A Prospective Clinical Pilot Study on the Level of Matrix Metalloproteinase-9 in Dental Pulpal Blood as a Marker for the State of Inflammation in the Pulp Tissue

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Abstract

Introduction: Differentiation between reversible pulpitis (savable pulp) and irreversible inflammation of the pulp tissue (nonsavable pulp) based on clinical and radiographic diagnoses has proven to be difficult. Pulp exposure allows for the collection of pulpal blood to quantitatively determine the level of inflammation markers or proteolytic enzymes, even with small samples. Pulpitis is associated with the invasion of neutrophil granulocytes and their release of matrix metalloproteinase-9 (MMP-9). Methods: Forty-four patients (aged 18–74 years, mean = 35 years), each with 1 tooth with carious pulp exposure presenting with different stages of pulpitis, were included in this prospective, 2-center clinical study; 26 patients presented with irreversible pulpitis (groups 3 and 4), 10 with reversible pulpitis (group 2), and 8 with completely asymptomatic teeth with deep carious lesions (group 1). Six of the 26 patients with teeth diagnosed with irreversible pulpitis had not taken any nonsteroidal anti-inflammatory drugs and were evaluated as a separate group (group 4). Partial pulpotomy and blood sample collection from the pulp chamber were performed. The total levels of MMP-9 and tissue inhibitor of metalloproteinase-1 were assessed by fluorometric and colorimetric enzyme-linked immunosorbent assays, respectively. The Mann-Whitney U test and Spearman rank correlations were used to compare the MMP-9 levels with different stages of pulpal inflammation; significance was set at .05. Results: The MMP-9 levels in the asymptomatic teeth (group 1) were significantly different from those in the teeth with reversible pulpitis (group 2, P = .006) or irreversible pulpitis (group 4, \(P < .001\)). A statistically significant difference was also observed between the MMP-9 levels in group 1 and group 3 (\(P < .001\)) in which the patients had taken nonsteroidal anti-inflammatory drugs. Conclusions: These findings indicate that the MMP-9 levels in pulpal blood samples could be a useful ancillary diagnostic tool for distinguishing different stages of pulp tissue inflammation. (J Endod 2016;42:190–197)

Key Words
Clinical diagnosis, irreversible pulpitis, matrix metalloproteinase-9, pulp tissue inflammation, reversible pulpitis

Dentists are often faced with the problem of deciding on the best therapy after pulp exposure during caries removal. The crucial question is whether the extensive carious lesion has already caused an infection of a large part of the dental pulp resulting in irreversible pulpitis or whether only a small localized part of the pulp tissue close to the carious lesion has been affected. In the first scenario, root canal treatment is indicated (1, 2), whereas in the latter scenario, only a partial pulpotomy (3–5) and dressing of the exposed pulp tissue with a biocompatible material are needed to maintain pulpal vitality and health (6). Therefore, it is important to determine with as much precision as possible whether the inflammatory process is reversible (infection of a localized part of the dental pulp close to the carious lesion) or irreversible (infection of a large part of the dental pulp). Histologic examination of the pulpal tissue would allow an exact differentiation but is only possible after extraction (7–11). A recently published systematic review showed that, to date, none of the clinical parameters that were thought to determine the condition of exposed vital pulps in teeth with different types of damage could be validated by clinical studies (11). Radiographic changes are also rarely noted after irreversible inflammation of the pulp tissue (1). Therefore, distinguishing between the different stages of pulpal inflammation must be effected through clinical diagnostics (12) to decide whether to perform vital pulp therapy (eg, partial pulpotomy) or root canal treatment. The dental “history of pain” and the extent of hemorrhage after pulp exposure also give an indication of the severity of the inflammation (7, 8, 13).

