Lasers in Medical Science (2019) 34:1905–1916
https://doi.org/10.1007/s10103-019-02791-8

ORIGINAL ARTICLE

How standard deviation contributes to the validity of a LDF signal: a cohort study of 8 years of dental trauma

Herman J. J. Roeykens[1] · Peter De Coster[1] · Wolfgang Jacquet[2,3,4] · Roeland J. G. De Moor[1]

Received: 3 January 2019 / Accepted: 16 April 2019 / Published online: 16 May 2019

Abstract

The aim of this cohort study was to document the potential of laser Doppler flowmetry (LDF) for diagnosing tooth vitality in traumatized teeth in a population of 88 referred patients with dental trauma over a period of 8 years (2011–2018). The main reason to refer was tooth discoloration (26%), and the main trauma cause was a traffic injury (35%). Taking into account that referral for LDF is best given within the week after trauma, 66% of referrals were overdue. In 73% of cases, root canal treatment could be avoided, and in 65%, the presence of a fixed orthodontic retainer showed beneficial for pulp recovery. Data of 394 teeth were collected and submitted to multilevel modelling statistics. As a general conclusion, both LDF perfusion and concentration values proved to be highly reliable in the appreciation of tooth vitality. The difference of variability of standard deviation and range of LDF measurements involving intra-subject control teeth showed statistically significant in discriminating between vital and non-vital pulps. In non-vital teeth, the diagnostic combination of LDF, sensibility tests and peri-apical X-ray is advised.

Keywords Laser Doppler flowmetry · Validity · Variability · Reliability · Standard deviation (sd) · Dental trauma

Introduction

Recent reviews on pulp diagnosis have ascertained the accuracy of laser Doppler flowmetry (LDF) in assessing changes in pulp vitality as a complement to traditional diagnostic sensibility tests [1–3]. The LDF diagnostic protocol has been well described: the use of a probe positioning splint is advised as well as probe location between 2 and 3 mm above the gingival margin; simultaneous measurements with two double-channel probes are preferred whereas LDF cut-off values from 0.6 till 1.6 PU (perfusion units) in amplitude have been recommended [4–7]. However, a number of extrinsic (ambient light, room temperature) and intrinsic environmental factors (body temperature, blood pressure, tobacco use, drug intake, time of the day, etc.) might bias the measurement outcome. Therefore, these parameters should be carefully monitored [8, 9].

Successful treatment of traumatized teeth requires a correct diagnosis and implicates standardized diagnostic data sampling [1]. If not managed properly and treated accordingly, a dental trauma can present considerable physical and functional challenges to the patient. Diagnostic and clinical guidelines, based on the reference work by Andreasen et al. (1972) [10], are constantly being actualized in the recommendations of the International Association of Dental Traumatology (IADT) series in Dental Traumatology. Luxation injuries are classified into concussion, subluxation, extrusion, lateral luxation and intrusion. Each injury comes with a specific response in terms of mobility, percussion sensitivity, electric pulp test reactivity and discoloration. Thermal pulp tests, radiographic documentation (one occlusal and three eccentric peri-apical X-rays) and intra- and extra-oral digital imagery are highly recommended as additional diagnostic tools (25 December 2018) (http://www.iadt-dentaltrauma.org/1-9iadtguidelinescombined-lr-2011-5-2013.pdf). More recently, cone beam computed tomography (CBCT) has significantly expanded the

✉ Herman J. J. Roeykens
herman.roeykens@ugent.bec

1 Department of Reconstructive Dentistry and Endodontology, Ghent Dental Laser Centre, Ghent Dental Photonics Research Cluster, Dental School, Ghent University, Ghent University Hospital, C. Heymanslaan 10 - 1P8, 9000 Ghent, Belgium

2 Department of Periodontology & Oral Implantology, Dental School, Ghent University, Ghent University Hospital, Ghent, Belgium

3 Department of Oral Health Sciences ORHE, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Brussels, Belgium

4 Department of Educational Sciences EDWE-LOCI, Faculty of Psychology and Educational Sciences, Vrije Universiteit Brussel, Brussels, Belgium

diagnostic workout with respect to alveolar ridge and root fracture localization and root resorption [11–13]. However, special care should be taken as to the increased amount of radiation associated with tomography, more particularly in children and young adults. The most frequent reason for referral for CBCT involves differentiation between pathologies and physiological anatomical structures, always respecting the as low as reasonably achievable (ALARA) principle for exposure to radiation. For endodontic purposes, the use of small field-of-view (FOV) (85 kV, 5 mA, 40 × 40 mm) scanner settings is recommended as the reconstructed images have a higher spatial resolution than larger FOVs, and the effective radiation dose for the patient is reduced [14–16].

When considering administration of antibiotics, systemic antibiotics are indicated during the first week after a severe trauma. Tetracyclines are considered the first choice owing to their excellent absorption in bone, but should be strictly administered taking into account patient age and body weight. In order to avoid intrinsic tooth discoloration especially in youngsters up to 10 years of age, phenoxymethylpenicillin (PenV) or amoxicillin can alternatively be administered in an appropriate dose for age and weight [17, 18].

Dalya and co-workers [19] have reported a 90% efficiency of fixed orthodontic retainers in maintaining the alignment of the anterior teeth, the latter also having a limiting effect on the propagation of frontal dental trauma [20–22].

As hemodynamics in the human pulp reduces with age, to be interpreted as a change of the detectable area, age-related changes in the human pulpal blood were already examined [23].

Despite clear descriptions and defined protocols for diagnosis and follow-up, present-day techniques and mains do not allow making the crucial and reliable distinction between the vital and non-vital statuses of the pulp. Therefore, the aim of this cohort study was to evaluate the potential of LDF for the discrimination of the vital stage versus the non-vital stage of the pulp. The population consists of referred patients where parameters which can increase the reliability of the registrations were determined under highly standardized conditions. As referrals for LDF are not common, the second aim of this study was to determine why dentists chose this particular option.

Materials and method

Sample characteristics

A total of 394 teeth from 88 referred patients—46 males (170 teeth; mean age 26.7 ± 15.2 years) and 42 females (224 teeth; mean age 20.4 ± 12.2 years)—were screened over the period July 2011–July 2018 by the same investigator (H.R.). Figure 1 displays the reasons for referral, involving tooth discoloration (25%), subluxation (16%), luxation (11%), avulsion (11%), peri-apical cysts (6%), root fracture (5%), external resorption (5%), loss of sensibility (5%), internal resorption (4%), alveolar ridge fracture (4%), intrusion (4%), over occlusion (2%), ankyloses (1%) and pain following periodontal treatment (1%). Ten percent of patients were referred immediately after trauma, 9% 1 week after trauma, 8% after 2 weeks, 5% after 3 weeks, 7% after 1 month, 11% after 2 months, 7% after 3 months, 21% between 6 to 12 months after trauma and 22% at least one to several years after traumatic injury. With respect to arch location, 76% of traumatized teeth were located in the maxillary anterior region and 7% in the mandibular anterior region, whereas 5% involved the maxillary canines and 12% the mandibular molars and premolars.

All patients were referred by practitioners affiliated to the Ghent University Hospital Dental Clinic and outside general practitioners. Within the sample of 394 teeth, 129 were subject of trauma. The other 265 teeth were subdivided into 158 neighbouring teeth, and 107 teeth were in the same arch of the traumatized tooth which were assessed by LDF in function of diagnosis. The remaining 107 teeth comprised control teeth that could be two teeth away as it was not always possible to test the antimere. This control group ($N = 107$) was needed in order to make a comparison with non-traumatized and non-restored teeth: at least one neighbouring tooth to the traumatized tooth/teeth was kept as a buffer. This judgement was arbitrary as the literature on this topic is not decisive yet. The control group was further subdivided according to the patient's age: 7 –15 years, 16 –20 years, 21 –40 years and ≥ 40 years. All values were put into an Excel file, and mean and weighted mean (some teeth were measured several times due to the trauma follow-up) were calculated accordingly to the age bracket (Table 5).

