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# Development of a Root Caries Prediction Model in a Population of Dental Attenders

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## Keywords

Caries prediction · Prognostic study · Root caries

## Abstract

Root caries prevalence is increasing as populations age and retain more of their natural dentition. However, there is generally no accepted practice to identify individuals at risk of disease. There is a need for the development of a root caries prediction model to support clinicians to guide targeted prevention strategies. The aim of this study was to develop a prediction model for root caries in a population of regular dental attenders. Clinical and patient-reported predictors were collected at baseline by routine clinical examination and patient questionnaires. Clinical examinations were conducted at the 4-year timepoint by trained outcome assessors blind to baseline data to record root caries data at two thresholds – root caries present on any teeth (RC > 0) and root caries present on three or more teeth (RC ≥ 3). Multiple logistic regression analyses were performed with the number of participants with root caries at each outcome threshold utilized as the outcome and baseline predictors as the candidate predictors. An automatic backwards elimination process was conducted to select predictors for the final

model at each threshold. The sensitivity, specificity, and c-statistic of each model's performance was assessed. A total of 1,432 patient participants were included within this prediction model, with 324 (22.6%) presenting with at least one root caries lesion, and 97 (6.8%) with lesions on three or more teeth. The final prediction model at the RC >0 threshold included increasing age, having ≥9 restored teeth at baseline, smoking, lack of knowledge of spitting toothpaste without rinsing following toothbrushing, decreasing dental anxiety, and worsening OHRQoL. The model sensitivity was 71.4%, specificity 69.5%, and c-statistic 0.79 (95% CI: 0.76, 0.81). The predictors included in the final prediction model at the RC ≥ 3 threshold included increasing age, smoking, and lack of knowledge of spitting toothpaste without rinsing following toothbrushing. The model sensitivity was 76.5%, specificity 73.6%, and c-statistic 0.81 (95% CI: 0.77, 0.86). To the authors' knowledge, this is the largest published root caries prediction model, with statistics indicating good model fit and providing confidence in its robustness. The performance of the risk model indicates that adults at risk of developing root caries can be accurately identified, with superior performance in the identification of adults at risk of multiple lesions.

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## Background

Globally, dental caries is the fourth-most expensive disease to treat [Petersen, 2008], and there is evidence that the burden of untreated caries is now shifting from children to older adults. This peak in prevalence in adults around 70 years old is attributed to the development of root caries [Kassebaum et al., 2015]. Root caries prevalence is increasing as populations age and retain more of their natural dentition [Müller and Schimmel, 2007; HSCIC, 2011; Slade et al., 2014; Schwendicke et al., 2018]. There is a wide range in the reported prevalence of root caries for differing populations, ranging from 4 to 100% [Fejerskov et al., 1991; Christensen et al., 2015]. Root caries can be associated with pain, discomfort, and tooth loss [Fure and Zickert, 1997; Slade et al., 1997], the latter impacting most significantly upon the oral health-related quality of life (OHRQoL) of the elderly [Slade et al., 1996a, b].

Root caries is a preventable disease; however, approximately one-third of the older population bears the majority of the root caries burden [Petersson et al., 2003; Griffin et al., 2004]. However, there is generally no accepted practice to accurately identify individuals at high risk of root caries [Ritter et al., 2010]. Reported failure rates of root surface restorations are higher than coronal restorations [Hayes et al., 2016; Meyer-Lueckel et al., 2019], supporting the need for targeted disease prevention to avoid invasive treatment. Identification of individuals at increased risk of developing root caries would maximize the cost-effectiveness of delivering targeted prevention measures [Schwendicke and Göstemeyer, 2017]. Prediction models allow the inclusion of multiple risk factors to predict the future development of a disease [Collins et al., 2015].

Two systematic reviews have been conducted to investigate root caries predictors – one included longitudinal observational studies to investigate risk factors and the other included published root caries risk models. The systematic review of root caries predictors from longitudinal observational studies included 16 studies assessing the association between root caries development and a broad range of socio-demographic, general health, and oral health factors [Zhang et al., 2019]. The review found a positive correlation between age and root caries in four of the seven studies in which this variable was investigated. The three studies that did not find a positive correlation were conducted in populations over the age of 60 years which may not have allowed sufficient discrimination between participants of different ages. A positive correla-

tion was also found in all six of the studies investigating an association with previous root caries experience and in five of the seven studies investigating smoking.

The systematic review of root caries risk models included 13 published risk models and 95 tested predictors [Ritter et al., 2010]. The review reported the number of times a predictor was tested and the number of times each predictor was significantly associated with development of root caries. There was little overlap in the predictors included in risk models, with the predictors included in at least three separate models – root caries at baseline, number of teeth, and plaque index. However, the direction of association of number of teeth with root caries was not consistent and inconclusive. A significant association with root caries was found in two out of ten studies investigating age [Locker, 1996; Phelan et al., 2004] and one of nine studies investigating smoking [Phelan et al., 2004]. This review concluded there is a need for the development of root caries prediction models in well-conducted studies with a large sample size, representative population, and adequate follow-up time to better guide targeted prevention strategies [Ritter et al., 2010]. The aim of this study was therefore to develop a prediction model for root caries among a population of regular dental attenders.

## Methods

### *Source of Data*

This prognostic study was conducted within a randomized controlled trial set in UK primary care dental practices (INTERVAL Dental Recalls Trial). INTERVAL was a National Institute for Health Research (NIHR)-funded trial comparing the effects of different dental recall intervals on oral health which has been previously published [Clarkson et al., 2021]. Participants were randomized to attend for a routine dental check-up at one of three frequencies – 6-monthly, 24-monthly, or at a personalized risk-based frequency, ranging from 3 to 24 months, based on an individual's likely risk of developing oral disease (based on the NICE Clinical Guideline CG19) [National Institute for Health and Care Excellence (NICE), 2004]. Participants were recruited between July 2010 and July 2014, with follow-up completed on the 13th of August 2018. Data for this study were collected at two timepoints – predictors were collected at baseline from a clinical examination and patient-reported questionnaire which captured data on patient demographics and oral health characteristics. The clinical outcomes were collected at the 4-year post-randomization follow-up timepoint.

### *Participants*

Dentate adults aged over 18 years who received at least some of their dental treatment under the state-provided National Health Service (NHS) were recruited within the INTERVAL Trial. Participants underwent clinical examination by their primary care

dentist at trial entry to confirm inclusion and to provide the opportunity for participants to be made dentally fit. Patients with uncontrolled medical conditions or at increased risk of bleeding were excluded.