In recent years, matrix metalloproteinases (MMPs), which belong to the family of calcium- and zinc-dependent endopeptidases, have been increasingly studied for their role in the diagnosis of dental inflammatory processes. For instance, an increase in MMP-8 shows a high correlation with periodontal inflammation (14–16) and apical periodontitis (17). Several studies have evaluated the correlation between the inflammation of the dental pulp tissue and the levels of MMPs (MMP-1, -2, -3, -8, and -9) and other molecular markers (interleukins, prostaglandins, and so on) (17–26).
Of these studies, the results of Gusman et al (24) are of particular interest. This group evaluated the levels of MMP-1, MMP-2, MMP-3, and MMP-9 in clinically healthy and inflamed human dental pulp. The MMP-9 levels of inflamed pulps were significantly higher compared with the clinically healthy pulps. Interestingly, MMP-2 and MMP-3 levels were significantly lower in inflamed pulp compared with healthy dental pulp. The MMP-1 levels were below the detection levels in this study (24).

These results indicate a possible important role of MMP-9 in inflammation processes in dental pulp tissue (27), which is a known fact. The results evaluated by Gusman et al (24) were later supported by other results (25, 26). Tsai et al (25) focused on the examination of tissue obtained after extraction, whereas Zehnder et al (26) analyzed MMP-9 samples from the dentinal fluid of symptomatic human permanent teeth diagnosed with irreversible pulpitis and healthy counterparts.

To date, the use of the concentration of MMP-9 in the pulpal blood as a marker to differentiate between reversible and irreversible pulpitis has not been investigated so there are no reference values showing whether MMP-9 in pulpal blood indicates an irreversible inflammatory process and, if so, in what concentration. In pulpitis, the concomitant expression of the specific inhibitor of MMP-9 activity, the tissue inhibitor of metalloproteinase-1 (TIMP-1), is expected to occur in parallel to MMP-9 release (27). TIMPs reversibly inhibit the proteolytic activity of MMPs (14, 28, 29). Because of this close correlation of MMP-9 and TIMP-1 expression, a relatively high correlation between the MMP-9 and TIMP-1 levels evaluated from the same blood sample is to be expected, at least with regard to the blood samples of pulp with severe inflammation. In addition, a high correlation of all MMP-9 and TIMP-1 levels from the same blood sample could indirectly serve as a control for the accuracy of the parameters performed.

The aim of this prospective, 2-center clinical study was to assess the relationship between different degrees of inflammation in the dental pulp tissue (and thus the clinical diagnoses) and the MMP-9 levels in the pulpal blood. This study also investigated the correlation between the MMP-9 and TIMP-1 values in these blood samples.

**Materials and Methods**

The study population was composed of patients seeking dental treatment at the Department of Conservative Dentistry of the University Hospital of Heidelberg, Heidelberg, Germany, and patients from a private dental practice located nearby. The treatment provider in the private dental practice has a part-time position as a supervisor in the department. The study protocol of this prospective, 2-center clinical study was approved by the Ethics Committee of the University of Heidelberg (Ref. S-219/2012).

**Inclusion Criteria and the Assignment Pulpal Diagnoses**

Patients with a tooth showing clinical or radiographic evidence of a deep carious lesion extending close or into the pulp chamber were considered for potential inclusion. All patients who agreed to participate provided their informed consent before treatment and had the right to decline participation in the study at any time.

The teeth were assigned to different groups according to the clinical diagnoses. The diagnoses were based on strict clinical and radiographic criteria. When the dental pulp was exposed during caries removal, the following clinical and radiographic diagnostic criteria were applied:

1. **Group 1** (asymptomatic teeth): No clinical signs or symptoms of pulpitis, no history of pain, response to cold test (with carbon dioxide snow) within normal limits, no sensitivity to percussion or the bite test, bleeding time from the exposed pulp tissue less than 2 minutes, and no widening of the periodontal ligament space (periapical index [PAI] = 1)

2. **Group 2** (teeth with reversible pulpitis): Slight clinical symptoms of minor intensity, slightly exaggerated reaction to cold or sweet stimuli, no history of pain, response to cold test (with carbon dioxide snow) within normal limits, no sensitivity to chewing or percussion, bleeding time from the exposed pulp tissue less than 5 minutes, and no widening of the periodontal ligament space (PAI = 1)

3. **Group 3** (teeth with symptomatic irreversible pulpitis and anti-inflammatory medication [nonsteroidal anti-inflammatory drugs (NSAIDs)]): Patients who used long-acting NSAIDs before treatment. Signs or symptoms could be as follows: history of continuous moderate or severe pain, either provoked or spontaneous; prolonged pain initiated by provocation with carbon dioxide snow; tenderness to chewing or percussion; bleeding time from the exposed pulp tissue longer than 5 minutes; and widening of the peridental ligament space but no periapical periodontitis (PAI ≤ 2)

4. **Group 4** (teeth with symptomatic irreversible pulpitis without NSAID use): Patients did not use long-acting NSAIDs before treatment and clinical or radiographic signs or symptoms identical to those of group 3.