The reported trauma causes were bike accidents (35%), accidents during leisure or hobby time (17%), during domestic activities (16%) or school accidents (15%), following dental treatment (7%) and domestic violence (2%). In 8%, the origin of trauma was unknown to the patient (Fig. 2).

In total, 966 measurements were performed with on average 2.4 follow-up appointments (mean 1.0 ± 2.1).

No treatment was proposed in 73%, whereas in 26% of cases, one or two endodontic treatments were indicated; in 1%, an endodontic treatment combined with an apicectomy was prescribed (Fig. 3).

An orthodontic retainer was present in 16% of the patients, with eight males (13 teeth; 18.6 ± 8.0 years) and six females (9 teeth; 15.7 ± 7.2 years).

Antibiotics were prescribed by the referring dentist with 90% amoxicillin and 1% tetracycline (Doxycycline®). In 9% of the cases, no prescription was delivered.

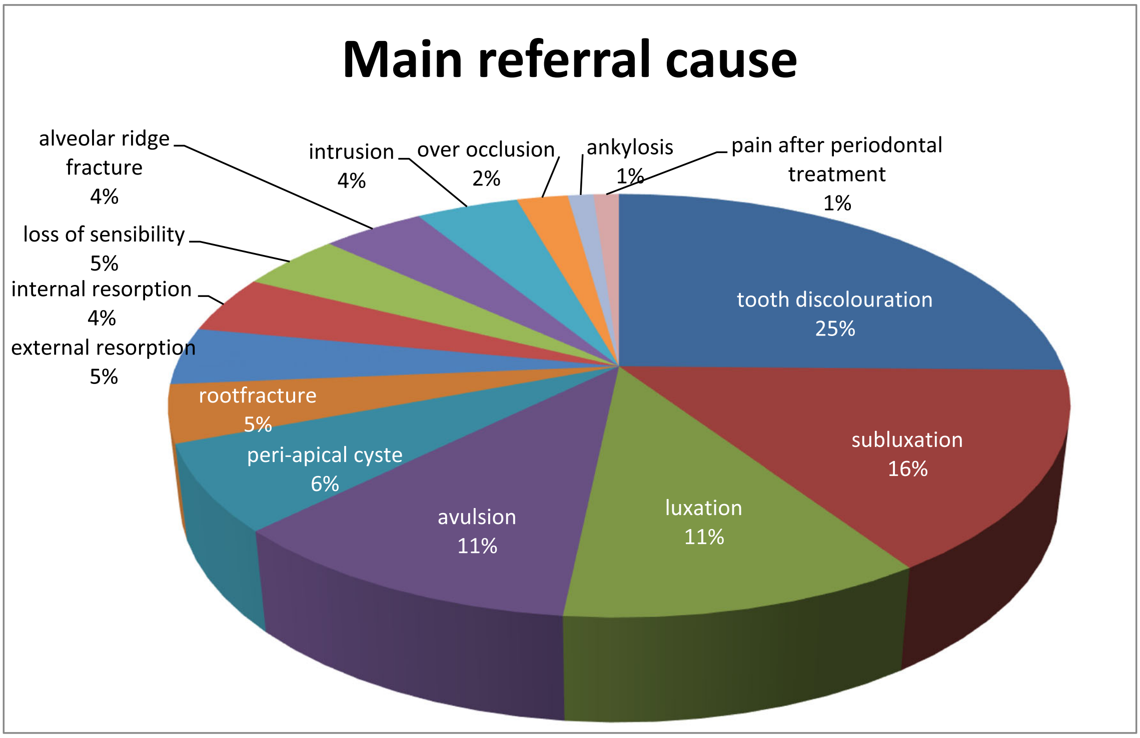

Fig. 1 Main referral cause

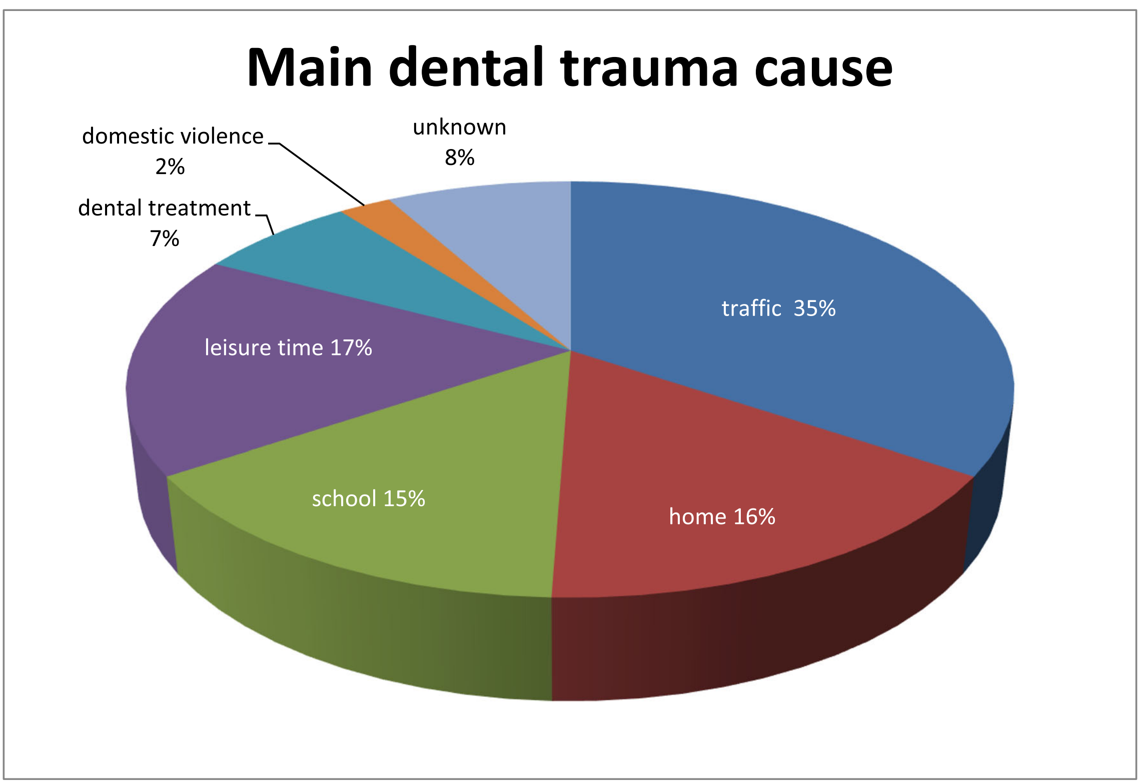

Fig. 2 Main dental trauma cause

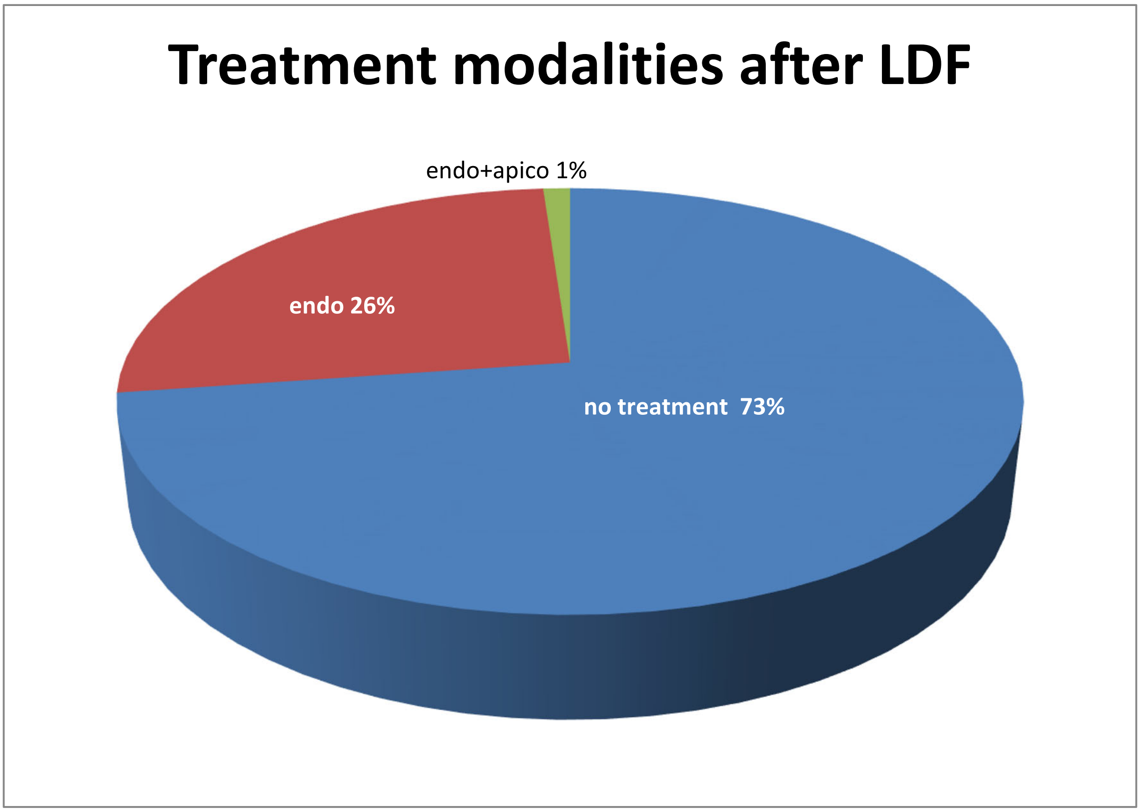

Fig. 3 Treatment modalities after LDF

Diagnostic protocol

An IADT guideline–based diagnostic protocol was followed for all patients [7]. These guidelines comprise the use of the following: sensibility tests (application of cold pellet − 50 °C; Miracold® Plus, Hager & Werken Inc., Duisburg, Germany; application of heated gutta-percha stick at 160 °C [24]); an electrical pulp test (Elements™ Diagnostic Unit, SybronEndo Inc., Kerr Corporation, 1717 West Collins, Orange, USA); an assessment of tooth mobility and percussion sensitivity, preferably in combination with a peri-apical X-ray (VistaScan Perio Plus Durr Dental, Bietigheim, Germany taken with EndoRay II film holder Dentsply Rinn); and digital extra-oral images. For each tooth, the sensitivity after thermal tests was appraised and rated by the patient on a dichotomous scale (yes/no) as was sensitivity to percussion. Tooth mobility was assessed using Miller's mobility index for routine clinical examination of tooth mobility (grades 0–3) [25], and electric pulp test outcomes were scored on an ordinal scale (0 to 80). In addition, a LDF measurement (moorVMS LDF II, Moor Instr. Axminster, England) was performed. All patients were in good health and seated semi-supine in a dental chair during each assessment. All measurements were performed by the same investigator (H.R.) over a period of 8 years (2011–2018) and recorded in the morning between 9 a.m. and 12 noon. with the exception of two measurements, which were performed in the afternoon between 3 p.m. and 6 p.m. The afternoon measurements were not included in the statistical analysis. In 6% of the cases (5/88), internal or external resorption occurred. In these instances, a cone-beam computed tomography (CBCT) was performed. Here, we used a Planmeca ProMax 3D Max, with voxel sizes of 200 μm, 96 Kv and 10 mA; exposure time 12.376 s; 549.1 DAP value; and a field of view of 29.6 × 29.6 × 29.6 mm and an effective dose of 43.9 μSv (Planmeca USA, Inc., Roselle, IL 60172, USA).

Pulpal blood flow registration