#### *Primary Outcomes*

Clinical examinations at the 4-year timepoint were conducted by trained outcome assessors blind to participant baseline data and intervention arm allocation. The root caries classification system used was a modification of the International Caries Detection and Assessment System (ICDAS II) [Ismail et al., 2007; International Caries Detection and Assessment System (ICDAS) Coordinating Committee, 2005]. Root caries was defined as a demarcated area on the root surface or at the cemento-enamel junction which is discoloured and cavitated (with loss of anatomical contour  $\geq 0.5$  mm), and soft on tactile assessment, with no resistance to gentle probing. Secondary caries around an existing root surface restoration was recorded in the same manner as a primary root caries lesion. Root caries was recorded as present or absent on each root surface of every tooth, excluding third molars. Given the evidence to support the cost-effectiveness of root caries prevention in high-risk adults [Schwendicke and Göstemeyer, 2017], two primary outcomes of interest were selected: participants with root caries present on at least one tooth ( $RC > 0$ ) and participants with root caries present on three or more teeth ( $RC \geq 3$ ) – a threshold used in previous studies to define high-risk individuals [Powell et al., 1991]. Prediction models were developed at both thresholds.

Training in the ICDAS clinical caries criteria and collection of caries clinical outcomes was delivered by an international cariologist (DR) involved in the development of the ICDAS criteria and experienced in the use of ICDAS in clinical research. Training in caries assessment included completion of a preliminary ICDAS e-learning course specifying the diagnostic criteria followed by didactic face-to-face training. This training involved use of clinical photographs and assessment of extracted teeth representing lesions at all severity and cavitation levels. This was carried out independently by all outcome assessors followed by discussion and consensus. This was followed by assessment of a cohort of 15 patient volunteers, specifically recruited for outcome assessor training, who were similar in age and dental attendance behaviour to the study population, with an emphasis on consistency of the examination process and agreement of diagnostic criteria. Assessor training was provided 1 month before the first trial outcome assessment and was repeated mid-way through the outcome assessment period. Outcome assessors also met regularly throughout the outcome assessment period to conduct the assessment protocol on extracted teeth with carious lesions at all stages of lesion progression and cavitation in order to enhance the consistency of the outcome assessment.

#### *Candidate Predictors*

All candidate predictors collected at baseline in INTERVAL were considered for inclusion in the model [Clarkson et al., 2021]. Predictors were selected following a scoping review to identify relevant reviews on the topic, critical consideration of the literature available, and knowledge of dental experts in cariology.

Demographic characteristics collected included participant age, sex, participant-reported dental attendance pattern (regular or irregular), time since their previous dental appointment (less than 1 year or more than 1 year), smoking status in the preceding 12 months (smoker or non-smoker), type of dental treatment re-

ceived (NHS, private, or a combination of NHS and private treatment), type of toothbrush used (electric or manual), and difficulty in travelling to dental appointments. Difficulty in travelling to their dentist was scored from 1 to 7 on a Likert scale, where lower scores reflected greater difficulty in travelling to the dentist.

Oral health-related attitude was measured using seven questions, each with a seven-point scale varying from 1 (strongly disagree) to 7 (strongly agree), with higher scores reflecting more positive oral health-related attitudes. The final score was the average of the individual item scores.

Oral health knowledge of the practices that should be performed was measured using three questions related to oral health – frequency of toothbrushing (twice a day or more, or once a day or less), duration of toothbrushing (2 min or more, or less than 2 min), and correct action following toothbrushing (rinse mouth with water, rinse mouth with mouthwash, or spit without rinsing). Current recommendations from Public Health England discourage rinsing with water or mouthwashes after toothbrushing and instead encourage spitting out excess toothpaste without rinsing ([Public Health England] Delivering Better Oral Health: an Evidence-based Toolkit for Prevention 3rd edition). Oral health behaviour was measured in a similar way, using three questions related to each participant's oral health behaviour.

Dental anxiety status was measured using the Modified Dental Anxiety Scale (MDAS) [Humphris et al., 2000]. MDAS is a recognized and validated psychological tool comprising five questions relating to different dental treatments and scenarios. The questions are scored on a scale from 1 (not anxious) to 5 (extremely anxious) and added together to produce a total score ranging from 5 to 25, with higher scores reflecting increasing dental anxiety. A score of 19 or higher is considered to reflect high dental anxiety [Humphris et al., 2000].

OHRQoL was measured using the Oral Health Impact Profile-14 (OHIP-14) tool [Slade, 1997]. The OHIP-14 is a 14-question oral health-specific patient-centred measure referring to symptoms in the past 12 months. The questions are scored from 0 (never) to 4 (very often) and added together to produce a total score ranging from 0 to 56, with higher scores reflecting worsening OHRQoL.

Prior to intervention allocation, primary care dentists provided baseline clinical data for each participant to record the number of restored teeth ( $<9$  or  $\geq 9$  restored teeth), gingival bleeding on probing (present or absent), and suitability to be recalled at an interval of 24 months between check-up appointments (yes/no) as an indicator of the participant's oral disease risk. Each participant's postcode was used to identify their index of multiple deprivation (IMD) quintile. The IMD is derived from patient postcode and is the official national measure of deprivation. It provides a combined measure of household income, education level, employment type, healthcare provision, crime, and living environment for the UK at small spatial levels. The use of these indices is commonplace in health research [Abel et al., 2016]. As participants were recruited from across all four UK nations, with each nation constructing different national-level IMDs, each nation's IMD was converted to a UK IMD to allow comparison between participants from across the UK [Abel et al., 2016]. Routinely collected NHS data were used to identify participants who qualified for exemption from paying for NHS dental treatment. The exemption status linked to the course of treatment closest to the trial entry date was utilized as the participant's exemption status during statistical analysis.



### Sample Size

Sample size was influenced by the number of participants in INTERVAL who attended a follow-up clinical examination. Only those participants with root caries data recorded at the clinical assessment at the 4-year follow-up timepoint were included in the model.

### Missing Data

A complete case analysis approach was used in developing the prediction model, where only participants with a complete set of predictors were included in the analysis. Sensitivity analysis with multiple imputation (MI) was utilized to assess the effect of missing data on the selection of predictors by imputing missing candidate predictors. Participants with a missing clinical outcome were excluded from all analyses.

### Statistical Analysis

Continuous candidate predictors were assessed for linearity between predictors and the log odds of both root caries outcomes, which confirmed all relationships to be linear. The deprivation quintile was assessed for linearity in the same way to confirm appropriateness of incorporating as a continuous variable. In order to develop a final prediction model for  $RC > 0$ , the following statistical analyses were undertaken, and this process repeated at the  $RC \geq 3$  threshold:

1. Univariate (outcome and single candidate predictor)
2. Multiple
  - Full model: including all potential predictors
  - Final model: including the selected predictors

Initially, univariate logistic regression analyses were performed with the number of participants with root caries at the  $RC > 0$  threshold as the outcome. The candidate predictors included as categorical variables were sex, suitability for a 24-month recall interval, number of restored teeth at baseline, gingival bleeding on probing, exemption from payment for NHS dental services, smoking status, participant-reported dental attendance pattern, time since participant's previous dental appointment, type of dental treatment received, type of toothbrush used, toothbrushing frequency behaviour, toothbrushing duration behaviour, after brushing behaviour, toothbrushing frequency knowledge, toothbrushing duration knowledge, and after brushing knowledge. Candidate predictors included as continuous variables were age, difficulty travelling to dental appointments, dental anxiety, OHRQoL, oral health attitude, and deprivation quintile.  $p$  values less than 0.05 were considered statistically significant.