For ethical reasons, there was no control group with blood samples from healthy teeth with no deep carious lesions.

**Exclusion Criteria**

The following exclusion criteria were defined in advance for all groups of teeth: teeth with a negative response to cold test (carbon dioxide snow), apical radiolucency (PAI > 2); condensing apical periodontitis, internal/external root resorption, history of trauma, longitudinal root fracture or evidence of a periodontal-endodontic lesion on the day of treatment, loss of function (eg, tooth mobility grade 3), and swelling in association with the treated tooth. Teeth that could not be treated using rubber dam isolation, teeth from which less than 2.5 μL blood could be obtained from the pulp, and teeth that could not be unequivocally assigned to 1 of the 4 study groups were also excluded. In addition, patients with a compromised immune status; patients who were pregnant at the time of treatment; and patients who had taken antibiotics, bisphosphonates, or statins within the last 4 weeks before treatment were not allowed to participate. For patients in group 3, the use of long-acting anti-inflammatory medications (NSAIDs) in the 14 days preceding treatment was allowed.

**Clinical Treatment Intervention and Sample Collection**

The flowchart diagram in Figure 1 gives a step-by-step overview of the treatment intervention, sample collection, and storage of the blood samples. All treatment interventions were performed under rubber dam isolation. The treatment providers were either dentists or supervised undergraduate students. Caries were removed by means of mechanical excavation with a slow-speed rose head bur. The departmental operating protocol states that the peripheral caries has to be removed before the carious dentin from the cavity walls near the pulp is excavated. If the pulp is exposed, a sterile cotton pellet soaked in sterile physiological saline must be placed on the exposed pulp tissue. At this point, 1 of the clinical investigators responsible for this study temporarily took over the treatment, which involved the verification of complete caries removal, partial pulpotomy, and blood sample collection and afterward the disinfection of the cavity with 0.12% chlorhexidine solution and dressing of the
exposed pulp using ProRoot MTA white (Dentsply Maillefer, Bal-
laigues, Switzerland). The treatment was always performed by an 
endodontically experienced treatment provider using either loupes 
or a dental operating microscope. Blood samples from the dental 
pulp were obtained using heparinized 10-
μL microcapillary tubes 
(Hirschmann, Eberstadt, Germany; Fig. 2). The cavities of the treated 
teeth were immediately restored by the direct placement of compos-
tite resin (Tetric EvoCeram; Vivadent, Schaan, Liechtenstein).

Transport and Storage of Blood Samples
The blood obtained from the exposed dental pulp was transferred 
immmediately from the microcapillary tubes into numbered reaction 
tubes (e-cups). These contained 50 μL sterile physiological saline so-
lution. The volume of the blood sample was precisely determined by 
measuring the penetration depth of the blood in the microcapillary 
tube and calculating in relation to the maximal volume capacity of 
the microcapillary tube. The e-cups were transferred to the laboratory 

Figure 1. Flowchart showing the step-by-step treatment procedures and blood sample collection.
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**logistic curve fit (Magellan Software package, Tecan).** Concentrations dance with the manufacturer's instructions.

- MMP-9 and TIMP-1 Assays

  Before measuring the MMP-9 and TIMP-1 levels, the frozen samples were thawed on ice. Blood clots and serum were separated by centrifugation at room temperature (15 minutes at 1000 rpm and stored at −70°C until the laboratory measurements were performed. A sample collection at the external study center (the private dental practice) was created under identical conditions. The blood samples were stored at −70°C and transferred on dry ice from the dental practice to the laboratory at the University of Heidelberg, where all measurements were performed by a single experienced biologist. The numbering of the e-cups ensured that the staff was blinded to the clinical diagnosis.

