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A longitudinal study of caries onset in initially caries-free children and baseline salivary mutans streptococci levels: a Kaplan–Meier survival analysis

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Abstract – *Objectives:* To apply survival analysis to a longitudinal study of the relationship between salivary mutans streptococci (MS) levels at baseline in initially caries-free children and caries onset in deciduous, mixed, and permanent dentition. *Methods:* The Kaplan–Meier survival analysis method was used to compare survival times to caries onset for initially caries-free children with low levels of MS at baseline with survival times to caries onset for initially caries-free children with high levels of MS at baseline. *Results:* Data from a 6-year longitudinal study of caries risk in initially caries-free children in Rochester and the Finger Lakes Region of western New York were utilized for this study. Of 464 children analyzed, 327 had a low level of MS and 137 had a high level of MS at baseline. Survival analyses showed that children with a low level of MS at baseline remained caries-free for a longer period than children with a high level of MS at baseline. Statistically significant relationships [hazard ratios (HR)] with onset of caries in deciduous, mixed and permanent teeth were found with high and low levels of salivary MS. *Conclusions:* Based on our analysis, we concluded that children who were caries-free at baseline and who had high salivary MS levels at baseline would be at greater risk, i.e. more susceptible to caries onset, at any given time than caries-free children who had low salivary MS levels at baseline. Survival functions for deciduous, mixed and permanent dentitions with their 95% confidence limits have been calculated. Survival analysis for the exploration of longitudinal caries studies has an advantage over traditional statistical methods, as it takes into account censored observations and incorporates the concept of risk over time. Hence, survival analysis is well suited for studying transitions from one health state to another, in this case, from a caries-free state to a caries-active state.

Keywords: caries onset; mutans streptococci; survival analysis

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Dental caries is a multifactorial disease. Epidemiologically, it can be modeled as an interplay among three primary factors: a favorable environment, microflora and, a susceptible host. All of these factors are essential for the initiation and progression of the disease (1). In this paper, we describe the use of survival analysis with respect to caries onset in initially caries-free children from Rochester, NY and the Finger Lakes region of western New York.

Although bacteria can be isolated from the oral cavity shortly after birth, organisms that require nonshedding surfaces for colonization will normally only be detected after the eruption of teeth;

e.g. mutans streptococci (MS) are strongly associated with the initiation and progression of dental caries and colonize primarily hard or nonshedding surfaces (2). Although MS are usually not detected prior to the eruption of the deciduous dentition (3, 4) recent reports suggest that colonization may actually occur earlier (5–7). Infants most likely become colonized from their parents or other individuals with whom they have close contact, and infants whose mothers harbor higher levels of MS in saliva become colonized more readily than infants of mothers with low salivary MS levels (8). In a follow-up study, 4-year-old children who had detectable MS at 2 years of age had significantly higher caries prevalence than children who were colonized later or who were not colonized at all (9). Further support for the role of MS in caries development was shown when higher proportions of MS were found on carious enamel than on adjacent caries-free enamel (2).

Many studies have shown that there is a positive correlation between the severity of caries experience and the level of MS in saliva or plaque (9–14). However, few studies have investigated the association of caries experience and the level of MS in saliva or plaque prior to the onset of the disease. For example, in the University of North Carolina Risk Assessment Study, although microbiological tests made a modest contribution to the caries risk assessment model, only a portion of children were caries-free at baseline (15). Similarly, in a small study by van Palenstein Helderman et al. (16) 68% of children were caries-free at baseline. However, the microbiological data did not add to the prediction model in the presence of past caries experience.

In an effort to extend our understanding of the relationship between salivary MS and caries, we examined the relationship between salivary MS levels at baseline in initially caries-free children and caries onset in deciduous, mixed and permanent dentition. Our aim was to show the longitudinal effect of salivary MS levels at baseline on caries onset by means of an analytical procedure that allowed us to describe caries onset longitudinally. Survival analysis is a robust analytic procedure and can be used to analyze data corresponding to the time between a well-defined starting point and the occurrence of a disease.

