Predicting Caries by Measuring Its Activity Using Quantitative Light-Induced Fluorescence in vivo: A 2-Year Caries Increment Analysis

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Abstract

The aim of this study was to analyse the predictive power of several clinical baseline parameters and the de-/remineralisation properties of in vivo etched sites measured with quantitative light-induced fluorescence (QLF) for subsequent 2-year caries increment. At baseline, in 44 children (8.23 ± 1.5 years) two areas (diameter 2 mm) of the buccal surface of a primary posterior tooth were etched with 36% phosphoric acid gel for 1 and 4 min, respectively. The etched sites were analysed immediately after etching ($\Delta Q1$) and 24 h ($\Delta Q2$) later by QLF. Additionally, caries status (deft/DMFT and initial caries), approximal plaque, bleeding on probing, and the patient's current use of fluorides were recorded. In the 2-year follow-up, 29 children were re-assessed. After clinical examination, the caries increment was calculated ($\Delta DMFT$) and correlated with the baseline clinical variables and the QLF readings. Results showed a significant positive correlation between $\Delta Q_{1\,min}$ and the $\Delta DMFT$ (r = 0.44, p = 0.02). The $\Delta DMFT$ was significantly correlated with the baseline deft (r = 0.56, p = 0.002), cavitated active caries lesions (r = 0.52, p = 0.003), and filled teeth (r = 0.53, p = 0.003). In a regression analysis the use of fluoridated salt (SC = –0.10) and fluoride gel (SC = –0.14) were negatively associated with $\Delta DMFT$. In conclusion, these findings suggest that the demineralisation properties of the etched sites and the outcome of the 24-hour measurements with QLF are significantly associated with caries increment. Previous caries experience strongly correlated with caries increment in this group of children.

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The ability to predict the risk of dental caries is one of the cornerstones for successful control and management of the disease. Due to its multifactorial aetiology, there is no known sole factor associated with caries initiation and development [Twetman and Fontana, 2009; Meurman and Pienihäkkinen, 2010]. Risk factors for dental caries include a variety of biological, environmental, and behavioural factors, which are taken into account when developing a caries profile of the patient [Fontana and Zero, 2006; Holgerson et al., 2009; Riley et al., 2010]. Although various modern tools for caries risk assessment have been developed and evaluated, notwithstanding extensive research, the sensitivity and specificity of individualised caries risk prediction are still suboptimal [Hausen et al., 1994; Hausen, 1997; Reich et al., 1999; Pienihäkkinen et al., 2004; Meller et al., 2006a, b].

The foremost purpose of caries prediction is to determine the probability of a caries lesion occurring, thereby

allowing the clinician to identify those individuals with an increased risk for future disease development and to institute a risk-specific preventive regime. Currently, the best predictor of future caries development is the patient's past caries experience and active existing caries lesions [Tinanoff and Reisine, 2009; Twetman and Fontana, 2009]. Thus, caries prediction is primarily made according to an already manifested disease. From a disease control point of view, this is an undesirable outcome, considering that the ultimate objective in caries management is to prevent even the earliest manifestations of the disease, even before its destructive effects become apparent [Meller et al., 2006a, b; Twetman and Fontana, 2009].

Due to the difficulties in finding a more accurate risk model for caries prediction, a method to measure the activity of de- and remineralisation with quantitative light-induced fluorescence (QLF) was recently proposed by Meller et al. [2006a]. A previous study [Meller et al., 2006b] using a colorimeter already showed positive results. The analysis is based on a method to assess de- and remineralisation in vivo as used in several previous studies [Möller et al., 1987; Ulbricht, 1989; Petzold and Poppe, 1992] concerning remineralisation speed. The method consists of minimal standardised enamel etching and measurement of the degree of demineralisation and subsequent remineralisation. Meller et al. [2006a] quantified these minute mineral changes with the help of QLF, a fluorescence inducing analysis, which has been proven to be suitable for in vivo detection and monitoring of small mineral changes in enamel lesions [van der Veen and de Josselin de Jong, 2000; Tranæus et al., 2001, 2002; Al-Khateeb et al., 2002]. The results of this attempt suggest that the degree of demineralisation of an etched test site measured immediately after etching and its subsequent behaviour measured 24 h later are associated with caries activity. The authors concluded that this method could be used for early assessment of caries activity [Meller et al., 2006a].

