SYSTEMATIC REVIEW

Validity of micro-CT for *in vitro* caries detection: a systematic review and meta-analysis

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Objective: To investigate the validity of micro-CT for *in vitro* caries detection in comparison with histology as the reference standard.

Methods: A systematic search was conducted in the databases Latin American and Caribbean Health Sciences (LILACS), LIVIVO, PubMed, Scopus and Web of Science from their inception to 16 January 2019. Grey literature was searched on Open Grey, ProQuest Dissertations and Theses Database and Google Scholar. *In vitro* studies assessing the validity of micro-CT for caries detection were included when compared with histology as the reference standard were included. Two authors independently collected the information and sensitivity, specificity, positive and negative likelihood ratios, as well as diagnostic odds ratios were calculated. The risk of bias of the included studies was assessed using the QUADAS-2 tool. Certainty of evidence was assessed with GRADE.

Results: A total of 270 papers were identified, and after a 2-phase selection, 12 studies were included in qualitative and three in quantitative synthesis. For enamel caries diagnostic, sensitivity values ranged from 29.0 to 84.0% indicating high variability while specificity varied from 88.0 to 95.0% indicating good to excellent micro-CT capability to identify the true negative. For dentine caries diagnostic, sensitivity values ranged from 61.0 to 77.0% indicating fair-to-good probability of micro-CT to identify the true positives, while specificity varied from 88.0 to 94.0%. The majority of the included studies presented low risk of bias and moderate certainty of evidence.

Conclusions: This study demonstrated the validity of micro-CT for *in vitro* caries detection in comparison with histology.

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Introduction

Studies have investigated the presence of caries lesions using different methods as reference standard, like confocal microscopy, transversal microradiography and microscopic examination. Histological inspection (usually stereomicroscopy) of sections is the most-used reference standard for caries lesion depth validation. However, all these methods require destructive sample preparation because sectioning of the sample is necessary for all these reference standards and remains a factor of variation.¹ Huysmans and Longbottom¹ reported since 2004 that if sectioning should be avoided, the future lies with non-destructive validating methods and the most appropriate in that moment was X-ray microtomography. They emphasized that this technique had reached acceptable resolution, however, it was very expensive and time-consuming.

Nowadays, the reference standard for validation has been predominantly focused on the histological extent of demineralization as seen in sections of extracted teeth.² However, in the last years the potential of micro-CT as a substitute for histological exam as the reference standard has been examined by several studies.^{3–12} Soviero *et al.*⁷ affirmed that micro-CT and histological analysis function are reference standard in a similar manner. The authors verified that micro-CT is very sensitive for the detection of caries lesions in enamel and for differentiation of lesions in enamel and dentin *in vitro*. Thus, the excellent performance in detecting initial lesions in enamel and with the differentiation of lesions in enamel and dentin strengthens the argument for micro-CT being used as the reference standard for the evaluation of proximal surfaces.^{7,8,13,14}

Controversially, a previous study reported that micro-CT, in spite of the high diagnostic performance achieved, cannot constitute an alternative to histological examination of tooth sections.^{4,5} Image noise and beam hardening artifacts are particularly important features in micro-CT imaging; the first can reduce the contrast resolution and both can degrade the image quality interfering in the final diagnosis. All the parameters and acquisition protocols are chosen with the intention of avoiding those two problems. Recently, a study evaluated the effect of beam hardening correction tool on micro-CT images used in the diagnosis of incipient caries lesions. The authors concluded that the beam hardening correction tool improves the diagnosis of incipient caries lesions in micro-CT images, especially when low-quality parameters aiming less acquisition and processing time are used. Using low-quality acquisition parameters consists in volumes reconstructed with less information, as a partial rotation combined with low numbers of frames and big rotation steps what can result in images of doubtful quality for the diagnosis of incipient caries lesions.¹⁵ Considering the fact that the results of published studies evaluating the potential of micro-CT as a substitute for histological examination as the reference standard are conflicting, the objective of this systematic review was to investigate the validity of

micro-CT for *in vitro* caries detection in comparison with histology as the reference standard.

Methods

The protocol of this systematic review was developed following recommendations of preferred reporting items for systematic review and meta-analysis (PRISMA-p).¹⁶ The PRISMA checklist¹⁷ was used to report this systematic review. A pre-registration of the protocol was not possible due to absence of register for studies of this nature to date. However, readers can request the corresponding author for a protocol copy.

Eligibility criteria and information sources

In vitro studies that evaluated the validity of micro-CT for *in vitro* caries detection in permanent and in primary teeth in comparison with histological examination as the reference standard were selected. Both caries detection in occlusal and proximal surfaces were accepted. The exclusion criteria were based on the following: (1) Studies assessing no incipient caries lesions; (2) Studies assessing only the validity of micro-CT; (3) Studies assessing only the validity of histological exam; (4) Reviews, letters, personal opinions, case reports, book chapters and conference abstracts.

An appropriate search strategy was developed for each of the following electronic database with the help of a health science librarian: Latin American and Caribbean Health Sciences (LILACS), LIVIVO, PubMed, Scopus and Web of Science from their inception to 16 January 2019. Grey literature was searched on Open Grey, ProQuest Dissertations and Theses Database and Google Scholar (Supplementary Material 1). Also, reference lists of included studies were searched for additional citations.

References were managed with electronic software (EndNote X7, Thomson Reuters; and Rayyan, Qatar Computing Research Institute).

Study selection and data collection

A two-phase process was adopted to study selection. First, two reviewers (LBO and CM) read titles and abstracts and after the full texts of the selected ones. The first and second authors (LBO, CM) performed the study selection and data collection independently. A third reviewer (MB) was consulted in the event of a disagreement that was not solved by a consensus discussion between the two reviewers.