Pulpal blood flow was registered with a VMS-LDF 2 (Moor Instr., Axminster, Great Britain, October 2009 serial no. 098) diode (785 nm) class 1 laser. The average power of the device was 1.0 mW (range 0.5 to 1.5 mW) at the probe tip (diameter = 1.5 mm). For measurements of tooth vitality, a 3-kHz bandwidth (broad spectrum, low blood volume) is recommended by the manufacturer. Values were recorded every 0.1 s, but shown twice with a frequency of 40 Hz over a minimum interval of 30 s. Two VP3 blunt-ended needle probes (SN 2185 and SN2187, Moor Instr., Axminster, UK) with one afferent fibre and one efferent fibre were used. In cases with spatial constraints due to cheek interposition in the molar region, two VP3 bended blunt-ended needle probes (SN2189 and SN2228) were an alternative. Both probe fibres were packed in a hypodermic stainless steel tube with an external diameter of 1.5 mm and a length of 20 mm (40 mm long, 90° bend, 10 mm to the tooth for the bended probes). The inter-fibre distance was 0.5 mm, and each fibre had a diameter of 200 μm. Each probe was calibrated prior to the measurement session in a PFS (i.e. Perfusion Flux Standard, Lot WO11824, good until 2019–05, Moor Instr., Axminster, UK) fluid.

Pulpal blood flow was recorded with the use of a polyvinyl polysiloxane splint (EXAFLEX, GC America Inc., Alsip, IL, USA) fabricated at the first measurement session. A small shaft with a 1.5-mm diameter was drilled in each splint at multiple tooth sites and with a probe location at 2 mm incisal to the enamel-cement border. Simultaneous measurements with two double-channel probes, one for the test and one for the reference tooth, were performed [26]. Values of perfusion (arbitrary units, AU) and concentration (CONC) were recorded. Data from each test tooth were compared to those of its control and to the last measurement before the final diagnosis of vital versus non-vital.

The protocol was approved by the Ghent University Hospital Ethical Committee: 2018/1098.

Statistics

Traumatized teeth were considered vital or non-vital based on clinical data available. In this evaluation of vitality, the variability of the measurements with respect to LDF and CONC was taken into account. At first, the data were analysed on the tooth level disregarding the patient but with respect to variability measures and with respect to the covariate gender and age. Chi^2 tests and the non-parametric Mann-Whitney U test have been used together with Kendal tau b correlation coefficients in order to make results robust for extreme values and deviations from normality.

Afterwards, the level of the patient was introduced through multilevel modelling statistics [27] in order to take into account variability due to the patient effect on the vitality of the teeth. A strategy moving from simple models to more complex models was adopted. Patient level as random effect on the intercept was considered to be the base model. Starting from this model, the covariate age and differences in variability measures were introduced as plausible predictors.

The difference in blood flow and concentration standard deviations and ranges between traumatized tooth and its antimere were considered. The level of significance was set at $p < 0.05$. For the effectiveness of a multilevel model, the Akaikei information criterion (AIC) was used. A Kendall rank correlation coefficient, commonly referred to as Kendall's tau coefficient, was used to measure the ordinal association between two measured quantities, here, concentration and sd. This test is a non-parametric hypothesis test for statistical dependence based on the tau coefficient.