The aim of the present clinical follow-up study was to analyse the predictive power of several clinical baseline parameters and the de-/remineralisation properties of etched test sites measured with QLF for the subsequent 2-year caries increment.

Subjects and Methods

Ethical Aspects
The present study was approved by the Research Ethics Committee of the University of Greifswald, under the protocol number III UV 61/02, and conducted in accordance with the principles for medical research involving human subjects described by the Declaration of Helsinki.

Subjects
Forty-four healthy patients attending the Paediatric Dentistry Department of the Greifswald Dental School with mixed dentition were included in the study (age range 5–11 years). Sample size had been estimated using the program nQuery Advisor version 4.0 for a two-sided analysis of the null hypothesis of the first cross-sectional survey with Fischer's z test at a power level of 80%. To compensate for possible loss of subjects to follow-up, 20% were added to the original sample size of 37 subjects. The clinical inclusion criterion was that participants had to have at least one sound vestibular surface of a deciduous molar. The parents of the participants were informed about the purpose of the study and gave written consent for their children to participate. Exclusion criteria were lack of cooperation and serious oral or general diseases.

Clinical Examination
A previously calibrated examiner (C.M.) performed all clinical examinations at the first (baseline) and the second (2-year follow-up) appointments. The study was done by blind design evaluation. The clinical examination included deft/DMFT scores [WHO, 1997], the carious lesion index [Nyvad et al., 1999] differentiating between active and inactive cavitated and non-cavitated lesions, the approximal plaque index (API) [Lange et al., 1977] after staining with 'Mira-2-Tone' (Hager and Werken GmbH, Duisburg, Germany), and the modified papilla bleeding index (PBI) [Saxer and Mühlemann, 1975]. Additionally, the use of several fluoride sources including the regular use of fluoridated toothpaste, gel (Elmex gelée, Gaba GmbH, Lörrach, Germany), tablets, and salt was documented (use = 1/no use = 0). A final fluoride score, as the sum of all frequently used fluorides, was calculated. Details of patient recruitment and follow-up are listed in figure 1.

Study Method
A clinically sound vestibular surface of a deciduous molar was selected, cleaned with a cup-shaped rotating brush and air-water spray in a slow-speed handpiece and then dried with the air syringe for 5 s. Subsequently, two areas of the cervical third of the vestibular surface were etched with 36% phosphoric acid gel (Dentsply DeTrey GmbH, Konstanz, Germany) for 1 and 4 min, respectively. These etching times were chosen as they allowed a moderate and strong etching mark in a pilot testing which also resulted in a difference in fluorescence. The etching gel was applied in a sufficient quantity to form an etched circle about 1–2 mm in diameter. After etching, the acid was washed out with air-water spray for 60 s.

The etched sites were analysed immediately after the etching ($\Delta Q1$) and again after 24 h ($\Delta Q2$) with QLF (fig. 2). Participants were explicitly asked not to brush their teeth during the 24-hour test period in order to avoid possible abrasive-induced changes on the etched areas. The QLF clinical system version 3.0.3.21 (Inspektor Research Systems BV, Amsterdam, The Netherlands) was used to analyse the images for quantification of mineral loss (ΔQ) in the demineralised areas.