**MMP-9 and TIMP-1 Assays**

- Before measuring the MMP-9 and TIMP-1 levels, the frozen samples were thawed on ice. Blood clots and serum were separated by centrifugation at room temperature (15 minutes at 1000 rpm). Serum was removed, diluted with the appropriate diluents, and assayed immediately in duplicate. MMP-9 was evaluated using a fluorometric assay (R&D, Wiesbaden, Germany) according to the manufacturer’s instructions. The assay uses a fluorogenic substrate, which is cleaved by MMP-9 to liberate the quencher molecule from the fluorophore. A fluorescence plate reader (Tecan, Crailsheim, Germany) was used to determine the relative fluorescence units (excitation wavelength: 320 nm, emission wavelength: 405 nm). Pro–MMP-9 was activated using amino phenyl mercuric acetate to assess the total level of MMP-9. The active MMP-9 levels in the serum samples are approximately 7 to 10 times lower than the total MMP-9 levels. Because of the limited sample volume, we focused on the total MMP-9 in order to remain within the measurement range of the assay. A minimum sample volume of 2.5 μL was determined empirically to allow for reproducible measurements in duplicates for both MMP-9 and TIMP-1. TIMP-1 was assessed using a colorimetric enzyme-linked immunosorbert assay (R&D) in accordance with the manufacturer’s instructions.

  Standard curves were created for both assays using a 4-parameter logistic curve fit (Magellan Software package, Tecan). Concentrations extrapolated from the standard curves were corrected for the dilutions and different initial sample volumes (Excel, version 2010; Microsoft, Redmond, WA) and are given in ng/mL of collected blood.

- **Standardization of Clinical Intervention and Initial Recording of Clinical Findings**

  All study investigators at the university hospital and in the private practice were instructed by the principal investigator (J.M.) to follow the same treatment protocol and to perform the same evaluation procedures and documentation of all information regarding the treated tooth and patient.

  The treatment procedures, blood sample collection, and recording of the initial clinical findings were standardized as much as possible to ensure therapeutic equality. The mandatory steps of both procedures are summarized in a flowchart diagram (Fig. 1) and were discussed in detail with all clinical investigators. The step-by-step procedures were always performed by 2 clinical investigators according to the standardized treatment protocol (Fig. 1). The 2 investigators assisted each other as needed to keep the time of treatment procedures and sample collection and transport to the freezer as brief as possible. In this way, new clinical investigators were supervised and trained directly on the patient for every step of the procedure. This ensured that a standardized protocol was followed by all clinical investigators from the first partial pulpotomy and blood sample collection onward.

  All details of the patient’s complaints, signs, or symptoms were entered by the clinical investigators in a structured form especially designed for this study. This included all clinical information regarding the condition of the pulp and the periodontal situation of the treated tooth (ie, the response to the cold test, the sensitivity to percussion, or the bite test; the duration and quality of pain; the presence of any other pain or discomfort related to the treated tooth; the bleeding time of the exposed pulp; the presence of tooth mobility; the pocket depths and attachment loss; the presence of furcation involvement; the presence of a sinus tract; radiographic findings; and the type and quality of the restoration). The form also contained information on the patient’s medical history such as use, duration, and dosage of NSAIDs; use of antibiotics, bisphosphonates, or statins; and information regarding any previous history of pain or dental trauma related to the tooth of interest.

- **Radiographic Calibration and Assessment**

  Before evaluating the study radiographs, 1 designated examiner (J.P.) was calibrated using the PAI calibration kit of 100 periapical radiographs (30). Intraexaminer reliability and interexaminer agreement with the calibration kit’s “gold standard” were assessed using the Cohen kappa statistic. In addition, radiographs were evaluated independently in a random sequence by 2 examiners (J.P. and J.M.) The radiographic examination aimed to determine the presence or absence of any pathologic changes adjacent to the treated teeth (eg, internal or external root resorption, condensing apical periodontitis, or the presence of a periodontal-endodontic lesion). In cases in which they did not agree, the examiners discussed the radiographic findings until a consensus was reached. All radiographs were evaluated in a darkened room using a 21.3-inch RadiForce LCD Medical Display monitor (EIZO RadiForce R22; EIZO GmbH, Karlsruhe, Germany) with a screen resolution of 1600 × 1200 pixels (pixel pitch 0.270 × 0.270 mm) and a contrast ratio of 550:1.

