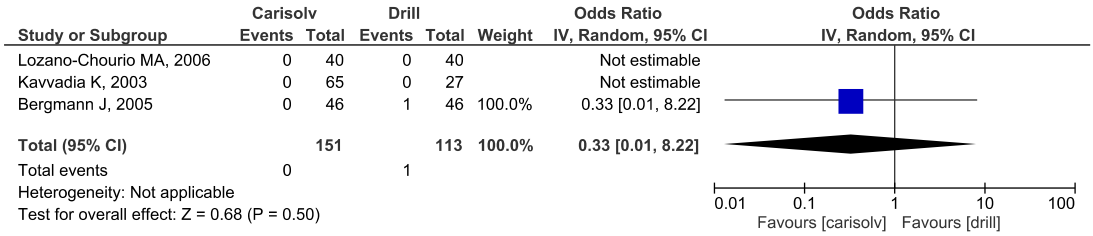


Fig. 29 Forest plot of comparison: Individual and overall Odds Ratio in the comparison of clinical efficacy between the Carisolv group and the rotary instrument group.

Data on the time required (seconds) to complete the procedure (mean ± SD) was obtained from seven studies (Bohari MR *et al.*, 2012; Kavvadia K *et al.*, 2003; Peric T *et al.*, 2009; Bergmann J *et al.*, 2005; Lozano-Chourio MA *et al.*, 2006; Maragakis GM *et al.*, 2001; Peters MC *et al.*, 2006) with a total of 480 teeth involved. The maximum time required for caries removal was 648 s for Carisolv and 206.7 s for the rotary instrument, whereas the minimum time of treatment was 402 s for the chemo mechanical removal and 80.7 s with the use of drills. The chi- square value was 48.38, with six degrees of freedom (df) and $p < 0.01$. The treatment with Carisolv required a statistically significant greater time amount than that required with the use of rotary instruments. The z-test for overall effect for the Carisolv group vs. rotary instruments was $z = 10.49$, $p < 0.01$ (*fig. 30*).

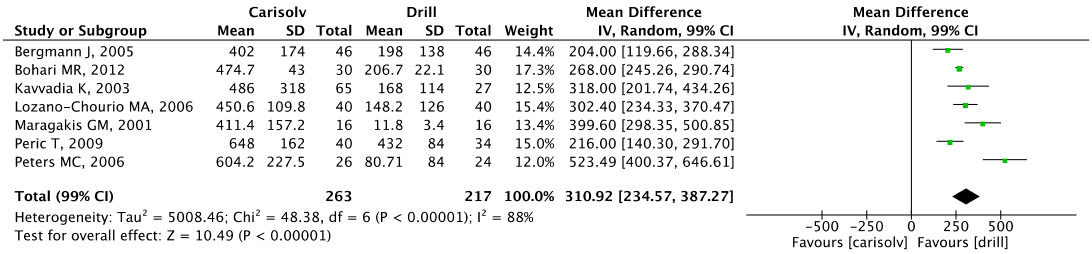


Fig. 30 Forest plot of comparison: Individual and overall Mean Difference in the comparison of time taken between the Carisolv group and the rotary instrument group.

Finally, data regarding the pain threshold were obtained from four studies only (Kavvadia K *et al.*, 2003; Lozano-Chourio MA *et al.*, 2006; Maragakis GM *et al.*, 2001; Peters MC *et al.*, 2006) with a total of 222 teeth involved. With the Carisolv system, 4% of the children requested local anaesthesia, while 26.8% used the conventional method. When data were combined in meta-analysis, the summary OR was 0.09 (95% CI = 0.01–1.07) with a difference between two types of treatment near to statistical significance ($z = 1.91$, $p = 0.06$), with fewer patients who needed local anaesthesia in the Carisolv group (*fig. 31*).

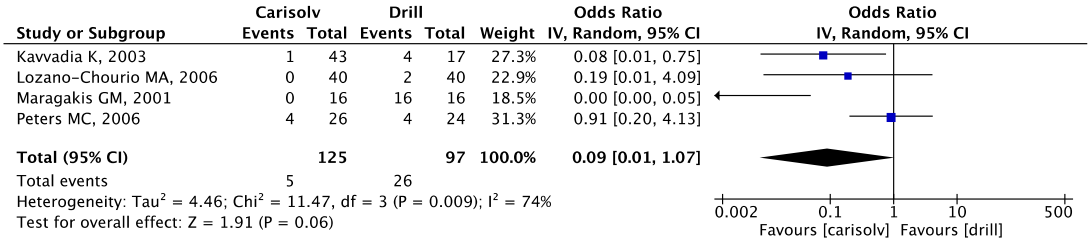


Fig. 31 Forest plot of comparison: Individual and overall Odds Ratio in the comparison of need for anaesthesia between the Carisolv group and the rotary instrument group.

Paper IV

A total of 47 patients (25 females and 22 males with a mean age of 9,3 range 7-12) were included for the study. We treated a total of fifty primary teeth. In all teeth treated were achieved the complete caries removal during the first visit.

The ANOVA analysis showed a difference in term of time treatment between groups ($p<0.001$) (*tab. 6*). The caries removal with Carisolv was slower than the other technique tested. (*fig.32*).

The increment of cavity size was similar in the five groups ($p= 0.363$) (*tab. 6*). The quantity of tissue removal was less in Cerabur group, however we found no difference between groups (*fig. 33*).

Removal technique	n	Time mean ± SD (sec)	Size cavity increment mean ± SD (mm^3)
Group A	10	150.5 ± 96.7	8.0 ± 5.2
Group B	10	261.7 ± 171.6	5.8 ± 3.6
Group C	10	954.4 ± 347.9	9.3 ± 7.2
Group D	10	430.2 ± 120.2	9.1 ± 6.0
Group E	10	487.7 ± 291.6	12.0 ± 10
p value		*<0.001*[a]	*0.363*[a]
[a]*ANOVA*			

Tab. 6 Descriptive and ANOVA analysis of time taken and size cavity increase

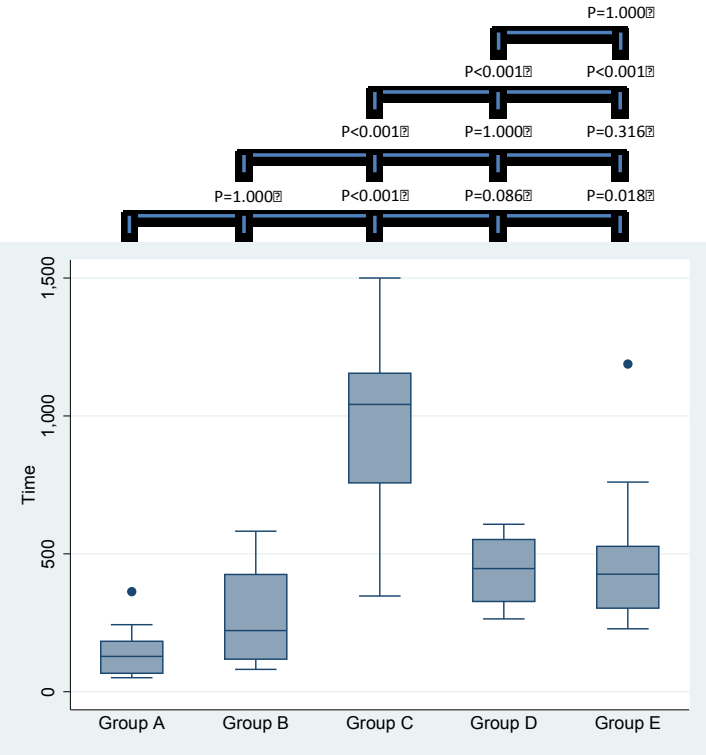


Fig. 32 Pairwise comparison between time taken. Statistical significance level (Bonferroni correction): p-value: 0.005

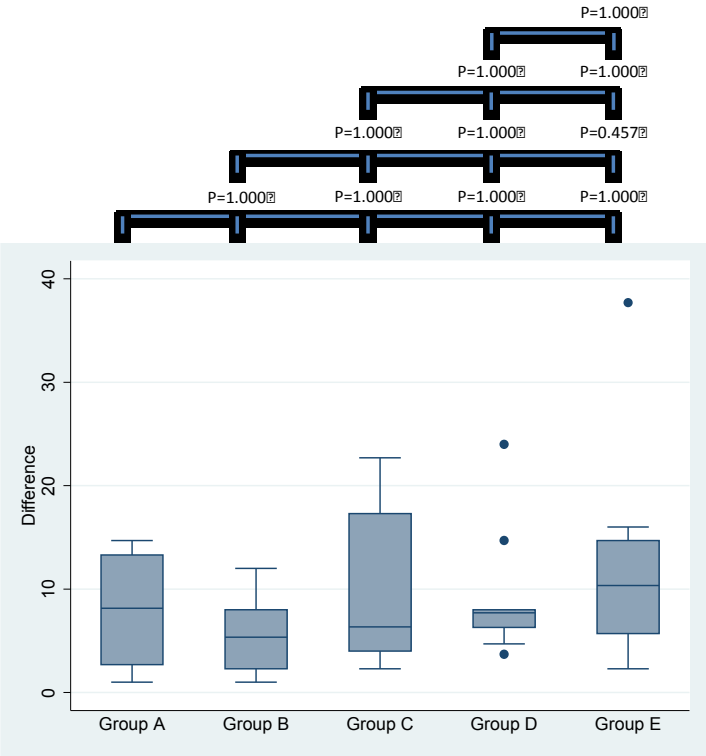


Fig. 33 Pairwise comparison between cavity size increment. Statistical significance level (Bonferroni correction): p-value:0.005

In pre-operative sample for each strain studied we found no difference between groups. In the experimental group the microbial reduction was achieved for almost all bacterial species studies. The mutans streptococci and the non-mutans streptococci species showed the less decrease (*tab. 7*). In post-operative sample a significant difference in bacterial count for the species A. Odontolyticus, A.Oris, A. Parvula, R. Dentocariosa, B. Dentium and P. Migra was found (*tab. 8*). Although the reduction of these bacterial species was significantly for all technique tested; the Carisolv method shows the strongest antibacterial effect than the traditional technique and Cerabur.

Bacterial species	Group A	Group B	Group C	Group D	Group E
S. Mutans median difference between pre- and post-operative	2.5 (<0.001)	1.0 (<0.001)	1.5 (<0.001)	1.5 (0.002)	2.5 (<0.001)
S. Sobrinus median difference between pre- and post-operative	2.0 (<0.001)	1.0 (0.102)*	2.0 (<0.001)	1.0 (0.012)	1.0 (0.005)
S. Sanguinis median difference between pre- and post-operative	0.5 (0.397)*	0.0 (1.000)*	2.0 (0.002)	1.0 (0.166)*	1.5 (0.012)
S. Salivarius median difference between pre- and post-operative	0.5 (0.573)*	0.5 (0.442)*	0.5 (0.744)*	1.0 (0.384)*	1.0 (0.042)
S. Gordonii median difference between pre- and post-operative	0.0 (1.000)*	0.0 (1.000)*	1.0 (0.188)*	1.0 (0.049)	1.0 (0.036)
S. Mitis median difference between pre- and post-operative	1.0 (0.100)*	0.0 (1.000)*	0.5 (0.726)*	0.0 (0.837)*	0.0 (1.000)*
L. Casei median difference between pre- and post-operative	2.5 (<0.001)	2.0 (0.004)	3.0 (<0.001)	1.0 (0.011)	3.0 (<0.001)
L. Fermentum median difference between pre- and post-operative	2.0 (0.005)	2.5 (<0.001)	2.5 (<0.001)	2.5 (<0.001)	2.5 (<0.001)
L. Salivarius median difference between pre- and post-operative	2.0 (<0.001)	3.0 (<0.001)	3.0 (<0.001)	3.5 (<0.001)	4.0 (<0.001)
A. Odotontolyticus median difference between pre- and post-operative	3.5 (<0.001)	2.0 (<0.001)	4.5 (<0.001)	3.0 (<0.001)	2.0 (0.003)
A. Oris median difference between pre- and post-operative	3.5 (<0.001)	2.5 (<0.001)	5.0 (<0.001)	4.0 (<0.001)	4.0 (<0.001)
V. Parvula median difference between pre- and post-operative	2.0 (0.001)	3.5 (<0.001)	4.0 (<0.001)	3.5 (<0.001)	4.0 (<0.001)
R. Dentocariosa median difference between pre- and post-operative	3.0 (<0.001)	3.0 (<0.001)	4.0 (<0.001)	3.0 (<0.001)	3.0 (<0.001)
B. Dentium median difference between pre- and post-operative	3.0 (<0.001)	3.0 (0.002)	4.5 (<0.001)	3.5 (<0.001)	3.5 (<0.001)
P. Migra median difference between pre- and post-operative	3.5 (<0.001)	3.5 (<0.001)	4.5 (<0.001)	3.5 (<0.001)	4.0 (<0.001)
(p value) Mann-Whitney U test *Difference:difference between median between pre- and post-operative* ** no significative*					

Tab. 7 Difference of bacterial score between pre- and post-operative time. Significance level: p-value: 0.05

Bacterial species	Group A median (IQR)	Group B median (IQR)	Group C median (IQR)	Group D median (IQR)	Group E median (IQR)	*p* value
S. Mutans	1.5 (1.0-2.0)	2.5 (1.0-3.0)	1.5 (1.0-4.0)	2.0 (1.0-3.0)	1.5 (1.0-2.0)	*0.766*[a]
S. Sobrinus	1.0 (1.0-2.0)	2.0 (1.0-3.0)	1.0 (1.0-2.0)	2.0 (1.0-2.0)	2.0 (1.0-2.0)	*0.380*[a]
S. Sanguinis	2.0 (1.0-3.0)	2.0 (1.0-3.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-1.0)	*0.094*[a]
S. Salivarius	2.0 (1.0-3.0)	1.5 (1.0-2.0)	1.5 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	*0.363*[a]
S. Gordonii	2.0 (1.0-2.0)	2.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	*0.066*[a]
S. Mitis	2.0 (1.0-3.0)	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.5 (1.0-2.0)	1.0 (1.0-2.0)	*0.448*[a]
L. Casei	2.0 (1.0-2.0)	2.0 (1.0-4.0)	1.5 (1.0-2.0)	3.0 (1.0-4.0)	2.0 (1.0-3.0)	*0.596*[a]
L. Fermentum	2.0 (1.0-4.0)	1.5 (1.0-3.0)	1.0 (1.0-2.0)	1.5 (1.0-3.0)	1.5 (1.0-2.0)	*0.801*[a]
L. Salivarius	2.0 (1.0-2.0)	2.0 (1.0-3.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)	1.0 (1.0-3.0)	*0.417*[a]
A. Odotontolyticus	1.5 (1.0-2.0)	2.0 (1.0-3.0)	0.5 (0.0-1.0)	1.0 (1.0-2.0)	2.0 (1.0-3.0)	*0.003*[a]
A. Oris	1.5 (1.0-2.0)	2.5 (1.0-3.0)	0.0 (0.0-1.0)	1.0 (1.0-2.0)	1.0 (1.0-3.0)	*<0.001*[a]
V. Parvula	3.0 (1.0-4.0)	2.0 (1.0-2.0)	1.0 (0.0-1.0)	1.5 (1.0-2.0)	1.0 (1.0-2.0)	*0.002*[a]
R. Dentocariosa	2.0 (1.0-2.0)	2.0 (1.0-3.0)	1.0 (1.0-1.0)	2.0 (1.0-3.0)	2.0 (1.0-2.0)	*0.001*[a]
B. Dentium	2.0 (1.0-2.0)	2.0 (1.0-4.0)	0.5 (0.0-1.0)	1.5 (1.0-2.0)	1.5 (1.0-3.0)	*0.001*[a]
P. Migra	2.0 (1.0-3.0)	2.0 (1.0-3.0)	0.5 (0.0-1.0)	1.5 (1.0-3.0)	1.0 (1.0-2.0)	*0.001*[a]
IQR inter-quartile range *[a]Kruskall-Wallis test*						

Tab. 8 Descriptive and inferential analysis of bacterial score in post-operative sample. p-value: 0.05

DISCUSSION

Polyols efficacy in caries prevention
(Paper I)

In the literature, there is no availability of meta-analysis on the efficacy of polyols in caries prevention. Hence, this study was performed in an attempt to gain further insight into the reliability in caries prevention of the polyols in chewing gums, candies, tablets and lozenges. Twenty-three studies were included, with a total of 5464 patients involved.
Mutans S. is considered to be the main pathogen responsible for dental caries. Numerous studies have shown an association between the number of carious lesions and the levels of Mutans S. in both adults and children. Also, a significant correlation between caries and Mutans S. was found (ElSalhy M *et al.*, 2012). Regarding the effects of polyols on Mutans S. we found studies that tested or xylitol, sorbitol and erythritol, vs. or control gum with or no intervention group. The vectors used to administer polyols were gum, candies and tablets.
In the comparison between xylitol gum and control gum in short terms we have found, after comparison of data extracted from six studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011; Hildebrandt GH *et al.*, 2000; Holgerson PL *et al.*, 2007; Ly KA *et al.*, 2006; Milgrom P *et al.*, 2006), a higher reduction of salivary S. Mutans count in patients which consumed xylitol gum. This trend was not confirmed in the medium term where we have analysed data from two studies (Campus G *et al.*, 2009; Holgerson PL *et al.*, 2007) and we found no difference. In the long term we evaluated only one study (Campus G *et al.*, 2013) that showed a significant reduction of salivary S. Mutans count in subject that consumed xylitol gum. One study (Runnel R *et al.*, 2013) included in this meta-analysis showed a significant difference in salivary MS count between the control group with sorbitol candies and the experimental group with xylitol candies. For the comparison between xylitol tablets and control tablets we included in the meta-analysis one study (Mäkinen KK *et al.*, 2005) that showed a significant reduction of salivary MS count in xylitol group. These results reflected the properties of two polyols; sorbitol, even if it has not effect on the growth of dental plaque it stimulates the growth of some strains of mutans streptococci (Mäkinen KK, 2011).
In the comparison between xylitol gum vs. no intervention group we founded three studies (Hildebrandt GH *et al.*, 2000; Martinez-Pabòn MC *et al.*, 2014; Simons D *et al.*, 2002) that showed in the short term a larger reduction in salivary S. Mutans count in subject allocated in xylitol group. In the medium and long terms the only study (Simons D *et al.*, 2002) analysed showed no difference between xylitol gum

and no intervention group. These results were confirmed also in the studies that tested xylitol tablets vs. no tablets: in the medium term the subjects in the xylitol group showed a significant reduction of salivary S. Mutans count (Mäkinen KK *et al.*, 2005). Numerous studies have demonstrated that habitual xylitol consumption decrease count of mutans streptococci several mechanisms may explain the phenomenon: growth inhibition, a decrease in the amount of plaque, elevated pH in the mouth, a decrease of adhesive polysaccharides produced by mutans streptococci (Söderling E *et al.*, 2015). Xylitol has been used as a substitute for refined white sugar for more than 30 years, and is thought to have an inhibitory action on the major causative bacteria in dental caries, S. Mutans. Xylitol and can also promote an ecological shift, resulting in less cariogenic environment (Holgerson PL *et al.*, 2007). These results would seem confirm the antimicrobial effect against S. Mutans of xylitol (Deshpande A *et al.*, 2008) when was administrated via gum, tablets, candies or lozenges.
The erythritol was tested against S. Mutans in two studies. In one paper (Makinen KK, *et al.* 2005) tablets were used as vectors and no intervention group and sorbitol tablets were used as control. In the other study (Runnel R *et al.*, 2013) candies were used as vectors. In the study that test tablets (Mäkinen KK *et al.*, 2005) in the medium term the salivary S. Mutans count was significantly smaller in erythritol group compared both whit no intervention group and with the control group. The same result has been found at long term in the study that test erythritol candies (Runnel R *et al.*, 2013). Information on oral biological effect of erythritol has been scant. However the few reports on erythritol suggest that this sugar alcohol may be of significant dental benefit (Mäkinen KK *et al.*, 2005).
The two studies included in this review investigated on the efficacy against S. Mutans of sortibol gum vs. no gum and sorbitol tablets vs. no tablets, showed no difference between two groups. These results reflected the little data reported in literature: sorbitol might stimulate the growth of some strains of mutans streptococci (Mäkinen KK, 2011).

Regarding the protective effects of polyols on dental caries in terms of DMFS/dmfs increment we found studies that tested or xylitol, sorbitol and erythritol, vs. or control gum or no intervention group, or sorbitol. The vectors used to administer polyols were gum, lozenges, candies and tablets.
For the comparison between xylitol gums vs. control gums we found one study (Machiulskiene V *et al.*, 2001). This paper showed a significantly smaller DMFS increment at 2 years follow-up in subjects that consumed sorbitol gum while at 3 years follow-up the analysis of data indicated a significantly smaller DMFS increment in the xylitol gums group. In a study that compared xylitol candies vs. control candies (Honkala S *et al.*, 2014) only the number of surface decay and not the DMFS index were analysed. The comparison of data showed no difference. The data

analysis and the little literature suggest that xylitol had no greater cariostatic effect than sorbitol when dispensed with gums or candies (Machiulskiene V *et al.*, 2001; Honkala S *et al.*, 2014).
For the comparison between xylitol gums vs. no intervention group we found three studies (Machiulskiene V *et al.*, 2001; Peng B *et al.*, 2004; Alanen P *et al.*, 2000). The comparison showed a protective effect in terms of DMFS increment at 2 and 3 years follow-up with xylitol gum. For the comparison between xylitol lozenges and no intervention group we included one study (Stecksen-Blicks C *et al.*, 2008) that showed no protective effect in terms of DMFS score. In the study (Alanen P *et al.*, 2000) that compared xylitol candies vs. no intervention group, the DMFS increase was significantly smaller in the experimental group at 3 years follow-up. One paper (Oscarson P *et al.*, 2006) investigated xylitol tablets vs. no intervention group in term of caries preventive effect on deciduous teeth: no difference was found in dmfs increase. The scientific evidence of the anticariogenic effects of the xylitol has been under debate (Stecksen-Blick C *et al.*, 2008; Oscarson P *et al.*, 2006). This meta-analysis shows that the caries preventive effect of xylitol was obtained when this polyol was administer via gums. The singles studies on candies, tablets and lozenges containing xylitol did not showed clearly protective effects in terms of DMFS and dmfs increment when compared with no intervention. The results do not lend support to the claims made in a number of previous studies that xylitol acts as a therapeutic or cariostatic agent. Rather, the results indicate that the caries preventive effect of gum could relate to an effect of increased salivary secretion resulting in higher salivary pH, buffer capacity and glucose clearance (Machiulskiene V *et al.*, 2001)
In literature we found only one study (Honkala S *et al.*, 2014), included in this meta-analysis that investigated the caries preventive effect of erythritol. This paper compared erythritol candies vs. sorbitol candies (control group) and for the statistical comparison the number of surface decayed was used. The results of this study suggested that the erythritol could have a greater protective effect against dental than sorbitol. Erythritol has been reported to be a totally safe and promising sweetener and to have several advantages, being non-caloric, less laxative than any other polyol, including sorbitol and xylitol (Honkala S *et al.*, 2014). In the short term erythritol could reduce dental plaque and S. mutans (Mäkinen KK *et al.*, 2005)
For the comparison between sorbitol gums vs. no intervention group we included three studies (Beiswanger BB *et al.*, 1998; Machiulskiene V *et al.*, 2001; Szöke J *et al.*, 2001). The analysis of data showed a smaller increment in DMFS score in experimental group at 2 and 3 years follow-up. This data confirmed the anti-caries benefits of sorbitol gums vs. no intervention described in literature (Szöke J *et al.*, 2001).

Regarding the AUC of pH we included in the meta-analysis studies that test xylitol in gum and lozenges vs. control group with sorbitol. For the comparison between

xylitol gums vs. control gum we included two studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011). At baseline we found difference among the experimental and the controls group. However the $AUC_{5.7}$ and $AUC_{6.2}$ of pH is greater in xylitol group. In the short and in the medium term there was a reversal in the $AUC_{5.7}$ and $AUC_{6.2}$ of pH value.
These results could reflect the ability of mutans streptococci to ferment sorbitol while the xylitol cannot be metabolised by mutans streptococci (Oscarson P *et al.*, 2006). Indeed sorbitol should be considered a low-cariogenic sweetener rather than a non-cariogenic one. Mutans streptococci could ferment sorbitol increasing the plaque acidity (Campus G *et al.*, 2009).
In the study (Splieth CH *et al.*, 2009) that compared xylitol lozenges and sorbitol lozenges we found a difference between two groups at baseline. At control there was no reversal of pH value.