For data extraction, the same process was followed with two reviewers independently collecting data and comparing afterwards. A third reviewer, an experienced radiologist, collected data regarding specific aspects of micro-CT (ACO), and one reviewer (LBO), cross-checked for precision.

The following structured information was recorded: author, year, country, sample size, observers, classification systems, statistical analysis, diagnostic agreement and authors' main conclusions. Furthermore, prevalence, sensitivity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), diagnostic odds ratio (DOR) and Youden's index were also collected.

Risk of bias in individual studies

Two reviewers (LBO and CM) independently assessed risk of bias with the Revised Tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2).¹⁸ For domain four, "Flow and Timing", the signaling question one, "Was there an appropriate interval between the index test and reference standard?" was judged to be not applicable for the kind of the design of the included studies so the decision was made to submit the question. All others questions in all domains, patient selection; index test; reference standard and flow and timing, were completed. The software Revman v.5.3 (Review Manager 5.3, The Cochrane Collaboration) was used to generate figures.

Summary measures and synthesis of the results

The diagnostic sensitivity and specificity of micro-CT to accurately identify enamel caries lesion and dentine caries lesion from sound tissues was evaluated by means of meta-analysis.

All the studies that presented these data in a similar way were planned to be grouped together in the meta-analysis. If a study did not assess sound tissue, enamel and dentin caries lesions or did not present sensitivity and specificity, it was excluded from meta-analysis. Both, permanent and primary teeth were planned to be part of meta-analysis. A receiver operating characteristic (ROC) graph and a forest plot were developed with the aid of Revman v.5.3 (Review Manager 5.3, The Cochrane Collaboration). The Cochrane guidelines were followed for data analysis.¹⁹

Additional analyses

PPV, NPV, LR+, LR-, DOR and Youden Index were calculated as additional analyses. To interpret the results cutoffs were used (Supplementary Material 2).

Risk of bias across studies

The overall quality of evidence assessment was planned using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE).²⁰

Results

Study selection

A detailed flowchart describing the search and selection with the number of articles included and excluded is shown in Figure 1. The search identified 270 records

and after removing duplication, 187 remained being 13 selected to full-text analysis. In total, 12 studies were selected^{3-12,21,22} and from these, three studies^{4,10,11} were retained to meta-analysis.

Study characteristics

Studies were conducted from 2009^{3,22} to 2018¹² and evaluated from 20⁴ to 140,¹⁰ permanent teeth,^{3-6,9-12,21,22} primary teeth⁷ or both permanent and primary teeth.⁸ Six studies analyzed the presence of caries in occlusal surface,^{3,5,6,10,21,22} five in proximal^{4,7,8,11,12} and one in both, occlusal and proximal surfaces¹⁰ (Table 1).

Risk of bias within studies

The study methods were heterogeneous especially regarding the index text, as it was expected. According to QUADAS-2,¹⁸ risk of bias or limited applicability was considered as low in half of the included studies. The main concerns found in the other studies were regarded to poor reporting of domain 1 ("patient selection") given that studies did not describe inclusion/exclusion criteria for sample selection or included restored teeth. There are also concerns related to domains 2 ("index text") and 3 ("reference standard") where some studies did not report if the crowns were separated from the roots before the analysis or the sectioning were performed in the middle of the lesions before analysis. The domain 4 ("flow and timing") also presented concerns, not all teeth received both the index test and the reference standard. More information about the risk of bias of included studies can be found in Figure 2A and B (summarized assessment).

Results of individual studies

From the 12 included studies, eight reported sensitivity and specificity values,^{3,4,7,8,10,12,21,22} from these, four reported also positive predictive value and negative predictive value^{4,7,10,12} and four informed accuracy.^{4,7,10,21}

Overall, results from the included studies showed micro-CT as accurate as histology for diagnose caries lesion from sound tissue and for depth of caries lesion.^{3,6-11,21,22} However, in some studies micro-CT was not accurate to assess enamel lesions,⁵ did not classify adequately the depth of the lesion¹² and showed moderate agreement with histology for enamel caries detection.⁴

Synthesis of results

Studies that evaluated data in a similar way were pooled in meta-analysis (Al Jamaan, 2016; Boca et al. 2017; Mitropoulos et al. 2010). Seven studies were excluded from meta-analysis because there was lacking specific quantitative data (lacking specificity and sensibility data^{5,6,9}; different thresholds to classify dental caries lesions^{7,21,22}; evaluated different protocols of images reconstruction¹² and in two studies there was lacking validation for both methods in all sample^{3,8} (Supplementary Material 3).

¹ Adapted from PRISMA.

Figure 1

All assessed sound tissue, enamel and dentin caries lesions in proximal surface in permanent teeth. Only permanent teeth were clustered together. Two different meta-analyses were performed with the three included studies, one for enamel and another one for dentine. One author provided separate data from two different observers; therefore, Mitropoulos et al.⁴ article is showed twice (Figure 3). The units of analysis are teeth surfaces.

The results are also presented in ROC curves in order to allow a graphic representation improving visualization (Figure 4).

For enamel caries diagnostic, sensitivity values ranged from 29.0 to 84.0% indicating high variability, while specificity varied from 88.0 to 95.0% indicating

good-to-excellent micro-CT capability to identify the true negative. Only one study reported excellent sensitivity for enamel detection in permanent teeth proximal surface with micro-CT.¹⁰

For dentine caries diagnostic, sensitivity values ranged from 61.0 to 77.0% indicating fair-to-good capability of micro-CT to identify the true positives, while specificity varied from 88.0 to 94.0% indicating good-to-excellent capability to identify the true negative.

For primary teeth, it was not possible to perform meta-analysis due to the number of studies presenting these data; only one study Soviero et al.⁷ The same occurred for occlusal surfaces.

