Comparison of a new chair-side test for caries risk assessment with established methods in children

Key words: caries risk assessment, children, lactic-acid production, mutans streptococci, lactobacilli

Introduction

Over the last decades, a notable caries decline and an increase in the number of caries-free dentitions have taken place in highly industrialized countries which use preventive programs (Marthaler 2004; Pieper & Schulte 2004). The studies using the Significant Caries Index (SiC) showed that there is still a small group of children with high numbers of caries lesions in populations with low caries prevalence (Nishi et al. 2002, Morgan et al. 2005). Thus, the objective of further efforts is an early identification of children with high caries risk and then to enrol them in intensive preventive programs.

However, caries is a complex process, and the multitude of causative factors makes the assessment of the caries risk difficult. The past caries experience was described as a useful and also simple predictor of the future caries activity in children. Several variables like the number of decayed, missing and filled teeth/surfaces in deciduous and permanent dentitions, caries in primary molars and in fissures of permanent first molars, as well as the low number of sound primary teeth and pre-cavity lesions on permanent molars are possibilities to assess the past caries experience (Steiner et al. 1992, Van Palenstein Helderman et al. 2001, Leroy et al. 2005, Zhang & Van Palenstein Helderman 2006). Also behavioural host factors like quality and frequency of oral hygiene measures, dietary habits, especially the regular usage of sugar-containing drinks, and frequency of snack intake and lack of systemic fluoride supplements were confirmed as risk factors for caries (Vanobbergen et al. 2001, Marshall et al. 2005, Leroy et al. 2005). On the other hand, subclinical factors like flow rate, buffering ca-

Summary

Purpose: The aim of this study was to compare the results of a new method, measurement of lactic-acid production on the tongue (LAP), with established methods of caries risk assessment in children.

Methods: One hundred nineteen children (6–10 years old) participated in the study. Data collection included number of carious lesions (D–T) and filled teeth (F–T), approximal plaque index (API), LAP, buffering capacity (BC), counts of mutans streptococci (MS) and lactobacilli (LB) in stimulated saliva. According to caries presence, the children were divided into low risk (LR group; D–T =0) and high risk (HR group; D–T ≥ 1) groups. Statistical analysis was performed using Chi-square test, Spearman’s test for nonparametric correlations, uni- and multivariate regression analysis.

Results: Seventy-two children (F–T =0.4 ± 1.4) were in the LR and 47 (D–T= 2.5 ± 2.7; F–T= 1.5 ± 1.9) in the HR group. The correlation analysis verified statistically significant correlations between D–T and the salivary counts of MS/LB and between D–T and F–T. API correlated with F–T and LB, while F–T and LB also correlated with each other significantly. The counts of MS and LB showed also a significant correlation. The LAP showed a significant correlation only to F–T. Significances in univariate regression analysis were found for F–T, counts of LB and MS in saliva, and for LAP. The multivariate regression analysis indicated significances only for F–T and LB in saliva, but not for LAP.

Conclusion: LAP might be useful only as a supplementary screening tool for caries risk assessment, but not as a sole predictor.
pacity and pH of resting or stimulated saliva, the microbiological, organic and inorganic composition of saliva and plaque were determined as related factors of caries development and also verified as further parameters to predict future caries risk (Loesche et al. 1975, Zoiopoulos et al. 1997, Raftio et al. 1996, Vehkalahti et al. 1996, Brambilla et al. 1999, Gabris et al. 1999, Pearce et al. 2002). The variability of influencing factors has lead to the development of caries prediction and risk models, combining clinical and subclinical parameters (Raftio et al. 1996, Vanobbergem et al. 2001, Hänsel Petersson et al. 2002, Brathall & Hänsel Petersson 2005). In such models, the past caries experience and plaque amount, salivary properties, contents and frequency of intake of the cariogenic diet as well as the bacterial composition of saliva and plaque were considered in different combinations; but the accuracy of these models is still low (Raftio et al. 1996, Vanobbergem et al. 2001).