Reliability of DIFOTI device in caries detection
(Paper II)

The main findings of this study are that the DIFOTI device (DIAGNOcam) proved to be consistent to clinical examination for the detection of lesions on the occlusal surface and to bite-wing x-ray for dentinal lesions on approximal surfaces; a higher number of enamel lesions was detected by DIAGNOcam compare to x-ray, especially in premolars. In the calibration process, no statistical significant difference was observed between benchmark and examiners and no systematic bias between examiners' scores was noted. The level of concordance among dental professionals respect to DIAGNOcam analysis result derived from the first part of the study, was really high in both examinations (EVA1/EVA2); furthermore the intra-examiner reliability of the dental professionals was quite good since in 40% of the examiners the level of agreement was moderate or less.
The DIFOTI device used in this study, KaVo DIAGNOcam 2170, is a non-invasive real-time recording tool that was developed for regular practice use with no exposure of ionizing radiations to the patient. The device was designed to be useful to identify lesions at initial stage that can be re-evaluated at a higher frequency compared to the radiographic technique (American Dental Association, 2012).
The DIFOTI method has been shown to be more sensitive than radiography to detect early changes in enamel (Young DA, 2005; Bin-Shuwaish *et al.* M, 2008; Astvaldsdottir A *et al.*, 2012). Overall, the use of DIFOTI along with radiographic analysis is able to improve the diagnostic accuracy, identify the early approximal lesions respect to presence and size (Bin-Shuwaish M *et al.*, 2008). The outcome of this paper confirms these findings. The DIAGNOcam has shown to identify a higher number of approximal lesions in enamel than the radiographic technique, allowing an earlier detection of incipient lesions than traditional detection methods. Proper and

early caries detection is crucial for an optimal treatment decision, helping the clinician to choose between a restorative treatment or a chemically remineralisation, as in the early stages of caries development (Young DA, 2005).
Intraoral radiographs are, in addition to clinical evaluation, considered the first choice for caries detection. Nevertheless, radiographs are unable to detect initial demineralization of the tooth resulting in low sensitivity, since 40 to 60% of tooth decalcification is needed to produce a radiographic imaging of caries resulting in false-negative test (Machiulskiene V *et al.*, 1999; Chong MJ *et al.*, 2003; Yang J *et al.*, 2005). Conversely, the use of DIFOTI method might lead to an overdetection as the device has a lower specificity respect to radiograph method (Young DA, 2002). In this in vivo trial the DIAGNOcam findings were compared to radiographs, used as golden standard. The "true" status of the lesion was not evaluated since the teeth were not extracted after the in vivo evaluation. Regarding occlusal surfaces the DIAGNOcam was able to detect the presence of carious lesions but not to determinate the extension of the lesion since the device is able to capture the light emerging from the tooth surface that is closest to the digital camera as specified by the manufacturer instructions.
Over diagnosis can be occurred owing to lower specificity of DIFOTI compared with bite-wing radiographs. Dark areas on the images can be attributed to scatter and the absorption of light as it passes through demineralized enamel, consequently white spots can be confused for cavitations (Schneiderman A *et al.*, 1997).
Some strengths of the present study should to be considered. The DIFOTI device was compared to clinical examination for the occlusal surface and to bitewing for the approximal, and this is the first study comparing in vivo the DIAGNOcam to traditional caries detection methods. The study outline may be seen as a limit since the findings may not compete with the "surface/teeth true status", as only in vitro study can assess.
The reliability of the DIFOTI among professionals showed a quite good intra-examiner concordance, even if an important shift to an over detection (EVA1 vs. EVA2) was noted. Since only images in doubt are tested in the clinic practice, the probability of a detection of a lesion will be increased (Chu CH *et al.*, 2010) with a high specificity and a low sensitivity. Moreover, a bias is probably ascribed to the study design, as the first evaluation was performed with a strict time limit while the second evaluation was more "free". Examiners received an email with the images and no time limit was provided. A stochastic drift might be also postulated as the misclassification performed by examiners in EVA2 happened unconsciously leading to a higher inter-examiner concordance. A further weak point might be ascribed to study design: EVA2 was designed to be carried out one month later EVA1, and this might have affected the results; otherwise the results of EVA2 still showed a good concordance with EVA1 and the DIAGNOcam results derived from the first part of the study.

The results of this paper allow stating that the DIAGNOcam can be help in everyday clinical practice. However, the usefulness regarding cost effectiveness of DIFOTI method in community dentistry surveys may be seen as a concern as an average of at least 15 minutes is required to examine the whole dentition. Therefore the advantage of DIFOTI is particularly related to an early lesion detection and caries monitoring in clinical management.

Efficacy and reliability of different technique of caries removal (Paper III and Paper IV)

A multitude of technique and materials are proposed in the dental market to use in restorative dentistry and so the need of a strong scientific evidence for the 'new' methods is essential before their use in everyday practice.
Carisolv was introduced in the dental market (Sweden) in 1998 (Bohari MR *et al.*, 2012) and during the last 15 years it has been used almost exclusively in paediatric dentistry, as the use of Carisolv in clinical practice might be limited because of the material cost (Kathuria V *et al.*, 2013). Recently a new bur made of a special alumina-based ceramic with stabilized zirconia (ZrO2: 76%; Al2O3: 20%; Y2O3: 4%), was introduced in the dental market (CeraBur, K1SM, Komet). In literature there were not in vivo studies that tested the clinical efficacy of this new bur (Dammaschke T *et al.*, 2008).
The parameter to evaluate the effectiveness of the Carisolv against rotary instruments, both in clinical trial and in meta-analysis, was the caries removal rate as clinically estimated. This evaluation method may appear empirical and inaccurate, however it is the main and simplest approach to check the caries removal (Schwendicke F *et al.*, 2015). This method only required a visual estimation and a tactile evaluation using a sharp probe. The results in term of complete caries removal of Carisolv method obtained in clinical trial were in agreement with the data reported in several studies (Lozano-Chourio MA *et al.*, 2006; Kavvadia K *et al.*, 2003; Bergamann J *et al.*, 2005; Peric T *et al.*, 2009; Ericson D *et al.*, 1999; Bohari MR *et al.*, 2012). The meta-analysis showed that no statistically significant difference exists between the Carisolv system and the traditional method with drill in terms of caries removal efficacy (Lozano-Chourio MA *et al.*, 2006; Kavvadia K *et al.*, 2003; Bergmann J *et al.*, 2005). In literature any studies (Maragakis GM *et al.*, 2001; Peters MC *et al.*, 2006) reported an incomplete caries removal with Carisolv. However these results might depend on limited setting time of 15 minutes to complete caries removal.
In literature there were no studies in vivo that evaluated the efficacy of ceramic bur (CeraBur by Komet). In our clinical trials the complete caries removal using CeraBur was achieved in groups B, D and E. The results obtained by our study were in agreement with data of an in vitro study (Dammaschke T *et al.*, 2008) that evaluated

the efficacy of CeraBur. No data regarding the combined use of CeraBur and Carisolv system was available.

In the meta-analysis outcomes regarding the time required to complete the procedure were reported in five of seven studies selected (Bergmann J *et al.*, 2005; Bohari MR *et al.*, 2012; Kavvadia K *et al.*, 2003; Lozano-Chourio MA *et al.*, 2006; Maragakis GM *et al.*, 2001; Peric T *et al.*, 2009; Peters MC *et al.*, 2006). There was a significant difference regarding time required by the Carisolv procedure and the conventional drilling: treatment time was statistically significantly longer using Carisolv than drilling. In our clinical study the traditional method resulted the faster method than the other technique tested. The data regarding time taken obtained with Carisolv method were accordance with reported in several study (Bergamann J *et al.*, 2006; Bohari MR *et al.*, 2012; Kavvadia K *et al.*, 2003; Lozano-Chourio MA *et al.*, 2006; Maragakis GM *et al.*, 2001; Peric T *et al.*, 2009; Peters MC *et al.*, 2006). Study investigating this aspect in teeth treated with Carisolv combined with CeraBur was no found. One in vitro study (Dammaschke T *et al.*, 2008) on CeraBur showed no difference in average time to excavate a cavity between ceramic burs and tungsten carbide burs.

Pain is commonly reported when removing dental caries and the use of local anaesthesia is often required. Data on pain threshold or need of local anaesthesia were reported in four papers (Kavvadia K *et al.*, 2003; Lozano-Chourio MA *et al.*, 2006; Maragakis GM *et al.*, 2001; Peters MC *et al.*, 2006). From our meta-analysis Carisolv seems to reduce the use of local anaesthesia and this difference may be related to the use, together with Carisolv gel, of sharp hand instruments.

In our clinical trial we found no difference between the techniques of caries removal in terms of increase of cavity size. With regard to increasing of cavity size using Carisolv system the data in literature were debatable: one study (Lonzano-Chourio MA *et al.*, 2006) showed that size increase with Carisolv was less than in control group with a rotating instrument; another study (Fure S *et al.*, 2000) showed no difference between the chemo-mechanical method and the conventional method. In our trial the cavity size increase of teeth treated with CeraBur was less than in the control group, however this difference was not significantly. No clinical trial described an increase of cavity size after treatment with CeraBur, Carisolv and CeraBur.

The use of detecting dyes is source of controversy in the literature (Fure S *et al.*, 2000). The use in our clinical trial of caries dye to check cavity after treatment with combination of Carisolv and CeraBur did not lead to smaller cavities.

The presence of chloramines in Carisolv gel might reduce the bacterial load. The study on primary dention have confirmed that Carisolv® system reduce the bacterial count in cavity cleaned with this system (El-Tekeya M *et al.*, 2012).

Although several studies reported a strong anti-bacterial effect of Carisolv technique (El-Tekeya M *et al.*, 2012; Lager A *et al.*, 2003; Azrak B *et al.*, 2004; Baysan A *et al.*, 2000) in our study all technique tested loaded a significantly bacterial decrease after treatment for all almost species tested. However we found strong anti-bacterial effect,

against A. Odontolyticus, A.Oris, A. Parvula, R. Dentocariosa, B. Dentium and P. Migra in Carisolv tecnique.

CONCLUSIONS

The main conclusions from this thesis are that:

- The use of xylitol via gum, tablet, candy or lozenge could inhibit the growth of MS especially in short-term period. The data on the effect of erythritol on MS are poor, however this polyol seems to inhibit the MS growth. The use of xylitol or sorbitol when administer via gum could reduce the increase of DMFS index. Sorbitol should be considered a low-cariogenic sweetener, however MS can ferment sorbitol could increase the plaque acidogenicity respect xylitol.

- DIAGNOcam might be a useful device for early caries detection, especially for early non-cavitated lesions on approximal surfaces. The DIFOTI images are quite easy to decode even for professionals without any experience of the use of the method. More information is needed to standardize the scoring of the lesions to help the clinicians to give an accurate interpretation of the images.

- Carisolv and Cerabur, together or separately, were a valid alternative of traditional method in caries removal in primary dentition. The techniques tested seem to be as aggressive as the traditional method and have a similar anti-bacterial effect. Data analysis in meta-analysis and in our trial suggest that the difference in terms of time taken was statistically significant: the Carisolv system takes more time that the traditional method to remove dental caries. Regarding patient's comfort the systematic review indicates that the Carisolv system can reduce the use of local anaesthesia.

REFERENCES

- American Dental Association: Dental radiographic examinations: recommendations for patient selection and limiting radiation exposure. 2012 Available at: http://www.ada.org/~/media/.../Dental_Radiographic_Examinations_2012.ashx (accessed June 23, 2015).

- Alanen P, Isokangas P, Gutmann K: Xylitol candies in caries prevention: results of a field study in Estonian children; *Community Dent Oral Epidemiol* 2000; 28: 218-224.

- Astvaldsdòttir A, Ahlund K, Holbrook WP, de Verdies B, Tranæus S: Approximal caries detection by DIFOTI: In vitro comparison of diagnostic accuracy/efficacy with film and digital radiography. *Int J Dent*, 2012: 326401.

- Azrak B, Callaway A, Grundheber A, Stender E, Willer- shausen B. Comparison of the efficacy of chemomechanical caries removal (Carisolv) with that of conventional excava- tion in reducing the cariogenic flora. *Int J Pediatr Dent* 2004;14:182-91.

- Bader JD, Shugars DA, Bonito AJ: A systematic review of the performance of methods for identifying carious lesions. *Journal of public health dentistry* 2002; 62; 4: 201-213.

- Balciuniene I, Sabalaite R, Juskiene I: Chemomechanical caries removal for children. *Stomatologija* 2005; 7: 40-44.

- Baysan A, Whiley R, Lynch And. Antimicrobial assessment of Carisolv on primary root decay of an ex-living creature. *J Dent Res* 2000; 79: 1296

- Beiswanger BB, Boneta AE, Mau MS, Katz BP, Proskin HM Stookey GK: The effect of chewing gugar-free gum after meals on clinical caries incidence. *J Am Dent Assoc* 1998; 129: 1623-1626.

- Bergmann J, Leitão J, Kultje C, Bergmann D, Clode MJ: Removing dentine caries in deciduous teeth with Carisolv: a randomised, controlled, prospective study with six-month follow-up, comparing chemomechanical treatment with drilling. *Oral Health Prev Dent* 2005; 3: 105-111.

- Bin-Shuwaish M, Yaman P, Dennison J, Neiva G: The correlation of DIFOTI to clinical and radiographic images in Class II carious lesions. *J Am Dent Assoc* 2008; 139: 1374-1381.
- Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1: 307-310.
- Bohari MR, Chunawalla YK, Ahmed BM: Clinical evaluation of caries removal in primary teeth using conventional, chemomechanical and laser technique: An in vivo study. *J Contemp Dent Pract* 2012; 13: 40-47.
- Campus G, Cagetti MG, Cocco F, Sale S, Sacco G, Strohmenges L, Lingström P: Effect of a sugar-free chewing gum containing magnolia bark extract on different variables related to caries and gingivitis: a randomized controlled intervention trial. *Caries Res* 2011; 45; 393-399.
- Campus G, Cagetti MG, Sacco G, Solinas G, Mastroberardino S, Lingström P: Six month of daily high-dose xylitol in high-risk schoolchildren: a randomized clinical trial on plaque pH and salivary mutans streptococci. *Caries Res* 2009; 43: 455-461.
- Campus G, Cagetti MG, Sale S, Petruzzi M, Solinas G, Strohmenger L, Lingström P: Six months of a high-dose xylitol in high-risk caries subjects-a 2-year randomised, clinical trial. *Clin Oral Invest* 2013; 17: 785-791.
- Chong MJ, Seow WK, Purdie DM, Cheng E, Wan V: Visual-tactile examination compared with conventional radiography, digital radiography, and Diagnodent in the diagnosis of occlusal occult caries in extracted premolars. *Pediatr Dent* 2003; 25: 341-349.
- Chu CH, Chung BT, Lo EC: Caries assessment by clinical examination with or without radiographs of young Chinese adults. Int Dent J 2008;58:265-268.
- Cohen J: A coefficient of agreement for nominal scales. *Educ Psychol Meas* 1960; 20: 37-46.
- Dammaschke T, Vesnic A, Schafer E: In vitro comparison of ceramic burs and conventional tungsten carbide bud burs in dentin caries excavation. *Quintessence Int* 2008; 39: 495-499.

- Deshpande A, Jadad AR: The impact of polyol-containing chewing gums on dental caries: a systematic review of original randomized controlled trials and observational studies. *J Am Dent Assoc* 2008; 139: 1602-1614.
- El-Tekeya M, El-Habashy L, Mokhles N, El-Kimary E: Effectiveness of 2 Chemomechanical Caries Removal Methods on Residual Bacteria in Dentin of Primary Teeth. *Pediatr Dent* 2012; 34: 325-330.
- ElSalhy M, Sayed Zahid I, Honkala E: Effects of xylitol mouthrinse on Streptococcus mutans. *J Dent* 2012; 40: 1151-1154.
- Ericson D, Zimmerman M, Raber H, Götrick B, Bornstein R: Clinical evaluation of efficacy and safety of a new method for chemo mechanical removal of caries. *Caries Res* 1999; 33: 171-177.
- Fure S, Lingström P: Evaluation of the chemomechanical removal of dentine caries in vivo with a new modified Carisolv gel. *Clin Oral Invest* 2004; 8: 139-144.
- Hildebrandt GH, Sparks BS: Maintaining mutans streptococci suppression with xylitol chewing gum. *J Am Dent Assoc* 2000; 131: 909-916.
- Holgerson PL, Sjöström I, Stecksén-Blicks C, Twetman S: Dental plaque formation and salivary mutans streptococci in schoolclidren after use of xylitol-containing chewing gum. *Int J Paediatr Dent* 2007; 17: 79-85.
- Honkala E, Runnel R, Honkala S, Olak J, Vahlberg T, Saag M, Mäkinen KK: Measuring Dental Caries in the Mixed Dentition by ICDAS. *Int J Dent* 2011:150424.
- Honkala S, Runnel R, Saag M, Olak J, Nõmmela R, Russak S, Mäkinen PL, Vahlberg T, Falony G, Mäkinen K, Honkala E: Effect of erythritol and xylitol on dental caries prevention in children. *Caries Res* 2014; 48: 482-490.
- International Caries Detection and Assessment System Coordinating Committee: Criteria Manual. International Caries Detection and Assessment System (ICDAS II) Workshop. Baltimore, Md, USA, March 2005.
- Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, Pitts NB: The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dent Oral Epidemiol* 2007; 35: 170-178.

- Kathuria V, Ankola AV, Hebbal M, Mocherla M. Carisolv- an innovative method of caries removal. *J Clin Diagn Res* 2013; 7: 3111–3115.
- Kavvadia K, Karagianni V, Polychronopoulou A, Papagiannouli L: Primary teeth caries removal using the Carisolv chemomechanical method: a clinical trial. *Pediatr Dent* 2004; 26: 23-28.
- Keem S, Elbaum M: Wavelet representations for monitoring changes in teeth imaged with digital imaging fiber-optic transillumination. *IEEE Trand Med Imaging* 1997; 16: 653-663.
- Landis JR, Koch GG: The measurement of observer agreement for categorical data. *Biometrics* 1997; 33: 159-174.
- Lager A, Thornqvist E, Ericson D. Cultivable bacteria indentin after caries excavation using rose-bur or Carisolv. *Caries Res* 2003; 37: 206-211.
- Lenkkeri AM, Pienihäkkinen K, Hurme S, Alanen P: The caries-preventive effect of xylitol/maltitol and erythritol/maltitol lozenges: results of a double-blinded, cluster-randomized clinical trial in area of natural fluoridation. *Int J Paediatr Dent* 22 (2012): 180-190.
- Lodlow JB, Koch GG: The measurement of observer agreement for categorical data. *Biometrics* 1997; 33: 159-174.
- Lozano-Chourio MA, Zambrano O, Gonzalez H, Quero M: Clinical Randomized controlled trial of chemomechaninal caries removal (Carisolv™). *Inte J of Paediatr Dent* 2006; 16: 161-167.
- Ly KA, Milgrom P, Roberts MC, Yamaguche DK, Rothen M, Mueller G: Linear response of mutans streptococci to increasing frequency of xylitol chewing gum use: a randomized controlled trial; *BMC Oral Health* 2006; 6:6.
- Machiulskiene V, Nyvad B, Baelum V: A comparison of clinical and radiographic caries diagnoses in posterior teeth of 12-year-old Lithuanian children. *Caries Res* 1999; 33: 340-348.
- Machiulskiene V, Nyvad B, Baelum V: Caries preventive effect of suger-substituted chewing gum. *Community Dent Oral Epidemiol* 2001; 29: 278-288.
- Mäkinen KK: Sugar Alcohol Sweeteners as Alternatives to Sugar with Special Consideration of Xylitol . *Med Princ Pract* 2011; 20: 303-320.

- Mäkinen KK, Pemberton D, Mäkinen PL, Chin CY, Cole J Hujoel PP, Lopatin D, Lambert P: Polyol-combinant saliva stimulants and oral health in Veterans Affairs patients - An exploratory study. *Spec Care Dentist* 1996; 16: 104-115.
- Mäkinen KK, Saag M, Isotupa KP, Olak J, Nõmmela R, Söderling E, Mäkinen PL: Similary of the effects of erythritol and xylitol on some risk factors of dental caries. *Caris Res* 2005; 39: 207-215.
- Maragakis GM, Hahn P, Hellwig E: Clinical evaluation of chemomechanical caries removal in primary molars and its acceptance by patients. *Caries Res* 2001; 35: 205-210.
- Martinez-Pabòn MC, Duque-Agudelo L, Díaz-Gil JD, Isaza-Guzmaán DM, Tobón-Arroyave SI: Comparison of the effect of two sugar-substituted chwing gums on different caries- and gingivitis-related variables: a double-blind, randomized, controlled clinical trial. *Clin Oral Invest* 2014; 18: 589-598.
- Milgrom P, Ly KA, Roberts MC, Rothen M, Mueller, Yamaguchi DK: Mutans Streptococci dose response to xylitol chewing gum. *J Dent Res* 2006; 85: 177-181.
- Moher D, Liberati A, Tetzlaff J, Altman DG: Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement . *PLos Med* 2009; 6: e1000097.
- Neves Ade A, Coutinho E, Cardoso MV, de Munck J, Van Meerbeek B: Micro-tensile bond strength and interfacial characterization of an adhesive bonded to dentin prepared by contemporary caries-excavation techniques. *Dent Mater* 2011; 27: 552-562.
- O'Mullane DM, Kavanagh D, Ellwood Rp, Chester Rk, Schafer F, Huntington E, Jones PR: A three-year clinical trial of a combination of trimetaphosphate and sodium fluoride in silica tooth paste-pastes. *J Dent Res* 1997; 76 : 1776-1781.
- Oscarson P, Holgerson PL, Sjöström I, Twetman S, Stecksén-Blicks C: Influence of a low xylitol-dose on mutans streptococci colonisation and caries development in preschool children. *Eur Arch Paediatr Dent* 2006; 7: 142-147.
- Peng B, Petersen PE, Bian Z, Tai B Jiang H: Can school-based oral health education and a sugar-free chewing gum program improve oral health? Results fro a two-year study in PR China. *Acta Odontol Scand* 2004; 62: 328-332.

- Peric T, Markovic D, Petrovic B: Clinical evaluation of a chemomechanical method for caries removal in children and adolescents . *Acta Odontol Scand* 2009; 67: 277-283.
- Peters MC, Flamenbaum MH, Eboda NN, Feigal RJ, Inglehart MR: Chemomechanical caries removal in children: Efficacy and efficiency.» *J Am Dent Assoc* 2006; 137: 1658-1666.
- Rethman MP, Beltrán-Aguilar ED, Billings RJ, Hujoel PP, Katz BP, Milgrom P, Sohn W, Stamm JW, Watson G, Wolff M, Wright JT, Zero D, Aravamudhan K, Frantsve-Hawley J, Meyer DM, e American Dental Association Council on Scientific Affairs Expert Panel on Nonfluoride Caries-Preventive Agents: Nonfluoride caries-preventive agents: executive summary of evidence-based clinical recommendations. *J Am Dent Assoc* 2011; 142: 1065-1071.
- Runnel R, Mäkinen KK, Honkala S, Olak J, Mäkinen PL, Nõmmela R, Vahlberg T, Honkala E, Saag M: Effect of three-year consumption of erythritol, xylitol and sorbitol candies on various plaque and salivary caries-related variables. *J Dent* 2013; 41: 1236-1244.
- Schneiderman A, Elbaum M, Shultz T, Keem S, Greenebaum M, Driller J: Assessment of dental caries with Digital Imaging Fiber-Optic Transillumination (DIFOTI): in vitro study. *Caries Res* 1997; 31: 103-110.
- Schutzbank SG, Galaini J, Kronman JH, Goldman M, Clark RE: A comparative in vitro study of GK-101 and GK-101E in caries removal. *J Dent Res* 1978; 57: 861-864.
- Schwendicke F, Paris S, Tu YK. Effects of using different criteria for caries removal: A systematic review and network meta-analysis. *J Dent* 2015; 43: 1–15.
- Seki M, Karakaa F, Kawato T, Tanaka H, Saeki Y, Yamashita Y: Effect of xylitol gum on the level of oral mutans streptococci of preschoolers: block-randomised trial. *Int Dent J* 2011; 61: 274-280.
- Selwitz RH, Ismail AI, Pitts NB: Dental Caries. *Lancet* 2007; 369: 51-59.
- Senna A, Campus G, Gagliani M, Strohmenger L: Socio-economic influence on caries experience and CPITN values among a group of Italian call-up soldiers and cadets. *Oral Health Prev Dent* 2005; 3: 39-46.