Table 1 Summary of descriptive characteristics of included articles that evaluated micro-CT and histology for caries detection ($n = 12$)

Author, year, country	Sample size and dentition/teeth/surface	Observers number/type	Histological evaluation and criteria description	Statistical analysis	Results and diagnostic agreement value	Main conclusions
Al Jamaan, 2016, Scotland	140 teeth permanent/premolars and molars/171 sites on the occlusal surface and 229 proximal surfaces	two experienced examiners/ dentists	Downer Histological Classification Criteria (Downer, 1975) ERK Histological Classification Criteria (Ekstrand et al, 1997). Histology with serial sections were between 250–350µm thick at serial sections were between 250–350µm thick.	κ , Descriptive statistics, Spearman's correlation tests, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Accuracy.	Occlusal Surfaces - Downer criteria: Percentage agreement = 61%, Spearman rank correlation coefficient = 0.85, $p < 0.001$. Occlusal Surfaces - EKR criteria: Percentage agreement = 68%, Spearman rank correlation coefficient = 0.84, $p < 0.001$. Proximal Surfaces - Downer criteria: Percentage agreement = 88%, Spearman rank correlation coefficient = 0.86, $p < 0.001$. Proximal Surfaces - EKR criteria: Percentage agreement = 88%, Spearman rank correlation coefficient = 0.86, $p < 0.001$.	The high diagnostic performance of Micro-CT achieved, when compared with histology accurate and conventional gold standard at D1 and D3 diagnostic thresholds, would suggest that Micro-CT has the potential to be used as an alternative to conventional histology for occlusal and proximal caries detection.
Arslan et al., 2014, Turkey	60 teeth Permanent/ Premolars and molars/ occlusal surfaces	two experienced examiners/ radiologists	The scores of histological examination were sound, enamel caries and dentin caries. Two to four sections were obtained for each tooth with crown slices with a thickness of 80–100µm were examined under a light microscope (Leica Dental Corp, Wetzlar, Germany).	IC (Information content) and PRDU (Proportion reduction in diagnostic uncertainty)	Micro-computed tomography examination was the best method among the diagnostic techniques for the diagnosis of occlusal caries in terms of both IC and PRDU. M-CT examination supplied the maximum diagnostic information about the diagnosis of occlusal caries in the first (IC: 1.056; $p < 0.05$), (PRDU: 70.5%) and second evaluation (IC: 1.105; $p < 0.05$), (PRDU: 73.8%) for the first examiner. M-CT examination was the best method among the diagnostic techniques for the second examiner in both the first (IC:1.105; $p < 0.05$), (PRDU:73.8%) and second evaluation (IC:1.061; $p < 0.05$), (PRDU:70.8%).	The present study demonstrates that IC and PRDU can be used to evaluate diagnostic test performance when multiple disease states are being evaluated. Micro-computed tomography showed the best performance for both examiners in both evaluations, according to PRDU. It might have a role as an alternative to the histological method.

(Continued)

Table 1 (Continued)

<i>Author, year, country</i>	<i>Sample size and dentition/teeth/surface</i>	<i>Observers number/type</i>	<i>Histological evaluation and criteria description</i>	<i>Statistical analysis</i>	<i>Results and diagnostic agreement value</i>	<i>Main conclusions</i>
Boca et al., 2017, Belgium	60 teeth Permanent/Premolars and molars/approximal surfaces	four observers/unclear	Downer Histological Classification Criteria (Downer, 1975). one or two sections per tooth depending on the size and position of the lesion). Each cut surface was photographed with reflected light using a digital camera (Nikon) equipped with a macro lens. The exact magnification was determined from the image of a calibrated grid (10 lines/mm), placed on the histological sample at an identical focal distance and exhibiting the same magnification.	Cohen's κ , Spearman's rank correlation, Student's t test, Fleiss' κ statistics, Bland-Altman plots	Spearman's correlation coefficients (0.738 to 0.829, $p < 0.0001$) showed a good agreement between signs of carious lesions in the identical region obtained with both methods. Bland-Altman plots showed that 90.76% of the data points were within the limits of agreement.	Micro-CT imaging was shown to provide an interesting alternative to histological sectioning as detection method for carious lesions.
de Souza et al., 2014, Brazil	109 teeth; 38 primary teeth, 71 permanent teeth/molars, Premolars, and incisors/proximal surfaces	two experienced examiners/ dentists	CAST – Caries Assessment and Treatment index (De Souza et al., 2012) Downer Histological Classification Criteria (Downer, 1975). each half of the tooth Table under the same lighting conditions, using a Nikon D70 digital camera fitted with a Sigma 105 mm lens plus a close-up lens +10 (Polaroid, Minnetonka, MN, USA). Photographs were standardized for size, color, and contrast, using Adobe Photoshop CS5.	Validation was determined through calculation of the sensitivity, specificity, and Youden index for two categories of carious lesions examined visually, with histology and micro-CT as gold standards.	For the category 'healthy' vs 'diseased' (CAST codes 0–2 vs CAST codes 3–6), sensitivity, specificity, and Youden index values of 100%, 92.9%, and 93%, respectively, for micro-CT, and 96.6%, 86%, and 83%, respectively, for histology, were obtained. For the category 'dentine' vs 'non-dentine lesions' (CAST codes 0–3 vs CAST codes 4–6) sensitivity, specificity, and Youden index values of 90%, 100%, and 90%, respectively, for micro-CT, and 81.4%, 100%, and 81%, respectively, for histology, were obtained.	The two examiners employed in reading the histological and micro-CT images showed a very high level of agreement.