All analyses except the multilevel analyses were carried out using SPSS for windows version 22.0 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). Multilevel modelling was performed through R version 3.4.0 (April 21, 2017) package lme4 version 1.1–13.

Results

Table 1 shows the analysis of values at the tooth level regardless of age, gender and location of the tooth in the mouth. At the patient's level, the differences in distribution of vital versus non-vital are not significantly different between male and female as the significance index is 0.655. There is a significant age difference of approximately 5.4 years in average between male and female. The average age when comparing vital versus non-vital is not significant disregarding the patient level (0.1905). When age classes are applied, the relation is significant though (0.0497). This significant result in age classes disappears when a multilevel model is introduced with random intercept (Table 4). The differences (between traumatized tooth and antimere) of the variability measures, standard deviation and range for LDF and CONC were tested with respect to the vitality classification.

The distribution of LDF sd and range is significantly different between vital and non-vital teeth, $p \leq 0.05$ ($p = 0.0171$). This difference between vital and non-vital teeth remains significant for the differences in sd for the CONC ($p = 0.017$); the range of difference is not significant ($p = 0.166$) (Table 2, Fig. 1).

The differences in measures of variability between traumatized tooth and antimere are significant ranging from a moderate $t = 0.26$ up to a strong correlation of $t = 0.65$ between the sd for CONC and its range. Also for LDF, the differences in sd are strongly correlated with the differences in range ($t = 0.59$) (Table 3).

When human behaviour is modelled, context can be terribly important. Individual action may be determined by independent variables operating at different levels. Therefore, consecutive multilevel models [26] introducing the covariate age and differences in variability measures (for sd and range from LDF and CONC with a random intercept modelling the patient level) are proposed. In the first step, age was introduced at patient level but it did not significantly improve the model ($p = 0.2568$). The difference in LDF variability between traumatized tooth and antimere expressed as a standard deviation as well as range was found to contribute significantly to the model (AIC = 161.8 and $Chi^2 = 34.39$). With respect to the CONC, the difference in variability between traumatized tooth and antimere expressed as a sd as well as range was also found to contribute significantly to the model (AIC = 176.1 and $Chi^2 = 20.09$) (Table 4).

Table 1 The distribution of gender, age and vital versus non-vital. Exploration of gender and age on the vitality of traumatized teeth

Gender	***N***	**Vital**	**Non-vital**			**Chi²**	***df***	**Sig.**
Male	170	87.5%	12.5%			0.221	1	0.655
Female	224	85.9%	14.1%					
Age	**Mean**	**sd**	**Min**	**Max**	**Skewness**	**Mann-Whitney *U***	***z***	**Sig.**
Male	26.7	15.2	7.0	55.0	0.7	14,612.5	− 4.0	0.0001***
Female	20.4	12.2	7.0	60.0	1.5			
Vital	22.9	14.0	7.0	60.0	1.1	7890.5	− 1.3	0.1905
Non-vital	24.7	13.3	9.0	56.0	1.0			
Age classes	**Vital**		**Non-vital**		**Chi²**	***df***	**Sig.**	
	n	***%***	***n***	***%***				
7–15	130	38.0%	13	25.0%	7.83	3	0.0497*	
16–20	59	17.3%	17	32.7%				
21–40	109	31.9%	15	28.8%				
> 40	44	12.9%	7	13.5%				

$^*p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$

This contribution was significantly lower for the concentration than for the LDF (AIC = 161.8 versus 176.1). The contribution of the LDF standard deviation contributes the most as can be observed from the gain in information (AIC) as well as *p* value when comparing with the base model.

The introduction of the difference in CONC sd does not contribute significantly to the model although all coefficients are highly significant. The model exploration of the introduction of difference in CONC sd between reference and test (traumatized) tooth to the LDF sd failed to converge and is thus unreliable (Table 4).

Within the sample of 394 teeth, 129 teeth had been referred after traumatic injury by the patient's general dentist. In this cohort, 16% of the patients had an orthodontic treatment at the time of the trauma, with an average of 1.6 ± 0.8 traumatized teeth. Within the group of patients wearing an orthodontic appliance or retainer at the time of trauma, endodontic treatment was advised in 28%, endodontic treatment followed by an apicectomy in 7%, whereas in 65% of cases, no endodontic intervention was needed. Among those patients needing an endodontic treatment, 50% presented with maxillary brackets, 25% with mandibular brackets and 25% with a maxillary retainer. Subsequent endodontics and apicectomy involved upper lateral incisors exclusively (Fig. 4).

Referring to the age bracket (Table 5), only teeth 12, 13 and 22 were represented in all categories. With a weighted mean for tooth 12 of 27.7 ± 3.2 AU and for tooth 22 of 28.6 ± 3.3 AU, the age group from 7 years until 15 years (group I) differs from the second age group (16–20 years = group II, tooth 12 = 24.5 ± 2.4 AU and tooth 22 = 23.5 ± 3.1 AU), the third age group (21–40 years = group III, tooth 12 = 27.6 ± 3.6 AU and tooth 22 = 26.0 ± 2.8 AU) and the fourth age group (41 years = group IV, tooth 12 = 18.6 ± 1.5 AU and tooth 22 = 15.4 ± 1.6 AU). Values of LDF lower with age, especially between groups I and IV (− 41%), but also between groups III and IV (− 36%).

Values of teeth 41 and 31 for group I (tooth 41 = 32.4 ± 2.7 AU and tooth 31 = 26.0 ± 2.3 AU) remain high compared

Table 2 Differences in variability measures between traumatized and reference tooth

		Mean	sd	Min	Max	Skewness	Mann-Whitney *U*	*z*	Sig	
Sd LDF	Vital	0.2	2.5	− 5.1	35.7	9.3	3581.0	− 6.88	0.0000	***
	Non-vital	− 3.2	7.4	− 35.7	4.7	− 3.6				
Sd CONC	Vital	0.5	11.1	− 71.3	78.1	1.4	6840.0	− 2.38	0.0171	*
	Non-vital	− 6.8	21.3	− 78.1	24.8	− 2.0				
Range LDF	Vital	− 1.9	24.1	− 284.6	137.7	− 5.1	2462.0	− 4.21	0.0000	***
	Non-vital	22.4	57.4	− 24.7	284.6	3.9				
Range CONC	Vital	− 3.3	77.9	− 458.3	367.0	− 1.0	4139.0	− 1.39	0.1661	
	Non-vital	38.5	152.2	− 212.4	458.3	1.2				

The differences between traumatized tooth and antimere of the variability measure standard deviation and range for LDF and concentration were tested with respect to the vitality classification

$^*p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$)

Table 3 correlation of standard deviation and range

Kendall's tau *b* rank correlation test			
	sd CONC	Range LDF	Range CONC
Sd LDF	0.37	0.59	0.26
Sd CONC		0.40	0.65
Range LDF			0.46

to those of group III (tooth 41 = 32.3 ± 2.7 AU and tooth 31 = 27.7 ± 3.0 AU).

Reference values for the four groups can be observed for teeth 12 and 22. As a result, reference value for a lateral incisor in the upper jaw is for group I = 28.2 ± 3.2 AU, for group II = 24.0 ± 2.7 AU, for group III = 26.8 ± 3.2 AU and for group IV = 17.1 ± 1.5 AU (Table 5).