- Simons D, Brailsford SR, Kidd EAM, Beighton D: The effect of medicated chewing gums on oral health in frail older people: a 1-year clinical trial. *J Am Geriatr Soc* 2002; 50: 1348-1353.
- Söderling E, ElSalhy M, Honkala E, Fontana M, Flannagan S, Eckert G, Kokaras A, Paster B, Tolvanen M, Honkala S: Effects of a short-term xylitol gum chewing on the oral microbiobiome. *Clin Oral Invest* 2015; 19: 237-244.
- Soni HK, Sharma A, Sood PB: A comparative clinical study of various methods of caries removal in children. *Eur Arch Paediatr Dent* 2015; 16: 19-26.
- Splieth CH, Alkilzy M, Schmitt J, Berndt C, Welk A: Effect of xylitol and sorbitol on plaque acidogenesis. *Quintessence Int* 2009; 40: 279-285.
- Stecksén-Blicks C, Holgerson PL, Twetman S: Effect of xylitol and xylitol-fluoride lozenges on approximal caries development in high-caries-risk children. *Int J Paedriatr Dent* 2008; 18: 170-177.
- Szöke J, Bánóczy J Proskin HM: Effect of after-meal sucrose-free gum-chewing on clinical caries. *J Dent Res* 2001; 80: 1725-1729.
- The Cochrane Collaboration. Review Manager (RevMan) - computer program. Version 5.3. Prod. The Nordic Cochrane Centre. Copenhagen, 2012.
- Virajsilp V, Thearmontree A, Aryatawong S, Paiboonwarachat D: Comparison of proximal caries detection in primary teeth between laser fluorescence and bitewing radiography. *Pediatr Dent* 2005; 27: 493-499.
- Yang J, Dutra V: Utility of radiology, laser fluorescence, and transillumination. *Dent Clin North Am* 2005; 49: 739-752.
- Young DA: New caries detection technologies and modern caries management: merging the strategies. *Gen Dent* 2002; 50: 320-331.
- Young DA, Featherstone JD: Digital imaging fiber-optic trans-illumination, F-speed radiographic film and depth of approximal lesions. *J Am Dent Assoc* 2005; 136: 1682-1687.

II

Title: The use of polyols in caries prevention: a systematic review and meta-analysis

Lai Gianfranco[1], gilai@uniss.it
Lara-Capi Cynthia[1], clcapi@uniss.it
Cocco Fabio[1,2,3], fcocco@uniss.it
Cagetti Maria Grazia[2], maria.cagetti@unimi.it
Campus Guglielmo[1,2] gcampus@uniss.it

[1]Department of Surgery, Microsurgery and Medical Sciences, School of Dentistry, University of Sassari, Italy
[2]WHO Collaborating Centre for Epidemiology and Community Dentistry, University of Milan
[3]Department of Chemistry and Pharmacy, University of Sassari, Italy

Corresponding author
Gianfranco Lai
Department of Surgery, Microsurgery and Medical Sciences
School of Dentistry University of Sassari
Viale San Pietro 43/C 07100 Sassari, SS, Italy
tel. +39079228540
gilai@uniss.it

Key word: polyols, xylitol, sorbitol, mannitol, erythritol, maltitol, streptococcus mutans, dental caries, pH

ABSTRACT

Aim: to analyze the reported scientific evidence indicating the beneficial effects of polyols on caries prevention and to evaluate the effects on caries risk factors and clinical outcomes.

Methods: A meta-analysis evaluated compared sorbitol and/or mannitol and/or maltitol or no intervention group, in terms of ΔDMFS/dmfs, salivary count of Mutans S. (MS) and plaque pH. Randomized controlled trials assessing chewing gums, tablets, candies, lozenges, containing or xylitol or erythritol or maltitol or sorbitol or mannitol, were tested versus a control group (sorbitol and/or mannitol and/or maltitol) or versus no intervention group.

Results: We found 518 studies involving 5464 patients (1975 to 2015); fifty-one papers were analysed and twenty-one met eligibility criteria. Three were not included. We clustered groups as A) MS count: 1) Xilitol gum versus sorbitol gum; 2) Sorbitol gum versus no gum; 3) Xylitol tablet versus no tablet; 4) Xylitol tablet versus control tablet; 5) Erythritol tablet versus no tablet; 6) Erythritol tablet versus control tablet; 7) Sorbitol tablet versus no tablet; 8) Xylitol candy versus no candy; 9) Erythritol candy versus no candy. B) DMFS: 1) Xylitol gum versus sorbitol gum; Xylitol gum versus no gum; Sorbitol gum versus no gum; Xylitol lozenges versus no lozenges; Xylitol candy versus no candy. C) DMFS + dmfs: 1) Xylitol candy versus no candy; 2) Erythritol candy versus no candy. D) dmfs: 1) Xylitol tablet versus no tablet. E) plaque pH: 1) Xylitol gum versus sorbitol gum – $AUC_{5.7}$; 2) Xylitol gum versus sorbitol gum – $AUC_{6.2}$ and 3) Xylitol lozenges versus sorbitol lozenges – $AUC_{7.0}$.

Conclusions: This meta-analysis showed that the use of xylitol via gum, tablet candy or lozenge could inhibit the MS growth, especially in short-term period. The data on the effect of erythritol on MS are poor however this polyol seem to inhibit the MS growth.

The xylitol and sorbitol gum could reduce the increase of DMFS index.

Sorbitol should be considered a low-cariogenic sweetener. However sorbitol fermented by MS could increase the plaque acidogenicity respect xylitol.

Introduction

The prevention of caries is a public health goal. The use of sugar substitutes as "polyols" or "sugar alcohols" has been implemented to halt the high consumption of sugar that has increased over the decades. The frequent use of sugar substitutes like polyols has demonstrated to have a role in the decrease in oral bacteria and dental plaque growth (Deshpande, 2008, Thaibuis, 2013).
Chewing gums, lozenges or candies, are the primary vehicles of administration of polyols. (Fontana, 2012)
The polyols most frequently used are: xylitol, sorbitol, maltitol, mannitol and erythritol (Lingstrom, 2003, Makinen, 2011, Antonio, 2011). Many global INO health promotion institutions and organizations have promoted the use of xylitol produtcs particularly chewing gums in caries prevention across the world, especially in Europe and the US. (Makinen, 2011). The use of chewing gums, candies or/lozenges has been recommended for at least three times/day for a daily quantity that oscillates from 5 to 11 grams (Rethman, 2011; Campus, 2011).
Although many studies have shown a benefit effect on the use of polyols, further analysis needs to be address to verify the scientific evidence and understand the specific role in the prevention of caries (Fontana, 2012).

In this meta-analysis, the scientific evidence indicating the beneficial effects of polyols on caries prevention was investigated, evaluating the efficacy as well as the effects on caries risk factors and on clinical outcomes.

Methods

Focused PICO Question
What is the efficacy in caries prevention, of polyols compared to the sorbitol and/or mannitol and/or maltitol or no intervention group, in terms of ΔDMFS/dmfs, salivary count of Mutans S. and plaque pH?

Eligibility Criteria
The papers included in this systematic review were randomized controlled trials (RCT) assessing the efficacy in caries prevention of chewing gums, tablets, candies and lozenges containing polyols. We selected the studies that involved both children and adults in which gums, tablets, candies or lozenges, contained xylitol, erythritol, maltitol, sorbitol or mannitol, were tested either against control group (sorbitol

and/or mannitol and/or maltitol) or versus no intervention group. In addition, we have included studies where experimental agents other than polyols were tested.
We considered as primary outcome:

- Dental caries increment.
- Level of S. Mutans in the saliva.
- Plaque pH.

We excluded studies where the control group used sucrose in pellets, candies, tablets or lozenges. As well, we excluded studies where subjects had disabilities, wore orthodontics appliances or were pregnant. The studies in which the follow-up was performed under 4 weeks were excluded. For the statistical comparison of incidence of caries the minimum follow-up of 2 years was determined. For the rest of the variables no timing was settled. The length of the experimental period was classified in short-term (between 1 - 5 months), medium-term (between 6 – 11 months) and long-term (more than 12 months). When controls were performed more than one time in the short term, in the medium term or in the long term we have considered the last data performed in the same period. If the polyols were tested in different way with regard to dose and frequency of administration, we choose the data from the group in which the polyols were administer according to the guidelines (Rethman MP *et al.*, 2011). If the follow-ups were longer than the administration period of polyols, we extracted only the data of the three primary outcomes until the last control.
Considering that the dental caries increment could be reported differently in different trials, we decided to include in the meta-analysis only two types of data: decayed-filled-missing tooth (ΔDMFS – Δdmfs) or the data of decayed surface increment. Furthermore, considering that in the studies the clinical examination to determine the presence of caries could have been made according to different methods and the lesions could have been classified in different ways, we established a-priori how to designate the data: data from "combined clinical and radiological examination" were chosen over data from "separated clinical and radiological" and as a second choice we included "only clinical" data when radiological examination was not performed. Data for non-cavitated lesions combined with cavitated lesions was chosen over cavitated-only lesion; when more than one follow-up performed over 2 years was present, we included the data for each follow-up performed.
For the data of S. Mutans count in the saliva we considered for the meta-analysis only the data expressed in CFU/ml. Finally, data of the plaque pH contemplated for the meta-analysis was only from areas under the plaque pH curve for each pH cut-off value presented in the paper. The studies that satisfied the inclusion criteria but data was not serviceable, were included only in the systematic review.

Data Analysis

The outcomes considered in the studies were: the dental caries increment (continuous and dichotomous), salivary S. Mutans count (continuous) and plaque pH (continuous). When the raw data was not present in the text or tables, single authors were contacted to obtain such information. If the authors did not answer the petition, we extracted the information from the graphs. The data comparison of the primary outcome was done separately for the gums, lozenges, tablets and candies. The comparison of DMFS and dmfs index was done separately and if that was not possible we used to comparison the number of new surface or teeth decayed. Within each vector (gum, lozenges, tablets and candies) and for each primary outcome we compared separately data between control group and/or no intervention and experimental polyols group. To compare dichotomous data, a calculation of the Odd Ratio (OR) along with 95% Confidence Intervals (CIs) was used, whereas, for continuous data, the Mean Difference (MD) with the 95% Confidence Intervals (CIs) was calculated. Also, for each comparison the Z-test was used. A Fixed-effect model was applied to reassess all data extracted from the included studies. We compared the data of salivary count of S. Mutans and plaque pH at baseline in the short- medium- and at long term. For the dental caries increment we have compared data only at follow-ups. Data of gums, lozenges, tablets and candies were compared separately. Analysis was performed using Review Manager 5.3 software provided by the Cochrane Collaboration (The Cochrane Collaboration, 2012).

For the identification of studies to be included or considered for the review we developed two search strategies: one was used in two electronic databases (PUBMED and EMBASE) (*tab. 1*) and the other was used in SCOPUS (*tab. 2*). We did not place any restriction on language or date of publication when searching the electronic database.

Search Strategy

We searched the following electronic databases:

• MEDLINE via PUBMED (to March 2015)
• EMBASE (to March 2015)
• SCOPUS (to March 2015)

A comparison of the different searches was carried out to exclude the repeated studies. Then, two authors, tasked with to evaluating the eligibility of the papers, examined independently all abstracts and titles of the studies found. If the information contained in abstract or in the title was no enough to determinate if the studies met inclusion criteria, the full paper was obtained. All studies that appeared to meet inclusion criteria were obtained in the full text format. The two authors

assessed the papers independently to establish whether the studies met the inclusion criteria. Disagreements were resolved by discussion.

Results

A total of 518 studies published from 1975 to 2015 were found. Fifty-one papers were analysed and twenty-one met the eligibility criteria. Three papers (Mäkinen KK *et al.*, 1996; Seki M *et al.,* 2011, Lekkeri AM *et al.*, 2011) were not included in the meta-analysis but only in the systematic review because primary outcome data was missing. (fig.1). The trials admitted in the review involved a total of 5464 patients. One of the studies included in the meta-analysis (Spieth CH *et al.*, 2009) did not specify the randomization method of the sample. All the other studies had a randomization clinical trial with parallel arms design. Five studies (Honkala S *et al.*, 2014; Mäkinen KK *et al.*, 2005; Alanen P *et al.*, 2000; Peng B *et al.*, 2004; Machiulskiene V *et al.*, 2001) used a cluster-randomized design. Eleven studies (Milgrom P *et al.*, 2006; Honkala S *et al.*, 2014; Mäkinen KK *et al.*, 2005; Stecksen-Blicks C *et al.*, 2008; Martinez-Pabòn MC *et al.*, 2014; Runnel R *et al.*, 2013; Holgerson PL *et al.*, 2006; Campus G *et al.*, 2013; Campus G *et al.*, 2011; Campus G *et al.*, 2009, Splieth CH *et al.*, 2009) were double blind and one single blind (Oscarson P *et al.*, 2006). Seven studies were performed in adults (Hildebrandt GH *et al.*, 2000; Milgrom P *et al.*, 2006; Simons D *et al.*, 2002; Martinez-Pabòn MC *et al.*, 2014; Ly KA *et al.*, 2006; Campus G *et al.*, 2011; Splieth CH *et al.*, 2009), eleven in patients with mixed dentition, aged between 6 and 13 years old, (Szöke J, 2001; Honkala S *et al.*, 2014; Beiswanger BB *et al.*; 1998; Runnel R *et al.*, 2013; Holgerson PL *et al.*, 2007; Campus G *et al.*, 2013; Campus G *et al.*, 2009; Stecksen-Blicks C *et al.*, 2007; Machiulskiene V *et al.*, 2001; Alanen P *et al.*, 2000; Peng B *et al.*, 2004) and one in children with deciduous dentition (Oscarson P *et al.*, 2006).
In fifteen studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011; Campus G *et al.*, 2013; Holgerson PL *et al.*, 2007; Ly KA *et al.*, 2006; Simons D *et al.*, 2002; Martinez-Pabon MC *et al.*, 2014; Machiulskiene V *et al.*, 2001; Peng B *et al.*, 2004; Beiswanger BB *et al.*, 1998; Szöke J *et al.*, 2001; Milgrom P *et al.*, 2006; Hildebrandt GH *et al.*, 2000) were used gums and in thirteen of these studies were tested xylitol and in eight paper was used as control gum with sorbitol and/or mannitol or maltitol, while in the other 5 the control group did not received gum. In two studies (Szoöke J *et al.*, 2001; Beiswanger BB *et al.*, 1998) was tested a combination of sorbitol and mannitol versus a control group with no gum. In two study (Hildebrandt GH *et al.*, 1998, Mäkinen KK *et al.*, 2005) as control group were present both sorbitol and no intervention group. From two studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011), which tested gums, we could extract two types of data: salivary count of S. Mutans and plaque pH. From seven studies (Campus G *et*

al., 2013; Holgerson PL *et al.*, 2007; Ly KA *et al.*, 2006; Simons D *et al.*, 2002; Martinez-Pabòn MC *et al.*, 2014; Milgrom P *et al.*, 2006, Hildebrandt GH *et al.*, 2000) we extracted data of S. Mutans count whereas from four studies (Szöke J *et al.*, 2001; Beiswager BB *et al.*, 1998; Machiulskiene V *et al.*, 2001; Peng B *et al.*, 2004) we gained data of dental caries increment. From study of Ly et al. the data of salivary count of S. Mutans at baseline was no present in the text. We contact the authors to obtain this data but we had no answer. The data of follow-up were deduced from graph in the papers. In also we extracted the data of salivary count of S. Mutans from the graph in the paper text from Simons et al., 2006 study.
In two studies included in the meta-analysis were used lozenges (Splieth CH *et al.*, 2009; Stecksen-Blicks C *et al.*, 2007) and in both studies were tested xylitol. In the study of Splieth et al., was used as control group lozenges with sorbitol while in Stecksen-Blicks et al. paper in the control group was not administrated any lozenges. From Splieth et al. study we extracted data of plaque pH while from Stecksen-Blicks et al. paper we gained data of ΔDMFS.
In two studies included in the meta-analysis were used tablets (Mäkinen KK *et al.*, 2005; Oscarson P *et al.*, 2006) as device. In the paper of Oscarson et al. was tested xylitol while in the study of Mäkinen et al. the polyols tested were two: erythritol, xylitol and sorbitol. In both studies in the control group was not administrated any tablets. From one study (Mäkinen KK *et al.*, 2005) was gained data of salivary count of S. Mutans while from the other study (Oscarson P *et al.*, 2006) we extracted data of Δdmfs.
In two studies included in the meta-analysis were used candies (Runnel, 2013; Honkala S *et al.*, 2014) as device. In both studies were tested erythritol and xylitol while in control group were used candies with sorbitol. From one studies (Honkala S *et al.*, 2014) we extracted data of number of decayed surface while in the other we gained data of salivary count of S. Mutans. From the study of Honkala et al. the data of decayed surface interest both the primary teeth and permanent teeth.
In the study of Alanen et al. were used both candies and gum. In the study was tested xylitol while in the control group the subject did not received gum or candy. From this study were extracted data of ΔDMFS.

Xylitol gum versus control gum - MS count
For this comparison we considered seven studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011, Campus G *et al.*, 2013; Hildebrandt GH *et al.*, 200; Holgerson PL *et al.*, 2007; Milgrom P *et al.*, 2006; Ly KA *et al.*, 2006). At baseline and in the short term we found data in six studies, in medium term we extracted data from two studies and in long term only one study presented data of MS (Mutans S.) salivary count. At baseline we have no found difference in MS salivary count (mean difference (MD) 0.01, 95% confidence interval (CI) -0.04 to 0.06, P value = 0.78). In the short term (tab. 3) the MS salivary count is significantly lower in the Xylitol group (MD -0.20,

95% CI -0.28 to -0.12, P value = 0.002). In the medium term (tab. 3) was not found difference between gum with xylitol and gum with sorbitol in terms of reduction of MS salivary count (MD -0.16, 95% CI -0.32 to 0.01, P value = 0.26. In the long term (tab. 3) from the analysis of Campus et al., 2013 study, we found that the MS salivary count is smaller in the xylitol gum group than in the control group with sorbitol gum (MD -0.70, 95% CI -1.31 to -0.09, P =0.02).

Xylitol gum versus no gum – MS count

For this comparison we included three studies (Hildebrandt GH *et al.*, 2000; Martinez-Pabòn MC *et al.*, 2014; Simons D *et al.*, 2002). At baseline and in the short term we found data in all studies whereas for the comparison in medium and long terms the only one study (Simons D *et al.*, 2002) was used to extract MS salivary count. At baseline we found no difference in MS salivary count (MD -0.17; 95% CI -0.58 to 0.24, P value = 0.42). in the short term (tab. 3) MS salivary count was significantly lower in the Xylitol group (MD -0.70, 95% CI -1.14 to -0.25, P = 0.002). In the medium and long term we found no difference in MS salivary count: at medium term P value = 0.08 and at long term P value = 0.85 (tab. 3).

Sorbitol gum versus no gum – MS count

For this comparison we extracted data from one study (Hildebrandt GH *et al.*, 2000). In this study was present control at baseline and in the short term. At baseline there was not difference in terms of MS salivary count (MD 0.10, 95% CI -0.47 to 0.67, P = 0.73). At control in the short term period we found no difference between sorbitol gum and control group without chewing gums (MD 0.30, 95% CI -0.21 to 0.81, P = 0.25)

Xylitol tablet versus no tablet – MS count

For this comparison we included only one study (Mäkinen KK *et al.*, 2005) and we have data at baseline and in the medium term. At baseline there was not difference in MS salivary count (MD 0.32, 95% CI -0.71 to 0.07, P = 0.10) whereas in the medium term salivary presence of MS is significantly lower in the xylitol group than in the control group (MD -0.70, 95% CI -1-12 to -0.28, P = 0.001) (tab. 3).

Xylitol tablet versus control tablet– MS count

For this comparison we included only one study (Mäkinen KK *et al.*, 2005) and we have data at baseline and in the medium term. No significant difference was found at baseline in MS salivary count (MD -0.03, 95% CI -0.40 to 0.34, P = 0.87) whereas in the medium term the MS salivary count resulted higher in the xylitol group than in the control group (MD -0.61, 95% CI -1.01 to -0.21, P = 0.003) (tab. 3).

Erythritol tablet versus no tablet – MS count

For this comparison we included only one study (Mäkinen KK *et al.*, 2005) and we have data at baseline and in the medium term. At baseline there was not difference in MS salivary count (MD -0.04, 95% CI -0.48 to -0.40, P = 0.86) whereas in the medium term salivary presence of MS is significantly lower in the xylitol group than in the control group (MD -0.85, 95% CI -1.26 to -0.44, P < 0.0001) (tab. 3).

Erythritol tablet versus control tablet – MS count

For this comparison we included only one study (Mäkinen KK *et al.*, 2005) and we have data at baseline and in the medium term. No significant difference was found at baseline in MS salivary count (MD 0.25, 95% CI -0.18, 0.68, P = 0.25) whereas in the medium term the MS salivary count resulted higher in control group than erythritol group (MD -0.76, 95% CI -1.15 to -0.37, P = 0.0001) (tab. 3).

Sorbitol tablet versus no tablet – MS count

For this comparison we included only one paper (Mäkinen KK *et al.*, 2005) and we have data at baseline and in the medium term. No significant difference in terms of MS salivary count was found at baseline (MD -0.29, 95% CI -0.71 to 0.13, P = 0.17) and in the medium term (MD -0.09, 95% CI -0.52 to 0.34, P = 0.68).

Xylitol candy versus control candy – MS count

For this comparison we found one study (Runnel R *et al.*, 2013) and we in the paper were presented data at baseline and in the long term. At baseline the MS salivary count was significantly higher in the xylitol group (MD 0.11, 95% CI 0.09 to 0.13, P < 0.00001) whereas at control in the long term the situation that we found was the opposite: the MS salivary count was lower in the xylitol group (MD -0.18, 95% CI -0.20, -0.16, P < 0.0001).

Erythritol candy versus control candy – MS count

For this comparison we included only one study (Runnel R *et al.*, 2013) and we have data at baseline and in the long term. At baseline there was not difference in MS salivary count (MD 0.02, 95% CI -0.00 to -0.04, P = 0.08) whereas in the medium term (tab. 1) salivary presence of MS is significantly lower in xylitol group than in the control group (MD -0.44, 95% CI -0.46 to -0.42, P < 0.0001) (tab. 3).

Xylitol gum versus control gum – DMFS

For this comparison we included one study (Machiulskiene V *et al.*, 2001) and we have data at 2 years and at 3 years of follow-ups. The data comparison of ΔDMFS at two years showed, in the control group with sorbitol gum, a significantly smaller increase of decayed surface (MD 2.45, 95% CI 2.20 to 2.70, P < 0.00001. At 3 years

the increment of DMFS is higher in the control group with sorbitol gum (MD -0.90, 95% CI -1.35 to 0.45, P < 0.0001) (tab. 4).

Xylitol gum versus no gum – DMFS
For this comparison we included three studies (Machiulskiene V *et al.*, 2001; Peng B *et al.*, 2004; Alanen P *et al.*, 2000); from one studies (Machiulskiene V *et al.*, 2001) we had extracted data at 2 and at 3 years follow-ups whereas from studies of Peng et al. and Alanen et al. the follow-up was performed respectively at 2 and 3 years. At 2 years the increase of DMFS was significantly lower in the xylitol group (MD -0.01, 95% CI -0.17 to -0.02, P = 0.01). This trend was confirmed at 3 years of follow-up: the ΔDMFS was smaller in the xylitol group than in the control group (MD -0.69, 95% CI -1.08 to 0.30, P = 0.0005) (tab. 4).

Sorbitol gum versus no gum – DMFS
For this comparison we included three studies (Machiulskiene V *et al.*, 2001; Sköze J *et al.*, 2001; Beiswanger BB *et al.*, 1998); from two studies (Machiulskiene V *et al.*, 2001, Beiswanger BB *et al.*, 1998) we extracted data at 2 and at 3 years follow-ups whereas from studies of Szöke et al. the follow-up was performed only at 2 years. At 2 years the increase of DMFS was significantly lower in the xylitol group (MD -0.01, 95% CI -0.17 to -0.02, P = 0.01). This trend was confirmed at 3 years of follow-up: the ΔDMFS was smaller in the xylitol than in the control group (MD -0.69, 95% CI -1.08 to 0.30, P = 0.0005) (tab. 4).

Xylitol lozenges versus no lozenges – DMFS
For this comparison we found data in one study (Stecksen-Blick C *et al.*, 2008) and the follow-up was performed at 2 years. We found no difference in terms of ΔDMFS between xylitol lozenges and group without lozenges (MD 1.00, 95% CI -0.42 to 2.42, P = 0.17).