Other investigators have taken a similar approach using regression techniques, but these studies did not incorporate microbiological factors. For instance, Steiner et al. (17), considered clinical conditions in the assessment of future caries risk. All variables were tested as individual predictors in simple logistic regression analyses and showed that variables based on the primary dentition were good predictors of high caries increment. Statistically significant variables in the simple model were further studied using multiple logistic regression analyses. A similar study conducted by Helfenstein et al. (18), aimed to predict future high caries increment on the basis of the state of primary teeth and first molars. Data in this study were analyzed using multiple regression technique and showed that sound primary teeth were good predictors of future caries activity.

Recently, Ansai et al. (19), utilized the Kaplan–Meier method to investigate the relationship of MS to caries onset in initially caries-free preschool children. The study, conducted on a small sample of 60 children in Japan aged 0.5–6 years who were followed up for 24 months, concluded that caries onset in the deciduous dentition correlated significantly with high salivary MS levels at baseline.

In our study we extended the use of the Kaplan–Meier method to model transitions from a caries-free state to a caries-active state, i.e. from sound to caries, in an older population of caries-free children who were followed up for a longer period of time. Survival functions for caries-free children with high salivary MS levels at baseline were compared with caries-free children with low salivary MS levels at baseline for the deciduous, mixed and permanent dentition by means of the Kaplan–Meier method. The hypothesis tested was that caries-free children with low levels of MS at baseline would remain caries-free for a longer period than caries-free children with high levels of MS at baseline.

Materials and methods

Clinical and ambulatory procedures

A cohort of 631 initially caries-free children, 6–7 years of age from Rochester and the Finger Lakes region of western New York formed the study population for this analysis. Each child was examined at 6-month intervals for up to 6 years. The data collected on each subject included dental caries and salivary microbiological profiles.

Clinical caries was defined using a modified version of the visual-tactile criteria of Radike (20). Fiberoptic lights, plane mirrors, and no. 23 pianowire explorers were used to conduct clinical

examinations. The explorer was used to clean the tooth surface as necessary and to detect dental sealants. Radiographs were not used. All saliva samples were obtained at least 2 h after oral hygiene procedures were last performed and before lunch. Approximately 1 ml of whole stimulated saliva was collected for microbiological analysis.

All saliva samples were stored on ice in heavy-duty styrofoam containers and transported to the laboratory for processing. Microbiological assays were started within 24 h. All laboratory analytical procedures were performed without knowledge of caries status or place of residence. At the time of collection, all saliva samples were identified solely by date, exam number and subject ID. MS colonies were enumerated using MSB agar (21).

To confirm their identity, cells from colonies grown on MSB were (a) gram-positive cocci in chains when grown in fluid thioglycollate broth, (b) mannitol positive, and (c) produced glucan from sucrose.

At baseline, children were categorized after testing for MS as having low levels of MS or high levels of MS in saliva. A high level of salivary MS, for the purpose of this study, was defined as $\geq 10^6$ colony forming units per milliliter (CFU/ml) of saliva (22). A low level of salivary MS was defined as $<10^6$ CFU/ml (22). Additionally, we analyzed our data for the cut-off point of 10^4, $10^{4.5}$, 10^5 and $10^{5.5}$ CFU/ml of saliva and obtained similar results as for the cut-off point of 10^6 CFU/ml of saliva. However, when a cut-off point of 10^5 was chosen, at baseline 50% of the children fell into a low MS category and 50% fell into a high MS category. For the cut-off point of 10^6 CFU/ml of saliva, 70% of the children fell into a low MS category and 30% fell into a high MS category at baseline (Fig. 1). Given these observations and for historical reasons the cut-off point of 10^6 CFU/ml of saliva between high and low salivary MS levels was chosen (22).