All QLF readings were done by one experienced and trained operator (C.M.) under identical physical conditions in a dental unit located in a darkened room. Blind image analysis was conducted as to whether the areas were demineralised for 1 or 4 min
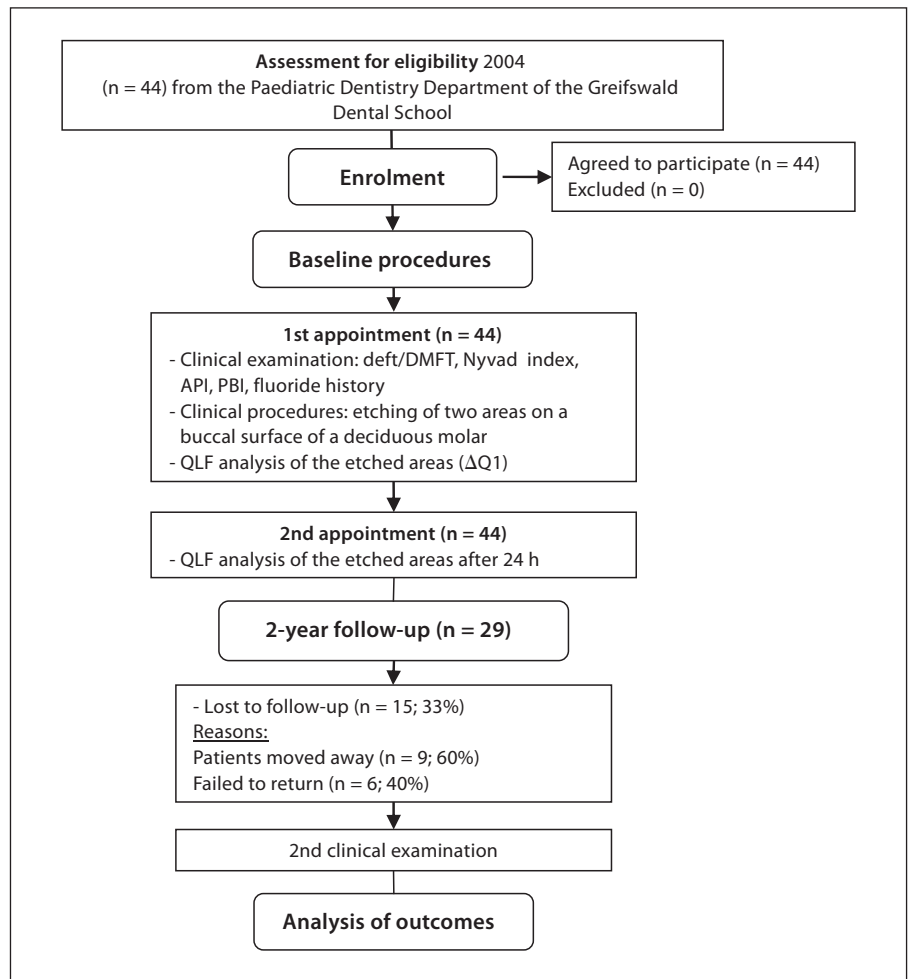
Fig. 1. Flow diagram of the study.
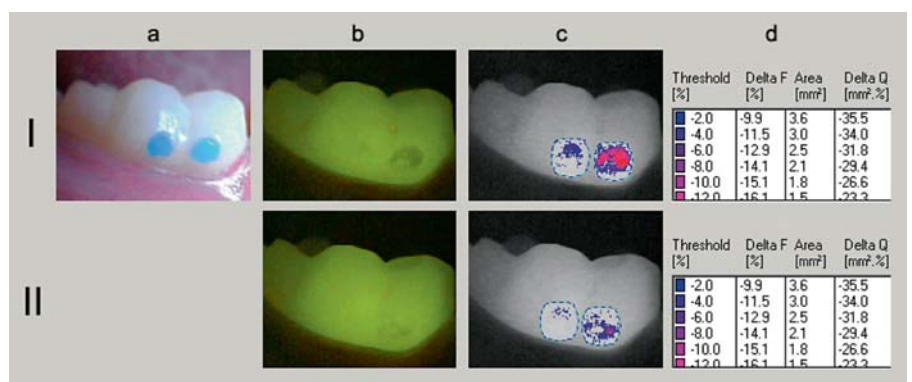
Fig. 2. Example of enamel etching (**a**) for measurement of fluorescence loss (**b**) immediately after demineralisation (I) and 24 h later (II), after computerised lesion reconstruction in relation to intact enamel (**c**) and quantified for statistical analysis (**d**).

and without reference to the patient's data. The custom-made computer program Inspektor QLF version 2.00f (Inspektor Research Systems) was used to quantify the mineral loss (ΔQ) in the etched areas.

After 2 years, 29 children were re-examined by the same examiner (C.M.) who was blinded to baseline data following the same clinical examination protocol already described.