All candidate predictors were included in a full model using multiple logistic regression analysis. Finally, an automatic backwards elimination process was conducted to select predictors for the final reduced model. All candidate predictors were initially included, then the predictor with the  $p$  value closest to 1 was removed, and the model rerun. This process was repeated sequentially until no other non-significant candidate predictor could be removed.  $p$  values less than 0.05 were considered statistically significant. Each model's performance was assessed using measures of discrimination including sensitivity, specificity, and c-statistic. Sensitivity and specificity were based on the prevalence of root caries at each outcome threshold within the study population. Model performance was also assessed by visual inspection of calibration.

As the statistical analysis was conducted in a population within a randomized controlled trial, sensitivity analysis was conducted, incorporating the intervention arm each participant was randomized to, in order to test the effect of this intervention on the accuracy of the risk model [Groenwold et al., 2016]. The same process was followed for the development of the prediction model at the  $RC \geq 3$  threshold.

## Results

A total of 2,372 patient participants were recruited from 50 primary care dental practices within the INTERVAL Trial, with clinical outcome assessment at the 4-year follow-up point. Of this recruited sample, 1,624 participants attended a clinical follow-up assessment appointment (68.5% of the total randomized). Presence or absence of root caries was recorded for 1,432 participants for the  $RC > 0$  teeth threshold, and 1,430 for  $RC \geq 3$ , with missing outcome data for two participants at this threshold. The flow of participants through the study is outlined in Figure 1.

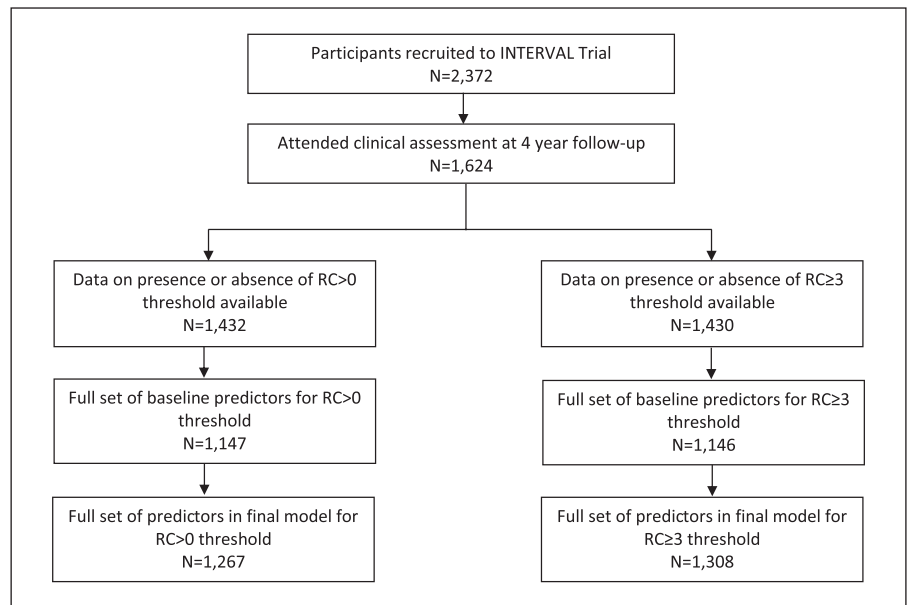
A total of 324 (22.6%) participants had at least one root caries lesion, and 97 (6.8%) participants had root caries lesions on 3 or more teeth. Of these 324 participants, the mean number of teeth with root caries lesions was 2.3 (SD 1.8, range 1–10). For the 97 participants with root caries lesions on 3 or more teeth, the mean number of teeth with root caries lesions was 4.5 (SD 1.8).

The characteristics of study participants are summarized in Table 1. The average age of participants was 50 years. The majority were women (57.1%) and self-reported regular dental attendees (88.1%).

Most participants performed toothbrushing twice a day or more (76.9%), and 62.1% reported taking 2 min or longer to brush. 50.8% reported rinsing with water following toothbrushing while 26.3% reported that following toothbrushing they spit toothpaste without rinsing with either water or mouthwash.

Mean OHIP-14 scores were low (5.3 [SD 6.6]) indicating good OHRQoL. Mean dental anxiety scores (10.0 [SD 4.3]) indicated low anxiety. In general, participants reported good knowledge and behaviour about the frequency and duration of toothbrushing; however, they were less informed about what to do after toothbrushing (i.e., spit do not rinse).

Missing data rates for each predictor are presented in the online supplementary Table S1 (for all online supplementary material, see [www.karger.com/doi/10.1159/000526797](http://www.karger.com/doi/10.1159/000526797)). The predictor with the highest percentage of missing data was type of dental treatment received (10.1%) and overall, the rates of missing data were low.



**Fig. 1.** Flow of participants from recruitment to collection of clinical outcomes and presence of full set of baseline predictors.

#### Development of a Prediction Model for $RC > 0$

Table 2 presents the univariate analysis at the  $RC > 0$  threshold. Participants had higher odds of disease where they had  $\geq 9$  restored teeth at baseline (OR 2.3 [95% confidence interval [CI]: 1.7, 3.0],  $p < 0.001$ ), were smokers (OR 1.8 [1.3, 2.5],  $p = 0.001$ ), reported the behaviour rinsing with water after toothbrushing (OR 1.8 [1.3, 2.6],  $p < 0.001$ ) or mouthwash (OR 1.7 [1.1, 2.6],  $p = 0.012$ ) rather than to spit toothpaste and not rinse, or where they reported knowledge that they thought correct behaviour following toothbrushing should be to rinse with mouthwash (OR 2.0 [1.4, 2.9],  $p < 0.001$ ), or with water (OR 2.6 [1.9, 3.7],  $p < 0.001$ ). Increased root caries risk was associated with ageing (OR 1.07 [1.06, 1.08],  $p < 0.001$ ) and worsening OHRQoL (OR 1.03 [1.01, 1.05],  $p = 0.001$ ). Increased dental anxiety was associated with lower risk of root caries (OR 0.93 [0.90, 0.96],  $p < 0.001$ ).