  Radiographic assessment was undertaken on the day of treatment using the parallel technique with an RWT film holder (KKD GmbH, Ellwangen, Germany) and a photostimulable phosphor image plate system (VistaScan PSP System; Dürr Dental GmbH, Bissingen, Germany).

- **Statistical Analysis**

  Descriptive statistics comprising the mean, median, and standard deviation were calculated as well as the minimum, maximum, absolute, and relative frequencies. In addition, box and whisker plots are presented, which show the dispersion and the median of the MMP-9 and TIMP-1 values within the 4 different groups of pulpal diagnoses. Because of the nature of the MMP-9 and TIMP-1 values, pair-wise comparisons were conducted using Mann-Whitney U tests. Spearman rank
correlations were subsequently conducted to investigate correlations between the paired MMP-9 values within the pulp diagnosis groups. Given the exploratory nature of the study, no adjustment was made for multiple testing, and test results of more than a 5% confidence level were interpreted as statistically significant. The Kappa-Cohen test was used for the PAI calibration according to the recommendations of Ørstavik et al (30). The data were statistically analyzed using SAS 9.3 software (SAS Institute Inc, Cary, NC) and SPSS 21.0 (SPSS Inc, Chicago, IL).

Results

Study Group

Forty-four patients, 19 men and 25 women aged 18–74 years (mean = 55 years, first quartile = 26.75, third quartile = 49.75 years), each having a tooth with a deep carious lesion reaching the pulp chamber fulfilled the inclusion criteria for this study. Twenty-six of these patients presented as emergencies with a diagnosis of symptomatic irreversible pulpitis. Only 6 of the 26 patients had not taken any NSAIDs (group 4). Eleven patients presented with the diagnosis of reversible pulpitis (group 2), and 8 patients presented with completely asymptomatic teeth despite the deep carious lesion (group 1). Five teeth had to be excluded from the study because it was not possible to unequivocally assign a pulpal diagnosis according to the strict clinical and radiographic criteria of this study (see Materials and Methods section). The study sample was comprised of 11 maxillary premolars, 13 maxillary molars, 2 maxillary anterior teeth, 7 mandibular premolars, and 11 mandibular molars.

Radiographic Calibration Process

The intraexaminer reliability for the PAI calibration results was \( \kappa = 0.86 \), indicating “almost perfect agreement”; the interexaminer agreement (examiner scores vs the calibration kit “authorized scores”) was \( \kappa = 0.80 \), indicating “substantial agreement” (31).

MMP-9 Levels

The distribution of the MMP-9 levels in ng/mL, including the mean values for all diagnosis groups, is given as a box plot in Figure 3.

The MMP-9 levels in group 1 (asymptomatic teeth) ranged from 267 ng/mL–807 ng/mL (median = 386 ng/mL). In group 2 (teeth with reversible pulpitis), the levels of MMP-9 ranged from 300 ng/mL–1396 ng/mL (median = 938 ng/mL). In group 3 (teeth with irreversible pulpitis not influenced by the intake of NSAIDs), the MMP-9 levels were from 389 ng/mL–7991 ng/mL (median = 1051 ng/mL). In group 4 (teeth with irreversible pulpitis not influenced by NSAIDs), the range of MMP-9 in the blood was between 1011 ng/mL and 3055 ng/mL (median = 1701 ng/mL).