(Continued)

Table 1 (Continued)

<i>Author, year, country</i>	<i>Sample size and dentition/teeth/surface</i>	<i>Observers number/type</i>	<i>Histological evaluation and criteria description</i>	<i>Statistical analysis</i>	<i>Results and diagnostic agreement value</i>	<i>Main conclusions</i>
Kamburoğlu K et al., 2011, Turkey	21 teeth Permanent/ molars/ occlusal surfaces	three experienced examiners/ radiologists	Lesion depth was measured. Teeth were embedded in acrylic and serially sectioned mesio-distally in parallel to the long axis. For each tooth, the section with the deepest carious lesion was scanned using a high-resolution scanner (Epson Perfection V750-M Pro Scanner) at 600 dpi resolution and ×15 magnification and viewed using the AxioVision Rel. 4.7 image software (Carl Zeiss Imaging Solutions, Göttingen, Germany).	Intra-rater and inter-rater reliabilities were assessed according to the Bland/Altman method by calculating Intraclass Correlation Coefficients (ICCs). Mean/median values obtained with intraoral systems were lower than those obtained with 3-D and histological images for all observers and both readings. ANOVA	Intra observer ICC values for all observers were highest for histology and micro-CT.	Micro-CT was found to be the best imaging method for the <i>ex vivo</i> measurement of occlusal caries depth.
Kawato et al., 2009, Japan	27 teeth Permanent/ molars/ occlusal surfaces	one examiner /unclear	The extend of caries on the sliced images was scored on the following scale: 0 = sound; 1 = enamel caries limited to the outer half of the enamel; 2 = caries extending into the inner half of the enamel; 3 = caries penetrating the dentino-enamel junction; 4 = caries involving the inner half of the dentine. A histological examination was performed using a stereomicroscope.	Spearman's rank correlation coefficient, sensitivity, specificity	The extent of caries progression diagnosed with micro-Ct was the same as that revealed by the results of the histological examination in all six specimens.	The authors concluded that micro-Ct is useful for non-destructive examination in the evaluation of occlusal caries.

(Continued)

Table 1 (Continued)

<i>Author, year, country</i>	<i>Sample size and dentition/teeth/surface</i>	<i>Observers number/type</i>	<i>Histological evaluation and criteria description</i>	<i>Statistical analysis</i>	<i>Results and diagnostic agreement value</i>	<i>Main conclusions</i>
Mitropoulos et al., 2010, Greece	20 teeth Permanent/ Premolars and molars/ proximal surfaces	two experienced examiners/ dentists	ICDAS II The carious lesions were classified according to the grade disease: 0 = no lesion; E1 = caries extended to the outer half of the enamel; E2 = caries extending to the inner half of the enamel; D1 = caries extending to dentin. Tooth sections by the use of an optical microscope (20 magnification) (DM4000B, Leica, Wetzlar, Germany).	Cohen's κ , Spearman's coefficient, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Accuracy, McNemar test	The agreement between the histological and micro-CT examination. Both validation methods presented perfect agreement at scale 0. They showed substantial agreement at E2 and D1 scale, but they showed moderate agreement at scale E1. MCT did not agree with histological validation at each disease severity scale.	MCT is not capable of constituting a reliable alternative to histological examination for caries research.
Özkan et al., 2015, Turkey	60 teeth Permanent/ Premolars and molars/ occlusal surfaces	two experienced examiners/ unclear	ICDAS II Micro-CT: Scores of 1 (D1), and 2 (D3) were the cut-off points for the defined criteria Histological examination: Downer's criteria (Downer, 1975) Crown slices of ~100 μ m thickness were examined under a light microscope (Leica Dental Corp., Wetzlar, Germany)	Sensitivity, Specificity, Accuracy, AUC values, McNemar test Receiver operator characteristic (ROC) analysis was also performed for each method (Z-test; $p < 0.05$).	Besides showing a high correlation with histology results, micro-CT yielded the greatest values at the D3 threshold; moreover, accuracy and area under the ROC curve (AUC) values were greatest at the D1 threshold.	The results indicate that micro-CT performs as well as or better than histological examination for the purpose of comparing methods for caries detection.
Rathore, 2009, USA	45 teeth Permanent/ Premolars and molars/ occlusal surfaces	three observers / unclear	Presence or absence of caries was considered, and the results were compared with histology of the sectioned teeth. 250 μ m thin sections and viewed under a stereomicroscope.	κ scores, Sensitivity, Specificity,	Sensitivity and specificity for micro-CT were established using histology as the gold standard. Sensitivity was 0.88 and specificity 0.81. Interobserver agreement was calculated using κ and was reported as moderate to substantial (0.47–0.65) and the intraobserver agreement was substantial (0.62–0.73).	The initial data suggest that micro-CT can detect occlusal lesions in good agreement with histology. This may provide a viable alternative to histology in caries diagnosis.

(Continued)

Table 1 (Continued)