Data Analysis

Data analyses were performed using the SPSS software (version 17.0) for Windows. Caries increment was calculated (ΔDMFT) and correlated with the clinical baseline variables and the QLF readings ($\Delta Q1$ and $\Delta Q2$). The Pearson correlation coefficient was used to measure the degree of association between caries increment (deft scores, and the Nyvad criteria) and baseline

Table 1. Caries-related Nyvad scores according to Nyvad caries criteria (means ± SD)

Active lesions			Inactive lesions			Fillings		
code 1 without cavitation	code 2 enamel discontinuity	code 3 cavitation	code 4 without cavitation	code 5 enamel discontinuity	code 6 cavitation	code 7 filling	code 8 related to an active lesion	code 9 related to an inactive lesion
0.84 ± 1.80	0.39 ± 1.20	1.05 ± 2.64	1.86 ± 2.64	0.16 ± 0.64	0.07 ± 0.25	1.63 ± 1.64	0.16 ± 0.57	0.18 ± 0.58

Table 2. Correlations (r) between ΔQ after etching for 1 min (ΔQ1/1 min), 4 min (ΔQ1/4 min), and after 24 h (ΔQ2/1 min, ΔQ2/4 min) with deft, API, PBI, active carious lesions and fluoride use

ΔQ		deft	API	PBI	Active lesions	Fluoride scores
ΔQ1/1 min	r	0.52**	0.38**	0.34*	0.57**	−0.37*
	p	<0.001	0.009	0.21	<0.001	0.012
ΔQ1/4 min	r	0.52**	0.37*	0.40**	0.42**	−0.53**
	p	<0.001	0.012	0.007	0.004	0.001
Δ Q2/1 min	r	0.39**	0.38**	3.11*	0.59**	−0.31*
	p	0.008	0.010	<0.40	<0.001	0.037
Δ Q2/4 min	r	0.49**	0.39**	0.50**	0.57**	−0.44**
	p	0.001	0.008	<0.001	<0.001	0.003

* p < 0.05, ** p < 0.01.
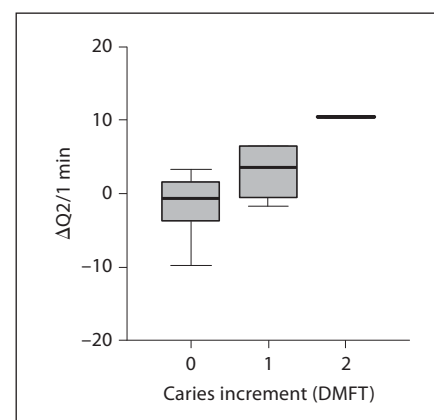
Fig. 3. ΔQ after etching for 1 min and subsequent remineralisation after 24 h associated with the caries increment (ΔDMFT) after 2 years.

QLF readings (ΔQ) as well as API, PBI, and fluoride scores. Additional linear regression analyses were performed to investigate the relationship between caries increment (ΔDMFT >0 over the 2-year period) and baseline variables. The level of significance was defined as $p < 0.05$.

Results

Baseline Findings

At baseline (sample size n = 44, 45.5% females, 54.5% males) the mean age of the sample was 8.22 (±1.4) years, mean dmft was 3.9 (±4.5) and mean DMFT 0.05 (±0.4). Mean values for the caries-related Nyvad scores are shown in table 1.

The results of the baseline correlation analysis are shown in table 2. The deft scores showed a very strong positive and statistically highly significant correlation with the presence of active carious lesions (r = 0.70, p < 0.001). After 1 min of demineralisation, mineral loss (ΔQ1/1 min, 5.4 ± 7.7) correlated also strongly with deft scores (r = 0.52, p < 0.001) and with the number of active lesions (r = 0.57, p < 0.001). Likewise, ΔQ after 24 h in both cases, after 1 min (ΔQ2/1 min, 3.4 ± 6.8) and after 4 min of demineralisation (ΔQ1/4 min, 16.0 ± 11.5), significantly correlated with baseline deft scores. Fluoride scores, API, and PBI also showed a statistically significant correlation with the de- and/or remineralisation measured in the QLF analysis, however the values were considerably lower.