The presence of lactic-acid producing bacteria, mainly mutans streptococci and lactobacilli in saliva and in plaque was related with the caries experience and increment in children (Loesche et al. 1975, Raftio et al. 1996, Brambilla et al. 1999, Campus et al. 2000). A correlation between the caries experience and the number of these bacteria in plaque and in saliva samples was also verified in different studies (Roeters et al. 1995, Zoiopoulos et al. 1997). Thus, the qualitative and quantitative detection of these bacteria in saliva was applied in the caries risk assessment; simplified techniques with valuable results were developed for chair-side tests (Köhler & Bratthall 1979, Jensen & Bratthall 1989, Davenport et al. 1992, Spleith & Bernhardt 1999, Pinelli et al. 2001). However, in several studies quantitatively detecting mutans streptococci and lactobacilli, the sensitivities of the tests were lower (0.41–0.82) than the specificities (0.59–0.85). Apparently, they seem to be better indicators for low than for high caries risk (Sullivan & Schröder 1989, Vehkalahti et al. 1996, Spleith & Bernhardt 1999; Pinelli et al. 2001). Further investigations analysing the bacterial colonization and the pH-drop in plaque and saliva demonstrated that also non-mutans streptococci and other acidogenic bacteria could affect the pH-drop and caries prevalence (Babaahmady et al. 1998, Seki et al. 2006). In addition, genotypic diversity of Streptococcus mutans strains and their virulence were verified comparing them in caries-free and caries-active individuals (Napimoga et al. 2004, Kamiya et al. 2005). These findings can probably explain the low sensitivity of the tests identifying mutans streptococci and lactobacilli separately.

Thus, new methods for quantitative evaluation of acidogenic bacteria causing a pH-drop in plaque as caries risk predictor are required. Although it was shown in healthy subjects that some species are site specific (Aas et al. 2005), a positive relationship between S. mutans on the tooth surfaces and on the dorsum of the tongue was shown in studies in young children with high caries activity (Tanner et al. 2002). Based on these findings, a new chair-side test (Clinpro Cario™ L-Pop™, 3M Espe, Seefeld, Germany) measuring the lactic-acid production on the tongue dorsum as an indicator for metabolic activity of cariogenic bacteria was developed. It was claimed by the manufacturer to be a semi-quantitative test to determine the individual caries risk. Schifferner & Torres-Quintero (2005) evaluated the reproducibility of this test and found a reproducibility of 82% if all conditions remained stable. However, if there were changes in the oral conditions the reproducibility was reduced to 60%. Gerardu et al. (2006) also demonstrated a low reproducibility of 37%. They found no correlations to the lactic-acid concentrations in plaque samples but observed a change in acidogenicity of the tongue samples after use of an antimicrobial toothpaste and mouthwash.

The aim of the present study was to compare the results of this new method, measurement of lactic-acid production on the tongue, with established clinical and subclinical methods of caries risk assessment in children.

Material and methods

One hundred nineteen children (6–10 years old, 65 girls, 54 boys), enrolled in a preventive program, participated in this cross-sectional study. The healthy subjects showed no enamel or dentin defects and they had not received any medication in the last three months. The study meets the ethical requirements of the Declaration of Helsinki. Written informed consent was obtained from all parents.

As the clinical caries risk predictors, past caries experience and oral hygiene scores were chosen. After drying the tooth surfaces, the dental examination was performed by one experienced examiner using a dental mirror and probe. The numbers of decayed (D) and filled (F) teeth (T) were recorded for the determination of the past caries experience. Teeth with initial and secondary lesions were also termed carious. Also, the primary molars were included in the determination because of the mixed dentition in this age group. The approximal plaque index (API, Lange 1977) using a disclosing solution (Plaviso®, Voco, Germany) was used for the evaluation of the oral hygiene score (OH-1 = API ≤ 25%; OH-2 = 25% < API ≤ 50%; OH-3 = 50% < API ≤ 75%; OH-4 = 75% < API ≤ 100%).