Statistical procedures

Survival probabilities, i.e. time to caries onset, were estimated by the Kaplan–Meier method, also known as the product-limit estimate of the survival function (23). Survival probabilities for caries-free children with low MS levels at baseline were compared with survival probabilities for caries-free children with high MS levels by means of the Wilcoxon and log-rank tests for the deciduous, mixed and permanent dentition (23). Cox proportional hazards ratio (HR) were calculated for caries

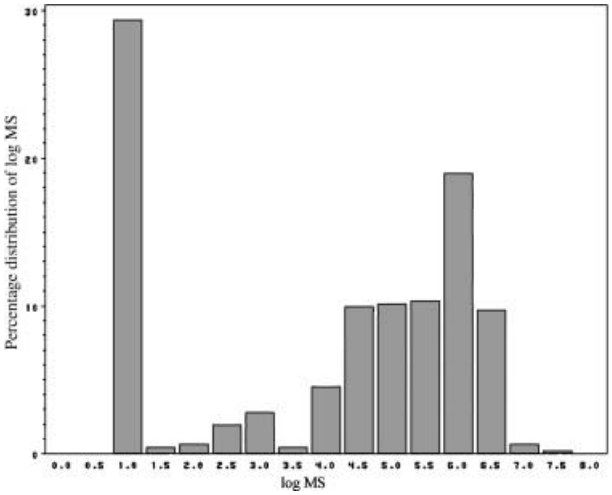

Fig. 1. Frequency of log salivary MS levels for all children at baseline.

onset in the deciduous, mixed and permanent dentition with respect to salivary MS levels. The rationale to differentiate deciduous, mixed and permanent dentition was to assess differences in survival rates among different dentitions for children with low and high MS levels at baseline.

The deciduous dentition was defined as the 20 primary teeth. The mixed dentition or transitional dentition is that period between the eruption of the first permanent molars at approximately 6 years of age and the exfoliation of the last primary tooth at approximately 12 years of age. The permanent dentition was defined as the 28 permanent teeth, excluding 3rd molars.

Survival probabilities and Cox proportional HR were calculated for children with DFMS = 0 and dfs = 0 at baseline. To analyze a homogenous population of children and to make the analysis more conservative, we excluded children younger than 6.5 and older than 7.5 years. Children with restorations that were not preceded by a diagnosis of caries in the prior exam were also excluded as were children who participated only in the baseline examination. A total of 167 children were excluded from the analysis, thus resulting in a final sample size of 464 children.

A key characteristic that distinguishes survival analysis from other areas in statistics is that survival data are usually censored (23). Censoring occurs when incomplete information is available about survival time for some individuals. Thus, survival analysis has an advantage over conventional statistical methods, as it includes censored observations in the analysis of the data. Censored observations are comprised of those subjects who

did not reach a disease endpoint, i.e. did not become caries-active during the 6-year period of the study or who were lost to follow-up. The strength of the Kaplan–Meier method over conventional methods of analysis is that it accounts for censored observations and includes them in the data analysis. Typically, only subjects who are present at the final examination are included in the analyses. The traditional analytic methods would generally only include those children who completed all examinations. In contrast, survival analysis makes it possible to use information from children who had at least two examinations. We assumed that censoring was noninformative, i.e. subjects who were censored had the same underlying survival curve after censoring as subjects who were not censored (23).

The analyses were performed using SAS version 8.2 statistical package. Statistical significance was set at $P < 0.05$ (5%). The outcome variable evaluated was time to caries onset in the deciduous, mixed and permanent dentition.

Results

A total of 464 children comprised the study group at baseline. The average age of the subjects at the baseline examination was 7.2 years (95% CI 7.21–7.24). The average level of log MS at baseline for the children characterized as having low MS levels was 3.22 (95% CI 3.01–3.44) and 6.44 (95% CI 6.39–6.49) for the children characterized as having high levels of MS. All children were caries-free at baseline. The maximum follow-up for each child was 6 years. A child completing the study would have had 12 examinations. A total of 160 children completed all 12 examinations. The study had an approximate 18.5% annual attrition rate primarily because of subject relocation outside of the study area. The highest attrition rate occurred in the sixth year of the study when children matriculated from elementary school to middle school and were no longer available for follow-up. Caries onset was used as an outcome measure in the analyses performed. Our outcome variable was measured by two calibrated and experienced clinicians. Both inter- and intra-examiner reliability were in excellent agreement, with a kappa value of 0.97 for intra-examiner agreement for both examiners and 0.96 for inter-examiner agreement.