Discussion

Referral of 88 patients with a dental trauma has yielded the largest described database of LDF measurement outcomes to date**.**

The aim of this cohort study was not to demonstrate the relationship between LDF and sensibility tests as such. Both were already well described in several reviews [3–5, 28]. This cohort study shows the contribution from the standard deviation as well from the blood flow as from the concentration to the validity of the real LDF signal. The standard deviation (sd) from LDF and the CONC were compared. Both for LDF and CONC, standard deviation had a significant predictive value for vitality. This contribution was significantly lower for the CONC than for LDF with respect to the difference in variability between traumatized tooth and antimere (i.e. sd LDF vital 0.2 ± 2.5 versus sd CONC vital 0.5 ± 11.1) (Table 2, Fig. 5).

The use of SD should be predictive because it will be greater for cases with good pulsatility and vasomotion but could also be susceptible to movement [29]. This is demonstrated by the variability of differences. Clinically, this statement leads us to the use of LDF as the golden standard for vitality testing. The latter is confirmed in a recent systematic review and meta-analysis as LDF scores are already the best without sd analysis for sensitivity (97.5%), specificity (95.0%) and negative predictive value (99.7%) [3].

There is no gender distribution difference for vital versus non-vital. And although a difference in age of 5.4 years between male and female was found in this survey, this was not significant on the patient level.

The principal referral reason for traumatized teeth happened to be tooth discoloration, not pain. With 25% of all referrals for LDF, tooth colour remains an important issue in pulpal health state diagnosis. As female patients are more into healthcare issues than male patients, this discoloration will be more noticed by women (68%) versus men (32%) [30].

The time of the day matters for LDF blood flow measurements [31]. Roeykens et al. [9, 31] found that pulpal

Table 4 Multilevel modelling

AIC	Fixed effects					Random effects	Comparison with base multilevel model		
		Estimate	sd	*z*-value	*p* value	sd	Chi^2	*df*	*p* value
Patient level as random effect on the intercept									
194.2	Intercept	− 1.60	0.19	− 8.44	< 2e−16	0.47			
Patient level as random effect with age as covariate at the patient level									
194.9	Intercept	1.84	0.31	− 5.93	0.0000	0.48	1.29	1	0.2568
	Age (patient level)	0.01	0.01	1.11	0.2650				
Patient level as random effect with difference in LDF sd as covariate on tooth level									
161.8	Intercept	− 2.13	0.47	− 4.56	0.0000	0.77	34.39	1	0.0000***
	Diff. LDF Std	− 0.49	0.15	− 3.36	0.0008				
Patient level as random effect with difference in LDF range as covariate on tooth level									
176.1	Intercept	− 1.81	0.27	− 6.79	0.0000	0.59	20.09	1	0.0000***
	Diff. LDF range	0.02	0.01	3.40	0.0007				
Patient level as random effect with difference in CONC sd as covariate on tooth level									
188.4	Intercept	− 1.66	0.20	− 8.22	< 2e−16	0.49	7.80	1	0.0052**
	Diff. CONC range	− 0.02	0.01	− 2.61	0.0091				
Patient level as random effect with difference in CONC range as covariate on tooth level									
189.6	Intercept	− 1.65	0.20	− 8.37	< 2e−16	0.47	6.59	1	0.0103*
	Diff. CONC sd	0.00	0.00	2.43	0.0151				
Exploration of the introduction of difference in CONC sd between ref and test (traumatized) tooth to LDF sd									
160.9	Intercept	− 2.27	0.00	− 1035.50	< 2e−16	0.87	2.87	1	0.0900
	Diff. LDF sd	− 0.62	0.00	− 284.40	< 2e−16				
	Diff. CONC sd	0.03	0.00	13.80	< 2e−16				

Consecutive multilevel models introducing the covariate age and differences in variability measures standard deviation and range for LDF and concentration with random intercept modelling the patient level

**p* ≤ 0.05; ***p* ≤ 0.01; ****p* ≤ 0.001

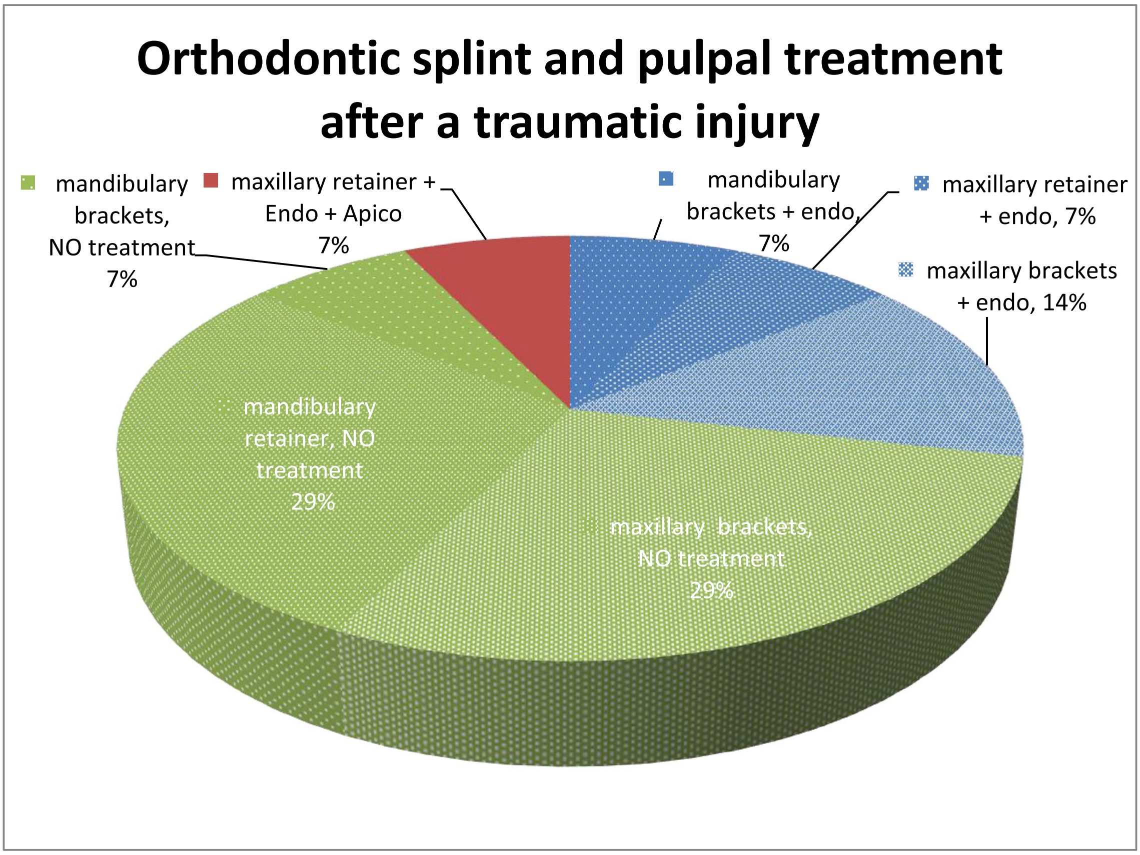

Fig. 4 Orthodontic splint and pulpal treatment

blood flow is subjected to a diurnal or circadian rhythm, producing differences of 50 to 70% between two measurement points in time. Moreover, pulpal blood flow will decrease over the years and will vary according to the age of the patient. That is for vital pulps, LDF value differences up to 41% were found between children and adults older than 40 years in the same time bracket of the day. This may have been overlooked in previous investigations of laser Doppler flowmetry. Since the measurements in the present cohort were all performed in the morning, between 9 and 12 am, experimental bias resulting from diurnal variations in blood flow was avoided. The use of standardized LDF settings additionally helped to minimize bias.

A traffic accident with a bicycle came out as the main cause of dental trauma. The latter was already observed in literature [32].