TIMP-1 Levels

The distribution of TIMP-1 levels in ng/mL is depicted as a box plot in Figure 4, which includes the mean values for all diagnostic groups. The TIMP-1 levels in group 1 (asymptomatic teeth) ranged from 11 ng/mL–96 ng/mL (median = 42 ng/mL). In group 2 (teeth with reversible pulpitis), the TIMP-1 levels ranged from 15 ng/mL–163 ng/mL (median = 57 ng/mL), and in group 3 (teeth with irreversible pulpitis influenced by the intake of NSAIDs), they ranged from 41 ng/mL–2006 ng/mL (median = 186 ng/mL). In group 4 (teeth with irreversible pulpitis not influenced by anti-inflammatory medication), the TIMP-1 levels ranged from 45 ng/mL–1565 ng/mL (median = 209 ng/mL).

Correlation between the MMP-9 Levels and the Diagnosis Groups

Pair-wise comparisons (Mann-Whitney U test) revealed that the MMP-9 levels from the pulpal blood of asymptomatic teeth (group 1) differed significantly from all other pulp diagnosis groups (group 1 vs 2, \( P < .001 \); group 1 vs 3, \( P < .001 \); group 1 vs 4, \( P < .001 \)). There was a statistically significant difference in the MMP-9 levels when groups 2 and 4 were compared (\( P = .005 \)). The observed differences between groups 2 and 3 and between groups 3 and 4 did not reach statistical significance (\( P = .65 \) and \( P = .32 \), respectively). Details are provided in Table 1.

The Spearman rank correlation was used to evaluate the correlation between all MMP-9 and TIMP-1 values independently of the groups. The MMP-9 and TIMP-1 levels showed a very highly significant correlation regardless of the group assignment (\( P < .001 \)) with a positive correlation coefficient (\( \rho = 0.58 \)).

Pulpal Diagnosis Assignment Based on the MMP-9 Levels

Based on the MMP-9 levels measured in this study, calculations regarding the assignment to the different pulpal diagnoses as a percentage were performed; these are summarized in Table 2. Because no reference values for MMP-9 concentrations in the pulpal blood exist in relation to the different stages of pulpitis, the cutoff points for the MMP-9 values in this table could not be defined in advance.

Figure 3. Box plot depicting MMP-9-values (ng/mL) for all 4 diagnosis groups: group 1, asymptomatic teeth; group 2, reversible pulpitis; group 3, irreversible pulpitis influenced by the intake of NSAIDs; and group 4, irreversible pulpitis not influenced by the intake of NSAIDs.

Figure 4. Box plot depicting TIMP-1 values (ng/mL) for all 4 diagnosis groups: group 1, asymptomatic teeth; group 2, reversible pulpitis; group 3, irreversible pulpitis influenced by the intake of NSAIDs; and group 4, irreversible pulpitis not influenced by the intake of NSAIDs.
Regarding the extent of pulpal inflammation/tissue destruction because common clinical tests do not provide enough information (nontreatable by vital pulp therapy [eg, partial pulpotomy]) slight clinical symptoms. Commonly used diagnostic tests provide limited information for distinguishing between reversible pulpitis (treatable by vital pulp therapy) and irreversible pulpitis only limited information for distinguishing between reversible pulpitis by partial pulpotomy (4, 5, 35, 36). Nevertheless, deciding on the options of partial pulpotomy or root canal treatment in those cases is often difficult, especially when there are only slight clinical symptoms. Commonly used diagnostic tests provide only limited information for distinguishing between reversible pulpitis (treatable by vital pulp therapy) and irreversible pulpitis (nontreatable by vital pulp therapy [eg, partial pulpotomy]) because common clinical tests do not provide enough information regarding the extent of pulpal inflammation/tissue destruction (11). Histologic examination of pulp tissues is a more reliable method for validating the clinically suspected degree of pulpal inflammation (12, 37, 38). However, histologic examination is only possible after the tooth has been extracted. Therefore, there is a great need for further diagnostic tools to confirm the suspected clinical diagnosis.

This also raised the most important question for this study project: Is the level of MMP-9 evaluated in the blood of cariously exposed pulps helpful in assessing the different stages of pulpal inflammation or the extent of pulp tissue destruction? If so, this would give the clinician valuable information for assessing the prognosis for the success of the therapeutic option of vital pulp therapy (eg, partial pulpotomy) in a specific case.