At baseline, a total of 327 children were classified as having low salivary MS levels and 137 children were classified as having high salivary MS levels. The distribution of log MS salivary levels at baseline is illustrated in Fig. 1. The summary of failures (conversion to a caries-active state) and censored observations (did not become caries-active during the period of follow-up or were lost to follow-up) across groups and dentitions are presented in Table 1. As shown in Table 1, among 327 children with low levels of MS at baseline, 69 (21.1%) children developed caries in the deciduous dentition, 120 (36.6%) developed caries in the mixed dentition and 80 (24.4%) children developed caries in the permanent dentition. Among 137 children with high levels of MS at baseline, 45 (32.8%) children developed caries in the deciduous dentition, 67 (48.9%) developed caries in the mixed dentition and 46 (33.6%) children developed caries in the permanent dentition.

The survival functions were calculated for two groups: the first group included children who were classified as having low levels of MS at baseline and the second group included children classified as having high levels of MS at baseline. The Kaplan–Meier curves for the deciduous, mixed and permanent dentition are shown in Figs 2–4. Children whose salivary MS levels were high at baseline were shown to be at a greater risk for caries onset than those children with low levels of salivary MS for the deciduous, mixed and perma-

Table 1. Summary of (failures)* and (censored observations)† across groups and dentitions

Dentition	Number of children at baseline	MS level at baseline	Number of failures (%)	Number censored
Deciduous	327	Low MS	69 (21.1)	258
	137	High MS	45 (32.8)	92
Mixed	327	Low MS	120 (36.6)	207
	137	High MS	67 (48.9)	70
Permanent	327	Low MS	80 (24.4)	247
	137	High MS	46 (33.6)	91

*Failures, number and proportion of children who transitioned from a caries-free state to a caries-active state.
†Censored observations, children who remained caries-free, i.e. did not transition from a caries-free state to caries-active state during the 6-year period of follow-up or children who were caries-free when lost to follow-up.

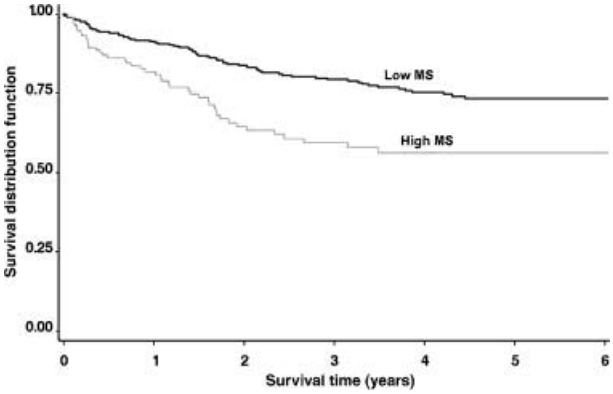

Fig. 2. Survival curves for the deciduous dention of children with low MS levels at baseline compared with children with high MS levels at baseline.

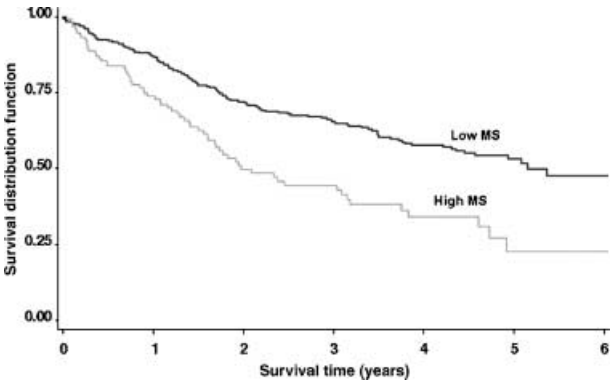

Fig. 3. Survival curves for the mixed dention of children with low MS levels at baseline compared with children with high MS levels at baseline.