Table 3 presents the results of the full model when all candidate predictors are included, as well as the predictors selected in the final prediction model. The predictors included in the final model were  $\geq 9$  restored teeth (OR 1.5 95% CI [1.07, 2.2],  $p = 0.020$ ), smoking (OR 2.6 95% CI [1.7, 3.9],  $p < 0.001$ ), lack of knowledge of spitting toothpaste without rinsing following toothbrushing in favour of rinsing with water (OR 1.8 95% CI [1.3, 2.7],  $p < 0.001$ ) or mouthwash (OR 2.0 95% CI [1.3, 3.0],  $p = 0.001$ ), increasing age (OR 1.08 95% CI [1.06, 1.09],  $p < 0.001$ ), decreasing dental anxiety (OR 0.95 95% CI [0.91, 0.98],

$p = 0.005$ ), and worsening OHRQoL (OR 1.05 95% CI [1.03, 1.07],  $p < 0.001$ ). The calibration plot of the final prediction model is presented in Figure 2. The model sensitivity was 71.4% (95% CI: 65.8, 76.4), specificity 69.5% (95% CI: 66.6, 72.3), and c-statistic was 0.79 (95% CI: 0.76, 0.81).

The model developed using MI resulted in the selection of the same predictors as the complete case model as well as an additional predictor, type of toothbrush used. The overall performance of the MI model was similar to the complete case model: sensitivity was 72.3%, specificity 67.7%, and c-statistic 0.79 (95% CI: 0.76, 0.81).

#### Development of a Prediction Model for $RC \geq 3$

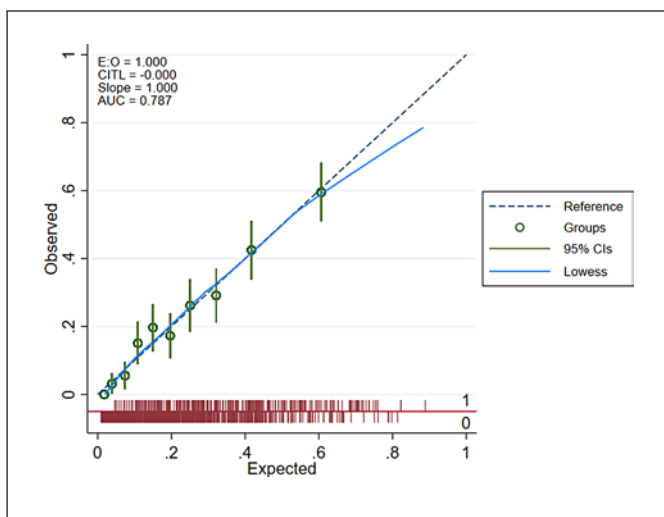
Table 4 presents the univariate analysis at the  $RC \geq 3$  threshold. Participants had higher odds of disease where they had  $\geq 9$  restored teeth at baseline (OR 2.1 [1.3, 3.4],  $p = 0.003$ ), were smokers (OR 3.9 [2.4, 6.2],  $p < 0.001$ ), were exempt from payment for dental services (OR 1.8 [1.1, 2.9],  $p = 0.01$ ), reported the behaviour rinsing with water (OR 2.5 [1.3, 4.7],  $p = 0.005$ ) or mouthwash (OR 2.8 [1.3, 5.8],  $p = 0.006$ ) after toothbrushing rather than spitting without rinsing, or where they reported knowledge that they thought correct behaviour following toothbrushing should be to rinse with mouthwash (OR 2.6 [1.4, 4.9],  $p = 0.003$ ), or with water (OR 2.6 [1.4, 4.7],  $p = 0.002$ ). Increased odds of root caries were associated with ageing (OR 1.06 [1.05, 1.08],  $p < 0.001$ ) and worsening OHRQoL (OR 1.04 [1.01, 1.06],  $p = 0.013$ ). Increased dental anxiety

**Table 1.** Characteristics of study participants

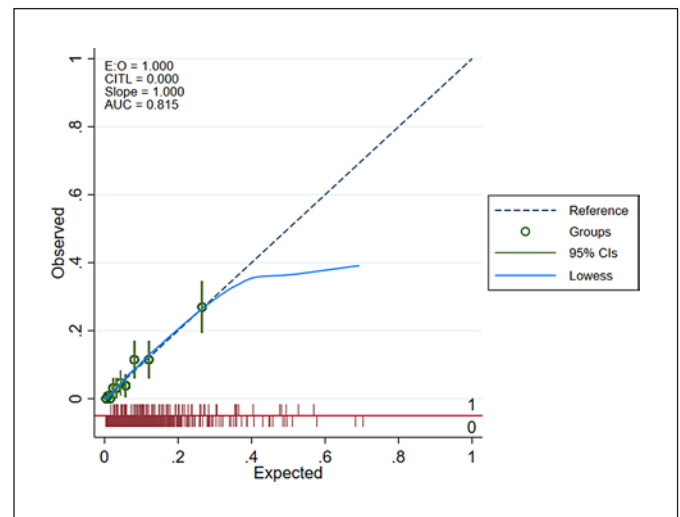
Categorical variables	N (%) (n = 1,432)
Sex	
Male	613 (42.8)
Female	819 (57.2)
Gingival bleeding on probing present at baseline	
Yes	1,007 (70.3)
No	425 (29.7)
Filled teeth at baseline	
≥9 teeth	900 (62.9)
<9 teeth	532 (37.2)
Smoked in the last 12 months	
Yes	204 (14.3)
No	1,140 (79.6)
Missing	88 (6.2)
Patient exempt from payment for dental services	
Yes	261 (18.2)
No	1,130 (78.9)
Missing	41 (2.9)
Regular attender (patient-reported)	
Regular	1,261 (88.1)
Irregular	75 (5.2)
Missing	96 (6.7)
Toothbrush	
Electric	519 (36.2)
Manual	825 (57.6)
Missing	88 (6.2)
Considered eligible for 24-month recall arm	
Eligible	366 (25.6)
Ineligible	1,064 (74.4)
Type of dental treatment received in last 12 months (patient-reported)	
NHS treatment	1,157 (80.8)
Private treatment	53 (3.7)
Combination of NHS and private treatment	78 (5.5)
Missing	144 (10.1)
Time since last visit to dentist	
Less than 1 year	1,253 (87.5)
More than 1 year	88 (6.2)
Missing	91 (6.4)
Frequency of toothbrushing behaviour	
Twice daily or more	1,101 (76.9)
Once daily or less	241 (16.8)
Missing	90 (6.3)
Duration of toothbrushing behaviour	
2 min or more	889 (62.1)
Less than 2 min	451 (31.5)
Missing	92 (6.4)
After toothbrushing behaviour	
Spit do not rinse	376 (26.3)
Rinse with mouthwash	237 (16.6)
Rinse with water	728 (50.8)
Missing	91 (6.4)
Frequency of toothbrushing knowledge	
Twice daily or more	1,313 (91.7)
Once daily or less	24 (1.7)
Missing	95 (6.6)
Duration of toothbrushing knowledge	
2 min or more	1,171 (81.8)
Less than 2 min	161 (11.2)

**Table 1** (continued)

Categorical variables	N (%) (n = 1,432)
Missing	100 (7.0)
After toothbrushing knowledge	
Spit do not rinse	475 (33.2)
Rinse with mouthwash	336 (23.5)
Rinse with water	505 (35.3)
Missing	116 (8.0)
Intervention arm	
Six-month recall	644 (45.0)
Risk-based recall	665 (46.4)
Twenty-four-month recall	123 (8.6)
Continuous variables	Mean (SD), n
Age	50.4 (14.3), 1,432
Deprivation quintile (1–5)	2.8 (1.4), 1,416
Oral health attitude (score 1–7)	4.1 (0.85), 1,345
OHIP-14 (score 0–56)	5.3 (6.6), 1,313
Anxiety (score 5–25)	10.0 (4.3), 1,341
Difficulty travelling to dentist mean (score 1–7)	6.4 (1.2), 1,342



**Fig. 2.** Calibration of final prediction model at RC > 0 threshold.



**Fig. 3.** Calibration of final prediction model at RC ≥ 3 threshold.

was associated with lower odds of root caries (OR 0.93 [0.88, 0.99],  $p = 0.022$ ).