The results of the present study indicate that the level of MMP-9 in the pulpal blood may indeed be a valuable tool to assess the degree of inflammation and destruction of the dental pulp tissue, perhaps serving as a useful ancillary diagnostic tool for deciding on the options of vital pulp therapy or root canal treatment after carious pulp exposure. Therefore, it is conceivable that MMP-9 levels may be used as an indirect parameter for assessing the prognosis after pulp exposure when striving to maintain the vitality of a tooth by partial pulpotomy. Yet, in view of the observed overlaps between the MMP-9 levels in the different diagnosis groups (Fig. 3), the MMP-9 levels in the pulpal blood can currently only serve as an indicator of the pulpal condition and not as the sole basis for the clinical diagnosis (Table 2). Table 2 shows a probability assessment of affiliation to one of the diagnosis groups based on the MMP-9 levels evaluated in this study.

Because all teeth in the study had deep carious lesions extending into the pulp chamber, localized inflammation in the pulp tissue close to the region of pulp exposure was to be expected in all groups, including the asymptomatic teeth of group 1 (32, 34, 38). This provided standardization because the comparisons between the 4 groups were based on the same initial condition.

Nevertheless, the MMP-9 levels in group 1 were significantly lower than those in the other 3 diagnostic groups (Fig. 3; Table 1). This finding was consistent with the results of previous studies showing significant differences in the MMP-9 levels between teeth with symptomatic pulpitis and teeth with healthy pulpal tissues (24, 26). NSAIDs are known to lower MMP-9 values (39, 40). Therefore, it is not surprising that the differences in the MMP-9 levels between groups 2 and 3 did not reach statistical significance (Table 1). The large variations in the MMP-9 levels in group 3 might be caused by different degrees of destruction in the dental pulp even when pulpitis has reached an irreversible stage. However, the degree of destruction can vary widely from the stage of initial damage in which initially only a limited area of the pulp tissue is involved to the stage of irreversible pulpitis, which affects the entire pulp tissue (33, 37, 41). This explains the great variation in the MMP-9 levels of group 3. In some of these cases, advanced inflammation and destruction of the pulp tissue likely resulted in very high MMP-9 levels. The degree of

### Table 1. Assignment of a Pulpal Diagnosis Based on the Matrix Metalloproteinase 9 (MMP-9) Levels Evaluated Within This Study Expressed in Percentages

<table>
<thead>
<tr>
<th>Probability of assignment to group</th>
<th>MMP-9 level (%)</th>
<th>MMP-9 level (%)</th>
<th>MMP-9 level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1000 (ng/mL)</td>
<td>≥1000–1400 (ng/mL)</td>
<td>&gt;1400 (ng/mL)</td>
</tr>
<tr>
<td>Group 1</td>
<td>36</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Group 2</td>
<td>23</td>
<td>50</td>
<td>0</td>
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<tr>
<td>Group 3</td>
<td>41</td>
<td>40</td>
<td>58.3</td>
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<tr>
<td>Group 4</td>
<td>0</td>
<td>0</td>
<td>41.7</td>
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Group 1, asymptomatic teeth; Group 2, teeth with reversible pulpitis; Group 3, teeth with irreversible pulpitis, patients with antiinflammatory medication (nonsteroidal anti-inflammatory drugs); Group 4, teeth with irreversible pulpitis, patients without anti-inflammatory medication.
destruction may not have been so advanced in other teeth in this group, but the individual perception of pain might have motivated some patients to take NSAIDs. Patients might start using painkillers at different stages of pain/pathology. Thus, the wide range of MMP-9 values observed in group 3 may on one hand result from MMP-9 decreasing the “intake of NSAIDs.”

A negative control group (without any inflammation inside the pulp tissue) was omitted in this study project because of the unbalanced risk-benefit ratio. MMP-9 levels of the pulp of healthy teeth without caries would most probably have shown MMP-9 levels below the detection limits of the available methods of measurement as reported by Gusman et al (24), but it would have been impossible to communicate to the patients of a healthy control group the necessity to access caries-free asymptomatic teeth to obtain this information and unacceptable for these patients, the treatment providers, and the ethics committee of our university.