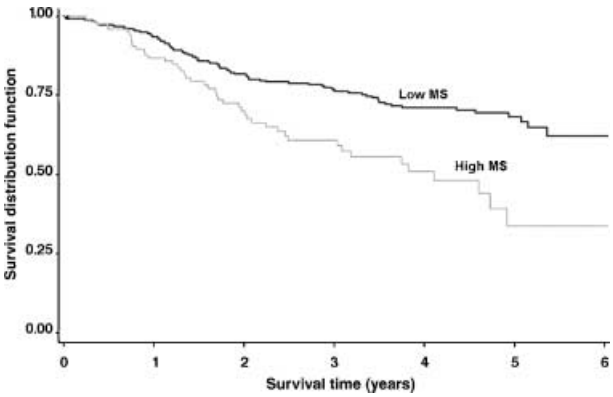

Fig. 4. Survival curves for the permanent dention of children with low MS levels at baseline compared with children with high MS levels at baseline.

nent dentition of the two groups of children as depicted in Figs 2–4.

The Kaplan–Meier survival function estimations and their 95% confidence intervals for the deciduous dentition at baseline and 1–5 years from baseline are shown in Table 2. Tables 3 and 4 show the estimation of survival functions for the mixed and permanent dentition respectively. As illustrated in Table 2, at 5 years, the deciduous dentition had a caries-free survival rate of 77.8% for children with low MS levels at baseline and 58.9% for children with high MS levels at baseline. As shown in Table 3, at 5 years, the mixed dentition had a caries-free survival rate of 53.2% for children with low MS levels at baseline and 22.6% for children with high MS levels at baseline. As shown in Table 4, at 5 years, the permanent dentition had a caries-free survival rate of 68.3% for children with low MS levels at baseline and 33.6% for children with high MS levels at baseline.

The differences in survival among groups and dentitions are summarized in Table 5. As shown in Table 5, the occurrence of caries in children with low levels of salivary MS was significantly different from children with high levels of MS for the deciduous, mixed and permanent dentition; i.e. children with low levels of MS remained caries-free for a longer period than children with high levels of MS. To compare survival curves, we used log-rank and Wilcoxon tests. For the deciduous dentition, the mean survival time for children with low MS levels was 3.75 years and for children with high MS levels, 2.5 years. These differences were statistically significant for both the log-rank and Wilcoxon tests: $P < 0.0001$ (Fig. 2). For the mixed dentition, the mean survival time for children with low MS levels was 3.75 years and for children with high MS levels was 2.6 years. These differences were statistically significant for both the log-rank and Wilcoxon tests: $P < 0.0001$ (Fig. 3). For the permanent dentition, the mean survival time for children with low MS levels was 4.31 years and for children with high MS levels was 3.35 years. These differences were statistically significant: $P = 0.0001$ (log-rank test) and $P = 0.0012$ (Wilcoxon test) (Fig. 4). The survival curves illustrate that low salivary MS levels at baseline have a significant effect on the longer survival times for the deciduous, mixed and permanent dentition.

Table 6 shows the results of the Cox proportional hazards regression analysis (HR).

Statistically significant HR with onset of caries in the deciduous dentition (HR = 2.08), mixed dentition (HR = 2.00) and permanent dentition (HR = 2.04) were found with high levels of salivary MS.

Table 2. Kaplan–Meier survival analysis for the deciduous dentition

	Low salivary MS at baseline (%)			High salivary MS at baseline (%)		
Years from baseline	% Survival	95% LCL	95% UCL	% Survival	95% LCL	95% UCL
0	100.0	100.0	100.0	100.0	100.0	100.0
1	93.6	90.4	96.9	83.4	78.4	88.4
2	87.8	83.3	92.3	68.8	62.2	75.4
3	83.8	78.6	89.0	63.3	56.2	70.4
4	80.4	74.6	86.2	58.9	51.4	66.5
5	77.8	71.5	84.1	58.9	51.4	66.5