<i>Author, year, country</i>	<i>Sample size and dentition/teeth/surface</i>	<i>Observers number/type</i>	<i>Histological evaluation and criteria description</i>	<i>Statistical analysis</i>	<i>Results and diagnostic agreement value</i>	<i>Main conclusions</i>
Rovaris et al., 2018, Brazil	122 teeth Permanent/Premolars and molars/proximal surfaces	Histological examination: two experienced examiners/unclear Micro-CT examination: three experienced examiners/radiologists	The presence or absence of caries was evaluated. 0 = absence 1 = presence In case of a score 1, depth was also registered: 1 = lesion involving only the external half of the enamel; 2 = lesion involving the internal half of the enamel, and 3 = lesion involving dentine. 1000 µm thick sections in the mesiodistal direction tooth sections using a light microscope Leica DMLP (Leica Microsystems Inc., Wetzlar, Germany)	κ scores, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, McNemar test McNemar-Bowker test	The intraobserver and interobserver agreement for both methods ranged from moderate to excellent. There was no difference between both micro-CT methods and histology for the presence of lesions ($p > 0.05$). However, both methods differed with the reference standard for depth ($p < 0.05$). The disagreement occurred mostly in cases of enamel lesions. Micro-CT performed well in detecting caries lesions compared to histology, meanwhile the classification of their depth presented lower values.	Both protocols (180° and 360°) of micro-CT tested presented an overall satisfactory performance in detecting proximal caries lesions; however, for the depth classification, the method was not accurate.
Soviero et al., 2012, Brazil	25 teeth Primary teeth/molars/proximal surfaces	two experienced examiners/ dentists	Micro-CT and histological analyses used caries scores: 0 = sound; 1 = outer enamel; 2 = inner enamel; 3 = not spread dentine; 4 = outer dentine; 5 = inner dentine. Three to four sections of 500 µm, sectioned mesio-distally, were obtained from each proximal surface, the sections were examined with x15 magnification under stereomicroscopy (Opton, Japan).	Spearman's rank correlation coefficient, Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value, Accuracy.	Inter examiner agreement ranged from 0.87 to 0.93 κ coefficient (k). Histological analysis revealed a frequency of sound tooth surfaces (18.8%) enamel carious lesions (E1) (48%) and dentine carious lesions (D1) (33.3%). Micro-CT showed high correlation with histology ($r(s)0.88$). At both diagnostic thresholds (E1 and D1), sensitivity and accuracy were higher for micro-CT. Inter device agreement between micro-CT and histology was $k = 0.81$.	Micro-CT can be used as a gold standard for detecting carious lesions in proximal surfaces in primary molars.

(Continued)

Table 1 (Continued)

Author, year, country	Sample size and dentition/teeth/surface	Observers number/type	Histological evaluation and criteria description	Statistical analysis	Results and diagnostic agreement value	Main conclusions
Taylor et al, 2010, England	69 teeth Permanent/Teeth unclear/occlusal surfaces	two examiners – Histology a third examiner – micro-CT data/ unclear	caries scores: Sound, indicating no disease; E1 or E2, indicating caries in the first or second half of enamel, respectively; EDJ, indicating that the lesion has reached, but not penetrated, the enamel-dentine junction; or dentine, indicating that the dentine has been affected. Teeth were trimmed until square using a dental model grinder, and then one edge was ground until dental tissue was exposed. The exposed section was polished on lapping paper, and photographed, using a Jai CV-M91 camera (Jai A/S, Copenhagen, Denmark).	The teeth were ranked by the automatically assigned caries progression (CP index) and this was compared to the ranks assigned by the histological and manual CT examinations using Spearman's ρ . The automatic measurement of the depth of dentine lesions was compared to the equivalent measurement based on histological and manual CT examinations using and intra class correlation coefficient.	The results of the automated analysis correlate well with histological assessment with a ρ of 0.80 ($p < 0.001$), and with manual CT assessment with a ρ of 0.85 ($p < 0.001$). The depth of dentine lesions correlated with histology with an intra class correlation coefficient of 0.82 ($p < 0.001$; $N = 45$) and with manual assessment with an ICC of 0.93 ($p < 0.001$; $N = 39$). Micro-CT is found to generally underestimate caries compared to histological assessment.	The algorithm presented can successfully segment micro-CT scans into occlusal enamel and dentine regions, and the results show that the depths of dentine caries lesions can be accurately and objectively measured automatically using micro-CT. However, if enamel caries is to be accurately assessed by a computer, better scans will be required than those used here.

Additional analysis

A diagnostic table (Table 2) was constructed with data provided from the included studies where prevalence and accuracy records for micro-CT (sensitivity, specificity, PPV (%), NPV (%), LR+, LR-, DOR and Youden's index) are shown.

Regarding PPV, the best results demonstrated that micro-CT is capable to distinguish enamel caries lesion from sound tissue in 81.5% of the times.^{10,11} Similar result was found for NPV where micro-CT discriminated sound enamel from enamel caries lesion in 95.4% of times.¹⁰

For dentine caries detection, micro-CT showed better performance than for caries in enamel with the higher results for PPV and NPV being 90.2 and 95.5% of times, respectively.

LR tests indicate the proportion of individuals with (+) the disease that tested positive or the proportion of individual without (-) the disease that tested negative for the disease. According to the results, LR+ was higher than 1.00 in most of the studies, which means that micro-CT assert the diagnostic of interest. LR- lower than 0.3 indicates acceptable diagnostic accuracy test and the results showed that only Al Jamaan et al¹⁰ presented this value for

both, enamel and dentine caries diagnostic in micro-CT, meaning low probability of the disease where it is absent.

Values of DOR showed that micro-CT has good discriminatory test performance, except in the study of mitropoulos that for enamel DOR was near or equal to zero.

Regarding Youden's index, Al Jamaan et al¹⁰ and Boca et al¹¹ showed high accuracy for enamel and for dentine caries lesion detection in micro-CT.

The selected cutoffs are presented in Supplementary Material 2. None of the values is validated or present consensus in the literature. Table 3 shows the summary of micro-CT technical aspects and image analysis of the included articles.

Confidence in cumulative evidence

Although the systematic review was performed with *in vitro* studies, the decision was made to apply the approach. A moderate quality of evidence for the outcome accuracy of micro-CT when compared to histology for caries detection was found for pooled results considering both, meta-analysis and qualitative synthesis. The main flaws were in "risk of bias" with

Figure 2

uncertainty of eligibility criteria and randomization of the teeth and uncertainty of the blinding of examiners. Besides, not all teeth were evaluated with both tests and interexaminer agreement was lacking in some studies. A summary of findings is presented on Supplementary Material 4.