Xylitol candy versus no candy – DMFS
For this comparison we found data in one study (Alanen P *et al.*, 2000) and the follow-up was performed at 3 years. At 2 years the increase of DMFS was significantly lower in the xylitol group (MD -1.65, 95% CI -2.67 to -0.63, P = 0.002) (tab. 4).

Xylitol candy versus no candy – DMFS + dmfs (number of decayed surface/total surface analysed)
For this comparison we found data in one study (Honkala S *et al.*, 2014) that performed the follow-up at 3 years. We found no difference between the xylitol group and the control group (OR 1.05, 95% CI 0.96 to 1.15, P = 0.26).

Erythritol candy versus no candy – DMFS + dmfs (number of decayed surface/total surface analysed)
For this comparison we found data in one study (Honkala S *et al.*, 2014) in which the follow-up was performed at 3 years. At 3 years the surface decayed in the control group were significantly higher than in the erythritol group (OR 0.83, 95% CI 0.75 to 0.91, P < 0.0001) (tab. 4).

Xylitol tablet versus no tablet – dmfs
For this comparison we found data in one study (Oscarson P *et al.*, 2006) and the follow-up was performed at 2 years. At 2 years we found no difference between the xylitol group and the control group (MD -0.42, 95% CI -1.12 to 0.28, P = 0.24).

Xylitol gum versus sorbitol gum – $AUC_{5.7}$ of plaque pH
For this comparison we included two studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011). In both studies we found data at baseline and in the short term whereas only one study performed (Campus G *et al.*, 2009) the control in the medium term. At baseline we found that the $AUC_{5.7}$ of plaque was significantly bigger in the xylitol group than in the control group (MD 0.98; 95% CI -0.82 to 1.14, P < 0.00001). In the short terms the $AUC_{5.7}$ of plaque was less big in xylitol group than control group (MD -1.28, 95% CI -1.43 to -1.13, P < 0.00001). Also in the medium terms we have found the $AUC_{5.7}$ in xylitol group was smaller than in the control group (MD -2.50, 95% CI -2.64 to -2.36, P<0.00001) (tab. 5).

Xylitol gum versus sorbitol gum – $AUC_{6.2}$ of plaque pH
For this comparison we included two studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011). In both studies we have found data at baseline and in the short term whereas only one study performed (Campus G *et al.*, 2009) the control in the medium term. At baseline comparing the data of two studies we found that the $AUC_{6.2}$ of plaque pH was significantly biggest in the xylitol group (MD 1.28; 95% CI 1.09 to 1.46, P < 0.00001). In the short terms the $AUC_{6.2}$ of plaque pH was smaller in xylitol group than control group (MD -6.22, 95% CI -6.37 to -6.07, P < 0.00001). Also in the medium terms we found the $AUC_{6.2}$ of plaque pH in the xylitol group was smaller than control group (MD -5.50, 95% CI -5.67 to -5.33, P<0.00001) (tab. 5).

Xylitol lozenges versus sorbitol lozenges – $AUC_{7.0}$ of plaque pH
For this comparison we included one study (Splieth CH *et al.*, 2009) in which the controls were performed at baseline and at short terms. At baseline comparing the data we found that $AUC_{7.0}$ of plaque pH was bigger in control group than experimental group with xylitol lozenges (MD -25.80, 95% -44.30 to -7.30). At

control in short term we found difference between two groups (MD -4.60, 95% CI -13.04 to 3.84, P =0.29).

Discussion

In the literature, there is no availability of meta-analysis on the efficacy of polyols in caries prevention. Hence, this study was performed in an attempt to gain further insight into the reliability in caries prevention of the polyols in chewing gums, candies, tablets and lozenges. Twenty-three studies were included, with a total of 5464 patients involved.

Mutans S. is considered to be the main pathogen responsible for dental caries. Numerous studies have shown an association between the number of carious lesions and the levels of Mutans S. in both adults and children. Also, a significant correlation between caries and Mutans S. was found (ElSalhy M *et al.*, 2012). Regarding the effects of polyols on Mutans S. we found studies that tested or xylitol, sorbitol and erythritol, vs. or control gum with or no intervention group. The vectors used to administer polyols were gum, candies and tablets.

In the comparison between xylitol gum and control gum in short terms we have found, after comparison of data extracted from six studies (Campus G *et al.*, 2009; Campus G *et al.*, 2011; Hildebrandt GH *et al.*, 2000; Holgerson PL *et al.*, 2007; Ly KA *et al.*, 2006; Milgrom P *et al.*, 2006), a higher reduction of salivary S. Mutans count in patients which consumed xylitol gum. This trend was not confirmed in the medium term where we have analysed data from two studies (Campus G *et al.*, 2009; Holgerson PL *et al.*, 2007) and we found no difference. In the long term we evaluated only one study (Campus G *et al.*, 2013) that showed a significant reduction of salivary S. Mutans count in subject that consumed xylitol gum. One study (Runnel R *et al.*, 2013) included in this meta-analysis showed a significant difference in salivary MS count between the control group with sorbitol candies and the experimental group with xylitol candies. For the comparison between xylitol tablets and control tablets we included in the meta-analysis one study (Mäkinen KK *et al.*, 2005) that showed a significant reduction of salivary MS count in xylitol group. These results reflected the properties of two polyols; sorbitol, even if it has not effect on the growth of dental plaque it stimulates the growth of some strains of mutans streptococci (Mäkinen KK, 2011).

In the comparison between xylitol gum vs. no intervention group we founded three studies (Hildebrandt GH *et al.*, 2000; Martinez-Pabòn MC *et al.*, 2014; Simons D *et al.*, 2002) that showed in the short term a larger reduction in salivary S. Mutans count in subject allocated in xylitol group. In the medium and long terms the only study (Simons D *et al.*, 2002) analysed showed no difference between xylitol gum and no intervention group. These results were confirmed also in the studies that

tested xylitol tablets vs. no tablets: in the medium term the subjects in the xylitol group showed a significant reduction of salivary S. Mutans count (Mäkinen KK *et al.*, 2005). Numerous studies have demonstrated that habitual xylitol consumption decrease count of mutans streptococci several mechanisms may explain the phenomenon: growth inhibition, a decrease in the amount of plaque, elevated pH in the mouth, a decrease of adhesive polysaccharides produced by mutans streptococci (Söderling E *et al.*, 2015). Xylitol has been used as a substitute for refined white sugar for more than 30 years, and is thought to have an inhibitory action on the major causative bacteria in dental caries, S. Mutans. Xylitol and can also promote an ecological shift, resulting in less cariogenic environment (Holgerson PL *et al.*, 2007). These results would seem confirm the antimicrobial effect against S. Mutans of xylitol (Deshpande A *et al.*, 2008) when was administrated via gum, tablets, candies or lozenges.
The erythritol was tested against S. Mutans in two studies. In one paper (Mäkinen KK *et al.,* 2005) tablets were used as vectors and no intervention group and sorbitol tablets were used as control. In the other study (Runnel R *et al.*, 2013) candies were used as vectors. In the study that test tablets (Mäkinen KK *et al.*, 2005) in the medium term the salivary S. Mutans count was significantly smaller in erythritol group compared both whit no intervention group and with the control group. The same result has been found at long term in the study that test erythritol candies (Runnel R *et al.*, 2013). Information on oral biological effect of erythritol has been scant. However the few reports on erythritol suggest that this sugar alcohol may be of significant dental benefit (Mäkinen KK *et al.*, 2005).
The two studies included in this review investigated on the efficacy against S. Mutans of sortibol gum vs. no gum and sorbitol tablets vs. no tablets, showed no difference between two groups. These results reflected the little data reported in literature: sorbitol might stimulate the growth of some strains of mutans streptococci (Mäkinen KK, 2011).

Regarding the protective effects of polyols on dental caries in terms of DMFS/dmfs increment we found studies that tested or xylitol, sorbitol and erythritol, vs. or control gum or no intervention group, or sorbitol. The vectors used to administer polyols were gum, lozenges, candies and tablets.
For the comparison between xylitol gums vs. control gums we found one study (Machiulskiene V *et al.*, 2001). This paper showed a significantly smaller DMFS increment at 2 years follow-up in subjects that consumed sorbitol gum while at 3 years follow-up the analysis of data indicated a significantly smaller DMFS increment in the xylitol gums group. In a study that compared xylitol candies vs. control candies (Honkala S *et al.*, 2014) only the number of surface decay and not the DMFS index were analysed. The comparison of data showed no difference. The data analysis and the little literature suggest that xylitol had no greater cariostatic effect

than sorbitol when dispensed with gums or candies (Machiulskiene V *et al.*, 2001; Honkala S *et al.*, 2014).
For the comparison between xylitol gums vs. no intervention group we found three studies (Machiulskiene V *et al.*, 2001; Peng B *et al.*, 2004; Alanen P *et al.*, 2000). The comparison showed a protective effect in terms of DMFS increment at 2 and 3 years follow-up with xylitol gum. For the comparison between xylitol lozenges and no intervention group we included one study (Stecksen-Blicks C *et al.*, 2008) that showed no protective effect in terms of DMFS score. In the study (Alanen P *et al.*, 2000) that compared xylitol candies vs. no intervention group, the DMFS increase was significantly smaller in the experimental group at 3 years follow-up. One paper (Oscarson P *et al.*, 2006) investigated xylitol tablets vs. no intervention group in term of caries preventive effect on deciduous teeth: no difference was found in dmfs increase. The scientific evidence of the anticariogenic effects of the xylitol has been under debate (Stecksen-Blick C *et al.*, 2008; Oscarson P *et al.*, 2006). This meta-analysis shows that the caries preventive effect of xylitol was obtained when this polyol was administer via gums. The singles studies on candies, tablets and lozenges containing xylitol did not showed clearly protective effects in terms of DMFS and dmfs increment when compared with no intervention. The results do not lend support to the claims made in a number of previous studies that xylitol acts as a therapeutic or cariostatic agent. Rather, the results indicate that the caries preventive effect of gum could relate to an effect of increased salivary secretion resulting in higher salivary pH, buffer capacity and glucose clearance (Machiulskiene V *et al.*, 2001)
In literature we found only one study (Honkala S *et al.*, 2014), included in this meta-analysis that investigated the caries preventive effect of erythritol. This paper compared erythritol candies vs. sorbitol candies (control group) and for the statistical comparison the number of surface decayed was used. The results of this study suggested that the erythritol could have a greater protective effect against dental than sorbitol. Erythritol has been reported to be a totally safe and promising sweetener and to have several advantages, being non-caloric, less laxative than any other polyol, including sorbitol and xylitol (Honkala S *et al.*, 2014). In the short term erythritol could reduce dental plaque and S. mutans (Mäkinen KK *et al.*, 2005)
For the comparison between sorbitol gums vs. no intervention group we included three studies (Beiswanger BB *et al.*, 1998; Machiulskiene V *et al.*, 2001; Szöke J *et al.*, 2001). The analysis of data showed a smaller increment in DMFS score in experimental group at 2 and 3 years follow-up. This data confirmed the anti-caries benefits of sorbitol gums vs. no intervention described in literature (Szöke J *et al.*, 2001).

Regarding the AUC of pH we included in the meta-analysis studies that test xylitol in gum and lozenges vs. control group with sorbitol. For the comparison between xylitol gums vs. control gum we included two studies (Campus G *et al.*, 2009;

Campus G *et al.*, 2011). At baseline we found difference among the experimental and the controls group. However the $AUC_{5.7}$ and $AUC_{6.2}$ of pH is greater in xylitol group. In the short and in the medium term there was a reversal in the $AUC_{5.7}$ and $AUC_{6.2}$ of pH value.
These results could reflect the ability of mutans streptococci to ferment sorbitol while the xylitol cannot be metabolised by mutans streptococci (Oscarson P *et al.*, 2006). Indeed sorbitol should be considered a low-cariogenic sweetener rather than a non-cariogenic one. Mutans streptococci could ferment sorbitol increasing the plaque acidity (Campus G *et al.*, 2009).
In the study (Splieth CH *et al.*, 2009) that compared xylitol lozenges and sorbitol lozenges we found a difference between two groups at baseline. At control there was no reversal of pH value.

Conclusions

This meta-analysis shown that the use of xylitol via gum, tablet, candy or lozenge could inhibit the growth of MS especially in short-term period. The data on the effect of erythritol on MS are poor, however this polyol seems to inhibit the MS growth. The use of xylitol or sorbitol when administer via gum could reduce the increase of DMFS index. Sorbitol should be considered a low-cariogenic sweetener, however MS can ferment sorbitol could increase the plaque acidogenicity respect xylitol.

References

• Alanen P, Isokangas P, Gutmann K: Xylitol candies in caries prevention: results of a field study in Estonian children; *Community Dent Oral Epidemiol* 2000; 28: 218-224.

• Antonio AG, Pierro Vs, Maia LC: Caries preventive effect of xylitol-based candies and lozenges: a systematic review. *J Public Health Dent* 2011; 71: 117-124.

• Beiswanger BB, Boneta AE, Mau MS, Katz BP, Proskin HM Stookey GK: The effect of chewing gugar-free gum after meals on clinical caries incidence. *J Am Dent Assoc* 1998; 129: 1623-1626.

• Campus G, Cagetti MG, Cocco F, Sale S, Sacco G, Strohmenges L, Lingström P: Effect of a sugar-free chewing gum containing magnolia bark extract on different variables related to caries and gingivitis: a randomized controlled intervention trial. *Caries Res* 2011; 45: 393-399.

• Campus G, Cagetti MG, Sacco G, Solinas G, Mastroberardino S, Lingström P: Six month of daily high-dose xylitol in high-risk schoolchildren: a randomized clinical trial on plaque pH and salivary mutans streptococci. *Caries Res* 2009; 43: 455-461.

• Campus G, Cagetti MG, Sale S, Petruzzi M, Solinas G, Strohmenger L, Lingström P: Six months of a high-dose xylitol in high-risk caries subjects-a 2-year randomised, clinical trial. *Clin Oral Invest* 2013; 17: 785-791.

• Deshpande A, Jadad, AR: The impact of polyols-containing chewing gums on dental caries: a systematic review of original randomized controlled trials and observational studies. *JADA* 2008; 139: 1602-1614.

• ElSalhy M, Sayed Zahid I, Honkala E: Effect of xylitol mouthrinse on Streptococcus mutans. *J Dent* 2012; 40: 1151-1154.

• Fontana M, González-Cabezas C: Are we ready for definitive clinical guidelines on xylitol/polyol use? *Adc Dent Res* 2012; 24: 123-128.

• Hildebrandt GH, Sparks BS: Maintaining mutans streptococci suppression with xylitol chewing gum. *J Am Dent Assoc* 2000; 131: 909-916.

• Holgerson PL, Sjöström I, Stecksén-Blicks C, Twetman S: Dental plaque formation and salivary mutans streptococci in schoolclidren after use of xylitol-containing chewing gum. *Int J Paediatr Dent* 2007; 17: 79-85.

• Honkala S, Runnel R, Saag M, Olak J, Nõmmela R, Russak S, Mäkinen PL, Vahlberg T, Falony G, Mäkinen K, Honkala E: Effect of erythritol and xylitol on dental caries prevention in children; *Caries Res* 2014; 48: 482-490.

• Lenkkeri AM1, Pienihäkkinen K, Hurme S, Alanen P: The caries-preventive effect of xylitol/maltitol and erythritol/maltitol lozenges: results of a double-blinded, cluster-randomized clinical trial in an area of natural fluoridation. *Int J Paediatr Dent* 2012; 22: 180-190.

• Lingström P, Holm AK, Majàre I, Twetman S, Söder B, Norlund A, Axelsson S, Lagerlöf F, Nordenram G, Petersson LG, Dahlgren H, Källestål C: Dietary factors in the prevention of dental caries: a systematic review. *Acta Odontol Scand* 2003; 61: 331-340
• Ly KA, Milgrom P, Roberts MC, Yamaguche DK, Rothen M, Mueller G: Linear response of mutans streptococci to increasing frequency of xylitol chewing gum use: a randomized controlled trial; *BMC Oral Health* 2006; 6:6.
• Machiulskiene V, Nyvad B, Baelum V: Caries preventive effect of suger-substituted chewing gum. *Community Dent Oral Epidemiol* 2001; 29: 278-288.
• Mäkinen KK: Sugar alcohol sweeteners as alternative to sugar with special consideration of xylitol. *Med Princ Pract* 2011; 20: 303-320.
• Mäkinen KK, Pemberton D, Mäkinen PL, Chin CY, Cole J Hujoel PP, Lopatin D, Lambert P: Polyol-combinant saliva stimulants and oral health in Veterans Affairs patients - An exploratory study. *Spec Care Dentist* 1996; 16: 104-115.
• Mäkinen KK, Saag M, Isotupa KP, Olak J, Nõmmela R, Söderling E, Mäkinen PL: Similary of the effects of erythritol and xylitol on some risk factors of dental caries. *Caries Res* 2005; 39: 207-215.
• Martinez-Pabòn MC, Duque-Agudelo L, Díaz-Gil JD, Isaza-Guzmaán DM, Tobón-Arroyave SI: Comparison of the effect of two sugar-substituted chwing gums on different caries- and gingivitis-related variables: a double-blind, randomized, controlled clinical trial. *Clin Oral Invest* 2014; 18: 589-598.
• Milgrom P, Ly KA, Roberts MC, Rothen M, Mueller, Yamaguchi: Mutans Streptococci dose response to xylitol chewing gum. *J Dent Res* 2006; 85: 177-181.
• Oscarson P, Holgerson PL, Sjöström I, Twetman S, Stecksén-Blicks C: Influence of a low xylitol-dose on mutans streptococci colonisation and caries development in preschool children. *Eur Arch Paediatr Dent* 2006; 7: 142-147.
• Peng B, Petersen PE, Bian Z, Tai B Jiang H: Can school-based oral health education and a sugar-free chewing gum program improve oral health? Results fro a two-year study in PR China. *Acta Odontol Scand* 2004; 62: 328-332.
• Rethman MP1, Beltrán-Aguilar ED, Billings RJ, Hujoel PP, Katz BP, Milgrom P, Sohn W, Stamm JW, Watson G, Wolff M, Wright JT, Zero D, Aravamudhan K, Frantsve-Hawley J, Meyer DM; American Dental Association Council on Scientific Affairs Expert Panel on Nonfluoride Caries-Preventive Agents. Nonfluoride caries-preventive agents: executive summary of evidence-based clinical recommendations. *J Am Dent Assoc* 2011; 142: 1065-1071.
• Ricketts D, Lamont T, Innes NP, Kidd E, Clarkson JE: Operative caries management in adults and children. *Cochrane Database Syst Rev* 2013; 3:CD003808.
• Runnel R, Mäkinen KK, Honkala S, Olak J, Mäkinen PL, Nõmmela R, Vahlberg T, Honkala E, Saag M: Effect of three-year consumption of erythritol, xylitol and

sorbitol candies on various plaque and salivary caries-related variables. *J Dent* 2013; 41: 1236-1244.
• Seki M, Karakaa F, Kawato T, Tanaka H, Saeki Y, Yamashita Y: Effect of xylitol gum on the level of oral mutans streptococci of preschoolers: block-randomised trial. *Int Dent J* 2011; 61: 274-280.
• Simons D, Brailsford SR, Kidd EAM, Beighton D: The effect of medicated chewing gums on oral health in frail older people: a 1-year clinical trial. *J Am Geriatr Soc* 2002; 50: 1348-1353.
• Söderling EM, Hietala-Lenkkeri AM: Xylitol and erythritol decrease adherence of polysaccharide-production oral streptococci. *Curr Microbiol* 2010; 60: 25-29.
• Splieth CH, Alkilzy M, Schmitt J, Berndt C, Welk A: Effect of xylitol and sorbitol on plaque acidogenesis. *Quintessence Int* 2009; 40: 279-285.
• Stecksén-Blicks C, Holgerson PL, Twetman S: Effect of xylitol and xylitol-fluoride lozenges on approximal caries development in high-caries-risk children. *Int J Paedriatr Dent* 2008; 18: 170-177.
• Szöke J, Bánóczy J Proskin HM: Effect of after-meal sucrose-free gum-chewing on clinical caries. *J Dent Res* 2001; 80: 1725-1729.
• Thabuis C, Cheng CY, Wang X, Pochat M, Han A, Miller L, Wils D, Guerin-Deremaux L: Effect of maltitol and xylitol chewing-gums on parameters involved in dental caries devlopment. *Eur J Paediatr Dent* 2013; 14: 303-308
• The Cochrane Collaboration. Review Manager (RevMan) - computer program. Version 5.3. Prod. The Nordic Cochrane Centre. Copenhagen, 2012.

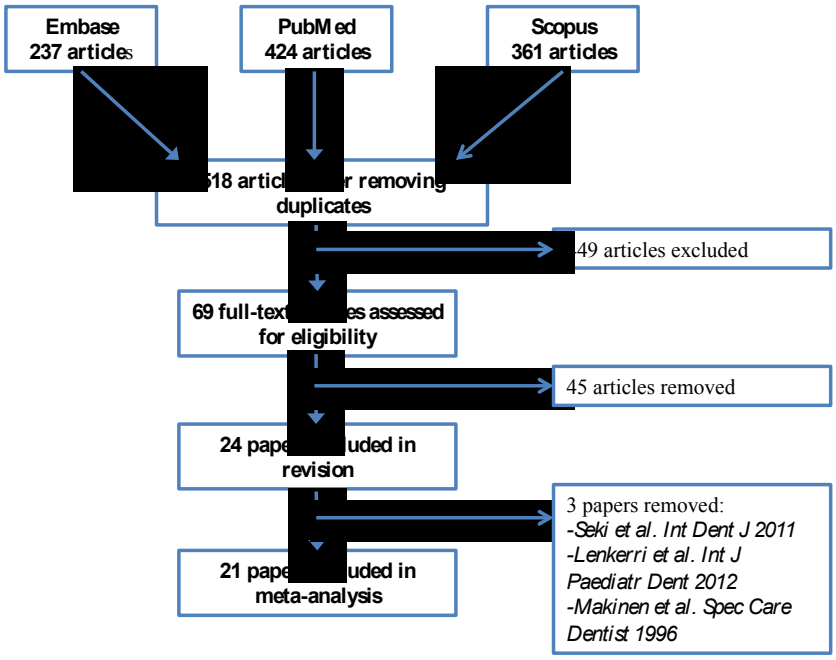


Fig. 1 Flow chart of search strategy

#1 randomized clinical trial [pt]
#2 dental caries AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR plaque pH OR streptococcus mutans OR lactobacillus) [tiab]
#3 dmft AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols) [tiab]
#4 lactobacillus AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
5# streptococcus mutans AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
6# plaque pH AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab]
#7 #2 OR #3 OR #4 OR #5 OR #6

Tab. 1 Search strategy used in MEDLINE and EMBASE database

#1 randomized clinical trial [tiab] #2 dental caries AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR plaque pH OR streptococcus mutans OR lactobacillus) [tiab] #3 dmft AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols) [tiab] #4 lactobacillus AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab] 5# streptococcus mutans AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab] 6# plaque pH AND (candies OR chewing gums OR lozenges OR mannitol OR maltitol OR erythritol OR sorbitol OR xylitol OR sugar alcohols OR polyols OR) [tiab] #7 #1 OR #2 OR #3 OR #4 OR #5 OR #6