Table 3. Kaplan–Meier survival analysis for the mixed dentition

	Low salivary MS at baseline (%)			High salivary MS at baseline (%)		
Years from baseline	% Survival	95% LCL	95% UCL	% Survival	95% LCL	95% UCL
0	100.0	100.0	100.0	100.0	100.0	100.0
1	87.0	83.3	90.8	74.0	66.2	81.8
2	72.1	66.9	77.4	49.8	39.9	59.7
3	65.4	59.7	71.1	44.5	34.3	54.6
4	57.7	51.5	63.9	34.1	23.4	44.8
5	53.2	46.5	60.0	22.6	9.6	35.6

Table 4. Kaplan–Meier survival analysis for the permanent dentition

	Low salivary MS at baseline (%)			High salivary MS at baseline (%)		
Years from baseline	% Survival	95% LCL	95% UCL	% Survival	95% LCL	95% UCL
0	100.0	100.0	100.0	100.0	100.0	100.0
1	93.7	91.0	96.4	86.9	80.7	93.1
2	81.8	77.2	86.3	70.1	60.9	79.3
3	76.7	71.6	81.8	60.8	50.6	71.0
4	71.1	65.4	76.8	50.9	39.2	62.7
5	68.3	62.0	74.7	33.6	17.1	50.1

Table 5. Summary statistics for survival caries-free for the deciduous, mixed and permanent dentition

Dentition	MS level at baseline	Mean survival time in years	Log-rank test	Wilcoxon test
Deciduous	Low MS	3.75	<0.0001	<0.0001
	High MS	2.5		
Mixed	Low MS	3.75	<0.0001	<0.0001
	High MS	2.6		
Permanent	Low MS	4.31	0.0001	0.0012
	High MS	3.35		

Table 6. Cox proportional hazards analysis for caries onset

Variable	Dentition	Hazard ratio (95% CI)	*P*-value
MS level	Deciduous dentition	2.08 (1.43–3.04)	<0.0001
MS level	Mixed dentition	2.00 (1.48–2.71)	<0.0001
MS level	Permanent dentition	2.04 (1.41–2.94)	<0.0001

Discussion

Although many investigators have studied risk factors for caries, only one that we are aware of has studied the relationship of MS to caries onset in children who were initially caries-free (19). Ansai and colleagues investigated how salivary MS levels at baseline influenced caries hazard in preschool children. Their study concluded that caries HR correlated significantly with salivary MS levels at baseline (RR = 1.7). Their results were based on a small sample of 60 caries-free children aged 0.5–6 years who were followed up for 24 months. Our study, based on a somewhat older and larger cohort of initially caries-free children, followed up 464 subjects for up to 6 years. HR in this study were found to be statistically significant with high levels of salivary MS at baseline for the deciduous,

mixed and permanent dentition. These results suggest that once children are identified as harboring high salivary MS levels, they have a greater chance to develop caries than children with low levels of MS. Our approach to the analysis of caries risk factors suggests that assessing the microbiological profile of the caries-free child prior to caries onset, as part of a multifactorial risk assessment protocol, could be a useful adjunct to standard measures of caries risk assessment, i.e. calculating caries indexes, evaluating diets, fluoride exposure or oral habits of an individual. A caries risk assessment protocol must involve the use of measures that are easily obtained, widely accepted, simple to use, reproducible and cost-effective. At the individual level, microbiological screening for MS in saliva has great potential as one of a variety of measures that may be considered in multivariate caries risk assessment protocols, while at the population level, past caries experience is probably the best predictor for the assessment of caries risk (24). In our longitudinal study, as all of the children were caries-free at baseline, prior caries experience was not incorporated into the survival analysis.

We used survival analysis to determine survival time to caries onset and the Cox proportional hazards model to calculate the HR. Survival analysis is a robust analytic procedure for data corresponding to the time between a well-defined 'time origin' and the occurrence of a specified event, called 'endpoint' (23). In this study 'time origin' was the baseline examination and the 'endpoint' was caries onset. The special feature of survival data that makes standard methods inappropriate is that survival times are often censored. For instance, an individual is censored when the 'endpoint' of interest has not been observed for that person or it might not be known because the individual has been lost to follow-up.