An important statement can be made. For 73% of all cases, no treatment was advised after LDF control/follow-up. With a good diagnosis, an invasive treatment is at least postponed, probably not advised [1]. The latter refers also to a correct referral. Referral time was only in 37% of all cases within a month after trauma. With pulp degeneration already occurring after 3 weeks, 63% of all referrals were too late [33].

An orthodontic splint at the moment of trauma is a benefit. In this cohort of trauma cases, only 16% of the patients were wearing an orthodontic retainer or brackets. An orthodontic retainer (43%) or brackets (57%) in the lower jaw (43%) versus upper jaw (57%) at the time of a traumatic injury is beneficial looking at the outcome as (1) less teeth (8%) were involved in a trauma and (2) with a retainer or brackets in 65% of the cases no treatment was suggested.

As a result of the higher radiation dose, there are no applications of CBCT for caries diagnosis in paediatric dentistry nor evidence in reconstructive dentistry [34]. Therefore, CBCT, beside peri-apical X-rays, was only used in those cases where internal of external resorption was suspected or occurred (6%).

Epidemiological studies indicated that the annual incidence of dental trauma globally is at about 4.5% [35]. In certain groups, dental trauma was almost as high as one-fifth of all bodily injuries [36]. The prevalence of traumatic dental injury (TDI) in children (0–18 years old) was variable. Based on the geographical area, TDI was estimated at 17.5% with a higher prevalence for boys and with falling at home (toddlers) and enamel fracture as the most common parameter analysed [37]. With 15- to 18-year-old students in Taiwan, TDI increased even up to 19.9%. Here, sports and leisure events were the highest causes (30.8%) of TDI and more prevalent within boys [38]. So, dental trauma follow-up and as a consequence LDF

Table 5 107 not traumatized teeth, LDF values

Tooth	7 –15 years				16–20 years				21–40 years				≥40 years			
	Mean	teeth, *n*	Weighed mean	Measurements, *n*	Mean	Teeth, *n*	Weighed mean	Measurements, *n*	Mean	Teeth, *n*	Weighed mean	Measurements, *n*	Mean	Teeth, *n*	Weighed mean	Measurements, *n*
13	19.5 ± 2.3	2	19.5 ± 2.3	2	27.6 ± 3.2	1	27.6 ± 3.2	1	19.2 ± 2.3	5	19.4 ± 2.3	1	119.7 ± 1.5	1	19.7 ± 1.5	6
12	26.5 ± 3.0	8	27.7 ± 3.2	24	24.5 ± 2.4	1	24.5 ± 2.4	5	31.8 ± 3.9	7	27.6 ± 3.6	30	17.5 ± 1.6	2	18.6 ± 1.5	8
11	29.3 ± 3.7	8	27.8 ± 3.6	11	–	0	–	0	20.3 ± 2.6	3	21.8 ± 3.0	8	–	0	–	0
21	24.6 ± 3.0	7	26.1 ± 3.2	22	23.0 ± 2.5	6	21.9 ± 2.4	30	32.2 ± 3.0	3	31.0 ± 3.1	5	–	0	–	0
22	26.8 ± 3.3	9	28.6 ± 3.3	17	28.7 ± 3.3	2	23.5 ± 3.1	8	25.7 ± 3.0	4	26.0 ± 2.8	12	15.8 ± 1,7	2	15.4 ± 1.6	7
23	15.4 ± 1.8	2	15.4 ± 1.8	2	–	0	–	0	23.8 ± 2.1	4	22.8 ± 2.1	7	16.7 ± 2.5	2	17.0 ± 1.7	7
47	–	0	–	0	–	0	–	0	17.4 ± 3.8	2	17.4 ± 3.8	2	16.5 ± 1,5	2	16.5 ± 1.5	2
46	–	0	–	0	–	0	–	0	–	0	–	0	15.4 ± 2.0	1	15.4 ± 2.0	1
45	–	0	–	0	–	0	–	0	–	0	–	0	26.7 ± 2.9	1	26.7 ± 2.9	1
44	–	0	–	0	–	0	–	0	24.8 ± 4.5	2	24.8 ± 4.5	2	–	0	–	0
43	–	0	–	0	18.8 ± 2.2	2	18.8 ± 2.2	2	–	0	–	0	–	0	–	0
42	–	0	–	0	32.8 ± 3.9	2	24.8 ± 3.3	11	–	0	–	0	–	0	–	0
41	32.4 ± 2.7	1	32.4 ± 2.7	1	–	0	–	0	32.3 ± 2.7	1	32.3 ± 2.7	1	–	0	–	0
31	26.0 ± 2.3	1	26.0 ± 2.3	2	–	0	–	0	27.7 ± 3.0	1	27.7 ± 3.0	6	–	0	–	0
32	25.8 ± 2.4	1	25.8 ± 2.4	2	22.3 ± 3.2	1	22.3 ± 3.2	9	20.2 ± 2.0	1	20.2 ± 2.0	6	–	0	–	0
33	–	0	–	0	20.3 ± 2.3	2	20.3 ± 2.3	2	17.7 ± 1.9	1	17.7 ± 1.9	6	–	0	–	0
34	–	0	–	0	–	0	–	0	27.6 ± 2.9	1	27.6 ± 2.9	1	–	0	–	0
35	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0
36	–	0	–	0	–	0	–	0	10.1 ± 1.4	1	10.1 ± 1.4	1	15.7 ± 2.0	1	15.7 ± 2.0	1
37	–	0	–	0	32.6 ± 6.4	2	32.6 ± 6.4	2	–	0	–	0	15.4 ± 1.7	1	15.4 ± 1.7	1