Table 5 presents the results of the full model and the predictors selected in the final prediction model. The predictors included in the final model at the  $RC \geq 3$  threshold were smoking (OR 6.5 [3.8, 11.1],  $p < 0.001$ ), lack of knowledge of spitting toothpaste without rinsing following toothbrushing in favour of rinsing with water (OR 1.8 [0.97, 3.4],  $p = 0.063$ ) or mouthwash (OR 2.5 [1.3, 5.0],  $p = 0.007$ ), and increasing age (OR 1.08 [1.06, 1.10],  $p <$

0.001). The calibration plot of the final prediction model is presented in Figure 3. The model sensitivity was 76.5% (95% CI: 66.4, 84.3), specificity 73.6% (95% CI: 71.0, 76.0), and c-statistic was 0.81 (95% CI: 0.77, 0.86). As a sensitivity analysis, the addition of dental check-up frequency as a candidate predictor resulted in no changes to the predictors selected and negligible changes to the sensitivity, specificity, and c-statistic of the final model at either threshold.



**Table 2.** Univariate analysis at RC > 0 threshold (n = 1,432)

Categorical variable	Category	% with root caries, n (%)	% without root caries, n (%)	Univariate odds ratio (95% CI), p value
Gender	Male	163 (26.6)	450 (73.4)	Reference
	Female	161 (19.7)	658 (80.3)	0.68 (0.53, 0.87), 0.002
Suitability for 24-month arm	Yes	66 (18.0)	300 (82.0)	Reference
	No	258 (24.2)	808 (75.8)	1.5 (1.1, 2.0), 0.015
Filled teeth at baseline	<9	76 (14.3)	456 (85.7)	Reference
	≥9	248 (27.6)	652 (72.4)	2.3 (1.7, 3.0) <0.001
Gingival bleeding on probing at baseline	Yes	245 (24.3)	762 (75.7)	Reference
	No	79 (18.6)	346 (81.4)	0.71 (0.53, 0.94), 0.018
Patient exempt from payment for dental services	No	252 (22.3)	878 (77.7)	Reference
	Yes	66 (25.3)	195 (74.7)	1.2 (0.86, 1.6), 0.30
	Missing	6 (14.6)	35 (85.4)	
Smoked in the last 12 months	No	235 (20.6)	905 (79.4)	Reference
	Yes	64 (31.4)	140 (68.6)	1.8 (1.3, 2.5), 0.001
	Missing	25 (28.4)	63 (71.6)	
Regular attender (patient-reported)	Yes	284 (22.5)	977 (77.5)	Reference
	No	14 (18.7)	61 (81.3)	0.79 (0.44, 1.4), 0.44
	Missing	26 (27.1)	70 (72.9)	
Dental treatment received (patient-reported)	NHS treatment	257 (22.2)	900 (77.8)	Reference
	Private treatment	11 (20.8)	42 (79.3)	0.92 (0.47, 1.8), 0.80
	Combination of NHS and private treatment	19 (24.4)	59 (75.6)	1.1 (0.66, 1.9), 0.66
	Missing	37 (25.7)	107 (74.3)	
Time since last visit to dentist (patient-reported)	Less than 1 year	288 (23.0)	965 (77.0)	Reference
	More than 1 year	10 (11.4)	78 (88.6)	0.43 (0.22, 0.84), 0.014
	Missing	26 (28.6)	65 (71.4)	
Toothbrushing frequency behaviour	Twice a day or more	247 (22.4)	854 (77.6)	Reference
	Once a day or less	51 (21.2)	190 (78.8)	0.93 (0.67, 1.3), 0.67
	Missing	26 (28.9)	64 (71.1)	

**Table 2** (continued)

Categorical variable	Category	% with root caries, n (%)	% without root caries, n (%)	Univariate odds ratio (95% CI), p value	
Toothbrushing duration behaviour	2 min or more	197 (22.2)	692 (77.8)	Reference	
	Less than 2 min	99 (22.0)	352 (78.0)	0.99 (0.75, 1.3) 0.93	
	Missing	28 (30.4)	64 (69.6)		
After brushing behaviour	Spit not rinse	58 (15.4)	318 (84.6)	Reference	
	Rinse with water	183 (25.1)	545 (74.9)	1.8 (1.3, 2.6), <0.001	
	Rinse with mouthwash	56 (23.6)	181 (76.4)	1.7 (1.1, 2.6), 0.012	
	Missing	27 (29.7)	64 (70.3)		
Toothbrushing frequency knowledge	Twice a day or more	289 (22.0)	1,024 (78.0)	Reference	
	Once a day or less	6 (25.0)	18 (75.0)	1.2 (0.46, 3.0), 0.73	
	Missing	29 (30.5)	66 (69.5)		
Toothbrushing duration knowledge	2 min or more	255 (21.8)	916 (78.2)	Reference	
	Less than 2 min	38 (23.6)	123 (76.4)	1.1 (0.75, 1.6), 0.60	
	Missing	31 (31.0)	69 (69.0)		
	Spit not rinse	63 (13.3)	412 (86.7)	Reference	
After brushing knowledge	Rinse with water	145 (28.7)	360 (71.3)	2.6 (1.9, 3.7), <0.001	
	Rinse with mouthwash	80 (23.8)	256 (76.2)	2.0 (1.4, 2.9), <0.001	
	Missing	36 (31.0)	80 (69.0)		
	Manual	198 (24.0)	627 (76.0)	Reference	
Type of toothbrush	Electric	102 (19.7)	417 (80.4)	0.77 (0.59, 1.0), 0.063	
	Missing	24 (27.3)	64 (72.7)		
	Patients with root caries – mean (SD), count				
Continuous variables	Patients without root caries – mean (SD), count				
	Overall – mean (SD), count				
	Age	59.8 (11.4), 324	47.6 (13.9), 1,108	50.4 (14.3), 1,432	1.07 (1.06, 1.08), <0.001
	Difficulty accessing dental services (Likert scale 1–7)	6.4 (1.2), 299	6.4 (1.2), 1,043	6.4 (1.2), 1,342	1.01 (0.91, 1.13), 0.79
	Dental anxiety (score from minimum anxiety 5–25)	9.0 (3.8), 297	10.2 (4.5), 1,044	10.0 (4.3), 1,341	0.93 (0.90, 0.96), <0.001
	OHIP-14 score (score from minimum OHRQoL 0–56)	6.5 (7.7), 291	5.0 (6.3), 1,022	5.3 (6.6), 1,313	1.03 (1.01, 1.05), 0.001
	Oral health attitude (score from 1 to 7) 7 most positive attitude)	4.1 (0.9), 299	4.1 (0.84), 1,046	4.1 (0.85), 1,345	0.98 (0.84, 1.1), 0.77
Deprivation quintile (1–5)	2.8 (1.4), 320	2.8 (1.4), 1,096	2.8 (1.4), 1,416	1.01 (0.92, 1.10), 0.85	