Tab. 2 Search strategy used in SCOPUS database

Tab. 3 Significative results in MS salivary count

Type of comparison	**Authors**	**Short term**				**Medium term**				**Long term**			
Xylitol gum vs. control gum		**Xylitol**	**Control**	**Intervals**	**p value**	**Xylitol**	**Control**	**Intervals**	**P value**	**Xylitol**	**Control**	**Intervals**	**P value**
	Campus 2009	5.28 (0.6) – 80	5.36 (0.2) – 85	-0.08 [-0.22, 0.06]		5.25 (0.6) – 78	5.36 (0.5) – 85	-0.11 [-0.28, 0.06]					
	Campus 2011	5.29 (0.3) – 40	5.4 (0.2) – 39	-0.11 [-0.22, 0.00]									
	Campus 2013									1.9 (0.8) - 74	2.6 (2.4) - 66	-0.70 [-1.31, -0.09]	
	Hildebrandt 2000	3.6 (1.2) – 42	4.7 (1.2) – 47	-1.10 [-1.60, -0.60]									
	Holgerson 2007	4.18 (4.54) – 64	4.92 (4.25) – 64	-0.74 [-2.26, 0.78]		4.3 (1.2) – 27	5.2 (1.3) - 28	-0.90 [-1.56, -0.24]					
	Ly 2006	4.35 (0.4) - 33	4.95 (0.5) – 33	-0.60 [-0.82, -0.38]									
	Milgrom 2006	4.7 (1.1) – 30	5.3 (1.1) – 30	-0.60 [-1.16, -0.04]									
					P<0.01				P=0.06				P=0.02
Xylitol gum vs. no gum		**Xylitol**	**No gum**	**Intervals**	**p value**	**Xylitol**	**No gum**	**Intervals**	**p value**	**Xylitol**	**No gum**	**Intervals**	**p value**
	Hildebrandt 2000	3.6 (1.2) – 42	4.4 (1.3) – 46	-0.80 [-1.32, -0.28]									
	Martinez-Pabòn 2014	6.42 (6.4) – 46	6.97 (6.89) – 36	-0.55 [-3.46, 2.36]									
	Simons 2002	1.8 (2.3) - 57	2.2 (2.5) - 52	-0.40 [-1.30, 0.50]		1.8 (2.1) - 57	2.5 (2.1) - 52	-0.70 [-1.49, 0.09]		3.9 (2.3) - 37	4 (2) - 31	-0.10 [-1.12, 0.92]	

					P<0.01				P=0.08				P=0.85
		Xylitol	**No tablet**	**Intervals**	**p value**	**Xylitol**	**No tablet**	**Intervals**	**p value**	**Xylitol**	**No tablet**	**Intervals**	**p value**
Xylitol tablet vs. no tablet						0.77 (0.84) – 35	1.47 (0.88) – 30	-0.07 [-1.12, -0.28]					
	Makinen 2005												
									P<0.01				
		Xylitol	**Control**	**Intervals**	**p value**	**Xylitol**	**Control**	**Intervals**	**p value**	**Xylitol**	**Control**	**Intervals**	**p value**
Xylitol tablet vs. control tablet	Makinen 2005					0.77 (0.84) - 35	1.38 (0.89) - 36	-0.61 [-1.01, -0.21]					
									P<0.01				
		Xylitol	**Control**	**Intervals**	**p value**	**Xylitol**	**Control**	**Intervals**	**p value**	**Xylitol**	**Control**	**Intervals**	**p value**
Xylitol candy vs. control candy	Runnel 2013									1.47 (0.11) - 126	1.65 (0.1) - 165	-0.76 [-1.15, -0.37]	
													P<0.01
		Erythritol	**Control**	**Intervals**	**p value**	**Erythritol**	**Control**	**Intervals**	**p value**	**Erythritol**	**Control**	**Intervals**	**p value**
Erythritol tablet vs. control tablet	Makinen 2005					0.62 (0.79) - 36	1.38 (0.89) - 36	-0.76 [-1.15, -0.37]					
									P<0.01				
		Erythritol	**No tablet**	**Intervals**	**p value**	**Erythritol**	**No tablet**	**Intervals**	**p value**	**Erythritol**	**No tablet**	**Intervals**	**p value**
Erythritol tablet vs. no tablet	Makinen 2005					0.62 (0.79) - 36	1.47 (0.88) - 30	-0.85 [-1.26, -0.44]					
									P<0.01				
Erythritol candy vs control candy		**Erythritol**	**Control**	**Intervals**	**p value**	**Erythritol**	**Control**	**Intervals**	**p value**	**Erythritol**	**Control**	**Intervals**	**p value**
	Runnel 2013									1.21 (0.1) – 122	1.65 (0.1) - 126	-0.44 [-0.46, -0.42]	
													P<0.01

Tab. 4 Significative results in ΔDMFS, Δdmfs increment

Type of comparison	**Authors**	**2 years follow-up**				**3 years follow-up**			
Xylitol gum vs. control gum – DMFS		**Xylitol**	**Control**	**Intervals**	**p value**	**Xylitol**	**Control**	**Intervals**	**p value**
	Machiulskiene 2001	5.45 (0.95) – 105	3 (0.9) – 107	2.45 [2.20, 2.70]		8.1 (1.3) – 99	9 (1.6) – 71	-0.90 [-1.35, -0.45]	
					P<0.01				P<0.01
Xylitol gum vs. no gum –DMFS		**Xylitol**	**No gum**	**Intervals**	**p value**	**Xylitol**	**No gum**	**Intervals**	**p value**
	Alanen 2000					1.87 (2.55) – 115	4.42 (4.36) – 146	-2.55 [-3.40, -1.70]	
	Machiulskiene 2001	5.5 (1.1) – 107	5.4 (1.3) – 102	0.10 [-0.23, 0.43]		8.1 (1.3) – 99	8.3 (1.6) – 80	-0.20 [-0.63, 0.23]	
	Peng 2004	0.15 (0.42) – 363	0.26 (0.67) – 410	-0.11 [-0.19, -0.03]					
					P=0.01				P<0.01
Sorbitol gum vs. no gum – DMFS		**Sorbitol**	**No gum**	**Intervals**	**p value**	**Sorbitol**	**No gum**	**Intervals**	**p value**
	Beiswager 1998	5.71 (4.72) – 874	6.05 (5.15) – 944	-0.34 [-0.79, 0.11]		8.1 (6.07) – 657	8.63 (6.54) – 746	-0.53 [-1.19, 0.13]	
	Machiulskiene 2001	3 (0.9) – 105	6.7 (1.2) – 102	-3.70 [-3.99, -3.41]		9 (1.6) – 80	12.4 (1.35) - 68	-3.40 [-3.88, -2.92]	
	Szoke 2001	0.814 (0.102) - 269	1.327 (0.105) - 278	-0.51 [-0.53, -0.50]					
					P<0.01				P<0.01
Xylitol candy vs. no candy – DMFS		**Xylitol**	**No candy**	**Intervals**	**p value**	**Xylitol**	**No candy**	**Intervals**	**p value**
	Alanen 2000					2.77 (3.05) – 66	4.42 (4.36) – 146	-1.65 [-2.67, -0.63]	
									P<0.01
Erythritol candy vs. no candy – DMFS + dmfs *		**Erythritol**	**No candy**	**Odds Ratio**	**p value**	**Erythritol**	**No candy**	**Intervals**	**p value**
	Honkala 2014					860 (18763)	1022 (18596)	0.83 [0.75, 0.91]	
									P<0.01

III

Caries Research

Digital Imaging Fiber-optic Transillumination Device versus Radiographic and Clinical Examination in the Detection of Dental Caries

Journal:	*Caries Research*
Manuscript ID	201506020.R1
mstype:	Original Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Lara-Capi, Cynthia; University of Sassari, Surgery, Microsurgery and Medicine Sciences Lingstrom, P.; Institute of Odontology, Department of Cariology Lai, Gianfranco; University of Sassari, Surgery, Microsurgery and Medicine Sciences Cagetti, Maria Grazia; Universty of Milan, Department of Health, CCWHO for Epidemiology and Community Dentistry of Milan Science Cocco, Fabio; University of Sassari, Department of Chemistry and Pharmacy Simark, Charlotte; Institute of Odontology, Department of Cariology Campus, Guglielmo; University of Sassari, Surgery, Microsurgery and Medicine Sciences
Keyword:	Caries detection, DIFOTI, ICDAS, Radiographs

SCHOLARONE™
Manuscripts

Digital Imaging Fiber-optic –Transillumination Device versus Radiographic and Clinical Examination in the Detection of Dental Caries

Cynthia Lara-Capi[a], Peter Lingström[bc], Gianfranco Lai[a], Maria Grazia Cagetti[cd], Fabio Cocco[ace], Charlotte Simark-Mattsson[b] and Guglielmo Campus[ac]

[a]Department of Surgery, Microsurgery and Medical Sciences, School of Dentistry, University of Sassari, Sassari, Italy;

[b]Department of Cariology, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[c]WHO Collaboration Centre for Community Dentistry and Epidemiology, University of Milan, Italy

[d]Department of Helth Science, School of Dentistry, University of Milan, Milan, Italy;

[e]Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy

Running head

Caries detection using DIAGNOcam

Key Words

Caries detection - DIFOTI - ICDAS - Radiographs

Corresponding author

Cynthia Lara Capi

Department of Biomedical Sciences

School of Dentistry, University of Sassari, Italy

Viale San Pietro 43/C, I-07100 Sassari

For Peer Review

Digital Imaging Fiber-optic Transillumination Device versus Radiographic and Clinical Examination in the Detection of Dental Caries

Cynthia Lara-Capi[a], Peter Lingström[bc], Gianfranco Lai[a], Maria Grazia Cagetti[cd], Fabio Cocco[ace], Charlotte Simark-Mattsson [b] and Guglielmo Campus[ac]

[a]Department of Surgery, Microsurgery and Medical Sciences, School of Dentistry, University of Sassari, Sassari, Italy;

[b]Department of Cariology, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[c]WHO Collaboration Centre for Community Dentistry and Epidemiology, University of Milan, Italy

[d]Department of Helth Science, School of Dentistry, University of Milan, Milan, Italy;

[e]Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy

Running head

Caries detection using DIAGNOcam

Key Words

Caries detection - DIFOTI - ICDAS - Radiographs

Corresponding author

Cynthia Lara Capi

Department of Biomedical Sciences

School of Dentistry, University of Sassari, Italy

Viale San Pietro 43/C, I-07100 Sassari

tel +39-324-9030047

email clcapi@uniss.it

Abstract

Aim: To evaluate the reliability of a Digital Imaging Fiber-Optic Transillumination device (DIFOTI) for the detection of caries lesions and compare it with the results of clinical or radiographic examinations. In addition, the reliability of DIFOTI method was evaluated in a group of dental professionals. Methods: 52 selected subjects were included into the study. Two calibrated dentists evaluated premolars and molars using DIFOTI (DIAGNOcam) and a clinical examination (CE) for assessing caries lesions on occlusal surfaces (CAMo), and DIAGNOcam and a radiographic examination (BW) for caries in approximal surfaces (CAMa). Forty-eight trained dental professionals evaluated thirty randomly selected surfaces (EVA1) derived from CAMo/a images analysis. One month later, the same dentists re-evaluated the same images (EVA2). Cohen's Kappa was used to evaluate the grade of accordance while Intra-Class Correlation coefficients (ICC) for the reproducibility for each surface. ***Results***: The number of detected occlusal caries lesions was similar for CAMo and CE (Kappa=0.99). DIAGNOcam identified a higher number of approximal lesions compared to BW (Kappa=0.91). The same number of lesion in dentine (Kappa=1) was identified by the two detection methods, while in enamel a low agreement was found with more lesions detected by CAMa (Kappa=0.24). For EVA1, 87.5% of the participants had high concordance of Cohen's Kappa compared to DIAGNOcam images and an higher concordance in EVA2. The intra-examiner reliability was substantial/almost perfect in 59.4% of the participants. ***Conclusion***: DIAGNOcam images may be useful for early caries detection on approximal surfaces. The device seems easy to decode for professionals without experience.

INTRODUCTION

Caries clinical management is linked to the number of teeth and surfaces affected, as well as the severity (depth) and the activity (progression or new development) of the lesions. Caries detection, including assessment of non-cavitated and cavitated carious lesions, is an important issue in operative dental practice [Pitts, 2004; Piovesan et al., 2013]. Radiographic examination is a highly sensitive method to detect carious lesions on surfaces that can not be inspected visually, such as approximal surfaces. However, limitations in its sensitivity to diagnose early lesions have been reported [Bader et al., 2002]. In addition, the risk related to radiographic exposure needs to be taken into consideration [Ludlow et al., 2008].

There is a need for improvement of the current methods for caries detection. As a complementing aid to visual examination, a Digital Imaging Fiber-Optic Transillumination Device (DIFOTI) was designed with the task to support clinicians in the identification of caries lesions in different stages [Keem and Elbaum, 1997, Schneiderman et al., 1997; Astvaldsdóttir et al., 2012]. Using the specific optical properties of a carious tissue, transillumination of the teeth with DIFOTI amplifies the change in scattering and absorption of light photons and thereby, makes the lesion appear as a dark shadow [Astvaldsdóttir et al., 2012]. DIFOTI was developed to facilitate in real time the detection, localization and quantitative characterization of lesions [Schneiderman et al., 1997]. The major advantage of the method is that it is non-invasive and therefore can be used as frequently as needed, providing an immediate digital image capture that can be stored and compared with previously acquired images [Astvaldsdóttir et al., 2012]. Caries lesion activity may be monitored by quantification of the changes in mineral content of the lesion over time using the comparison of DIFOTI images acquired at different time points. The detection of early lesions is extremely relevant from clinical point of view as implies an uplift caries activity and

the need for additional non-invasive intervention [Keem & Elbaum, 1997; Astvaldsdóttir et al., 2012].

Although it is subjective, the interpretation of the DIFOTI images seems to be relatively easy to learn. In literature, clinical studies that compare the *in situ* depth of carious lesions with DIFOTI *versus* radiographs are quite limited [Bin-Shuwaish et al., 2008]. A recent *in vitro* study used the transillumination device to identify approximal carious lesions and compared the diagnostic accuracy/efficacy of the device with both traditional and digital x-ray examination, finding that DIFOTI identified a higher number of enamel caries by detecting lesions at an earlier stage than radiographs, providing more accurate results. In contrast, radiographs showed a better sensitivity in deeper lesions, this is, DIFOTI identified a higher number of incorrect dentin lesions. Radiography is able to identify great change in lesion depth although small changes in the mineral content are not detectable. Moreover, DIFOTI and film radiography showed a high intra-examiner concordance [Astvaldsdóttir et al., 2012]. The International Caries Detection and Assessment System (ICDAS) is a more a visual scoring systems than tactile, developed to assess the caries lesions at both initial and manifest thresholds [International Caries Detection and Assessment System Coordinating Committee, 2005; Honkala et al., 2011].

Meticulous and reliable data collection is vital for success in all fields of research [Lesaffre et al, 2004]. The training of the examiners is fundamental, and it can be defined, according to the Guidance on the Statistical Aspects of Training and Calibration of Examiners for Surveys of Child Dental Health by British Association for the Study of Community Dentistry (BASCD) [Pine et al, 1997; Assaf et al., 2006; Agustsdottir et al., 2010], as teaching the agreed interpretation of the diagnostic criteria.

The main aim of this study was to evaluate the effectiveness and reliability of the DIAGNOcam. The null-hypothesis was that the reliability of a DIFOTI device (KaVo

DIAGNOcam 2170) for the detection of caries lesions did not differ from that obtained through the clinical or radiographic examinations. To validate this hypothesis, an observational study was designed and evaluated as well in a group of dental professionals. In the first part of the study, the DIAGNOcam was compared with a clinical examination appraising the occlusal surfaces and with x-ray bitewings assessing the approximal surfaces. In the second part, the reproducibility of image evaluation using DIAGNOcam was determined in a group of dental professionals.

Materials and Methods

The study was approved by the Ethical Committee at the University of Sassari (authorization number 389/2013) and it was conducted over 6 weeks from June 9th to July 15th 2014.

Study design

The study was designed in two parts: the first was a comparison among three detection methods (DIFOTI, bitewing radiographs and clinical examination), the second was a reliability study among dental professionals using the DIFOTI imagines derived from the first part.

Comparison among three detection methods

The new KaVo DIAGNOcam 2170 is a camera system that reads the tooth's structure to verify occlusal, approximal and secondary caries lesions when the tooth is transilluminated. A digital video camera records the image and displays it on a computer screen.

For the radiographic examination, Planmeca intraoral radiographic equipment (Planmeca, Helsinki, Finland) and Kodak UltraSpeed DF42 films, with settings of 70 kV and 7 mA and an exposure time of 0.25 s, were used for bitewing radiographs. The radiographs were manually developed via conventional standard conditions and standard processing times, and examined according to O'Mullane criteria [O'Mullane et al., 1997].

The clinical examinations were performed under standard conditions. The subjects were seated in a dental unit and the teeth were examined using a plan mirror (Hahnenkratt, Königsbach, Germany) and the WHO CPITN ballpoint probe (Asa-Dental, Milan, Italy) under optimal light.

Calibration of the examiners

Calibration exercises for all the three methods (DIAGNOcam unit visual, clinical caries diagnostic system (ICDAS) and radiographic examination) were carried out by two dentists before the start of the study. One of the authors (GCampus) acted as benchmark, training and calibrating the two examiners. The calibration process was divided for each diagnostic method in four steps:

- lectures regarding the disease and the method (*i.e.* DIAGNOcam, ICDAS, x-ray) for eight hours;
- first examination, no discussion was allowed between the examiners and the dental advisors as to the interpretation of the criteria during the calibration sessions;
- re-evaluation by the examiners after 72 hours (clinical examination) and one week (DIAGNOcam and x-ray)
- evaluation of the agreement or disagreement and statistical analysis.

Fifty volunteers were clinically examined for presence of caries lesions in a dental chair using the ICDAS criteria and re-examined after 72 hours. Intra- and inter-examiner reliability was calculated through percent agreement and Cohen's Kappa statistics. Good inter-examiner reliability was found with no significant difference from benchmark values ($p=0.15$) and a low mean square of error (0.47). The Pearson's correlation coefficient between the two examiners was high ($r = 0.83$, $p < 0.01$, $R^2 = 0.71$). Intra-examiner reliability was also high, Cohen's $K=0.88$. !

147! Forty extracted human teeth (10 premolars and 30 molars), in total 80 approximal and 40
148! occlusal surfaces, were selected for the calibration of the DIFOTI device and the radiographic
149! examination. The teeth were selected from a pool of extracted teeth from the Department of
150! Oral Surgery at the University of Sassari. The teeth were cleaned, any remaining soft tissues
151! and calculus were removed, and they were subsequently frozen at -20° until used. Selection
152! criteria match the line of the first evaluation.! Evaluations were carried out at one-week
153! interval; Kappa values for inter- and intra-examiner agreement were high for both methods
154! (0.79 for DIFOTI and 0.83 for x-ray). The Pearson's correlation coefficient for the two
155! examiners was high ($r = 0.84$, $p < 0.01$, $R^2 = 0.74$). The clinical examiner did not have the
156! opportunity to look at DIAGNOcam (CAMo/a) or BW images for the entire period.!

157! *Study population*

158! The study population consisted of students of the School of Medicine of the University of
159! Sassari, Italy. To be suitable for enrolment, subjects had to meet these inclusion criteria: no
160! missing teeth, no secondary caries and no fillings in premolars or molars. The exclusion
161! criteria were subjects wearing fixed orthodontic appliances and subjects unable to be
162! exposed to x-rays for medical/specific reasons. All students (n=1145) attending the School of
163! Medicine were invited to participate via email/leaflet where the aim of the study was
164! described in detail. A total of 678 students accepted and were examined (59.2% acceptance
165! rate) and 52 subjects (19-23 years, mean age 21.2±1.2) fulfilled the inclusion/exclusion
166! criteria.

167! Power analysis (G*Power 3 software) was performed to establish the number of subjects
168! needed to evaluate the estimated difference in caries diagnosis using DIFOTI and/or clinical
169! evaluation and x-ray. Data [Virajsilp et al., 2005] related to the reliability of two diagnostic
170! methods were used to calculate the sample size, even if data used were on primary teeth.
171! The standardized effect was set at 0.39 with a sample size of 48 subjects and an upper 95%

172! one- sided confidence limit of 0.52. All subjects (n=52) that fulfilled the inclusion/exclusion
173! criteria were enrolled. Each subject was codified with a number in order to protect his/her
174! identity. The flow chart of the study is displayed in Figure 1.

175! The DIFOTI device was used to assess caries lesions on occlusal surfaces (CAMo) and on
176! approximal surfaces (CAMa). In addition, a clinical examination of the occlusal surfaces (CE)
177! and a radiographic examination (BW) for approximal surfaces were performed.

178! Each tooth were cleaned for 30 seconds with a prophylaxis paste (Clinpro™ Prophy Paste:
179! 3M ESPE Dental Products, USA) and then rinsed by a water spray for 10 seconds. The
180! clinical examination was performed under standardized conditions describe above after
181! drying teeth for 5 seconds. The students were examined and analysed during the same day
182! by both examiners, first attending the clinical and radiographic examination and afterwards
183! they were asked to go to another room where the DIFOTI device was installed with a
184! computer in a dental chair. The International Caries Detection and Assessment System
185! (ICDAS) was recorded for both enamel and dentinal lesions [International Caries Detection
186! and Assessment System Coordinating Committee, 2005; Ismail et al., 2007; Honkala et al.,
187! 2011]. The radiographs were taken using an 8-inchround cone that was placed in contact
188! with the ring of the film-holding system (RINN XCP, Dentsply, York), which in turn was placed
189! in contact with the patient's cheek during exposure. Not perfectly clear or overlapping images
190! were taken a second time. Then the DIFOTI device was used according to the
191! manufacturer's instructions, placing the mouthpiece over the occlusal surfaces. The image
192! appeared in real time on the computer monitor, and the examiner saved it in the electronic
193! patient record. !

194! The DIAGNOcam was used for the detection of occlusal and approximal caries at enamel or
195! dentine. When a defined approximal shadow in the enamel was present, it was scored as 1
196! and when reaching into the dentine it was scored as 2. Due to the impossibility to measure

197! the lesion vertically all dark occlusal areas were scored as 1. The ICDAS scores were
198! performed on the occlusal surface. Radiographs were examined according to O'Mullane
199! criteria [O'Mullane et al., 1997] and mesial and distal surfaces were assessed.

200! *Reliability among dental professionals using DIFOTI*

201! Forty-eight Italian dental professionals with no experience of the DIFOTI device were asked
202! to participate in the second part of the study. Their professional experience was at least 7
203! years. On the day of the study they underwent at 60-minute training session!describing the
204! DIFOTI technology and the DIAGNOcam by one of the authors (CLC). Immediately after the
205! training session, each participant had to diagnose ten teeth images randomly obtained from
206! the first part of the study, analysing 10 occlusal, 10 mesial and 10 distal surfaces.
207! Participants were asked to fill in a form containing two possible answers (1 - presence of
208! caries, 2 - absence of caries) (EVA1). One month later, participants were contacted via email
209! and were asked to revaluate the same images with the same criteria (EVA2). These results
210! were compared with their previous answers.

211! *Statistical Analysis*

212! All data were analysed using STATA 13. For all analysis a p-value <0.05 was considered
213! statistically significant. The general grade of accordance between the different detection
214! methods was evaluated using the Cohen's Kappa [Cohen, 1960], while the reproducibility for
215! the two methods for each surface (occlusal or approximal) was assessed using Intra-Class
216! Correlation coefficients (ICC). ICC values equal to 0 represent agreement equivalent to that
217! expected by chance, while 1 represents full agreement.

218! The inter-examiner DIFOTI reliability among dental professionals compared to the results
219! derived from DIAGNOcam analysis was evaluated categorizing the kappa value of each
220! professional respect to DIAGNOcam following the criteria described by Landis and Koch

221! [1977], who characterized values <0 as indicating no concordance and 0-0.20 as slight, 0.21-
222! 0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1 as almost perfect
223! concordance. The method by Bland and Altman [1986] was used to display the variability of
224! the two examinations (EVA1 and EVA2) by each examiner and the plot of EVA1 respect to
225! the DIAGNOcam results, the plot of EVA2 respect to DIAGNOcam and the comparison
226! between EVA1 and EVA2. This method allows to investigate the existence of any systematic
227! difference between the measurements and to identify possible outliers.

228! **Results**

229! *Comparison among the three detection methods*

230! A total of 2496 surfaces (832 mesial, occlusal and distal, respectively) were analysed. The
231! occlusal surfaces were analysed using DIAGNOcam (CAMo) and Clinical Examination (CE),
232! while the approximal surfaces were analysed with DIAGNOcam (CAMa) and Bite-Wing
233! radiographs (BW). The total number of occlusal caries lesions detected was similar, 149
234! using CAMo and 152 with CE with a Cohen's Kappa of 0.99. The ICC for the occlusal, mesial
235! and distal surfaces of each tooth is reported in Figure 2. The mean ICC for the occlusal
236! surface was 0.93 with a lowest value for maxillary right second molar (ICC=0.78), while a
237! perfect agreement (ICC=1) was observed for several premolars. Approximal caries identified
238! using CAMa were 83 and 70 using BW (Cohen's Kappa of 0.91). CAMa and BW identified
239! the same number (31) of caries in dentine. The Cohen's Kappa was 0.24 for enamel lesions
240! with a low agreement, while a complete concordance (Kappa=1) was observed for dentinal
241! lesions (Table 1). The mean ICC for approximal surfaces was 0.97 for the distal and 0.95 for
242! the mesial surfaces (Figure 2). Regarding enamel lesions, 17 lesions in molars were detected
243! with CAMa, while 16 with the BW method (Cohen's kappa=0.97); 35 lesions were detected in
244! premolars with CAMa respect to 23 with BW (Cohen's kappa=0.21). Twenty-nine decayed
245! mesial surfaces were registered with CAMa respect to 23 with BW (Cohen's kappa=0.39).