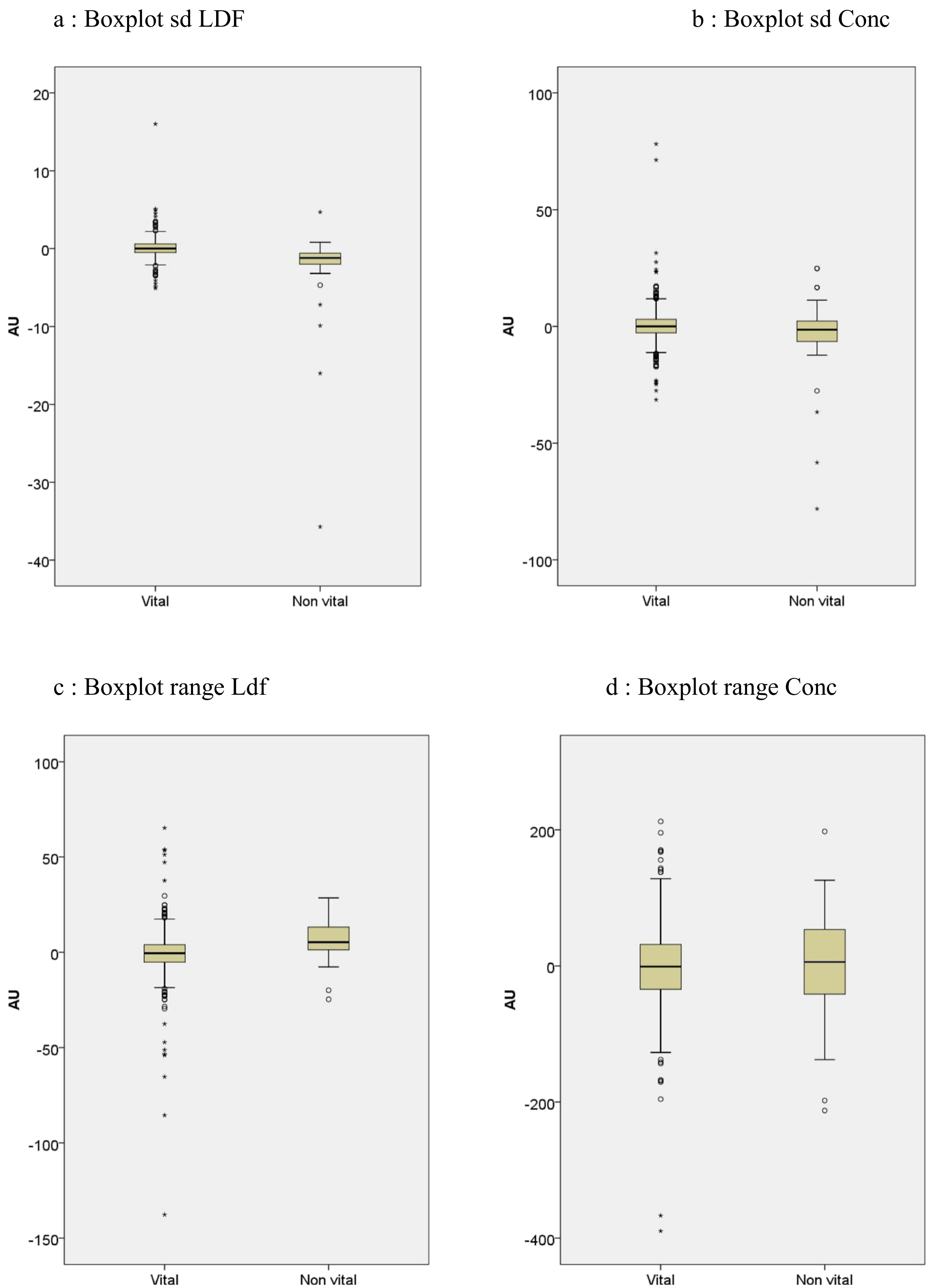

Fig. 5 Differences in variability measures between traumatized and reference tooth. **a** Boxplot sd LDF. **b** Boxplot sd CONC. **c** Boxplot range Ldf. **d** Boxplot range CONC

will occur most frequently in the front region of the upper jaw. In this survey with specific case referrals, this number increased even up to 76%. This high incidence of dento-alveolar trauma cases is also confirmed in the literature [35, 39, 40].

The referring dentists prescribed antibiotics according to the guidelines found in the dental trauma literature taking into account the patient's ages being younger than 10 years with more susceptibility for tooth discoloration [18]. Hammarström et al. (1986) reported that the immediate use of systemic antibiotics (penicillin and streptomycin) can prevent inflammatory resorption [41]. This might explain the large amount of prescribed systemic antibiotics after TDI. Nevertheless, the authors were aware of the severe impact of TDI on the pulp, the bone and surrounding tissue. Literature reports indicate that pulp necrosis and infection are more likely to occur with luxation injuries with severe displacement of the tooth [42]. According to Abbott (2016) [43], the differential diagnosis of external resorption should always be made following an avulsion, intrusion, lateral luxation with crown fracture and extrusion with crown fracture. In these cases, the root canal can become infected and mechanical damage to the cementum will occur, leading to external inflammatory resorption (EIR). Once inflammatory resorption has started, systemic antibiotics are of no value although intracanal antibiotics will arrest the resorption [41].

Sae-Lim et al. [44] recommended the use of systemic tetracycline following avulsion injuries as tetracycline inhibits clastic cells and has antiresorptive besides antibacterial properties. Moreover, tetracycline prevents endotoxin release in the absence of bacterial cell lysis. The combination of intracanal tetracycline and corticosteroids (Ledermix® paste, Haupt Pharma GmbH, Wolfratshausen, Germany) drops the external root surface resorption after 8 weeks with almost 74% and to zero for inflammatory resorption compared to no use of a medicament. Hence, if pulp necrosis is expected following an injury, immediate pulp removal and the placement of an appropriate medicament should be advised. Ledermix® paste (tetracycline 3% + triamcinolone 1%) shows significant less resorption, more healing (PDL) and more root mass compared to calcium hydroxide use [45] which has proven to induce ankyloses and replacement resorption [46]. As tetracycline chelates with Ca ions, tooth discolouration from yellow to dark brown and even purple black can occur with tetracycline molecules deposition into the dental hard tissue. This makes this material in areas of aesthetic concern less desirable even with paste application not above the cementoenamel junction (CEJ) [47, 48] and potential to bleach the tooth afterwards [49]. An alternative to tetracycline without tooth discolouration was given by Odontopaste® (clindamycin hydrochloride 5% + triamcinolone 1% + calcium hydroxide 2%) but did not reduce the occurrence of root resorption [50].

Conclusion

The use of both LDF and its standard deviation provides clearly more information on the vitality state of a vital, traumatized and non-vital tooth.

The use of a silicone isolation splint involving several teeth and a strict follow-up is strongly advised. Repeated evaluation with the same silicone splint (probes always in the same position) will enhance the validity of blood flow measurements.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors declare that the protocol of this article was approved by the Ghent University Hospital Ethical Committee: 2018/1098.

References

1. Andreasen FM, Kahler B (2015) Diagnosis of acute dental trauma: the importance of standardized documentation: a review. Dent Traumatol 31(5):340–349
2. Alghaithy RA, Qualtrough AJ (2017) Pulp sensibility and vitality tests for diagnosing pulpal health in permanent teeth: a critical review. Int Endod J 50(2):135–142
3. Mainkar A, Kim SG (2018) Diagnostic accuracy of 5 dental pulp tests: a systematic review and meta-analysis. J Endod 44(5):694–702
4. Chen E, Abbott PV (2011) Evaluation of accuracy, reliability, and repeatability of five dental pulp tests. J Endod 37(12):1619–1623
5. Karayilmaz H, Kirzioğlu Z (2011) Comparison of the reliability of laser Doppler flowmetry, pulse oximetry and electric pulp tester in assessing the pulp vitality of human teeth. J Oral Rehabil 38(5):340–347
6. Evans D, Reid J, Strang R, Stirrups D (1999) A comparison of laser Doppler flowmetry with other methods of assessing the vitality of traumatised anterior teeth. Endod Dent Traumatol 15(6):284–290
7. Ingólfsson AR, Tronstad L, Hersh EV, Riva CE (1994) Efficacy of laser Doppler flowmetry in determining pulp vitality of human teeth. Endod Dent Traumatol 10(2):83–87
8. Kouadio AA, Jordana F, Koffi NJ, Le Bars P, Soueidan A (2018) The use of laser Doppler flowmetry to evaluate oral soft tissue blood flow in humans: a review. Arch Oral Biol 86:58–71
9. Roeykens HJ, Deschepper E, De Moor RJ (2016) Laser Doppler flowmetry: reproducibility, reliability, and diurnal blood flow variations. Lasers Med Sci 31(6):1083–1092
10. Andreasen JO (1972) Traumatic injuries of the teeth. Munksgaard Publishers, Copenhagen **233p. pb Slr5600 ISBN 3-8055-2539**
11. Shokri A, Mortazavi H, Salemi F, Javadian A, Bakhtiari H, Matlab H (2013) Diagnosis of simulated external root resorption using conventional film radiography, CCD, PSP, and CBCT: a comparison study. Biom J 36:18–22
12. Cohenca N, Simon JH, Mathur A, Malfaz JM (2007) Clinical indications for digital imaging in dento-alveolar trauma. Part 2: root resorption. Dent Traumatol 23:105–113
13. May JJ, Cohenca N, Peters OA (2013) Contemporary management of horizontal root fractures to the permanent dentition: diagnosis-radiologic assessment to include cone-beam computed tomography. J Endod 39:520–525
14. Mota de Almeida FJ, Knutsson K, Flygare L (2014) The effect of cone beam CT (CBCT) on therapeutic decision-making in endodontics. Dentomaxillofac Radiol 43(4):20130137
15. European Commission. Evidence-based guidelines on cone beam CT for dental and maxillofacial radiology. Office for Official Publications of the European Communities. Radiation Protection