The highly significant \( P < .001 \) positive correlation \( (\rho = 0.58) \) between the MMP-9 and TIMP-1 levels of each blood sample was expected and indicates the correctness of the measurements performed. However, the results show that the TIMP-1 levels appear to be less sensitive for differentiating between different stages of inflammation in the pulp tissue than the MMP-9 levels (Figs. 3 and 4). Compared with MMP-9, the TIMP-1 levels showed a delayed increase during inflammatory processes in the pulp tissue, which is in line with the physiological function of TIMP-1. As a regulator for tissue remodeling, TIMP-1 prevents uncontrolled extracellular matrix turnover caused by (excessively) high MMP-9 levels (29). This specific down-regulating function of TIMP-1 could be the reason why TIMP-1 is expressed with some delay compared with MMP-9 (Figs. 3 and 4) and therefore seems to be a less sensitive protein marker in the early stages of pulp inflammation.

Because there are no standard values for MMP-9 levels in blood samples from dental pulp tissue to date, determination of the diagnoses regarding the pulp conditions in the current study had to be based on strict clinical and radiographic criteria. The validity of the clinical and radiographic criteria applied was confirmed in a recently published study (12). In that study, diagnostic criteria comparable with those used in the present study were used to ascertain diagnoses of normal pulp, reversible pulpitis, and irreversible pulpitis. The actual degree of inflammation was subsequently confirmed with histologic and histobacteriologic analyses of the pulp tissues and revealed good agreement (12). In addition to the clinical and radiographic criteria applied, we verified the pulp diagnoses by investigation of the medical and dental history, the intake of painkillers, and the “history of pain” (8). The last control to differentiate between reversible pulps (inflammation limited to a localized part of the dental pulp close to the carious lesion) and irreversible pulps (inflammation of a large part of the dental pulp) was the extent of hemorrhage after pulp exposure and partial pulpotomy. The extent of hemorrhage has also been shown to be a prognostic factor after carious pulp exposure (13).

The collection of pulpal blood samples using heparinized micro-capillary tubes (Fig. 2) has proven to be practical. The use of microcapillary tubes allows for the reliable determination of the collected blood volume. This method of blood sampling prevented the problems reported by Zehnder et al (26), who used cotton pliers to collect dentinal fluid from access cavities in the dentin wound rather than blood from the inflamed pulp tissue. In their study, the MMP-9 levels of more than half of the symptomatic teeth were below the detection limit of the assay used.

Most clinical studies assessing partial pulpotomy after carious pulp exposure have shown high success rates (5–55). Lower success rates were observed when the dental pulp was left untouched and only dressed with a direct pulp capping material (42–45). Partial pulpotomy with removal of the inflamed tissue after carious pulp exposure is therefore indicated, particularly in teeth with carious pulp exposure with or without symptoms of reversible pulpitis (groups 1 and 2), to maintain pulpal vitality and the health of the treated teeth. Not all pulpal inflammatory reactions inevitably result in permanent damage to the pulp tissue (33, 37). This line of research will be expanded to include a third study center in order to build a broader data foundation and to verify the results of the current project with a larger sample size.

**Conclusion and Future Perspective**

The results of the present study indicate that the degree of inflammation in the pulp tissue can be estimated from the MMP-9 levels in the blood of teeth with pulp exposure. This finding could be helpful in developing a diagnostic test assessing MMP-9 levels in pulpal blood samples. This would offer the dental practitioner a useful diagnostic tool after carious pulp exposure, especially to distinguish teeth with mild clinical symptoms and reversible inflammation localized to a part of the dental pulp close to the carious lesion from teeth with severe inflammation and extensive destruction of the dental pulp (nonsavable pulp). With this additional information, the dentist and patient can discuss the best treatment procedure for a tooth after carious pulp exposure (eg, vital pulp therapy [partial pulpotomy] vs root canal treatment).

The collection of more data regarding MMP-9 levels in different stages of pulp inflammation is strongly recommended to confirm the results of this study with larger sample sizes.

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The authors deny any conflicts of interest related to this study.

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