For the distal surfaces, 23 lesions were recorded with CAMa and 16 with BW (Cohen's kappa=0.34). A complete concordance was observed for dentinal lesions between the two methods.

Reliability among dental professionals using DIFOTI

Forty-eight dental professionals participated in the first evaluation (EVA1) and thirty-two (drop out rate 33.3%) in the second evaluation (EVA2). The Cohen's Kappa of each subject regarding the reliability between the two evaluations was categorized following the scale proposed by Landis and Koch [1977] (Table 3). Regarding inter-examiner reliability, in EVA1 the majority of the examiners (87.5%) had either a substantial (46.9%) or an almost perfect concordance (40.6%) compared to DIAGNOcam results, while in EVA2 a higher percentage had a substantial concordance (75.00%) and a lower percentage an almost perfect (18.8%), with a shift towards substantial concordance grade. Nineteen examiners (59.4%) showed a substantial/almost perfect agreement, while 13 examiners (40.6%) a fair/moderate agreement (Figure 3). The Bland-Altman plot showed a good intra-examiner (Figure 3a) and a higher over-rating of the number of the lesions in EVA2 (Figure 3c).

Discussion

The main findings of this study are that the DIFOTI device (DIAGNOcam) proved to be consistent to clinical examination for the detection of lesions on the occlusal surface and to bite-wing x-ray for dentinal lesions on approximal surfaces; a higher number of enamel lesions was detected by DIAGNOcam compared with x-rays, especially in premolars. In the calibration process, no statistically significant differences were observed between benchmark and examiners and no systematic bias between examiners' scores was noted. The level of concordance among dental professionals, with respect to the DIAGNOcam analysis result derived from the first part of the study, was really high in both examinations (EVA1/EVA2).

The intra-examiner reliability of the dental professionals was quite good even if in 40% of the examiners the level of agreement was moderate or less.

The DIFOTI device used in this study, KaVo DIAGNOcam 2170, is a non-invasive real-time recording tool that was developed for regular practice use with no exposure of ionizing radiations to the patient. The device was designed to be useful to identify lesions at the initial caries stage and the technique allows for more frequent re-evaluations of these diagnoses than what is feasible using radiographs [American Dental Association, 2012]. The DIFOTI method has been shown to be more sensitive than radiography to detect early changes in enamel [Young and Featherstone, 2005; Bin-Shuwaish et al., 2008; Astvaldsdottir et al., 2012]. Overall, the use of DIFOTI along with radiographic analysis is able to improve the diagnostic accuracy and to identify early approximal lesions with respect to presence and size [Bin-Shuwaish et al., 2008]. The outcome of this paper confirms these findings. The DIAGNOcam identified a higher number of approximal lesions in enamel than the radiographic technique, allowing an earlier detection of incipient lesions than traditional detection methods. Proper and early caries detection is crucial for optimal treatment decisions, helping the clinician to choose between a restorative treatment or chemically remineralisation, as that occurring in the early stages of caries development [Young and Featherstone, 2005].

Intraoral radiographs are, in addition to clinical evaluation, considered the first choice for caries detection. Nevertheless, radiographs are unable to detect initial demineralization of the tooth resulting in low sensitivity, since 40 to 60% of tooth decalcification is needed to produce a radiographic imaging of caries resulting in false-negative test [Machlulskiene et al., 1999; Chong et al., 2003; Yang et al., 2005]. Conversely, the use of the DIFOTI method might lead to an over-detection as the device has a lower specificity compared with radiographs [Young, 2002].In this *in vivo* trial the DIAGNOcam findings were compared to radiographs,

happened unconsciously leading to a higher inter-examiner concordance. A further weak point might be ascribed to study design: EVA2 was designed to be carried out one month later than EVA1, and this might have affected the results; otherwise the results of EVA2 still showed a good concordance with EVA1 and the DIAGNOcam results derived from the first part of the study.

The results of this paper suggest that the DIAGNOcam can be helpful in everyday clinical practice. However, the cost effectiveness of the DIFOTI method in community dentistry may be seen as a concern as at least 15 minutes is required to examine the whole dentition. Therefore, the DIFOTI technique may be particularly useful for early detection and monitoring of the progression of dental caries at individual sites.

Conclusion

DIAGNOcam might be a useful device for early caries detection, especially for early non-cavitated lesions on approximal surfaces. The DIFOTI images are quite easy to decode even for professionals without any experience of the use of the method. More information is needed to standardize the scoring of the lesions to help the clinicians to give an accurate interpretation of the images.

Authors' contributions:

Cynthia Lara-Capi: participated in the study design, performed the clinical examination and data collection;

Peter Lingström: design of the study, final revision of the paper;
Gianfranco Lai: participated in the study design and data collection;
Maria Grazia Cagetti: participated in the study design and drafting of the manuscript;
Fabio Cocco: participated in the study design, data and statistical analysis;

344! Charlotte Simark: participated in the study design and revision of the manuscript;

345! Guglielmo Campus: participated in the study design and drafting of the manuscript.

346!

347 **References**

348 Agustsdottir H, Gudmundsdottir H, Eggertsson H, Jonsson SH, Gudlaugsson JO,
349 Saemundsson SR, Eliasson ST, Arnadottir IB, Holbrook WP: Caries prevalence of permanent
350 teeth: a national survey of children in Iceland using ICDAS. Community Dent Oral Epidemiol
351 2010,38:299-309.

352 American Dental Association: Dental radiographic examinations: recommendations for
353 patient selection and limiting radiation exposure. 2012 Available at: http://
354 www.ada.org/~/media/.../Dental_Radiographic_Examinations_2012.ashx(accessed June 23,
355 2015).

356 Assaf AV, de Castro MM, Zanin L, Tengan C, Pereira AC: Effect of different diagnostic
357 thresholds on dental caries calibration - a 12 month evaluation. Community Dent Oral
358 Epidemiol 2006, 34:213-219.

359 Astvaldsdóttir A, Ahlund K, Holbrook WP, de Verdier B, Tranæus S: Approximal caries
360 detection by DIFOTI: In vitro comparison of diagnostic accuracy/efficacy with film and digital
361 radiography. Int J Dent 2012:326401.

362 Bader JD, Shugars DA, Bonito AJ: A systematic review of the performance of methods for
363 identifying carious lesions. J Public Health Dent 2002;62:201-213.

364 Bin-Shuwaish M, Yaman P, Dennison J, Neiva G: The correlation of DIFOTI to clinical and
365 radiographic images in Class II carious lesions. J Am Dent Assoc 2008;139:1374-1381.

366 Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of
367 clinical measurement. Lancet 1986;1:307-310.

368 Chong MJ, Seow WK, Purdie DM, Cheng E, Wan V: Visual-tactile examination compared
369 with conventional radiography, digital radiography, and Diagnodent in the diagnosis of
370 occlusal occult caries in extracted premolars. Pediatr Dent 2003;25:341-349.

371 Chu CH, Chung BT, Lo EC: Caries assessment by clinical examination with or without
372 radiographs of young Chinese adults. Int Dent J 2008;58:265-268.

373 Cohen J: A coefficient of agreement for nominal scales. Educ Psychol Meas 1960;20:37–46.

374 Honkala E, Runnel R, Honkala S, Olak J, Vahlberg T, Saag M, Mäkinen KK: Measuring
375 Dental Caries in the Mixed Dentition by ICDAS. Int J Dent 2011;2011:150424.

376 International Caries Detection and Assessment System Coordinating Committee: Criteria
377 Manual. International Caries Detection and Assessment System (ICDAS II) Workshop.
378 Baltimore, Md, USA, March 2005.

379 Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, Pitts NB: The International Caries
380 Detection and Assessment System (ICDAS): an integrated system for measuring dental
381 caries. Community Dent Oral Epidemiol 2007;35:170-178.

382 Keem S, Elbaum M: Wavelet representations for monitoring changes in teeth imaged with
383 digital imaging fiber-optic transillumination. IEEE Trans Med Imaging 1997;16:653-663.

384 Landis JR, Koch GG: The measurement of observer agreement for categorical data.
385 Biometrics 1997;33:159-174.

386 Ludlow JB, Davies-Ludlow LE, White SC: Patient risk related to common dental radiographic
387 examinations: the impact of 2007 International Commission on Radiological Protection
388 recommendations regarding dose calculation. J Am Dent Assoc 2008;139:1237-1243.

389 Machiulskiene V, Nyvad B, Baelum V: A comparison of clinical and radiographic caries
390 diagnoses in posterior teeth of 12-year-old Lithuanian children. Caries Res 1999;33:340-348.

O'Mullane DM, Kavanagh D, Ellwood RP, Chesters RK, Schafer F, Huntington E, Jones PR: A three-year clinical trial of a combination of trimetaphosphate and sodium fluoride in silica tooth-pastes. J Dent Res 1997;76:1776-1781.

Petersen PE: The World Oral Health Report 2003: continuous improvement of oral health in the 21st century—the approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol 2003;31(1 suppl):3-24.

Piovesan C, Moro BL, Lara JS, Ardenghi TM, Guedes RS, Haddad AE, Braga MM, Mendes FM: Laboratorial training of examiners for using a visual caries detection system in epidemiological surveys. BMC Oral Health 2013;13:49.

Pitts, NB: Are we ready to move from operative to nonoperative/preventive treatment of dental caries in clinical practice? Caries Res. 2004;38:294-304.

Schneiderman A, Elbaum M, Shultz T, Keem S, Greenebaum M, Driller J: Assessment of dental caries with Digital Imaging Fiber-Optic TransIllumination (DIFOTI): in vitro study. Caries Res 1997;31:103-110.

Virajsilp V, Thearmontree A, Aryatawong S, Paiboonwarachat D: Comparison of proximal caries detection in primary teeth between laser fluorescence and bitewing radiography. Pediatr Dent 2005;27:493-99.

Yang J, Dutra V: Utility of radiology, laser fluorescence, and transillumination. Dent Clin North Am 2005;49:739-752.

Young DA, Featherstone JD: Digital imaging fiber-optic trans-illumination, F-speed radiographic film and depth of approximal lesions. J Am Dent Assoc 2005;136:1682-1687.

Young DA: New caries detection technologies and modern caries management: merging the strategies. Gen Dent 2002;50:320-331.

414! **Figure legends**

415! Figure 1. Flow chart of the study design.

416! Figure 2. Comparison among the three detection methods. Intraclass Coefficient Correlation
417! between the DIAGNOcam and Clinical Evaluation for the occlusal surfaces (o) and between
418! DIAGNOcam and Bite-wing for the approximal surfaces; mesial (m) and distal (d) are
419! reported.

420! Figure 3. Reliability among dental professionals using DIAGNOcam. Intra-examiner reliability
421! using Bland-Altman plot of difference. Each small dot is the average value of one single
422! examiner observation, larger dots are the sum of two or more examiners. Shaded region
423! indicates 95% limits of agreement around the dashed line representing the mean.

Table 2. Comparison among the two detection methods, the DIAGNOcam readings and the Bitewing examination. Distribution of carious lesions in enamel and in dentine by type of tooth and surfaces is shown; Coehn's Kappa was calculated !

!

Lesions'for' teeth/surfaces!	Enamel' DIAGNOcam"" (CAMa)!n=52! n'(%)""	Bitewing" (BW)!n=39! n'(%)!	Cohen'skappa!! value'(SE)'95%'CI!	Dentine' DIAGNOcam" (CAMa)!n=31! n'(%)!	Bitewing" (BW)!n=31! n'(%)!	Cohen's!kappa!! value'(SE)'95%'CI!
Molars!	17!(32.7)!	16!(41.03)!	0.97!(0.03)!0.91<1.00!	13!(41.94)!	13!(41.94)!	1!
Premolars!	35!(67.3)!etc!	23!(58.97)!	0.21!(0.08)!0.05<0.36!	18!(58.06)!	18!(58.06)!	1!
Mesial!	29!(55.77)!	23!(58.97)!	0.39!(0.08)!0.23<0.56!	11!(35.48)!	11!(35.48)!	1!
Distal!!	23!(44.23)!	16!(41.03)!	0.34!(0.10)!0.15<0.54!	20!(64.52)!	20!(64.52)!	1!

!

!

Table 3. Reliability among dental professionals using the DIFOTI technique. Inter and Intra-examiner reliability categorized following the scale of the concordance degree proposed by Landis and Koch [1977] after two examinations (EVA1 and EVA2). n = 33

	Fair concordance *n (%)*	**Moderate concordance** *n (%)*	**Substantial concordance** *n (%)*	**Almost perfect concordance** *n (%)*
EVA 1	--	4 (12.50)	15 (46.87)	13 (40.63)
EVA 2	--	2 (6.25)	24 (75.00)	6 (18.75)
				χ^2=10.96 p<0.01
Drop-out after EVA1 *n=16*	--	4 (25.00)	6 (37.50)	6 (37.50)
Intra-examiners reliability EVA1/EVA 2	4 (12.50)	9 (28.12)	10 (31.25)	9 (28.13)

Gianfranco Lai
Prevention, diagnosis and minimally invasive treatment of dental caries.
Tesi di Dottorato in Odontostomatologia Preventiva, Università degli Studi di Sassari

III

Acta Odontologica Scandinavica. 2015; Early Online, 1–12

informa
healthcare

REVIEW ARTICLE

Comparison of Carisolv system vs traditional rotating instruments for caries removal in the primary dentition: A systematic review and meta-analysis

GIANFRANCO LAI[1], CYNTHIA LARA CAPI[1], FABIO COCCO[1,2,3], MARIA GRAZIA CAGETTI[2], PETER LINGSTRÖM[2,4], ULRICA ALMHÖJD[4] & GUGLIELMO CAMPUS[1,2]

[1]Department of Surgery, Microsurgery and Medical Sciences, School of Dentistry, University of Sassari, Sassari, Italy, [2]WHO Collaborating Centre for Epidemiology and Community Dentistry University of Milan, Milan, Italy, [3]Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy, and [4]Department of Cariology, Institute of Odontology, Sahlgrenska, Academy, University of Gothenburg, Gothenburg, Sweden

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

Abstract

Objective. The purpose of this systematic review and meta-analysis was to evaluate the reliability of the Carisolv system with respect to drilling regarding the full removal of decayed hard tissues in primary dentition. A systematic review of the literature was conducted to identify controlled trails, randomized controlled trials and clinical trials that compared the Carisolv system to the traditional mechanical caries removal in the primary dentition. Materials and methods. The main relevant databases were searched: MEDLINE via PUBMED, Web of Science and SCOPUS. Complete caries removal, length of working time and need of local anesthesia were the outcomes evaluated. Results. A total of 195 studies were identified and complete analysis of 28 studies was performed; finally, 10 papers were included. The trials included involved a total of 348 patients for 532 treated teeth. There was no significant difference in terms of clinical efficacy between the Carisolv and the rotary instrument ($z = 0.68$, $p = 0.50$), whereas the treatment with Carisolv was significantly longer in terms of time with respect to the rotary instruments ($z = 10.49$, $p < 0.01$). The chemo mechanical technique reduces the need for local anesthesia, with a difference between two types of treatment near to statistical significance ($z = 1.91$ $p = 0.06$). Conclusions. This systematic review indicates that the clinical efficacy of chemo-mechanical removal with Carisolv seems as reliable as the rotary instruments. However, the results should be interpreted cautiously due to the heterogeneity among study designs and to the shortage of available data. Further large-scale, well-designed randomized controlled trials are needed.

Key Words: carisdv, chemo mechanical caries removal, dental caries, primary dentition, rotating instruments

Introduction

According to the World Health Organization [1], dental caries is defined as a localized, post-eruptive, pathological process of external origin, involving softening of the hard dental tissues and proceeding to the formation of a cavitation. Dental caries is one of the most commonly occurring diseases worldwide and its treatment has considerable implications in term of economic resources and biological costs [2].

In the past, carious lesions operative treatment was related to the knowledge of the disease pattern and the restorative materials that were available at that time [3]. In the late nineteenth century, the principle of 'extension for prevention' was proposed: cavity preparation required the loss of sound tissue, extending to anatomical sites that might otherwise encourage plaque accumulation [4]. Due to a deeper knowledge of the caries evolving processes and the coming of adhesive restorative materials, the approach to the disease switched from the 'broaden to prevent' era to 'minimally invasive dentistry' [5]. Modern restorative dentistry offers alternatives to the traditional tissue removal using drilling instruments: a possible alternative is the chemo mechanical removal.

Correspondence: Professor Guglielmo Campus, Department of Surgery, Microsurgery and Medicine Sciences, School of Dentistry, University of Sassari, Viale San Pietro 43/C, I-07100 Sassari, Italy. Tel: +39 079 228540, +39 079 228508, +39 347 3394544. Fax: +39 079 228541. E-mail: gcampus@uniss.it

(Received 17 December 2014; accepted 23 February 2015)

ISSN 0001-6357 print/ISSN 1502-3850 online © 2015 Informa Healthcare
DOI: 10.3109/00016357.2015.1023353

RIGHTSLINK

In 1976, the possibility of removing decayed material chemically, using N-monochloroglycine, was reported [6]. A few years later, the Caridex system was introduced as the first chemo mechanical method for caries removal [7]. The chemo mechanical method allows for saving of healthy tissue, providing, at the same time, the patient's comfort [8].

In 1998, a gel-based system used with specially designed non-cutting hand instruments was developed, its name is Carisolv©. This product selectively removes infected carious dentine. When the gel of three amino acids (lysine, leucine and glutamic acid); 53mM and the gel containing 0.27M hypochlorite are mixed, amino acids bind chlorine and form chloramines at a pH of 11. This chlorination affects the secondary and/or quaternary structure of the collagen, by disrupting hydrogen bonding and, thus, brings about proteolytic reaction. It does not affect healthy dentine because amino acids act as homing devices for active chlorine. The chlorine atom of hypochlorite is transferred to the amino group of each amino acid and in this way it is made less reactive and less aggressive to healthy tissue [9]. The chemo mechanical method of caries removal is considered useful, especially in pediatric dental practice, reducing the noise, vibration and pain produced by the use of high- and low-speed rotary instrument [10]. Other products for the chemo mechanical caries removal based on papaya plant extract (Papacarie and Carie-care) were launched on the dental market, but scarce scientific data are present on these products [11–13].

Few in vivo studies evaluate Carisolv© efficacy: the results seem to support the reliability of the chemo mechanical caries removal [9,10,14–18].

Therefore, this study aimed at systematically evaluating the current literature by means of a meta-analysis. The primary outcome variable of interest was the clinical efficacy in primary caries removal and secondary parameters were the clinical efficiency (treatment time) and patient's comfort (need of local anesthesia).

Materials and methods

This systematic review was performed following the guidelines of the Transparent Reporting of Systematic Reviews and Meta-Analyses (PRISMA) [19].

Focused PICO question

In primary dentition, what is the efficacy of Carisolv in caries removal rate (clinically appreciated) compared to the traditional drill technique, the clinical efficiency (treatment time) and patient's comfort (need of anesthesia)?

Eligibility criteria

The studies included in the present review are Clinical Trials, Randomized Clinical Trials and Controlled Trials assessing the efficacy on the primary dentition of Carisolv compared to traditional mechanical caries removal (control) with drilling instruments. Only studies where total caries removal in each group was completed using Carisolv systems or rotary instruments used without any time limit were considered eligible. The studies including other experimental groups in addition to Carisolv and drilling were also included in this review. Studies assessing the complete caries removal different from clinical criteria (i.e. using a sharp probe) were excluded.

Search strategy

For the identification of studies to evaluate for this review, a unique search strategy to be applied for each database research was developed (Figure 1). The following key words were used: Carisolv and Chemo mechanical Caries Removal. No Mesh term match was found. The terms were searched following the Boolean term 'OR' for a total of three inquiries.

Database research:

- MEDLINE via PUBMED (from 1948 to December 2014);
- Web of Science (from 1948 to December 2014); and
- SCOPUS (from 1969 to December 2014).

A comparison of the different searches was carried out to delete the repeated studies. Then, two authors (GL and CLC), on charge to evaluate the eligibility of the studies, examined independently all abstracts of the selected papers. If an abstract didn't supply enough information to determine if the paper met the inclusion criteria, the full report was obtained. All studies which appeared to meet the inclusion criteria were obtained in the full text format. The two authors assessed the papers independently, to establish whether or not the studies met the inclusion criteria. Disagreements were resolved by discussion. If not possible, other authors were consulted.

Data analysis

The outcomes considered in the studies were: the caries removal rate clinically appreciated (binary yes/no), the time required to complete the tissue removal (continuous) and the pain threshold during the procedure, assessed through the need for local anesthesia by patients (binary yes/no). When raw data was not available in the text, tables or graphs, single authors were contacted to obtain such information. To compare dichotomous data, a calculation of the Odd Ratio

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

RIGHTSLINK

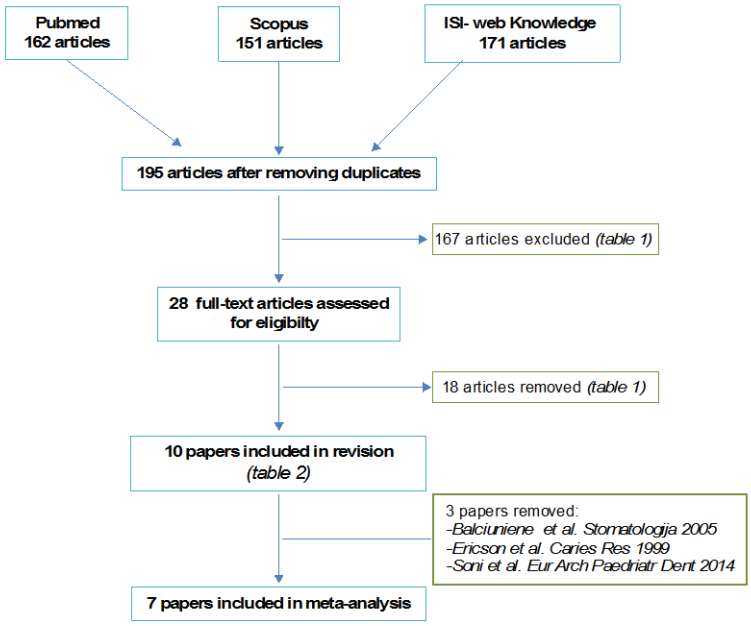


Figure 1. Flowchart of search strategy.

(OR) along with 95% Confidence Intervals (CIs) was used, whereas, for continuous data, the Mean Difference (MD) with 99% Confidence Intervals (CIs) was calculated. Also, for each comparison the Z-test was used. A random-effect model was applied to reassess all data extracted from the included studies.

Analysis was performed using Review Manager 5.3 software provided by the Cochrane Collaboration [20].

Results

A total of 195 studies published from 1999–2014 were identified and assessed (Table I). Twenty-eight papers were analyzed and 10 studies met the eligibility criteria (Table II).

The trials included in the review involved a total of 348 patients and 532 treated teeth. In three studies [14,16,21] it was not possible to extract the number of patients treated and so these studies were excluded.

From the selected studies, two were conducted in India [9,21], one in Venezuela [18], two in Greece [10,22], one in Lithuania [16], one in Serbia [15], one in Sweden [14], one in the US [23] and one in both Denmark and Portugal [17]. Two of the papers reported data from multi-center (Sweden; Denmark and Portugal) studies.

One of the studies had a crossover design [18], three were split mouth [16,17,22] and six had a parallel group design [9,10,14,15,21,23].

Most studies compared the Carisolv system [7] with the conventional rotary drill excavation for caries removal, but in three papers four different methods were reported [9,14,21].

In two studies no details about the operator and co-investigator were reported [9,21], in another two studies there was Testo one operator and one co-investigator [15,16], while in another one there was one operator and two co-investigators [18], in another one [10] two operators but no co-investigator and, finally, in two studies there was only one operator [21,23]. In one of the two multi-center studies there was one operator and one co-investigator for each center [14,17].

Six of the trials included only primary teeth [9,10,17,18,22,23] with participants' ages ranging from 28 months to 11 years. Four trials were carried out on permanent teeth also [14–16,21] and the ages of the subjects ranged from 30 months to 85 years.

In five trials [10,14–17] the teeth involved in the studies were molars and anterior primary teeth; in four studies [9,18,22,23] only primary molars with occlusal caries were treated; while in one study [21] primary molars were treated, but it was not mentioned which surfaces were treated.