Discussion

This systematic review has evaluated the validity of micro-CT for *in vitro* caries detection in comparison

with histology as the reference standard. Despite the histological inspection is the most-used reference standard for caries lesion depth validation, this method presents some disadvantages. During the preparation of the sections for the microscope examination, a substantial loss of material occurs, between 20 and 250 µm per slice. If we consider the difficulty in obtaining the donation of human teeth for research and the need to study incipient lesions, micro-CT is preferable for studies into demineralization and remineralization due to its non-destructive nature.^{1,8} Confocal microscopy and

Figure 3

transversal microradiography as a validation method also require destructive sample preparation. On the other hand, micro-CT has been increasingly used with *in vitro* studies due to the possibility of the non-destructive observation of the sample,²³ as well as other advantages

inherent to the method, such as: multiplanar and volumetric evaluation (3D) of the sample, preservation of the specimen's physical integrity as it does not need to be sectioned and it also provides mineral density evaluation.^{24,25}

Figure 4

Table 2 Diagnostic accuracy of the index test Micro-CT

Surface	Author	Sample	Prevalence	Sensitivity	Specificity	PPV (%)	NPV (%)	LR+	LR-	DOR	Youden's index
E	Al Jamaan 2016	203	0,21	0,83	0,95	81,52	95,46	16,60	0,18	92,76	0,78
E	Boca et al 2017	354	0,39	0,55	0,92	81,47	76,18	6,88	0,49	14,06	0,47
E	Mitropoulos et al 2010 examiner 1	22	0,27	0,5	0,87	58,72	82,47	0,00	0,57	0,00	0,37
E	Mitropoulos et al 2010 examiner 2	23	0,3	0,28	0,87	48,00	73,82	2,15	0,83	2,60	0,15
D	Al Jamaan 2016	192	0,16	0,77	0,94	70,97	95,55	12,83	0,24	52,45	0,71
D	Boca et al 2017	443	0,51	0,71	0,92	90,23	75,30	8,88	0,32	28,16	0,63
D	Mitropoulos et al 2010 examiner 1	34	0,52	0,61	0,87	83,56	67,31	4,69	0,45	10,47	0,48
D	Mitropoulos et al 2010 examiner 2	34	0,52	0,66	0,87	84,62	70,26	5,08	0,39	12,99	0,53

*Data not available in the original article. The authors calculated data from information available in the article.

Our main results demonstrate that for enamel caries diagnostic, sensitivity values presented high variability ranging from poor to excellent. On the other hand, specificity gave good-to-excellent micro-CT capability to identify the true negative. For dentine caries diagnostic, sensitivity values were less disparate indicating fair-to-good probability of micro-CT to identify the true positives, while specificity varied from good-to-excellent for caries detection on proximal surfaces in permanent teeth. For primary teeth, micro-CT showed to be a sensitive and accurate method for both enamel lesions and dentin.^{7,8} The authors found a high degree of accuracy (0.93 and 0.96)⁷ and an excellent sensitivity and specificity for both enamel and dentin lesions.⁸ Also, a very strong micro-CT association was observed with the polarized light microscope analysis, for both enamel and dentin lesions (0.96).⁷ In other words, they concluded that there is a response match with the verification of the progression of the caries lesion through micro-CT and polarized light microscope examination. Even if the test were negative, there is still an 88% chance of the proximal surface being healthy versus 12% having caries in the enamel. Thus, the excellent performance in detecting initial lesions in enamel and with the differentiation of lesions in enamel and dentin, strengthens the argument for micro-CT being used as the reference standard for the evaluation of proximal surfaces.^{7,8,13,14} Rovaris et al¹² concluded that micro-CT has shown a higher number of images compatible with caries lesions than histology, a conflicting data that can indicate loss of material containing affected tissue during the preparation of histological specimen or the presence of artifacts that mimics caries lesions.

A significant variation among diagnostic accuracy values was observed among included studies. The use of different validation methods and scores could result in a diversity of results and influence the accuracy of the tests being evaluated.²⁶ Some studies^{4,7,8} adopted the 5-point polarized light microscope scoring system conceived by Ekstrand et al²⁷ for polarized light microscope validation. In this study, scores were used for the absence and presence of carious lesions on proximal surfaces, in accordance with the standardized depth of the lesion for the modes tested, in a similar manner to the system adopted by Ekstrand et al,²⁷ however, a “pure”

comparison of the results of this study is “unviable” as the diagnostic studies classified carious lesions in micro-CT and polarized light microscope images, using different criteria, such as those of some authors^{27–30} as well as modified criteria.

Furthermore, validating diagnostic methods with ordinal variables or even continuous variables with a dichotomous or dichotomized reference standard is very controversial, because it reduces the continuous progression of the lesion to just two stages, even with the use of the ROC curve for the analysis. Gaalaas et al¹³ advocate the ROC curve analysis in relation to the presence or absence (dichotomous evaluation) of caries, often used to summarize the overall performance of the diagnostic test through the calculation of the AUC, as it removes the effect of different diagnostic options in the observers' decision-making process and it provides a better overall indication of the accuracy of the method to be compared with others. On the other hand, the studies to validate the caries diagnostic methods must compare the extent or the stage of the lesions with the corresponding stage of the reference standard.