The buffering capacity and the acidogenic bacterial composition of the stimulated saliva were the subclinical parameters of the caries risk assessment used in this study. In order to account for the circadian rhythm, the subjects were examined at the same time of day (10:00–11:00 a.m.), at least 120 minutes after the last meal (breakfast). For saliva collection, the subjects chewed on a piece of paraffin and the whole saliva was collected in a plastic cup for 5 minutes. For the determination of the buffering capacity (BC), the collected whole saliva was applied on an indicator (Clinpro buffer, Ivoclar Vivadent, Ellwangen, Germany) and the changes were estimated using a colour chart according to the instructions of the producer (BC-1 = pH > 6.60, high; BC-2 = pH = 4.5–5.5, medium; BC-3 = pH ≤ 4, low buffering capacity). The quantification of mutans streptococci (MS) and lactobacilli (LB) in saliva was performed using a chair-side test (CRT bacteria, Ivoclar Vivadent, Ellwangen, Germany). After the application of the collected saliva on the agar surfaces for MS and LB, the kits were incubated for 48 h at 37°C. The bacterial counts on the surfaces were determined always by the same examiner (MS1/LB1 = no bacterial growth; MS2/LB2 < 10^6 CFU/ml; MS3/LB3 > 10^5 CFU/ml).

Before the determination of lactic-acid production on the tongue, the subjects brushed their teeth with a provided toothbrush and toothpaste for two minutes (toothbrush: Oral B Stages 4; toothpaste: Elmex) as recommended by the producer. After 5 minutes, the sample collection was carried out with an indicator swab on the tongue by turning it for four times (LAP). Then the reagents in the blister were activated and the swab was inserted into the blister. After leaving it in place for two minutes, lactic-acid production was assessed using the colour chart (Fig. 1). Because of the irregular distribution of the colour on the swab, the assessment was performed always by the same examiner using the darkest zone on the cotton swab. The children were divided into three caries risk groups according
to the instructions of the producer: scores 1–3 for low caries risk (LAP-1), scores 4–6 for moderate caries risk (LAP-2), scores 7–9 for high caries risk (LAP-3).

After data collection, two study groups were built using the clinical caries predictors. Children without caries lesions (D–T = 0) were in the low caries risk group (LR) and children with carious teeth (D–T > 1) were in the high caries risk group (HR).

The statistical evaluation of the data was performed using the SPSS program (Version 12, SPSS Inc., Chicago, Illinois, USA) at the Institute for Medical Statistics, Epidemiology and Informatics, Johannes Gutenberg University, Mainz. Chi-square test was used to compare the clinical with the non-clinical findings. The correlations between the caries risk predictors were analyzed using the Spearman’s test for non-parametric correlations. The univariate regression analysis was used to identify predictor variables that were statistically significantly associated with the presence of carious lesions. The multivariate regression analysis was performed to determine stepwise the relevance of different caries risk predictors associated with the presence of carious lesions. After each step the most significant variable was removed to show the relevance of the remaining caries risk predictors. The significance level was set at p < 0.05.

### Results

The mean DF–T of the children (mean age: 7.7 ± 1.2) was 2.6 ± 3.1 and the mean number of carious teeth 1.5 ± 2.5. Caries-free dentitions (D–T = 0) were found in 72 children (61% LR group; 7.6 ± 1.4 years old; 41 girls, 31 boys); the mean number of fillings in these subjects was 0.4 ± 1.4 (median 0, range 0–6). The 47 children (39%; 7.7 ± 1.0 years old; 24 girls, 23 boys) with carious teeth (D–T = 2.5 ± 2.7; median 2, range 1–17) were in the HR group and they had a mean number of 1.5 ± 1.9 (median 1, range 0–7) fillings. The mean value of the API in the LR group was 37.5 ± 14.5% and in the HR group 42.6 ± 17.3%; the difference between the study groups was not significant (Tab. I).