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

RIGHTSLINK

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

Table I. List of excluded studies

Authors	Year	Journal	Reason for exclusion	Authors	Year	Journal	Reason for exclusion
Ammari et al.	2014	Braz Oral Res	Microbiological study	Amaral et al.	2011	Am J Dent	In vitro study
Boob et al.	2014	Int J Clin Pediatr Dent	In vitro study	Chang et al.	2011	Journal of Southern Medical University	Chinese language
Bussadori et al.	2014	Oral Health Prev Dent	In vitro study	Gugnani et al.	2011	J Conserv Dent	No group with Carisolv
Bussadori et al.	2014	J Contemp Dent Pract	No group with Carisolv	Imbronito et al.	2011	Int J Periodontics Restorative Dent	In vitro study
Garcia-Contreras et al.	2014	In vivo	In vitro study	Kochhar et al.	2011	J Clin Pediatr Dent	Evaluation with caries detector
Geetha Priya et al.	2014	J Indian Soc Pedod Prev Dent	No clinical evaluation	Li et al.	2011	J Dent	In vitro study
Gil-Montoya et al.	2014	Clin Oral Investig	Only permanent teeth	Neves Ade et al.	2011	J Dent	In vitro study
Hamama et al.	2014	Aust Dent J	Review	Neves Ade et al.	2011	Dent Mater	In vitro study
Hamama et al.	2014	J Endod	In vitro study	Neves Ade et al.	2011	J Dent	In vitro study
Jingarwar et al.	2014	J Clin Diagn Res	Review	Shadzendedar et al.	2011	J Contemp Dent Pract	In vitro study
Li et al.	2014	J Oral Rehabil	Review	Zawaideh et al.	2011	Pediatr Dent	In vitro study
Motta et al.	2014	J Appl Oral Sci	No group with Carisolv	Banerjee et al.	2010	J Dent	In vitro study
Pradeep Kumar	2014	Int J Pharma Bio Sci	No group with Carisolv	Gianini et al.	2010	Am J Dent	In vitro study
Schwendicke et al.	2014	J Dent	Review	Taenova et al.	2010	Folia Med	In vitro study
Aggarwal et al.	2013	Aust Dent J	In vitro study	Yamada et al.	2010	J Clin Pediatr Dent	In vitro study
Banerjee	2013	Br Dent J	Review	Allaker et al.	2009	Int J Antimicrob Agents	Review
Bijle et al.	2013	J Contemp Dent Pract	Statistical survey	Bertassoni et al.	2009	Scanning	In vitro study
Cecchin et al.	2013	Braz J Oral Sci	In vitro study	Fure et al.	2009	Oral Health Prev Dent	No control group
Goomer et al.	2013	J Int Oral Health	Clinical eval caries detector	Kato et al.	2009	J Clin Pediatr Dent	No group with Carisolv
Gupta et al.	2013	J Clin Pediatr Dent	No group with Carisolv	Martins et al.	2009	J Dent Child (Chic)	In vitro study
Hamama et al.	2013	Aust Dent J	In vitro study	Pal et al.	2009	J Conserv Dent	In vitro study
Juntavee et al.	2013	Int J Clin Pediatr Dent	In vitro study	Prabhakar et al.	2009	Pesqui Bras Odontopediatria Clin Integr	In vitro study
Kathuria et al.	2013	J Clin Diagn Res	In vitro study	Topaloglu-Ak et al.	2009	Clin Oral Investig	No control, no clin eval
Moldovanu et al.	2013	Rev Chim	In vitro study	Abdelnur et al.	2008	J Dent Child	Case report
Zenen et al	2013	Cumhuriyet Dent J	In vitro study	Berata et al.	2008	J Appl Oral Sci	Study in permanent teeth

RIGHTSLINK

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

Table I. (Continued).

Authors	Year	Journal	Reason for exclusion	Authors	Year	Journal	Reason for exclusion
Rajakumar et al.	2013	J Clin Pediatr Dent	No group with Carisolv	Bussadori et al.	2009	J Clin Pediatr Dent	Case report
Ramamoorthi et al.	2013	J Conserv Dent	In vitro study	Corra et al.	2009	J Clin Pediatr Dent	In vitro study
Venkataraghavan et al.	2013	J Int Oral Health	Review	Corra et al.	2009	Eur Arch Paediatr Dent	In vitro study
Viral et al.	2013	J Clin Pediatr Dent	In vitro study	Guer et al.	2009	Spec Care Dentist	No clinical evaluation
Yildiz et al.	2013	Eur J Paediatr Dent	In vitro study	Gurbuz et al	2009	Eur J Dent	In vitro study
Zhang et al.	2013	Aust Dent J	In vitro study	Hosein et al.	2009	J Coll Physicians Surg Pak	Only permanent teeth
Ahmed et al.	2012	Caries Res	In vitro study	Piva et al.	2009	Braz Oral Res	In vitro study
Anegundi et al.	2012	Contemp Clin Dent	No group with Carisolv	Perić et al.	2009	Srpski Arhiv za Celokupno Lekarstvo	No data
Arora et al.	2012	Eur Arch Paediatr Dent	In vitro study	Rupf et al.	2009	J Dent Res	In vitro study
Avinash et al.	2012	Jindian Soc Pedod Prev Dent	In vitro study	Subramaniam et al.	2009	J Clin Pediatr Dent	Microbiological study
Azzouz et al.	2012	SJKFU	Only permanent teeth	Tachibana et al.	2009	Lasers Med Sci	In vitro study
Banerjee	2012	Ann R Australas Coll Dent Surg	Review	Yamada et al.	2009	J Dent	In vitro study
Bhardwaj et al.	2012	RJPBCS	No clinical study	Corra et al.	2007	J Clin Pediatr Dent	In vitro study
El-Tekeya et al.	2012	Pediatr Dent	In vitro study	De Oliveira et al.	2007	Journal of Adhesion	In vitro study
Gallusean et al.	2012	Rev Chim	Clin evalu with caries detector	Giza	2007	Ann Acad Med Stetin	Polish language
Kumar et al.	2012	Indian J Dent Res	No control group	Inglehart et al.	2007	J Am Dent Assoc	No clinical evaluation
Schlafer et al.	2012	J Nat Prod	In vitro study	Kirzioglu et al.	2007	Clin Oral Investig	No control group
Singhal et al.	2012	Indian J Dent Res	In vitro study	Okida et al.	2007	Braz Oral Res	In vitro study
Sirin Karaarslan et al.	2012	J Dent	In vitro study	Pandit et al.	2007	J Indian Soc Pedod Prev Dent	No control group
Verma et al.	2012	Jindian Soc Periodontol	In vitro study	Perić T et al.	2007	Eur J Paediatr Dent	In vitro study
Yamada et al.	2012	J Clin Pediatr Dent	In vitro study	Topaloglu-Ak	2007	J Appl Oral Sci	Endodontic study
Zhao et al.	2012	Chinese Journal of New Drugs	No clinical evaluation	Topaloglu-Ak et al.	2007	J Appl Oral Sci	No control group
Yamada et al.	2007	J Clin Pediatr Dent	In vitro study	Kakaboura et al.	2003	Quintessence Int	Only permanent teeth
Antonio et al.	2006	J Oral Sci	Study on the bovine	Kinoshita et al.	2003	J Clin Laser Med Surg	In vitro study
Bonsor and Pearson	2006	Dent Update	Microbiological study	Leger et al.	2003	Caries Res	In vitro study

RIGHTSLINK

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

Table I. (Continued).

Authors	Year	Journal	Reason for exclusion	Authors	Year	Journal	Reason for exclusion
Clementino-Luedemann et al.	2006	Dent Mater J	In vitro study	Lumbau et al.	2003	Minerva stomatol	No clinical evaluation
Dammaschke et al.	2006	Aust Dent J	Study on the rats	Rafique et al.	2003	Caries Res	Only permanent teeth
De Magalhaes et al.	2006	Braz Dent J	In vitro study	Sakoolnamarka et al.	2003	Am J Dent	In vitro study
Grisi et al.	2006	Braz Dent J	In vitro study	Tonami et al.	2003	J Med Dent Sci	In vitro study
Lennon et al.	2006	Oper Dent	In vitro study	Yazici et al.	2003	J Oral Rehabil	In vitro study
Lenters M et al.	2006	Eur Arch Paediatr Dent	Full text not available	Arvidson et al.	2002	J Dent	In vitro study
Marquezan et al.	2006	Braz Oral Res	Review	Arvidson et al.	2002	Biomaterials	In vitro study
Meller et al.	2006	Eur J Paediatr Dent	In vitro study	Dammaschke et al.	2002	J Dent	Study on the rats
Mhaville et al.	2006	Eur Arch Paediatr Dent	Clinical evaluation with RX	Kubo et al.	2002	Oper Dent	In vitro study
Roeleveld et al.	2006	Eur Arch Paediatr Dent	No clinical evaluation	Nemes et al.	2002	Fogorvosi szemle	Review
Sabola et al.	2006	Oper Dent	No clinical evaluation	Sakoolnamarka et al.	2002	Aust Dent J	In vitro study
Bussadori et al.	2005	J Clin Pediatr Dent	No group with Carisolv	Yazici et al.	2002	Oper Dent	In vitro study
Dammaschke et al.	2005	Acta Odontol Scand	In vitro study	Arvidson et al.	2001	Gerodontology	In vitro study
El-Kholany et al.	2005	J Adhes Dent	In vitro study	Beeley et al.	2001	Ned Tijdschr Tandheelkd	Review
Flickger et al.	2005	J Dent	In vitro study	Dammaschk et al.	2001	J Dent	Study on the rats
Hosoya Y et al.	2005	J Dent	In vitro study	Hosoya et al.	2001	J Dent	In vitro study
Hu et al.	2005	Med J Wuhan Uni	In vitro study	Ilewicz et al.	2001	Acta Pol Toxicol	In vitro study
Lima et al.	2005	J Appl Oral Sci	Microbiological study	Maragakis et al.	2001	Int Dent J	Review
Morrow et al.	2005	Am J Dent	In vitro study	Munshi et al.	2001	J Clin Pediatr Dent	No control group
Rahman et al.	2005	Int Endod J	Study on the ovine	Nadanovsky et al.	2001	Caries Res	Only permanent teeth
Sakoolnamarka et al.	2005	Aust Dent J	In vitro study	Spliath et al.	2001	Clin Oral Investig	In vitro study
Sonoda et al.	2005	J Dent	In vitro study	Yamada et al.	2001	J Clin Laser Med Surg	In vitro study
Yamada et al.	2005	J Clin Pediatr Dent	In vitro study	Young et al.	2001	J Dent	Study on the rats
Yazici et al.	2005	Quintessence Int	In vitro study	Banerjee et al.	2000	J Dent	In vitro study
Zeeuwitz et al.	2005	Schweizer Monatt fr Zahnmedizin	German language	Beeley et al.	2000	Br Dent J	Review
Ziekind et al.	2005	Quintessence Int	Review	Fure et al.	2000	Caries Res	Only permanent teeth
Arvidsson	2004	Acta Odontol Scand	In vitro study	Heck et al.	2000	Eur J Oral Sci	In vitro study

RIGHTSLINK

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

Table I. (Continued).

Authors	Year	Journal	Reason for exclusion	Authors	Year	Journal	Reason for exclusion
Azrak et al.	2004	Int J Paediatr Dent	Microbiological study	Morrow et al.	2000	Dent Update	Full text not available
Berto et al.	2004	Gen Dent	Study on the rats	Yamada et al.	2000	J Clin Laser Med Surg	In vitro study
Bulut et al.	2004	J Dent	In vitro study	Cederlund et al.	1999	Acta Odontol Scand	In vitro study
Erhardt et al.	2004	Quintessence Int	In vitro study	Cederlund et al.	1999	Int J Periodontics Restorative Dent	In vitro study
Fure et al.	2004	Clin Oral Investig	Only permanent teeth	Hannig	1999	Clin Oral Investig	In vitro study
Grbz T	2004	Pain Clinic	No clinical evaluation	Banerjee et al.	2000	Caries res	In vitro study
Hahn et al.	2004	Caries Res	In vitro study	Banerjee et al.	2000	Br Dent J	In vitro study
Sepet et al.	2004	J Dent	In vitro study	Wennerberg et al.	1999	Eur J Oral Sci	In vitro study
Al-Kilani et al.	2003	Int Endod J	In vitro study	Lai	1990	Rivista italiana di odontoiatria infantile	
Ansari et al.	2003	J Oral Rehab	No clinical evaluation	Bianchi et al.	1999	Dental Cadmos	In vitro study
Beyth et al.	2003	Refuat Hapeh Vehashinayim	Review	Bianchi et al.	1999	Dental Cadmos	Only permanent teeth
Burrow et al.	2003	Aust Dent J	In vitro study	Scheutzel	1999	Deutsche zahnarztliche Zeitschrift	German language
Cehreli et al.	2003	J Dent	In vitro study	Goldman et al.	1986	J Pedod	In vitro study
Chaussain-Miller et al.	2003	Clin Oral Investig	Only permanent teeth	Anusavice et al.	1987	J Dent Res	No clinical evaluation
Fritz	2003	J Orofac Orthop	Review	Moriot	1986	Le Chirurgien-dentiste de France	Full text not available
Hosein et al.	2003	Oper Dent	In vitro study				

RIGHTSLINK

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

Table II. Summary of the characteristics of the included study.

			Interventions		Outcomes (Carisolv/drill)			
Author (year)	Patients characteristics	Carisolv/drill	Study design	Carisolv	Drill	Clinical efficacy	Time taken	Need for anesthesia
Soni et al. (2014)	120 patients (4–14 years); 120 primary and permanent teeth.	30/30	RCT parallel group	30 Carisolv/special hand instrument until cavity was hard on probing	Rotary instruments until the cavity clinically caries free			
Bohari et al. (2012)	120 patients (5–9 years); 120 primary teeth. Lesion dentine/ occlusal surfaces	30/30	RCT parallel group	30 Carisolv/special hand instrument until cavity was hard on probing	Rotary instruments until the cavity clinically caries free		474.7–43.0 s (30)/ 208.7–22.1 s (30)	
Perio (2009)	120 patients (3–17 years); 74 primary teeth. At least one primary carious lesion	40/34	RCT parallel group	30 Carisolv/special hand instrument until cavity was hard on probing	Rotary instruments until the cavity clinically caries free. The cavity checked by an operator		646–162 s (40)/ 432–84 s (34)	
Peters et al. (2006)	50 patients (6–11 years); 50 primary molars One primary occlusal carious lesion for each tooth.	26/24	RCT parallel group	30 Carisolv/special hand instrument until cavity was hard on probing. Time limit was 15 min	Rotary instruments until the cavity clinically caries free. Time limit was 15 min		604.2–227.5 s (26)/90.7–84 s(24)	
Lozano-Chourio (2006)	40 patients (7–9 years); 80 primary teeth. At least two caries in primary molars	40/40	RCT cross-over design	30 Carisolv/special hand instrument until cavity was hard on probing. The cavity checked by an operator	Rotary instruments until the cavity clinically caries free. The cavity checked by an operator	100% (40 of 40)/ 100% (40 of 40) caries free	450.6–109.8 s (40)/ 148.2–126 s (40)	0% (0 of 40)/ 5% (2 of 40)
Baldunlene (2005)	30 patients (2.5–13 years); 60 primary and permanent teeth. At least two lesions	30/30	Controlled clinical trial split mouth design	30 Carisolv/special hand instrument until cavity was hard on probing. The cavity checked by an independent examiner	Rotary instruments until the cavity clinically caries free. The cavity checked by an independent examiner			

RIGHTSLINK

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

Table II. (Continued).

			Interventions		Outcomes (Carisolv/drill)			
Author (year)	Patients characteristics	Carisolv/drill	Study design	Carisolv	Drill	Clinical efficacy	Time taken	Need for anesthesia
Kavvadia et al. (2004)	31 patients (26 months–9 years); 92 primary teeth. At least one primary carious lesion	65/27	Controlled clinical trial parallel group	30 Carisolv/special hand instrument until cavity was hard on probing. The cavity checked by two operators	Rotary instruments until the cavity clinically caries free. The cavity checked by two operators	100% (65 of 65)/ 100% (27 of 27) were caries free	496–318 ss (65)/ 166–114 s (27)	2.3% (1 of 43)/ 23.5% (4 of 17) requested anesthesia
Bergmann et al. (2005)	46 patients (4–11 years). At least two active dentinal caries lesions in primary teeth	46/46	RCT split mouth design. A multi-center study	30 Carisolv/special hand instrument until cavity was hard on probing. The cavity checked by an operator one for each center	Rotary instruments until the cavity clinically caries free. The cavity checked by an operator one for each center	100% (46 of 46)/ 97.8% (45 of 46) were caries free	402–174 s (46)/ 198–138 s (46)	
Maragakis et al. 2001	16 patients (97–109 months); two contralateral primary molar with occlusal primary decay	16/16	Controlled clinical trial split mouth design	Application of Carisolv/special hand instrument until cavity was hard on probing. The cavity checked by an operator. Time limit was 15 min	Rotary instruments until the cavity clinically caries free. Time limit was 15 min			
Ericson et al. (1999)	137 patients (3–65 years). At least one active dentinal primary caries	16/1	RCT parallel group multi-center study	20 Carisolv/special hand instrument until cavity was hard on probing. The cavity checked by an operator	Rotary instruments until the cavity clinically caries free. The cavity checked by an operator		616–336 s (16)/no data	

RIGHTSLINK

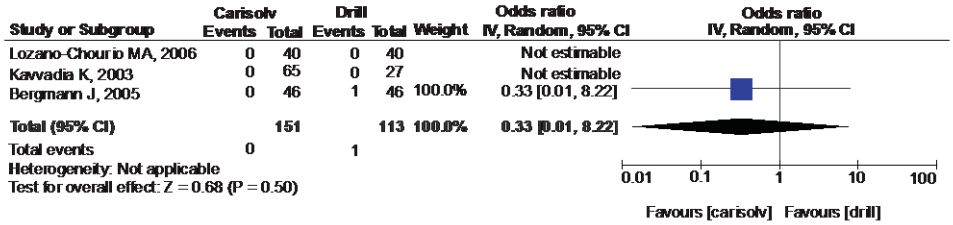


Figure 2. Forest plot of comparison: Individual and overall Odds Ratio in the comparison of clinical efficacy between the Carisolv group and the rotary instrument group.

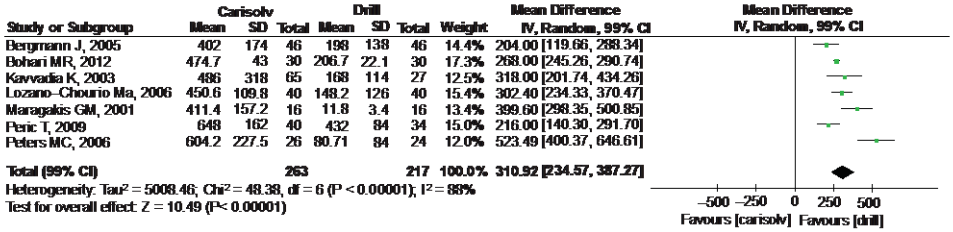


Figure 3. Forest plot of comparison: Individual and overall Mean Difference in the comparison of time taken between the Carisolv group and the rotary instrument group.

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

Data regarding the clinical efficacy in decayed tissue removal of the Carisolv system vs a control group were obtained from three papers [10,17,18], with a total of 264 analyzed teeth. Complete caries removal was obtained in 100% (151 of 151) of the teeth using Carisolv and 99.2% (112 of 113) using the drill. When data were combined in meta-analysis, the summary OR was 0.33 (99% CI = 0.00–22.65). On the basis of the available evidence, there was no statistically significant difference in caries removal between the chemo mechanical system (Carisolv) and the rotary instruments ($z = 0.68$ $p = 0.50$) (Figure 2).

Data on the time required (seconds) to complete the procedure (mean ± SD) was obtained from seven studies [9,10,15,17,18,22,23] with a total of 480 teeth involved. The maximum time required for caries removal was 648 s for Carisolv and 206.7 s for the rotary instrument, whereas the minimum time of treatment was 402 s for the chemo mechanical removal and 80.7 s with the use of drills. The chi-square value was 48.38, with six degrees of freedom (df) and $p < 0.01$. The treatment with Carisolv required a statistically significant greater time amount than that required with the use of rotary instruments. The z-test for overall effect for the Carisolv group vs rotary instruments was $z = 10.49$, $p < 0.01$ (Figure 3).

Finally, data regarding the pain threshold were obtained from four studies only [10,18,22,23] with a total of 222 teeth involved. With the Carisolv system, 4% of the children requested local anesthesia, while 26.8% used the conventional method. When data were combined in meta-analysis, the summary OR was 0.09 (95% CI = 0.01–1.07) with a difference between two types of treatment near to statistical significance ($z = 1.91$, $p = 0.06$), with fewer patients who needed local anesthesia in the Carisolv group (Figure 4).

Discussion

A multitude of technique and materials are proposed in the dental market to use in restorative dentistry and so the need of a strong scientific evidence for the 'new' methods is essential before their use in everyday practice.

Carisolv was introduced in the dental market (Sweden) in 1998 [9] and during the last 15 years it has been used almost exclusively in pediatric dentistry, as the use of Carisolv in clinical practice might be limited because of the material cost [24]. In the literature, there is no availability of systematic review on the efficacy of Carisolv system in caries removal in primary dentition. Hence, this meta-analysis review was performed in an attempt to gain further insight into the reliability of the Carisolv system. Seven studies were included, with a total of 450 primary teeth involved. The heterogeneity of the results generated by different studies on the use of Carisolv limits an overall correlation among outcome variables.

The parameter to evaluate the effectiveness of the Carisolv against rotary instruments was the caries removal rate, clinically appreciated. This evaluation

RIGHTSLINK

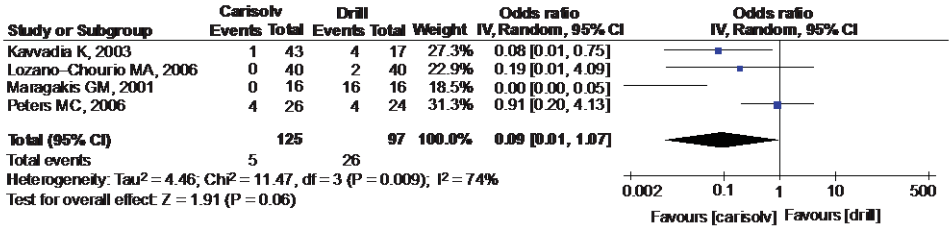


Figure 4. Forest plot of comparison: Individual and overall Odds Ratio in the comparison of need for anesthesia between the Carisolv group and the rotary instrument group.

method seems empirical and inaccurate, however it is the main and simple approach to check the caries removal [25]. This method only required a visual estimation and a tactile evaluation using a sharp probe. Other methods to evaluate the complete caries removal, like caries detectors, are a matter of controversy in the literature [26].

The comparison of the clinical evaluations data indicated that no statistically significant difference exists between the Carisolv group and the control group with rotary instruments in terms of caries removal efficacy.

Outcomes regarding the time required to complete the procedure were reported in five of seven studies selected. There was a significant difference regarding time required by the Carisolv procedure and the conventional drilling: treatment time was statistically significantly longer using Carisolv than drilling. This difference was related to the need of multiple applications of Carisolv gel, especially when big carious lesions were treated. Only one paper reported that the lesions in both groups were similar in terms of size, but the time taken for caries removal using Carisolv was 3-times longer [18]. A previous clinical investigation [27] found the depth of carious lesions was an important parameter for the excavation time with Carisolv.

Pain is a commonly reported phenomenon when removing dental caries and the use of local anesthesia is often required. Data on pain threshold or need of local anesthesia were reported in four papers. Carisolv seems to reduce the use of local anesthesia and this difference may be related to the use, together with Carisolv gel, of sharp hand instruments. However, it is necessary to consider that the four studies were heterogeneous in design and the Carisolv group was more numerous than the control group.