172, 2012. Cited May 2018: [about 154 pp.]. Available from: http://ec.europa.eu/energy/nuclear/radiation_protection/doc/publication/172.pdf
16. Pauwels R (2015) Cone beam CT for dental and maxillofacial imaging: dose matters. Radiat Prot Dosim 165(1–4):156–161
17. Andersson L, Andreasen JO, Day P, Heithersay G, Trope M, Diangelis AJ, Kenny DJ, Sigurdsson A, Bourguignon C, Flores MT, Hicks ML, Lenzi AR, Malmgren B, Moule AJ, Tsukiboshi M (2012) International Association of Dental Traumatology guidelines for the management of traumatic dental injuries: 2. Avulsion of permanent teeth. Dent Traumatol 28(2):88–96
18. Agence française de sécurité sanitaire des produits de santé AFSSAPS (2012) Prescription of antibiotics for oral and dental care. Med Mal Infect 42(5):193–202
19. Al-Moghrabi D, Pandis N, Fleming PS (2016) The effects of fixed and removable orthodontic retainers: a systematic review. Prog Orthod 17:24. **Published online 2016 Jul 26**. https://doi.org/10.1186/s40510-016-0137-x
20. Booth F, Edelman J, Proffit W (2008) Twenty-year follow-up of patients with permanently bonded mandibular canine-to-canine retainers. Am J Orthod Dentofac Orthop 1(33):70–76
21. Kalha AS (2014) Early orthodontic treatment reduced incisal trauma in children with class II malocclusions. Evid Based Dent 15(1):18–20
22. Owtad P, Shastry S, Papademetriou M, Park J (2015) Management guidelines for traumatically injured teeth during orthodontic treatment. J Clin Pediatr Dent 39(3):292–296
23. Ikawa M, Komatsu H, Ikawa K, Mayanagi H, Shimamauchi H (2003) Age-related changes in the human pulpal blood flow measured by laser Doppler flowmetry. Dent Traumatol 19:36–40
24. Briseño Marroquín B, Wolf TG, Schürger D, Willershausen B (2015) Thermoplastic properties of endodontic gutta-percha: a thermographic in vitro study. J Endod 41(1):79–82
25. Miller SC (1950) Textbook of periodontia. Blakiston Co, Philadelphia, p 91
26. Roeykens H, Van Maele G, De Moor R, Martens L (1999) Reliability of laser Doppler flowmetry in a 2-probe assessment of pulpal blood flow. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 87:742–748
27. Douglas AL (2004) Multilevel modeling. Sage Publications, Inc, California **ISBN 0-7619-2879-0 : 10-14**
28. Jafarzadeh H (2009) Laser Doppler flowmetry in endodontics: a review. Int Endod J 42:476–490
29. Todea C, Canjau S, Miron M, Vitez B, Noditi G (2016) Laser Doppler flowmetry evaluation of the microcirculation in dentistry. Microcirculation Revisited, Helena Lenasi, IntechOpen. DOI: https://doi.org/10.5772/64926
30. Haddad HJ, Jakstat HA, Arnetzl G, Borbely J, Vichi A, Dumfahrt H, Renault P, Corcodel N, Pohlen B, Marada G, de Parga JA, Reshad M, Klinke TU, Hannak WB, Paravina RD (2009) Does gender and experience influence shade matching quality? J Dent 37(Suppl 1):e40–e44
31. Roeykens HJ, De Moor RJ (2018) Diurnal variations and pulpal status: is there a need for FFT besides LDF? Lasers Med Sci 33:1891–1900
32. Amadori F, Bardellini E, Copeta A, Conti G, Villa V, Majorana A (2017) Dental trauma and bicycle safety: a report in Italian children and adolescents. Acta Odontol Scand 75(3):227–231
33. Andreasen FM, Kahler B (2015) Pulpal response after acute dental injury in the permanent dentition: clinical implications-a review. J Endod Mar 41(3):299–308
34. Dula K, Benic GI, Bornstein M, Dagassan-Berndt D, Filippi A, Hicklin S, Kissling-Jeger F, Luebbers HT, Sculean A, Sequeira-Byron P, Walter C, Zehnder M (2015) SADMFR guidelines for the use of cone-beam computed tomography/digital volume tomography. Swiss Dent J 125(9):945–953
35. Lam R (2016) Epidemiology and outcomes of traumatic dental injuries: a review of the literature. Aust Dent J 61 Suppl 1:4–20
36. Andersson L (2013) Epidemiology of traumatic dental injuries. J Endod 39:2–5
37. Azami-Aghdash S, Ebadifard Azar F, Pournaghi Azar F, Rezapour A, Moradi-Joo M, Moosavi A, Ghertasi Oskouei S (2015) Prevalence, etiology, and types of dental trauma in children and adolescents: systematic review and meta-analysis. Med J Islam Repub Iran 29(4):234
38. Huang B, Marcenes W, Croucher R, Hector M (2009) Activities related to the occurrence of traumatic dental injuries in 15- to 18-year-olds. Dent Traumatol 25(1):64–68
39. Bastos JV, Goulart EM, de Souza Côrtes MI (2014) Pulpal response to sensibility tests after traumatic dental injuries in permanent teeth. Dent Traumatol 30(3):188–192
40. Lin S, Pilosof N, Karawani M, Wigler R, Kaufman AY, Teich ST (2016) Occurrence and timing of complications following traumatic dental injuries: a retrospective study in a dental trauma department. J Clin Exp Dent 8(4):e429–ee36
41. Hammarström L, Blomlöf L, Feiglin B, Andersson L, Lindskog S (1986) Replantation of teeth and antibiotic treatment. Endod Dent Traumatol 2(2):51–57
42. Andreasen FM (1989) Pulpal healing after luxation injuries and root fracture in the permanent dentition. Endod Dent Traumatol 5:111–131
43. Abbott PV (2016) Prevention and management of external inflammatory resorption following trauma to teeth. Aust Dent J 61(1Suppl):82–94
44. Sae-Lim V, Wang CY, Trope M (1998) Effect of systemic tetracycline and amoxicillin on inflammatory root resorption of replanted dogs' teeth. Endod Dent Traumatol 14(5):216–220
45. Pierce A, Lindskog S (1987) The effect of an antibiotic/corticosteroid paste on inflammatory root resorption in vivo. Oral Surg Oral Med Oral Pathol 64(2):216–220
46. Lengheden A (1994) Influence of pH and calcium on growth and attachment of human fibroblasts in vitro. Scand J Dent Res 102(2):130–136
47. Kim ST, Abbott PV, McGinley P (2000) The effect of Ledermix paste on discolouration of mature teeth. Int Endod J 33:227–232
48. Krastl G, Allgayer N, Lenherr P, Filippi A, Taneja P, Weiger R (2013) Tooth discoloration induced by endodontic materials: a literature review. Dent Traumatol 29:2–7
49. Yogha-Padhma A, Jayasenthil A, Pandeeswaran R (2018) Tooth discolouration and internal bleaching after the use of ledermix paste with various bleaching agents – an in vitro study. J Clin Exp Dent 10(11):e1059–e1062
50. Lal R, Devi A, Abbott P (2013) A comparison of tooth discoloration when using Ledermix paste and odontopaste as medicaments. Am Int J Contemp Res 3(9):64–67