Follow-Up

After 2 years, 29 subjects (48.3% females and 51.7% males), representing 66% of baseline samples, were reassessed at a mean age of 10.1 (±1.3) years. The mean DMFT increment was 0.21 (±0.49). The correlations between ΔDMFT with the baseline variables are shown in table 3. In the correlation analysis, the ΔQ (1 min: 2.0 ± 3.7; 4 min: 6.0 ± 7.6) showed a statistically significant positive correlation with the ΔDMFT (r = 0.44, p = 0.02

Table 3. Correlations (r) between caries increment (ΔDMFT) with clinical baseline variables

ΔDMFT	$\Delta Q_{1\ min}$	deft	Active lesions	Fillings	Plaque (API)	Gingivitis (PBI)	F salt	F gel
r	0.44*	0.56*	0.53*	0.53*	0.24	0.04	−0.05	−0.18
p	<0.02	<0.02	<0.03	<0.03	0.83	0.21	0.58	0.78

* $p < 0.05$.

and r = 0.40, p = 0.03, respectively). Figure 3 shows the association between ΔQ and ΔDMFT for the follow-up period. ΔDMFT significantly correlated with baseline deft (r = 0.56, p = 0.002), cavitated active caries lesions (Nyvad 3; r = 0.53, p = 0.003), and filled teeth (decayed teeth, Nyvad 7; r = 0.53, p = 0.003). The plaque and bleeding on probing indices from baseline showed a weak or no correlation with caries increment (r = 0.24 and r = 0.04, respectively), which were far from statistical significance. The use of fluoridated gel and fluoridated salt showed very weak inverse correlations with the caries increment (ΔDMFT) without any statistical significance.

Multivariate Analysis

A regression analysis of single factors and the ΔDMFT as dependent variable showed that the use of fluoridated salt (standardised coefficient, SC = −0.10) and fluoride gel (SC = −0.14) were negatively associated with ΔDMFT. As all participants used fluoridated toothpaste the effect could not be analysed due to the lack of a control group. For the model considering the presence of active carious cavities (Nyvad 3) and the presence of fillings (Nyvad 7), both of them showed a clear, statistically significant positive association with the ΔDMFT (SC = 0.38, p = 0.02 and SC = 0.39, p = 0.02, respectively).

Discussion

This study was based on a previous investigation by Meller et al. [2006a], who addressed the question whether the degree of de- and remineralisation by artificial enamel etching was associated with the past and present caries activity in a cross-sectional examination. The present paper analyses the predictive power of this method for future caries development. In spite of higher attrition in the study sample, the results of this study clearly confirmed that the de-/remineralisation properties of artificially etched test sites and the changes revealed after 24 h

measured with QLF were significantly associated with caries increment after 2 years. Experience was gained from similar previous studies [Möller et al., 1987; Ulbricht, 1989; Petzold and Poppe, 1992; Meller et al., 2006b]. Statistically significant interrelations were established between caries activity and the rate of remineralisation of artificially induced demineralisations [Möller et al., 1987; Ulbricht, 1989; Petzold and Poppe, 1992]. What was problematic at first was the subjective evaluation of demineralisation using a staining procedure with a shade guide analysis. However, this was considerably objectified by the application of a colorimeter [Meller et al., 2006b].

The more powerful predictors of future caries development used today are the child's past caries experience and the presence of active caries lesions [Bratthall and Hänsel Petersson, 2005; Fontana and Zero, 2006; Riley et al., 2010], which both summarise all factors associated with de- and remineralisation. This was also confirmed in the present study by an association of the parameters decayed teeth and Nyvad 3 or 7 and the caries increment, but using QLF. The method applied in this study allows the prediction of caries probability at an earlier stage, that is before any caries-related damage can actually be detected. The measurement of de- and remineralisation on the buccal surfaces reveals the resistance to a standardised artificial demineralisation (etching) challenge and subsequent remineralisation, which is influenced by individual factors such as diet parameters, salivary properties and bacterial activity. Therefore, caries prediction is assessed on the basis of the patient's individual intraoral ability to resist caries activity.

Difficulties in caries prediction are most likely to occur when risk assessment is conducted at early ages, as young children do not have a long caries history which can be deducted from restorations or carious defects. In order to improve the accuracy of prediction of these patients, it has been suggested that the models should include several factors such as sociodemographic factors,