68% of the children had an oral hygiene score of 2 (OH-2) with an API value of 25 to 51%. In this group, the children with low (48%) and high (52%) caries risk were represented comparably (Fig. 2). As expected, in the groups with higher oral hygiene scores, the percentage of children with high caries risk was more than 75%.

The buffering capacity of the saliva did not differ significantly between the LR (2.6 ± 0.6, median 3, range 1–3) and HR (2.5 ± 0.6, median 3, range 1–3) groups. A high buffering capacity was found in 63% of the children; in all BC groups, 56–69% of the children belonged to the HR group.

A statistically significant difference was found in the bacterial counts of MS and LB in saliva between the risk groups (p < 0.05); 43% of the children in MS-1 and 36% of the children in LB-1, but 75% of the children in MS-3 and 73% of the children in LB-3 were in the HR group (Fig. 3). Between the study groups, also the lactic-acid production on the tongue was significantly different (p < 0.05). But this difference was only present in the children with LAP-3 (LR/HR = 26%/74%); in the groups with LAP-1 and LAP-2, the children with LR and HR were represented comparably (LAP-1: LR/HR = 44%/56%; LAP-2: LR/HR = 48%/52%).

The correlation analysis of the caries predictors without regard of the caries risk groups verified statistically significant correlations between the number of carious lesions and the number of the cariogenic bacteria (MS and LB) in saliva (p < 0.05). A marginal correlation was also found between the D–T and F–T (Tab. II). The oral hygiene scores of the children correlated with F–T and with the number of LB in saliva, while F–T and LB correlated also with each other significantly. The number of both cariogenic bacteria, MS and LB, in saliva showed also a significant correlation. The LAP showed a significant correlation to F–T, but was not associated with D–T or with counts of the cariogenic bacteria in saliva.
In the univariate regression analysis using the risk groups, the F–T, the counts of LB and MS in saliva and also LAP showed significances (Tab. III). The multivariate regression analysis indicated significances only for F–T and for the counts of LB in saliva, but not for LAP.

**Discussion**

The past caries experience, especially caries at the primary molars and first permanent molars was determined as a good predictor of the future caries risk in earlier investigations (Steiner et al. 1992, Van Palenstein Helderman et al. 2001, Leroy et al. 2005, Zhang & Van Palenstein Helderman 2006). Therefore, the actual caries activity was chosen as the clinical predictor of the future caries risk for the evaluation of the prognostic potential of various clinical and subclinical predictors in the present study. However, the caries activity might have been underestimated, since incipient approximal lesions can only be detected on radiographs.

Frequency of tooth brushing and plaque amount are strongly related with cavity formation and thus, the oral hygiene score can also be considered as a clinical predictor in caries risk assessment (vanobbergen et al. 2001, Leroy et al. 2005, Bratthall & Hansel Peterson 2005). In the present study, the plaque removal was assessed using the approximal plaque index and, according to its values, oral hygiene groups were built. A good plaque removal was detected in 81 children. However, according to caries presence, 52% of these, plus the three children with very good oral hygiene, were in the high caries risk group. On the other hand, 77–78% of the children with moderate
...buffering capacity was described as an important protective factor in oral health for neutralizing acids in saliva. A high buffering capacity of stimulated saliva was determined in 68% of our subjects. Only eight children (7%) had a low buffering capacity. Sánchez & Fernández de Prelisacco (2003) also found a low salivary buffering capacity in 8% of 4- to 10-year-old children, but the percentage of the children with a high buffering capacity was in their investigation with 47% lower than in the present study. Although the salivary buffering capacity was described as an important protective factor in oral health for neutralizing acids in saliva (Edgar 1992), in the present study the buffering capacity of the children with low (mean BC: 2.6±0.6) and high (mean BC: 2.5±0.6) caries risk did not differ significantly. However, a high variability in buffering capacity in children was demonstrated in earlier studies when measurements in the same individuals were repeated over several months (Tukia-Kulmala & Tenovuo 1993). Also Kirstila et al. (1998) concluded that none of the single properties of saliva, such as the buffering effect, has sufficiently strong power to have diagnostic value with respect to future caries.