Conclusion

Within the limitations of the available data, the clinical efficacy of chemo mechanical instruments in caries removal with Carisolv seems as reliable as that obtained using rotary instruments. Data analysis suggests that the difference in terms of time taken was statistically significant: the Carisolv system takes more time than the traditional method to remove dental caries. Regarding patient's comfort, this systematic review indicates that the Carisolv system can reduce the use of local anesthesia. However, these results should be interpreted cautiously due to the heterogeneity among study designs and to the shortage of data usable. To confirm these conclusions there is the need of further large-scale, well-designed RCTs.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] WHO expert Committee on Dental Health World Health Organization. Standardization of reporting of dental diseases and conditions: report of an expert committee on dental Health 1962. Available from http://apps.who.int/iris/handle/10665/38150.14-20 November 1961.
[2] Ricketts D, Lamont T, Innes NP, Kidd E, Clarkson JE. Operative caries management in adults and children. Cochrane Database Syst Rev 2013;3:CD003808.
[3] Banerjee A, Watson TF, Kidd EA. Dentine caries excavation: A review of current clinical techniques. Br Dent J2000;9:476–82.
[4] Osborne JW, Summitt JB. Extention for prevention: is it relevent today? Am J Dent 1998;11:189–96.
[5] Dalli M, Çolak H, Mustafa Hamidi M. Minimal intervention concept: A new paradigm for operative dentistry. J Investig Clin Dent 2012;3:167–75.
[6] Goldman M, Kronman JH. A preliminary report on a chemomechanical means of removing caries. J Am Dent Assoc 1976;93:1149–53.
[7] Schutzbank SG, Galaini J, Kronman JH, Goldman M, Clark RE. A comparative in vitro study of GK-101 and GK-101E in caries removal. J Dent Res 1978;57:861–4.
[8] Venkataraghavan K, Kush A, Lakshminarayana C, Diwakar L, Ravikumar P, Patil S, et al. Chemomechanical Caries Removal: A Review & study of an indigen-ously developed agent (Carie Care (TM) Gel) In Children. J Int Oral Health 2013;5:84–90.
[9] Bohari MR, Chunawalla YK, Ahmed BM. Clinical evaluation of caries removal in primary teeth using conventional,

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

RIGHTSLINK

chemomechanical and laser technique: An in vivo study. J Contemp Dent Pract 2012;13:40–7.
[10] Kavvadia K, Karagianni V, Polychronopoulou A, Papagiannouli L. Primary teeth caries removal using the carisolv chemomechanical method: A clinical trial. Pediatr Dent 2003;26:23–8.
[11] Kotb RM, Abdella AA, El Kateb MA, Ahmed AM. Clinical evaluation of Papacarie in primary teeth. J Clin Pediatr Dent 2009;34:117–23.
[12] Kochhar GK, Srivastava N, Pandit IK, Gugnani N, Gupta M. An evaluation of different caries removal techniques in primary teeth: A comparitive clinical study. J Clin Pediatr Dent 2011;36:5–9.
[13] Rajakumar S, Mungara J, Joseph E, Philip J, Shilpa Priya MP. Evaluation of three different caries removal techniques in children: A comparative clinical study. J Clin Pediatr Dent 2013;38:23–6.
[14] Ericson D, Zimmerman M, Raber H, Götrick B, Bornstein R. Clinical evaluation of efficacy and safety of a new method for chemomechanical removal of caries. Caries Res 1999;33:171–7.
[15 Peric T, Markovic D, Petrovic B. Clinical evaluation of a chemomechanical method for caries removal in children and adolescents. Acta Odontol Scand 2009;67:277–83.
[16] Balciuniene I, Sabalaite R, Juskiene I. Chemomechanical caries removal for children. Stomatologija 2005;7:40–4.
[17] Bergmann J, Leitão J, Kultje C, Bergmann D, João CM. Removing dentine caries in deciduous teeth with Carisolv: A randomised, controlled, prospective study with six-month follow-up, comparing chemomechanical treatment with drilling. Oral Health Prev Dent 2005;3:105–11.
[18] Lozano-Chourio MA, Zambrano O, Gonzalez H, Quero M. Clinical randomized controlled trial of chemomechanical caries removal (Carisolv). Int J Paediatr Dent 2006;16:161–7.
[19] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med 2009;6:e1000097.
[20] The Cochrane Collaboration. Review Manager (RevMan) - computer program. Version 5.3 Copenhagen: The Nordic Cochrane Centre; 2012.
[21] Soni HK, Sharma A, Sood PB. A comparative clinical study of various methods of caries removal in children. Eur Arch Paediatr Dent 2014. [Epub ahead of print].
[22] Maragakis GM, Hahn P, Hellwig E. Clinical evaluation of chemomechanical caries removal in primary molars and its acceptance by patients. Caries Res 2001;35:205–10.
[23] Peters MC, Flamenbaum MH, Eboda NN, Feigal RJ, Inglehart MR. Chemomechanical caries removal in children: Efficacy and efficiency. J Am Dent Assoc 2006;137:1658–66.
[24] Kathuria V, Ankola AV, Hebbal M, Mocherla M. Carisolv- an innovative method of caries removal. J Clin Diagn Res 2013;7:3111–15.
[25] Schwendicke F, Paris S, Tu YK. Effects of using different criteria for caries removal: A systematic review and network meta-analysis. J Dent 2015;43:1–15.
[26] McComb D. Caries-detector dyes-how accurate and useful are they? J Can Dent Assoc 2000;66:195–8.
[27] Chaussain-Miller C, Decup F, Domejean-Orliaguet S, Gillet D, Guigand M, Kaleka R, et al. Clinical evaluation of the Carisolv chemomechanical caries removal technique according to the site/stage concept, a revised caries classification system. Clin Oral Investig 2003;7:32–7.

Acta Odontol Scand Downloaded from informahealthcare.com by 85.119.43.29 on 03/15/15
For personal use only.

RIGHTSLINK

IV

Title: Clinical randomized controlled trial of four different techniques of caries removal in primary dentition.

Lai Gianfranco[1], gilai@uniss.it
Lingström Peter[2], peter.lingstrom@odontology.gu.se
Sale Silvana[1], sisale@uniss.it
Campus Guglielmo[1, 3] gcampus@uniss.it

[1]Department of Surgery, Microsurgery and Medical Sciences, School of Dentistry, University of Sassari, Italy
[2]Department of Cariology, Faculty of Odontology, Sahlgrenska Academy at Gothenburg University, Gothenburg, Sweden
[3]WHO Collaborating Centre for Epidemiology and Community Dentistry, University of Milan, Italy

Corresponding author
Gianfranco Lai
Department of Surgery, Microsurgery and Medical Sciences
School of Dentistry University of Sassari
Viale San Pietro 43/C 07100 Sassari, SS, Italy
Tel. +39079228540
gilai@uniss.it

Key words: Carisolv, Cerabur, dental caries, chemomechanical technique

ABSTRACT

Aim: to evaluate four different techniques for the removal caries in term of efficacy, treatment time and efficacy anti-bacterical.

Methods: Children had to have at least 1 caries lesion interesting either occlusal, interproximal or in cervical surface of first and second primary molars prone to exfoliation were invited to participate to study. The Carisolv with instrument dedicated, Cerabur, Carisolv plus Cerabur with and without caries detector were compared with traditional caries removal (drill). Before and after caries excavation dentine was sampled for the microbiological analysis. In all groups information about cavity size was recorded. This procedure was performed before and after excavation. Three different measurements were made using a periodontal probe. Treatment time related to the caries excavation was measured.

Results: A total of 47 patients (were included for the study. We treated a total of fifty primary teeth. In all teeth treated were achieved the complete caries removal during the first visit. The caries removal with Carisolv was slower than the other technique tested (954.4± 347.9 sec). The quantity of tissue removal was less in Cerabur group, however we found no difference between groups. In all techniques tested the microbial reduction was achieved for almost all bacterial species studies.

Conclusions: The result obtained in this study suggests that these four techniques were a valid alternative of traditional method in caries removal in primary dentition. The techniques tested seem to be as aggressive as the traditional method and have a similar anti-bacterial effect.

Introduction

Dental caries is one of the most prevalent chronic diseases of people worldwide and it affects the majority of individuals in all age groups during their lifetime (Selwitz RH et al., 2007). According to WHO this pathology is defined as a localized, post-eruptive, pathological process of external origin, involving softening of the hard dental tissue and proceeding to the formation of a cavitation (WHO, 1967).
The exclusive use of drill with conventional bur for excavation presents some disadvantages, since the drill removes both infected and non-infected dentine, it may cause an unnecessary weakness of the tooth structure, and also increases the possibilities of damaging pulpal tissue. The modern approach to caries treatment indicates the need to remove only dental tissue to the extent that is strictly necessary for treatment (Lozano-Chourio MA et al., 2006). The modern restorative dentistry offers alternatives to the traditional tissue removal using conventional drilling instrument: the possible alternative are the chemo mechanical removal and the new type of bur.
In 1999, a product from MediTeam group called Carisolv® was marketed. This contains sodium hypochlorite and three natural amino acids: lysine, leucine and glutamic acid. When the gel of three amino acids (lysine, leucine and glutamic acid); 53mM and the gel containing 0.27M hypochlorite are mixed, amino acids bind chlorine and form chloramines at a pH of 11. This chlorination affects the secondary and/or quaternary structure of the collagen, by disrupting hydrogen bonding and, thus, brings about a proteolytic reaction. It does not affect healthy dentine because amino acids act as homing devices for active chlorine. The chlorine atom of hypochlorite is transferred to the amino group of each amino acid and in this way it is made less reactive and less aggressive to healthy tissue (Bohari MR et al., 2012). In contrast with conventional excavators and drills used in the traditional caries removal, in Carisolv technique carious dentine is removed using specially designed instrument, all of which should reduce the risk of removing intact dentine.
The first in vitro investigation on the use of Carisolv, in primary and permanent teeth was published in 1998. It was reported that Carisolv has been compared in controlled clinical trials in permanent and in primary dentition to the conventional mechanical method and the removal of caries by hand instruments (Kavvadia K et al., 2004). In numerous clinical study was reported the reliability of Carisolv although this product needs significantly longer working time (Bergmann J et al., 2005; Kavvadia K et al., 2005; Lozano-Chourio MA et al., 2006).
The most conventional method of removing caries involves the use of steel or tungsten carbide burs mounted in a low-speed contra-angle. Although very efficient in term of time spent for caries removal, the decision to stop caries removal using these burs is very subjective, and basically depends on the operator's background

and clinical experience. The recently marketed CeraBur (Komer-Brasseler, Lemgo, Germany) is a self-limiting ceramic bur (alumina-based with stabilized zirconia), which according to the manufacture efficiently curt infected and soft dentin, while hardly acts on hard, sound tissue (Dammaschke T et al., 2008).
Caries detector dyes based on propylene glycol were developed in order to highlight alteration in dentine collagen structure but publications have shown that clinical and laboratory results produced are open to considerable user-interpretation (Neves Ade A et al., 2011).

Methods

This prospective, randomized and controlled clinical trial was performed at the Faculty of Medicine and Surgery, Sassari University between March 2013 and December 2014.

Experimental Design
Before starting the study, there was a preparation period. The training lasted 4 weeks. The operator (GL), who performed the clinical procedures of the study, reached a good clinical in vivo agreement with the benchmark operator (GC) about what constitutes a cavity with complete and incomplete caries removal.
The steps of the study were the following: a preliminary examination, informed consent, randomization of samples, recording of cavity characteristic, collect dentine sample, caries removal, cavity inspection, collect dentine sample and final restoration. The same operator performed both caries treatment and cavity examination.

Inclusion Criteria
Between all the patients who appeared for a regular dental examination, who met the inclusion criteria was invited to enter in the study. To be selected children had to have at least 1 caries lesion interesting either occlusal, interproximal or in cervical surface of first and second primary molars prone to exfoliation. The lesion considered in this study was between a D1 and D3 stage evaluated by radiographic examination. Teeth with pathological processes of dental tissue other than caries, or pulpal disease or with adjacent soft tissue lesion were excluded. The children with systemic disease were excluded.

Clinical Procedure
Each tooth treated was distributed among the five groups by computer randomization. The five treatments groups with a total of 50 teeth are (fig. 1):

A. Cavity preparation with traditional technique (control group)
B. Cavity preparation with CeraBur
C. Cavity preparation with Carisolv and hand instruments dedicate
D. Cavity preparation with Carisolv and CeraBur
E. Cavity preparation with Carisolv and CeraBur. Cavity inspection with caries detector

In all groups was registered information about cavity size. This procedure was performed before and after excavation. Three different measurement were made using a periodontal probe:

1. The outer diameter in buccal – lingual and in mesial – distal sense
2. The depth of the lesion (when possible before excavation)

To calculate the volume of cavity size before and after excavation we used pyramid as geometric model that could simulate the caries lesion shape. From cavity data we calculated the volume of geometric model that simulated the extension of caries lesions and clean cavity. Difference between post and pre-operative size was used to estimate the increment of cavity size.
Treatment time related to the caries excavation was measured. The clock was started when the first step of excavation or opening of the cavity began and stopped when the caries excavation and cavity preparation was completed. Time was measured in seconds.
In group A (control group) the carious lesions were treated using drills with two types of bur: Komet 880 314 012 to remove enamel and Komet H1SE 204 014 to eliminate tissue decay. In group B the enamel was removed using diamond bur (Komet 880 314 012) and ceramic bur was used to remove dentine decay. In group C Carisolv was used to remove caries: the gel was applied on dentine infected and after 30 seconds the softened tissue was removed using dedicated hand tools. This procedure was repeated until complete caries removal. When necessary enamel was removed using diamonds bur (Komet 880 314 012). In group D caries was removed using Carisolv gel.Finally ceramic bur (Komet Cerabur K1SM 2014 014) was used to finish the walls and the floor of the cavity. The procedure to remove tissue decay was the same in the D and E groups. In group E a caries detector was used for cavity inspection. In groups A, B, C and D the completion of caries removal was judged by standard clinical criteria, i.e. the probe did not stick in the remaining dentine. In the group E the complete caries removal was evaluated with caries detector. The data of complete caries removal was registered. After the cavity check the teeth were restored with ionomer glass cement.

Microbiological Analysis

After drying and isolation with cotton rolls, dentine was sampled from the cavity before and at the end of the cavity preparation using sterile excavator. Each sample was placed in an Eppendorf tube containing 150 µl of sterile TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6). Then 100 µl of 0.5 M NaOH was added to the dentine sample and the bacterial suspension was stored at -20°C pending further processing (Gellen LSS et al., 2007).

The analysis of bacterial species was performed using the checkerboard DNA-DNA hybridisation method (Wall-Manning GM et al., 2002). Whole genomic probes were prepared from the 15 bacterial strains known to be related to caries as shown in (tab. 1). An evaluation of the bacterial count in the samples was performed by comparing the obtained signals with the ones generated by the pooled standard samples containing a count of 10^6 and 10^5 of each bacterial species, respectively. The signals were coded on a scale from 0 to 5 as follows: 0 = no signal; 1 = a signal density weaker than that of the low standard ($<10^5$ bacteria); 2 = a signal density equal to that of the low standard ($=10^5$ bacteria); 3 = a signal density higher than that of the low standard but lower than that of the high standard ($>10^5$ but $<10^6$ bacteria); 4 = a signal density equal to that of the high standard ($=10^6$ bacteria) and 5 = a signal density higher than that of the high standard ($>10^6$ bacteria).

Statistical Analysis

Statistical difference in time taken and cavity size increment were performed using ANOVA analysis, adjusting statistical significance for the multiple comparisons (Bonferroni correction). For the analysis of microbiological data Shapiro-Francia normality test was used to assess the normality distribution of collect variables. Statistical difference in score of bacterial count was performed using the Kruskall-Wallis analysis of post-operative samples. In case of difference between groups comparisons were performed (Bonferroni correction). Statistical differences between pre- and post-operative in bacterial count, in each group and for each bacterial species, were calculated performing Mann-Whitney U test.

Statistical analysis was carried out using STATA®14.

Results

A total of 47 patients (25 females and 22 males with a mean age of 9,3 range 7-12) were included for the study. We treated a total of fifty primary teeth. In all teeth treated were achieved the complete caries removal during the first visit.

The ANOVA analysis showed a difference in term of time treatment between groups ($p<0.001$) (tab. 2). The caries removal with Carisolv was slower than the other technique tested. (fig.2).
The increment of cavity size was similar in the five groups ($p= 0.363$) (tab.2). The quantity of tissue removal was less in Cerabur group, however we found no difference between groups (fig. 3).
In pre-operative sample for each strain studied we found no difference between groups. In all techniques tested the microbial reduction was achieved for almost all bacterial species studies. The mutans streptococci and the non-mutans streptococci species showed the less decrease in all group (tab. 3). In post-operative sample a significant difference in bacterial count for the species A. Odontolyticus, A. Oris, A. Parvula, R. Dentocariosa, B. Dentium and P. Migra was found (tab. 4). Although the reduction of these bacterial species was significant for all technique tested; the Carisolv method shows the strongest antibacterial effect (fig. 5; fig. 6; fig. 7; fig. 8; fig. 9).

Discussion

In this study in all teeth treated was achieved complete caries removal. The results in term of complete caries removal of Carisolv method obtained in this study were in accordance with the data reported in several clinical trials (Lozano-Chourio MA et al., 2006; Kavvadia K et al., 2003; Bergamann J et al., 2005; Peric T et al., 2009; Ericson D et al., 1999; Bohari MR et al., 2012). The results of efficacy showed in this paper were in contrast what reported in two studies (Maragakis GM et al., 2001; Peters MC et al., 2006). Maragakis et al., 2001 reported a rate of complete caries removal of 62% and Peters et al., 2006 achieved success in 57.7% of cases. However these results might depend by time limit setting at 15 minutes to complete caries treatment. In literature there were no present studies in vivo that evaluated the efficacy of ceramic bur (CeraBur by Komet). The in vitro study that evaluated the efficacy of CeraBur confirmed the results obtained in this study (Dammaschke T et al., 2008).
In our study we found no difference between control group (Group A) and the other experimental group in terms of increment of cavity size. Relating the treatment with Carisolv the data in literature were debatable: one study (Lonzano-Chourio MA et al., 2006) showed that the cavities treated with Carisolv were smaller than in control group with rotating instrument; another study (Fure S et al., 2000) showed no difference between chemo mechanical method and conventional method. In this trial we found no difference between control group with traditional instrument and other experimental group. The cavity increment in Carisolv group is similar to the other group. In literature there is no clinical trial that describes increment of cavity

size after treatment with CeraBur, Carisolv and CeraBur. The use of detecting dyes is a matter of controversy in the literature (Fure S et al., 2000). In this study the use of caries dye to check cavity after treatment with combination of Carisolv and CeraBur did not brought to obtain smaller cavities.
The traditional method resulted the faster method than the other technique tested. The data obtained in Carisolv group were in accordance what reported in several studies (Bergamann J et al., 2006; Bohari MR et al., 2012; Kavvadia K et al., 2003; Lozano-Chourio MA et al., 2006; Maragakis GM et al., 2001; Peric T et al., 2009; Peters MC et al., 2006). Study that investigated this aspect in teeth treated with Carisolv combined with CeraBur was no found. One in vitro study (Dammaschke T et al., 2008) on CeraBur showed no difference in average time to excavate a cavity between ceramic burs and tungsten carbide burs. This trend was confirmed by our clinical trial.
The presence of chloramines in Carisolv gel might reduce the bacterial load. The study on primary dention have confirmed that Carisolv system reduce the bacterial count in cavity cleaned with this system (El-Tekeya M et al., 2012).
Although several studies reported a strong anti-bacterial effect of Carisolv technique (El-Tekeya M et al., 2012; Lager A et al., 2003; Azrak B et al., 2004; Baysan A et al., 2000) in this study all technique tested loaded a significantly bacterial decrease after treatment for all almost species tested. However we found strong anti-bacterial effect, against A. Odontolyticus, A.Oris, A. Parvula, R. Dentocariosa, B. Dentium and P. Migra in Carisolv tecnique.
In modern dentistry the use of composite resin in restoration of primary teeth was a common practice. A study in vitro showed that Carisolv system and CeraBur do not influence the bond strength than conventional caries removal with tungsten carbide burs (Neves Ade A et al., 2011). The use of chemo mechanical caries removal technique with Carisolv has no effect on bond strength of different adhesive system: total etch and self-etch (Banerjee A et al., 2012).
We have no collected data on patient's comfort. However our sensation suggests that the chemo mechanical caries removal with Carisolv can reduce the use of local anaesthesia. This idea was confirmed by many studies (Lozano-Chourio MA et al., 2006; Kavvadia K et al., 2003; Maragakis GM et al., 2001).

Conclusions

The result obtained in this study suggests that these four techniques were a valid alternative of traditional method in caries removal in primary dentition. The techniques tested seem to be as aggressive as the traditional method and have a similar anti-bacterial effect. The Carisolv results the slower method.

References

- Azrak B, Callaway A, Grundheber A, Stender E, Willer- shausen B. Comparison of the efficacy of chemomechanical caries removal (Carisolv) with that of conventional excava- tion in reducing the cariogenic flora. *Int J Pediatr Dent* 2004;14:182-91.
- Banerjee A, Kellow S, Mannocci F, Cook RJ, Watson TF. An in vitro evaluation of microtensile bond strengths of two adhesive bonding agents to residual dentine after caries removal using three excavation techniques. *J Dent* 2000; 38: 480-489.
- Baysan A, Whiley R, Lynch And. Antimicrobial assessment of Carisolv on primary root decay of an ex-living creature. *J Dent Res* 2000;79:1296
- Bergmann J, Leitão J, Kultje C, Bergmann D, Clode MJ: Removing dentine caries in deciduous teeth with Carisolv: a randomised, controlled, prospective study with six-month follow-up, comparing chemomechanical treatment with drilling. *Oral Health Prev Dent* 2005; 3: 105-111.
- Bohari MR, Chunawalla YK, Ahmed BM: Clinical evaluation of caries removal in primary teeth using conventional, chemomechanical and laser technique: An in vivo study. *J Contemp Dent Pract* 2012; 13: 40-47.
- Dammaschke T, Vesnic A, Schafer E: In vitro comparison of ceramic burs and conventional tungsten carbide bud burs in dentin caries excavation. *Quintessence Int* 2008; 39: 495-499.
- El-Tekeya M, El-Habashy L, Mokhles N, El-Kimary E: Effectiveness of 2 Chemomechanical Caries Removal Methods on Residual Bacteria in Dentin of Primary Teeth, *Pediatr Dent* 2012; 34: 325-330.
- Ericson D, Zimmerman M, Raber H, Götrick B, Bornstein R: Clinical evaluation of efficacy and safety of a new method for chemo mechanical removal of caries. *Caries Res* 1999; 33: 171-177.
- Fure S, Lingström P, Birkhed D: Evaluation of Carisolv™ for the chemo-mechanical removal of primary root caries in vivo. *Caries Res* 2000; 34: 275-280.
-Gellen LS, Wall-Manning GM, Sissons CH: Checkerboard DNA–DNA Hybridization Technology Using Digoxigenin Detection. In Methods in Molecular Biology. Totowa, NJ: *Humana Press Inc*; 2007:39-67
- Kavvadia K, Karagianni V, Polychronopoulou A, Papagiannouli L: Primary teeth caries removal using the Carisolv chemomechanical method: a clinical trial. *Pediatr Dent* 2004; 26: 23-28.
- Lager A, Thornqvist E, Ericson D. Cultivable bacteria indentin after caries excavation using rose-bur or Carisolv. *Caries Res* 2003; 37: 206-211
- Lozano-Chourio MA, Zambrano O, Gonzalez H, Quero M: Clinical Randomized controlled trial of chemomechaninal caries removal (Carisolv™). *Int J Paediatr Dent* 2006; 16: 161-167.

- Maragakis GM, Hahn P, Hellwig E: Clinical evaluation of chemomechanical caries removal in primary molars and its acceptance by patients. *Caries Res* 2001; 35: 205-210.
- Neves Ade A, Coutinho E, Cardoso MV, de Munck J, Van Meerbeek B: Micro-tensile bond strength and interfacial characterization of an adhesive bonded to dentin prepared by contemporary caries-excavation techniques. *Dent Mater* 2011; 27: 552-562.
- Peric T, Markovic D, Petrovic B: Clinical evaluation of a chemomechanical method for caries removal in children and adolescents . *Acta Odontol Scand* 2009; 67: 277-283.
- Peters MC, Flamenbaum MH, Eboda NN, Feigal RJ, Inglehart MR: Chemomechanical caries removal in children: Efficacy and efficiency. *J Am Dent Assoc* 2006; 137: 1658-1666.
- Selwitz RH, Ismail AI, Pitts NB: Dental Caries. *Lancet* 2007; 369: 51-59.
- Wall-Manning GM, Sissons CH, Anderson SA, Lee M: Checkerboard DNA-DNA hybridisation technology focused on the analysis of Gram-positive cariogenic bacteria. *J Microbiol Methods* 2002, 51:301-311
- WHO expert Committee on Dental Health World Health Organization. Standardization of reporting of dental diseases and conditions: report of an expert committee on dental Health 1962. Available from http://apps.who.int/iris/handle/10665/38150.14-20 November 1961.