According to Table 3, it is possible to verify that different technical parameters were adopted in micro-CT image analysis of the included articles. The heterogeneity among studies could be attributed to different micro-CT machines, X-rays energy parameters, detector characteristics, different sample sizes, beam hardening correction algorithm, calibration, segmentation, contrast, experience and number of examiners. The methodology of histological validation (Table 1) also shows large variations such as: the use of stereomicroscope or light microscope, reflected or transmitted light, magnification factor (from 2.5 to 100), dry or wet inspection, the use of dyes, the number of scores assigned (from 3 to 6 or measurement of depth) and the thickness of sections. Several of these factors may be expected to influence the detection of lesions, and the need for standardization is clear.¹

As the enamel is a high density tissue, micro-CT companies recommend the usage of machines that works with higher kVp and a full scan (360°) to permit the X-rays to be enough penetrating and pass through the teeth without generating too many artifacts. Despite the recommendations, even using 50 kVp the studies^{6,9,12,21}

Table 3

Author, year, country	Micro CT unit	Acquisition parameters	Other corrections	Reconstruction information	Analysis	Other considerations*
Al Jumaan, 2016, Scotland	µCT 40 Scanco system (Scanco Medical AG, Bassersdorf, Switzerland)	70 kVp 114 µA Voxel size 20 µm 500 projections 180° partial scan 0.36° rotation step two frames Scan time: two to 3.5 h Filter: 0.5 mm Al	Crowns were separated from the roots	Carried out by cone-beam algorithms in 1024 × 1024 pixels matrices All 1024 × 1024 pixel images were acquired in DICOM 16 bit images.	21 inch TFT computer screen. Image 1 (image selection dynamic, image analysis) JPEG static-slice (unclear), In order to compare Micro-CT images with histological sections only tomograms in the sagittal 3. The author related some loss of histological (mesio-distal) plane were examined. Gray scale of background noise eliminated (?) Contrast set at 20% (further enhance lesion)	(1) Teeth storage: saline 0.1% thymol crystals (2) Pairing of Micro-CT and histological images: composite resin right angled isosceles triangle 3. The author related some loss of histological sections during the sample sectioning (59 surfaces) (1) Calibration of the system: five different hydroxyapatite rods (0.11, 200, 400, 800 mg/cm3). Weekly basis (2) Sectioning: serial sections (1) Teeth storage: formalin (2) Sectioning: two to four sections were obtained for each tooth from the suspected area in the buccolingual direction (occlusal lesions)
Arslan et al., 2014, Turkey	SkyScan 1774 unit (SkyScan, Kontich, Belgium).	50 kV 800 µA Voxel size 15 µm 1° rotation step Scan time: 73 min. Filter - unclear Matrix 1024 × 1024	Tooth crowns separated from the roots to reduce ring artifacts.	NRecon for reconstruction	Coronal and sagittal images. No information regarding static or dynamic and evaluation mode.	(1) Teeth storage: formalin (2) Sectioning: two to four sections were obtained for each tooth from the suspected area in the buccolingual direction (occlusal lesions)
Boca et al., 2017, Belgium	SkyScan-1076 (SkyScan, Bruker, Kontich, Belgium)	100 kV 100 µA Voxel size 18 µm 293 projections 180° partial scan Rotation step 0.7° (20–100 keV, focal spot size 5 µm) Filter: 1 mm Al	Ring artifact reduction and beam-hardening correction	NRecon software, optimal contrast limits, ring artifact reduction and beam-hardening correction	Proprietary designed software (scoring) Observers calibration (15 training slides) 21° high-quality computer monitor Slices (enlarge slices of MCT and invert black and white)	(1) Teeth storage: moist environment (2) Pairing of Micro-CT and histological images: Lego home-made device and registration method. (3) The author related some loss of histological sections during the sample sectioning (five teeth). (4) Sectioning: one or two sections per tooth ("successive grinding").
de Souza et al., 2014, Brazil	SkyScan 1076; SkyScan, Kontich, Belgium	100 kV 100 µA Voxel size 9.05 µm 0.3° rotation step four frames. Mean scan time: 92 min Filter: 1 mm Al	The images were corrected for beam hardening (80%), smoothing (two levels), and ring-artefact (eighth to 11th position). The grey-scale range was set from 0.000198 to 0.059438 Hounsfield Units.	NRecon software (Kontich, Belgium).	13" liquid-crystal display (LCD) screen Axial and sagittal slices (dynamic?)	(1) Teeth storage: 0.1% thymol solution. (2) Teeth sealed and restored were also included.
Kamburoglu K et al., 2011, Turkey	SkyScan 1174 (SkyScan, Kontich, Belgium)	50 kV 800 µA Voxel size 18 µm	No information	No information	Mimics software (previous image calibration). Dynamic (axial, sagittal and coronal).	(1) Teeth storage: distilled water. Each tooth was embedded in a block of silicone impression material (2) Calibration of Micro CT image size, but not density. (3) Teeth with no restorations only. (6) Sectioning: the section with the deepest carious lesion.
Kawato et al., 2009, Japan	SMX 130 CT – Shimadzu Corporation, Kyoto, Japan	60 kV 60 µA Matrix 512 × 512 Voxel size 28 µm	No information. Very noisy low quality image.	No information.	Static slices in various angles (2d image).	1. Teeth storage: 10% neutral buffered formalin solution. 6. Sectioning: Only six teeth were randomly selected and cut "in the plane" of the µCT.
Mitropoulos et al., 2010, Greece	SkyScan 1702, SkyScan, Kontich, Belgium	100 kV 98 µA Voxel size 8.09 µm Matrix 1024 × 1024 180° partial rotation 0.23° rotation step two frames 6 s exposure time per step Scan time: from 1.5 to 2 h. Filter: 1 mm Al	No information.	No information.	17 in. TFT computer screen under the same lighting and viewing conditions, without image modification. Unclear if static or dynamic. Manufacturer supplied software (?) (TView, Skyscan, Kontich, Belgium)	(1) Teeth storage: tap water at 37 °C. (2) Unrestored premolars. (3) Slices in the center of the suspected carious lesion.
Özkan et al., 2015, Turkey	SkyScan 1774 unit (SkyScan, Kontich, Belgium).	50 kV 800 µA pixel size of 15 µm Matrix 1024 × 1024 1° rotation step Mean scan time: 73 min	Crowns were separated from their roots to (reduce ring artifacts?)	NRecon software	SkyScan Data Viewer software Dynamic.	1. Teeth storage: 10% neutral-buffered formalin solution.