Our study is unique in its nature, in that all children were caries-free at baseline, i.e. all children were free of the outcome variable of interest and they were followed for up to 6 years. We used survival analysis to describe longitudinally when caries onset occurs and whether or not the level of MS at baseline made a difference in the caries-free survival time. Based on our analysis, we concluded that caries-free children who had high salivary MS levels at baseline would have a greater risk of caries onset at any given time than caries-free children who had low salivary MS levels. The strength of our approach to data analysis is that survival analysis allows for the inclusion of censored observations. Thus, children who entered the study at baseline and completed at least one follow-up examination were included in the analysis.

The Kaplan–Meier method of estimating survival function is used in the presence of noninformative censoring, i.e. when the survival data are analyzed at a fixed interval of time, the prognosis for individuals who did not reach the disease 'endpoint' can be taken to be independent of the censoring (23). The usual aim of survival analysis is to characterize the distribution of survival for a given population and to compare this survival time among different groups, or to study the relationship between the survival time and existing variables. The Cox model is used to describe the relationship between the distribution of the survival time and the prognostic factors (23). It compares the hazard functions for individuals in two groups by means of HR.

In this study, survival analysis was used to evaluate survival time to caries onset in the deciduous, mixed and permanent dentition in a panel of initially caries-free children from Rochester and the Finger Lakes region of western New York. The hypothesis tested was that children with low levels of MS at baseline remained caries-free for a longer period than children with high levels of MS at baseline. Survival analyses demonstrated that caries-free survival probabilities were prolonged for children with low salivary MS levels at baseline compared with children with high salivary MS levels at baseline. The results from survival analyses demonstrated that there is a parallel relationship between salivary MS levels at baseline and caries onset in initially caries-free children, i.e. the estimated survival function for children with low salivary MS levels was always greater than that for children with high salivary MS levels for the deciduous, mixed and permanent dentition. This means that at any time t, the estimated probability of survival beyond time t was greater for children with low salivary MS levels, suggesting that the result of screening for the MS salivary level might be a useful prognostic indicator. Children whose salivary MS levels were high at baseline appeared to have a poorer prognosis for caries onset than those with low levels of salivary MS. The Kaplan–Meier estimates of survival functions for the deciduous, mixed and permanent

dentition of the two groups of children are plotted in Figs 2–4.

To explore the relationship between the survival experience of individuals and explanatory variables, we used the Cox proportional hazards model which unifies and extends the Kaplan–Meier method. The hazard function for individuals with low salivary MS levels at baseline was compared with the hazard function for individuals with high salivary MS levels by means of HR. The estimated values of the HR for our study are shown in Table 6. As the obtained HR of caries onset at time t for children with high salivary MS levels compared with children with low salivary MS levels were all >1, we concluded that children who had high salivary MS levels at baseline would have a greater risk of caries onset at any given time than children who had low salivary MS levels.

Assuming that the baseline screening for MS influenced the outcome variable (caries onset) significantly, it is important to design and implement effective strategies to prevent caries once high MS colonization is recognized. Salivary MS levels remained stable during the course of the study, i.e. a majority of children identified with low levels remained low and a majority of children identified with high levels remained high for the 6 years of the study. Based on our analysis of longitudinal caries data, we concluded that caries-free children who had high salivary MS levels at baseline would have a greater risk of caries onset at any given time than caries-free children who had low salivary MS levels at baseline.

In clinical trials where there is a sizeable attrition rate over time, considerable information may be lost. Survival analysis technique accounts for dropout rates and that is an advantage over standard statistical methods. In our case, by using survival analysis, we gained more accurate estimation on the timing of caries onset, at least in initially caries-free children. Use of survival time analysis in longitudinal studies has the additional benefit of incorporating the concept of 'risk' over time.

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