**Table 3.** Full model and final model odds ratios at RC > 0 threshold

	Category	Full model odds ratio (95% CI), <i>p</i> value	Final model odds ratio (95% CI), <i>p</i> value
<b>Categorical variable</b>			
Gender	Male	Reference	–
	Female	0.82 (0.59, 1.1), 0.24	–
Suitability for 24-month arm	Yes	Reference	–
	No	0.89 (0.59, 1.3), 0.59	–
Filled teeth at baseline	<9	Reference	Reference
	≥9	1.7 (1.2, 2.5), 0.005	1.5 (1.1, 2.2), 0.020
Gingival bleeding on probing at baseline	Yes	Reference	–
	No	0.81 (0.56, 1.2), 0.27	–
Patient exempt from payment for dental services	No	Reference	–
	Yes	1.2 (0.76, 1.9), 0.44	–
Smoked in the last 12 months	No	Reference	Reference
	Yes	2.4 (1.6, 3.8), <0.001	2.6 (1.7, 3.9), <0.001
Regular attender (patient-reported)	Yes	Reference	–
	No	0.71 (0.33, 1.5), 0.38	–
Dental treatment received (patient-reported)	NHS treatment	Reference	–
	Private treatment	1.4 (0.63, 3.1), 0.41	–
	Combination of NHS and private treatment	1.5 (0.77, 2.8), 0.24	–
Time since last visit to dentist (patient-reported)	Less than 1 year	Reference	–
	More than 1 year	0.49 (0.20, 1.2), 0.11	–
Toothbrushing frequency behaviour	Twice a day or more	Reference	–
	Once a day or less	0.78 (0.50, 1.2), 0.27	–
Toothbrushing duration behaviour	2 min or more	Reference	–
	Less than 2 min	0.98 (0.67, 1.4), 0.90	–
After brushing behaviour	Spit not rinse	Reference	–
	Rinse with water	0.99 (0.60, 1.6), 0.98	–
	Rinse with mouthwash	0.94 (0.50, 1.7), 0.84	–
Toothbrushing frequency knowledge	Twice a day or more	Reference	–
	Once a day or less	0.76 (0.24, 2.4), 0.65	–
Toothbrushing duration knowledge	2 min or more	Reference	–
	Less than 2 min	0.74 (0.43, 1.3), 0.28	–
After brushing knowledge	Spit not rinse	Reference	Reference
	Rinse with water	1.9 (1.2, 3.1), 0.01	1.8 (1.3, 2.7), 0.001
	Rinse with mouthwash	2.0 (1.2, 3.4), 0.01	2.0 (1.3, 3.0), 0.001

**Table 2** (continued)

	Category	Full model odds ratio (95% CI), <i>p</i> value	Final model odds ratio (95% CI), <i>p</i> value
Type of toothbrush	Manual	Reference	
	Electric	0.70 (0.50, 0.98), 0.038	
<b>Continuous variables</b>			
Age		1.08 (1.06, 1.09), <0.001	1.08 (1.06, 1.09), <0.001
Difficulty accessing dental services (Likert scale 1–7)		0.93 (0.82, 1.1), 0.30	–
Dental anxiety (score from minimum anxiety 5–25)		0.96 (0.92, 1.0), 0.04	0.95 (0.91, 0.98), 0.005
OHIP-14 score (score from minimum OHRQoL 0–56)		1.04 (1.02, 1.07), 0.001	1.05 (1.03, 1.07), <0.001
Oral health attitude ([score from 1 to 7] 7 most positive attitude)		1.1 (0.92, 1.3), 0.31	–
Deprivation		1.0 (0.90, 1.2), 0.77	–

The model developed using MI resulted in the selection of three additional predictors – number of filled teeth at baseline, deprivation quintile, and OHRQoL. The predictor after brushing knowledge was not selected in the MI model. Two predictors were selected in both the complete case analysis and in the MI model – smoking and age. Sensitivity of the MI model was 76.9%, specificity 71.2%, and *c*-statistic 0.81 (95% CI: 0.77, 0.85) – almost identical to the complete case model. When including only the predictors included in both models, smoking and age, the prediction model sensitivity is 72.4%, specificity 72.3%, and *c*-statistic 0.80 (95% CI: 0.76, 0.85).

There is some evidence of possible selection bias of predictors when comparing the complete case model with the MI model at both outcomes, with differences presented in the online supplementary Table S2. However, the model performance is very similar between both models at both thresholds.

## Discussion

To the author's knowledge, this root caries prediction model has the largest sample size of any published model and addresses a gap identified by a systematic review of 13 studies recommending development of root caries prediction models within well-designed studies with a large sample size, representative population, and adequate follow-up time [Ritter et al., 2010]. Development of this model has followed robust methods included in the most current recommendations and reporting guidance

[Collins et al., 2015], with inclusion of expert consensus to identify potentially relevant predictors. This study recruited participants receiving dental care in the primary care setting, with a wide variety in age and socio-economic status of included participants. The results are therefore generalizable to regular dental attenders in state-provided healthcare systems.

Age, filled teeth, smoking status, after brushing knowledge, anxiety, and OHRQoL were selected as predictors in the final diagnostic model at the  $RC > 0$  threshold. The predictors included in the model at the  $RC \geq 3$  threshold were age, smoking status, and after brushing knowledge. The performance of the MI model is similar due to the small percentage of missing data. The predictors within these two models are also found in other root caries risk models.

A systematic review assessing the association of smoking on coronal caries [Jiang et al., 2019] concluded that tobacco smoking increased caries risk. This may be due to the role of nicotine in promoting biofilm formation and metabolism of *Streptococcus mutans* [Huang et al., 2012] or the associated oral drying effect of smoking. Smoking may also result in an increased number of root surfaces at risk of caries as a result of poorer periodontal status and gingival recession in smokers [Heasman et al., 2017].