In our study, counts of salivary mutans streptococci and lactobacilli differed significantly between the two caries risk groups and correlated significantly with the number of carious teeth. Only the counts of the lactobacilli demonstrated significant correlations to the number of fillings, although the salivary counts of the two bacterial groups were highly correlated with each other. The results of the lactic-acid production on the tongue, which is supposed to assess the total count of lactic-acid producing bacteria in the oral cavity, differed statistically significantly between the caries risk groups. However, the mean levels of both groups (LR: 4.1±2.4; HR: 5.2±2.8) were within the range of moderate caries risk (LAP-2: scores 4–6), and thus this significance cannot be accepted as clinically relevant. This high significance was mainly a result of the distribution of the children in the LAP-3 group. 74% of 38 children in this group were in the HR-group. However, more than 50% of the children in the LAP-1 and LAP-2 had also a high caries risk according to caries presence.

The LAP showed a significant correlation to the number of filled teeth but not to the counts of salivary mutans streptococci and lactobacilli. The univariate regression analyses confirmed the relevance of the number of fillings, counts of lactobacilli and mutans streptococci but also the lactic-acid production on the tongue when using the caries risk groups. The multivariate regression analyses using the same criteria verified only the number of fillings and the counts of lactobacilli in saliva as relevant predictors. The count of mutans streptococci lost its relevance in the third step when the number of the filled teeth and the counts of lactobacilli were excluded. Càmpus et al. (2000) demonstrated in 6- to 8-year-old children significant differences for the scores of streptococci and lactobacilli in saliva between those with and without caries in primary teeth, but only for lactobacilli in the permanent dentition. Granath et al. (1994) found in 4- to 5-year-old children better explanatory potency for counts of lactobacilli than for mutans streptococci in saliva. In the present study, the number of fillings as well as the counts of LB correlated significantly with the API values of the children. Studies comparing gingival conditions and plaque growth on enamel surfaces and different tooth-coloured filling materials confirmed, independent of the material, a stronger plaque growth on such fillings and a worsening of gingival conditions (van Dijken & Sjöström 1998). Chang et al. (1999) determined also higher counts of lactobacilli three months after placement of fixed orthodontic appliances by increasing plaque index scores. Thus, the correlation between the plaque amount and the number of the filled teeth can explain the relevance of the counts of lactobacilli in the present study. Petti et al. (1997) verified in 6- to 7-year-old children an association between the number of decayed teeth and the detection of Streptococcus mutans. But in agreement with our results, they found no correlation between the detection of Streptococcus mutans and the number of filled teeth.

Tukia-Kulmala & Tenovuo (1993) demonstrated in 11-year-old children a high intra- and inter-individual variation in counts of mutans streptococci and lactobacilli in saliva, and...
they concluded that the developing dentition, behavioural and hormonal changes, but also dietary factors can make single point measurements of salivary factors unreliable. A similarly low reproducibility was also found for the lactic-acid production on the tongue (Schiffner & Torres-Quintero 2005, Gerardu et al. 2006). Gerardu et al. (2006) determined the reproducibility of this test by repeated measurements after four washout periods in which the subjects used only fluoride-free toothpaste without other antimicrobial additives. Only 11 scores of the 30 subjects were consistently in the same risk category. In addition to the discrepancies in the repeated measurements, Gerardu et al. (2006) found no correlations between the lactic-acid scores on the tongue and in the plaque. Schiffner & Torres-Quintero (2005) repeated the LAP measurements of 31 subjects after two weeks and determined the oral conditions by means of a questionnaire. In 82% of the 20 subjects with stable and in 60% of 11 patients with changes in oral conditions, the results of LAP were reproducible. They also compared the scores of the test swabs from a second examiner and found accordance only in 17 of 31 sticks. A reason for this low agreement could be the colour changes on the swabs, which are not uniform as described by Häberlein et al. (2003). In the present study, the LAP test swabs as well as the MS and LB tests were visually evaluated by the same examiner to avoid error by reading.