(Continued)

Table 3 (Continued)

Author, year, country	Micro CT unit	Acquisition parameters	Other corrections	Reconstruction information	Analysis	Other considerations*
Rathore, 2009, USA	Skyscan 1074h (Skyscan, Kontich, Belgium)	1000µA 40kVp 600ms Voxel size 20.4µm detector: 768 × 576 pixels, 8-bit, X-ray camera in on-chip integration mode with lens coupled to scintillator 200 projections 180° partial scan 0.9° rotation step	ring artifact reduction smoothing filter	NRecon software (dynamic range of 0.0500 to 0.69500)	Data viewer software: Dynamic.	(1) A dynamic range was established for the micro-CT using a set of 4 hydroxyapatite Rods (as phantoms to optimize the scan settings) - ranged in different densities from 50 mg/cc to 1000mg/cc. (2) Exclusion criteria: restorations (3) Sectioning: serial sections
Rovaris et al., 2018, Brazil	Skyscan 1174 device (Bruker, Kontich, Belgium)	50 kV 800 µA Voxel size 28.15µm Rotation step 0.4° four frames 360° full scan Scan time: 1 h 50 min Filter: 1.0mm Al	smoothing "1," ring artifacts reduction between "5" and "10," beam hardening correction "0," and automatic misalignment correction.	NRecon Software reconstruction in 180 and 360 modes 900 basis images(360) 450 images basis (180) Reconstruction parameters: smoothing "1," ring artifacts reduction between "5" and "10," beam hardening correction "0," and automatic misalignment correction.	Data Viewer Software Dynamic.	1. Teeth storage: distilled water (1) The author highlighted the weakness of histology: (2) Partial or total coronal destruction excluded. (3) Sectioning: teeth serially sectioned in mesio- distal direction - no reference was follow.
Soviero et al., 2012, Brazil	Skyscan 1076 Micro-CT (Skyscan, Kontich, Belgium)	100 kV 100 µA 9.05 µmvoxel size 0.38° rotation step four frames Mean time: 1 h 27 min Filter: 1.0mm Al	Beam-hardening and smoothing correction set at 80% and two levels, respectively. Ring artifact correction ranged from the eighth to the 11th position. Grey scale range was set from 0.000198 to 0.059438 HU.	NRecon software (Skyscan, Kontich, CT Analyzer software (Skyscan, Kontich, Belgium). Beam-hardening and smoothing correction set at 80% and two levels, contrast, respectively. Ring artifact correction ranged from when the contrast was set at 20% and the color mode was used).	(Skyscan, Kontich, Belgium). Dynamic: slice by slice, first with no alteration of contrast. (Shallow carious lesions in enamel were confirmed mode was used).	(1) Teeth storage: 0.1% thymol. (2) No restorations and no enamel developmental defects were selected for the study extensive cavities were also excluded. (3) Teeth Sectioning: First buccal-lingual section (two halves), and after three to four sections of 500mm, sectioned mesio-distally.
Taylor et al, 2010, England	SkyScan 1072 Microtomograph (Skyscan, Kontich, Antwerp, Belgium).	100kV 98 µA 400 projections 360° full scan 6.5s exposure Matrix up to 1024 × 1024×1024 voxels. Reduction to 128 × 128 × 128 voxels (Reduction of size on memory and processing time) Filter: 1mm Al	Artifact reduction and beam hardening correction.	NRecon (Sky- Scan) Artifact reduction and beam hardening correction.	Dynamic ("sliced" "manually") AND An algorithm to detect and measure demineralization	(1) Pairing of Micro-CT and histological images. Each photograph (section) was accompanied by a photograph of the occlusal surface and a measurement of the remaining resin block, to locate the section within the tooth. (2) Serial histology.

showed satisfactory results on caries diagnosis. Other authors^{4,10,11,22} that used 180° (partial scan) worked with high energy machines (70–100kVp) which may have minimized the expected and undesired effects of a partial scan of a dense structure. On the other hand, the study that compared partial and full scan used a weak X-ray source (50kVp) and did not find statistical difference between these protocols.¹²

Only Al Jamaan *et al.*¹⁰ reported excellent sensitivity for enamel detection in permanent teeth proximal surface with micro-CT, even using 70kVp. The authors, however, acquired the images in a different machine brand. It is worth mentioning that different machine brands were not included and compared using the same samples.

The risk of bias was considered as low in half of the included studies, however, our systematic review has some limitations that should be considered when interpreting the findings of the studies reviewed. Moreover, due to high methodological heterogeneity in the included studies the results should be considered with caution. Standardized methodology should be adopted in future studies in order to obtain homogeneity among studies. The risk of bias assessment demonstrated that some studies did not describe inclusion/exclusion criteria for sample selection or included restored teeth. Another point to be highlighted is related to the sample size. The sample size ranged widely from 20 to

140 teeth. The small sample sizes may impair statistics. In addition, some studies did not report if the crowns were separated from the roots before the analysis or the sectioning were performed in the middle of the lesions before analysis, experience and number of examiners, parameters which could influence the detection of lesions. We also found that studies using well-established scoring systems showed higher accuracy in the detection of lesions when compared with studies that did not report the method used. Standardize the methodology in micro-CT is a challenging task, taking into account the number of machines, engineering issues (*e.g.*, detector characteristics, algorithm solutions), heterogeneity of experimental conditions and different research questions. However, we speculate that for researches involving incipient caries lesions and micro-CT, some parameter should be minimally adopted in order to standardize methodology. Future studies should consider the points that affect the risk of bias of included studies in order to improve the level of scientific evidence regarding the validity of micro-CT for *in vitro* caries detection.

In conclusion, the current evidence demonstrated the validity of micro-CT for *in vitro* caries detection in proximal surfaces of permanent teeth in comparison with histology with moderate confidence. However, further future studies with standardized methodology are encouraged.

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