The predictive ability of the models in this study is much higher than Locker's and Sánchez-García's models which reported sensitivity of 9.2% [Locker, 1996] and 15.6% [Sánchez-García et al., 2011]. Powell reported the ability of root caries prediction models at similar thresholds of  $>0$  surfaces and  $\geq 3$  surfaces [Powell et al., 1991],

**Table 4.** Univariate analysis at RC  $\geq$  3 threshold ( $n = 1,430$ )

Categorical variable	Category	% with root caries <3 teeth, n (%)	% with root caries $\geq$ 3 teeth, n (%)	Univariate odds ratio (95% CI), p value
Gender	Male	565 (92.2)	48 (7.8)	Reference
	Female	768 (94.0)	49 (6.0)	0.75 (0.50, 1.1), 0.17
Suitability for 24-month arm	Yes	345 (94.3)	21 (5.7)	Reference
	No	988 (92.9)	76 (7.1)	1.3 (0.77, 2.1), 0.36
Filled teeth at baseline	<9	510 (95.9)	22 (4.1)	Reference
	$\geq$ 9	823 (91.7)	75 (8.4)	2.1 (1.3, 3.4) 0.003
Gingival bleeding on probing at baseline	Yes	930 (92.5)	75 (7.5)	Reference
	No	403 (94.8)	22 (5.2)	0.68 (0.41, 1.1), 0.12
Patient exempt from payment for dental services	No	1,060 (94.0)	68 (6.0)	Reference
	Yes	234 (90.0)	27 (10.3)	1.8 (1.1, 2.9), 0.01
Smoked in the last 12 months	Missing	39 (95.1)	2 (4.9)	Reference
	No	1,085 (95.3)	54 (4.7)	Reference
Regular attender (patient-reported)	Yes	171 (83.8)	33 (16.2)	3.9 (2.4, 6.2), <0.001
	Missing	77 (88.5)	10 (11.5)	Reference
Dental treatment received (patient-reported)	Yes	1,180 (93.7)	80 (6.4)	Reference
	No	68 (90.7)	7 (9.3)	1.5 (0.68, 3.4) 0.31
Time since last visit to dentist (patient-reported)	Missing	85 (89.5)	10 (10.5)	Reference
	NHS treatment	1,079 (93.3)	77 (6.7)	Reference
Toothbrushing frequency behaviour	Private treatment	51 (96.2)	2 (3.8)	0.55 (0.13, 2.3), 0.41
	Combination of NHS and private treatment	71 (91.0)	7 (9.0)	1.4 (0.61, 3.1), 0.43
Time since last visit to dentist (patient-reported)	Missing	132 (92.3)	11 (7.7)	Reference
	Less than 1 year	1,167 (93.2)	85 (6.8)	Reference
Toothbrushing frequency behaviour	More than 1 year	86 (97.7)	2 (2.3)	0.32 (0.08, 1.3), 0.12
	Missing	80 (88.9)	10 (11.1)	Reference
Toothbrushing frequency behaviour	Twice a day or more	1,030 (93.6)	71 (6.5)	Reference
	Once a day or less	223 (92.9)	17 (7.1)	1.1 (0.64, 1.9), 0.72
Toothbrushing frequency behaviour	Missing	80 (89.9)	9 (10.1)	Reference



**Table 4** (continued)

Categorical variable	Category	% with root caries <3 teeth, n (%)	% with root caries ≥3 teeth, n (%)	Univariate odds ratio (95% CI), p value
Toothbrushing duration behaviour	2 min or more	824 (92.8)	64 (7.2)	Reference
	Less than 2 min	428 (94.9)	23 (5.1)	0.69 (0.42, 1.1), 0.14
	Missing	81 (89.0)	10 (11.0)	
After brushing behaviour	Spit not rinse	363 (96.8)	12 (3.2)	Reference
	Rinse with water	673 (92.5)	55 (7.6)	2.5 (1.3, 4.7), 0.005
	Rinse with mouthwash	217 (91.6)	20 (8.4)	2.8 (1.3, 5.8), 0.006
	Missing	80 (88.9)	10 (11.1)	
Toothbrushing frequency knowledge	Twice a day or more	1,228 (93.6)	84 (6.4)	Reference
	Once a day or less	21 (87.5)	3 (12.5)	2.1 (0.61, 7.1), 0.24
	Missing	84 (89.4)	10 (10.6)	
Toothbrushing duration knowledge	2 min or more	1,092 (93.3)	78 (6.7)	Reference
	Less than 2 min	153 (95.0)	8 (5.0)	0.73 (0.35, 1.5), 0.41
	Missing	88 (88.9)	11 (11.1)	
After brushing knowledge	Spit not rinse	458 (96.6)	16 (3.4)	Reference
	Rinse with water	463 (91.7)	42 (8.3)	2.6 (1.4, 4.7), 0.002
	Rinse with mouthwash	308 (91.7)	28 (8.3)	2.6 (1.4, 4.9), 0.003
	Missing	104 (90.4)	11 (9.6)	
Type of toothbrush	Manual	765 (92.7)	60 (7.3)	Reference
	Electric	490 (94.6)	28 (5.4)	0.73 (0.46, 1.2), 0.18
	Missing	78 (89.7)	9 (10.3)	
Continuous variables	Patients with root caries <3 teeth – mean (SD), count	Patients with root caries ≥3 teeth – mean (SD), count	Overall – mean (SD), count	Odds ratio (95% CI), p value
	Age	49.6 (14.2), 1,333	61.0 (11.0), 97	50.4 (14.3), 1,430
Difficulty accessing dental services (Likert scale 1–7)	6.4 (1.2), 1,253	6.5 (1.0), 88	6.4 (1.2), 1,341	1.1 (0.90, 1.4), 0.33
	Dental anxiety (score from minimum anxiety 5–25)	10.0 (4.4), 1,254	8.9 (4.0), 86	10.0 (4.3), 1,340
OHIP-14 score (score from minimum OHRQoL 0–56)	5.2 (6.5), 1,226	7.1 (7.5), 86	5.3 (6.6), 1,312	1.04 (1.01, 1.06), 0.013
	Oral health attitude (score from 1 to 7) 7 most positive	4.1 (0.84), 1,256	4.1 (0.94), 88	4.1 (0.85), 1,344
Deprivation	2.7 (1.4), 1,318	3.1 (1.5), 96	1.4 (2.8), 1,414	1.2 (1.0, 1.4), 0.024