plaque accumulation, counts of mutans streptococci, and dietary habits [Holgerson et al., 2009]. In a Finnish study [Pienihäkkinen et al., 2004], which aimed to assess the caries prediction value of several indicators (presence of plaque, gingival bleeding, diet, mutans streptococci, and caries lesions), it was shown that the greatest accuracy in prediction was achieved by combining caries experience with some other predictors, e.g. diet or presence of bacteria. In addition, it was concluded that no single caries risk indicator in young children achieved the expected accuracy to predict further caries progression [Pienihäkkinen et al., 2004]. Thus, even though the other indicators increased the caries-predictive power in young children, caries experience was still indispensable to achieve a more accurate prediction. Although the idea of 'etching' a minor lesion and assessing its 24-hour remineralisation is at first sight an unconventional means of predicting caries, this method not only assesses the mineral quality and properties, but it also summarises the history of mineralization, saliva flow, fluoride exposure, prior biofilm demineralization and subsequent remineralisation. This actually describes how acid-resistant the enamel has become and, therefore, might also predict future caries development. Thus, the presented method performed on teeth with a considerable de- and remineralisation 'history' exceeds simple analysis of enamel solubility.

In the last years, QLF has been extensively used in several clinical studies to assess minimal quantitative changes in enamel mineral content. The basis of this technique is that decreased mineralisation results in a reduction of fluorescence with respect to adjacent sound enamel [de Josselin de Jong et al., 1995; Stookey, 2005]. Differences in the scattering of incident light and in the resulting amount of fluorescence enable us to quantify even small changes in the degree of demineralisation. QLF was successfully used in this study due to its ability to identify and quantify the demineralisation of an etched test site and to assess its remineralisation in a non-destructive or non-invasive manner longitudinally. The ability of QLF to longitudinally quantify and monitor enamel erosion has been validated against transverse microradiography, showing strong positive correlation for quantification of mineral loss [Pretty et al., 2004]. Furthermore, in the study by Meller et al. [2006a] the QLF images were visually analysed on the computer screen to ensure that the quantitative analysis of QLF images corresponded with that of the clinical situation. The results of that analysis showed a strong correspondence between the two interpretations. The present study showed that ΔQ after etching already correlated significantly with the deft scores at baseline. This statistically significant correlation remained valid in the follow-up analysis when the caries increment (ΔDMFT) was analysed. These findings provide the basis for a method of caries prediction which employs net changes in de- and remineralisation as a summary of individual factors in the caries process. From a clinical management perspective, this can be an important contribution for decision making in children with yet little caries experience. Children with a high caries risk could be identified and selected for intensified prevention to compensate for their higher caries risk.

In contrast to the robust and highly significant correlations between the QLF readings and the caries parameters, plaque showed a much weaker and statistically not significant correlation with caries increment. Although the presence of plaque is an aetiological prerequisite for dental caries [Kidd, 2004] and it is regularly included in caries risk assessments [Pienihäkkinen et al., 2004; Bratthall and Hänsel Petersson, 2005], the predictive power is often very low as it can easily be removed [Aleksejūniene et al., 2009].

Fluoride is considered as the cornerstone for caries control, as it changes the de- and remineralisation balance dramatically [Marinho et al., 2003; Bratthall and Hänsel Petersson, 2005]. In this study, the use of fluoridated gel and fluoridated salt resulted in a lower caries increment (ΔDMFT), which underlines the protective effect of fluorides. Still, the effect and its statistical solidity were low, possibly due to a relatively high fluoride use in the examined population, which leaves hardly any children with suboptimal fluoride levels. In addition, the real frequency, quantity and quality of fluoride use is difficult to verify.

In summary, the proposed method, which involves the QLF analysis of the de-/remineralisation of an etched test site, could be suitable for measuring caries activity. This is more consistent with the aetiological concept of caries as a disease process. The data suggest that the degree of demineralisation by etching and the subsequent changes over time are associated with both the current and future caries activity in children. Additionally, this method might be used for the early assessment of caries activity. Thus, much earlier diagnosis might become possible than with having to rely on parameters like past caries experience. In general, a method that assesses the caries activity in clinically caries-free patients satisfies the aim of primary prevention better than waiting for caries lesions to develop, when the

damage caused through caries becomes irreversible. This method can be used for caries prediction, especially in younger patients where highly predictive parameters such as previous caries experience cannot be applied. Also assessment of the de- and remineralisation process could be helpful in patients with contradictory risk parameters or in patients with caries experience that cannot be explained by conventional risk analysis. This way, patients with high caries risk can be identified early on and given intensive preventive care, enabling them to achieve an overall caries- and restoration-free healthy dentition.

Disclosure Statement

There are no conflicts of interest of neither of the authors.

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