In the present cross-sectional study, it was not possible to make a conclusion about the prognostic value of the new chair-side test measuring the lactic-acid production on the tongue dorsum for caries risk assessment. The comparison of its results with the other established clinical and subclinical predictors led only to minimal correlations. The number of filled teeth and the counts of lactobacilli were found to be relevant when using the caries presence as the main predictor of future caries development. Thus, the new test might be useful as a supplementary screening tool for caries risk assessment, but not as a sole predictor.

**Zusammenfassung**

Ziel: Ziel der vorliegenden Studie war es, die Ergebnisse einer neuen Methode, Bestimmung der Milchsäureproduktion auf der Zunge (LAP), mit etablierten Methoden zur Kariesrisikobestimmung bei Kindern zu vergleichen.

Methoden: 119 Kinder (6–10 Jahre alt) nahmen an der Studie teil. Als Parameter wurden Anzahl kariöser (D–T) und gefüllter Zähne (F–T), Approximal-Plaque-Index (API), LAP, Pufferkapazität (BC), Anzahl der Mutans-Streptokokken (MS) und Laktobazillen (LB) im stimulierten Speichel erhoben.

Die Kinder wurden nach dem Vorhandensein kariöser Läsionen in Gruppen mit niedrigem (LR-Gruppe; D–T = 0) und hohem (HR-Gruppe; D–T ≥ 1) Kariesrisiko eingeteilt. Die statistische Auswertung erfolgte mit dem Chi-Quadrat-Test, dem Spearman-Test für nichtparametrische Korrelationen und mit uni- und multivariaten Regressionsanalysen.


Schlussfolgerung: LAP könnte sich höchstens als zusätzlicher Test, aber nicht als alleiniger Prädiktor bei der Kariesrisikobestimmung als nützlich erweisen.

**Résumé**

Objectif: Le but de cette étude était de comparer les résultats d’une nouvelle méthode de mesure de la production de l’acide lactique sur la langue (LAP) avec des méthodes établies pour évaluer le risque carieux chez les enfants.

Méthodes: 119 enfants (6–10 ans) ont participé à cette étude. La collecte des données comprend le nombre des lésions carieuses (D–T) et des dents avec des obturations (F–T), l’indice de la plaque interproximale (API), LAP, le pouvoir tampon (BC), le nombre des mutants streptocoques (MS) et des lactobacilles (LB) dans la salive stimulée.

Selon la présence de caries, les enfants ont été classés en deux groupes (risque bas, groupe LR; D–T = 0; risque élevé, groupe HR; D–T ≥ 1). L’analyse statistique a été exécutée avec le test Chi carré, le test de Spearman pour les corrélations non paramétriques, les analyses par régression uni et multivariée.

Résultats: 72 enfants (F–T = 0,4 ± 1,4) étaient dans le groupe LR et 47 (D–T = 2,5 ± 2,7; F–T = 1,5 ± 1,9) dans le groupe HR. L’analyse de la corrélation a montré des corrélations statistiquement significatives entre D–T et le nombre de MS/LB dans la salive et entre D–T et F–T. API était corrélé avec F–T et LB, tandis que F–T et LB étaient aussi corrélés l’un avec l’autre significativement. Les nombres de MS et de LB ont montré aussi une corrélation significative. Le LAP a montré une corrélation significative seulement avec F–T. Des résultats statistiquement significatifs ont été trouvés avec l’analyse par régression uni variée pour F–T, le décompte de LB et MS dans la salive et pour LAP. L’analyse par régression multivariée indique une signification statistique seulement pour F–T et LB dans la salive, mais pas pour LAP.

Conclusion: LAP pourrait être utile comme outil supplémentaire de dépistage du risqué de lésions carieuses, mais pas comme un indicateur unique.

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References