**Table 5.** Full model and final model odds ratios at RC  $\geq$  3 threshold

	Category	Full model odds ratio (95% CI), <i>p</i> value	Final model odds ratio (95% CI), <i>p</i> value
<b>Categorical variable</b>			
Gender	Male		–
	Female	1.1 (0.64, 1.9), 0.74	–
Suitability for 24-month arm	Yes		–
	No	0.63 (0.33, 1.2), 0.17	–
Filled teeth at baseline	<9		–
	$\geq$ 9	2.1 (1.1, 4.1), 0.02	–
Gingival bleeding on probing at baseline	Yes		–
	No	0.85 (0.45, 1.6), 0.62	–
Patient exempt from payment for dental services	No		–
	Yes	1.3 (0.69, 2.6), 0.40	–
Smoked in the last 12 months	No		
	Yes	4.9 (2.6, 9.1), <0.001	6.5 (3.8, 11.1), <0.001
Regular attender (patient-reported)	Yes		–
	No	0.40 (0.12, 1.3), 0.13	–
Dental treatment received (patient-reported)	NHS treatment		–
	Private treatment	0.69 (0.15, 3.3), 0.64	–
	Combination of NHS and private treatment	2.1 (0.83, 5.2), 0.12	–
Time since last visit to dentist (patient-reported)	Less than 1 year		–
	More than 1 year	0.17 (0.02, 1.4), 0.10	–
Toothbrushing frequency behaviour	Twice a day or more		–
	Once a day or less	0.86 (0.42, 1.8), 0.68	–
Toothbrushing duration behaviour	2 min or more		–
	Less than 2 min	0.93 (0.50, 1.7), 0.83	–
After brushing behaviour	Spit not rinse		–
	Rinse with water	1.3 (0.52, 3.2), 0.58	–
	Rinse with mouthwash	1.1 (0.38, 3.2), 0.85	–
Toothbrushing frequency knowledge	Twice a day or more		–
	Once a day or less	1.4 (0.30, 6.3), 0.69	–
Toothbrushing duration knowledge	2 min or more		–
	Less than 2 min	0.55 (0.21, 1.5), 0.23	–
After brushing knowledge	Spit not rinse		
	Rinse with water	1.6 (0.66, 4.0), 0.29	1.8 (0.97, 3.4), 0.063
	Rinse with mouthwash	2.5 (1.0, 6.4), 0.05	2.5 (1.3, 5.0), 0.007
Type of toothbrush	Manual		–
	Electric	0.72 (0.41, 1.3), 0.25	–

**Table 5** (continued)

Category	Full model odds ratio (95% CI), <i>p</i> value	Final model odds ratio (95% CI), <i>p</i> value
<b>Continuous variables</b>		
Age	1.09 (1.06, 1.1) <0.001	1.08 (1.06, 1.10), <0.001
Difficulty accessing dental services (Likert scale 1–7)	1.05 (0.83, 1.3), 0.69	–
Dental anxiety (score from minimum anxiety 5–25)	0.96 (0.89, 1.03), 0.26	–
OHIP-14 score (score from minimum OHRQoL 0–56)	1.03 (1.00, 1.07), 0.08	–
Oral health attitude ([score from 1 to 7] 7 most positive attitude)	1.06 (0.80, 1.4), 0.68	–
Deprivation	1.07 (0.87, 1.3) 0.53	–

with accuracy of 67 and 94%, respectively, but inferior sensitivity of 45 and 67%.

The root caries risk model with the best performance was published by Scheinin, with a reported sensitivity of 77.6% and specificity of 76.6% [Scheinin et al., 1994]. Scheinin's model included 3 variables – past root caries experience, *Candida*, and *Lactobacilli* levels. Despite inclusion of salivary testing in Scheinin's model, the accuracy of Scheinin's model of 77% was inferior to that of the RC > O (79%) and RC ≥ 3 models (81%) reported in this study. Inclusion of salivary tests may improve the model sensitivity; however, it is important to consider model accuracy alongside cost-effectiveness of a diagnostic tool and ease of application and acceptability to patients. A strength of the models developed in this study is that no laboratory tests are required. Instead, the predictors can be obtained from a routine examination and patient self-report questionnaire. The models developed in this study provide a balance of high sensitivity and good specificity, both with superior sensitivity to specificity, and improved performance at identifying high-risk individuals at the RC ≥ 3 threshold.

This study included a large and representative sample of adult regular dental attenders from across the UK. A limitation of this study is that the model has not been externally validated. External validation of this model is required in a different population from those in which it was developed [Collins et al., 2015]. Authors of this study are also investigators in a separate clinical trial investigating the effect of 5000 ppm fluoride toothpaste on adults over 50 years old at high risk of dental caries (REFLECT clinical study) [Tickle et al., 2019]. This is an appropriate population in which to externally validate this model, where the greatest effectiveness and cost-effectiveness of preventive strategies may be observed [Schwendicke and

Göstemeyer, 2017], and where prevalence of root caries can be expected to be higher.

The use of different diagnostic criteria to define root caries within published risk models presents challenges in comparing the performance of these models. Agreement on the clinical criteria used to define root caries is needed to promote consistent reporting between studies [Ritter et al., 2010; Hayes et al., 2017; Fee et al., 2020]. This study relied on visual-tactile examination alone to detect root caries lesions. A systematic review on tests to detect root caries found very low certainty evidence to support the additional benefit of adjunctive diagnostic tests [Fee et al., 2020]. Visual-tactile examination therefore remains the mainstay of detection. Should adjunctive tests improve the accuracy of detection of non-cavitated lesions, their application may further improve model sensitivity and accuracy.

## Conclusions

To the authors' knowledge, this is the largest published root caries prediction model, providing confidence in the robustness of the model [Riley et al., 2020]. The model was developed within a relevant study population of contemporary dental attenders. The performance of the risk model suggests adults at risk of developing root caries can be accurately identified, with improved performance in the identification of high-risk adults. This model may be clinically useful in determining future root caries risk in regular dental attenders to target root caries preventive strategies. A key strength is that it will be easy and inexpensive to implement, requiring information obtained solely from routine examination and patient self-report. This study also highlights risk factors relating to patient

behaviours which appear to be key in predicting root caries. It is therefore important for dentists to routinely collect information on patient age, smoking status, number of filled teeth, and after brushing knowledge and provide their patients with appropriate advice. Given the large sample size and robust methods to develop this highly accurate risk model, it is unlikely a more predictive and generalizable model will be developed in future studies. Future research should instead focus on assessing the impact of implementing this model in a clinical setting through targeted clinician-delivered preventive interventions and prevention strategies to modify patient health behaviours.

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## Statement of Ethics

This study was conducted in accordance with the World Medical Association Declaration of Helsinki. Ethical approval was granted by the Fife and Forth Valley Research Ethics Committee (09/S0501/1) and written informed consent was obtained from each participant.

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## Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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## Author Contributions

Patrick A Fee designed and planned the study, collected the data, conducted the statistical analysis, wrote the manuscript, and approved the final version. Heather Cassie, Tanya Walsh, and Andrew F Hall critically reviewed the manuscript and approved the final version. Jan E Clarkson and David Ricketts designed and planned the study, critically reviewed the manuscript, and approved the final version. Beatriz Goulão designed and planned the study, collected the data, conducted the statistical analysis, critically reviewed the manuscript, and approved the final version.

## Data Availability Statement

All data generated or analysed during this study are included in this manuscript. Further enquiries can be directed to the corresponding author.

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