

# Presence of Aspartate Aminotransferase in Peri-Implant Crevicular Fluid With and Without Mucositis

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The aim of this study was to assess the presence of aspartate aminotransferase (AST) in peri-implant crevicular fluid, with or without clinical signs of mucositis, to determine its predictive diagnostic value, sensitivity, and specificity. The AST levels were determined (at a threshold of 1200  $\mu$ U/mL) for 60 clinically successful implants in 25 patients with or without peri-implant mucositis. Samples were taken prior (AST1) to peri-implant probing with a manual constant-pressure probe (0.2 N) and 15 minutes after probing (AST2). Clinical assessments included radiographic determination of preexisting bone loss, probing, and the evaluation of mucositis, plaque, and bleeding upon probing. Analysis was performed at both the level of the implant and the patient as a unit. We detected a significant difference between AST1 and AST2 at both levels. A significant difference was observed at AST1 between implants that bled upon probing and those that did not. However, when we considered the patient as a unit, there were no significant differences. The plaque index was not significant at either level. AST1 had high specificity and positive predictive diagnostic value (80%) for bleeding upon probing. Probing induces a greater release of AST from inflamed tissues compared with healthy tissues in situ but not at the systemic level. At the implant level, the implant position could be responsible for this difference. Aspartate aminotransferase was a reliable predictor of patients with mucositis.

**Key Words:** *peri-implant crevicular fluid, tissue damage enzymes, AST, aspartate aminotransferase, peri-implant probing*

## INTRODUCTION

The advent of systems that can diagnose periodontal and peri-implant breakdown based on proteins and enzymes present in the gingival fluid has opened new doors for in vivo research.<sup>1</sup> The analysis

of aspartate aminotransferase (AST) levels in crevicular fluid (CF) has the potential to facilitate the early diagnosis of periodontal and peri-implant tissue destruction.<sup>2-4</sup>

Aspartate aminotransferase is an enzyme that promotes the transfer of an amino group from glutamic acid to oxaloacetic acid. The enzyme was also called aspartate transaminase and glutamic-oxaloacetic transaminase. AST is normally confined to the cell and released to the extracellular environment upon cell death,<sup>2</sup> making it a nonspecific

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marker of cell death and tissue destruction. The greatest concentrations of AST are seen in the epithelial cells, fibroblasts, and polymorphonuclear cells following tissue injury.<sup>5</sup>

In 1984, Chambers et al<sup>6</sup> demonstrated the presence of AST in CF. The activity of AST has been reported to be strongly dependent on the degree of damage to the periodontal tissues<sup>5</sup> and has been closely associated with the severity of experimental gingivitis in humans during both the development and healing phases.<sup>7,8</sup> Some authors have demonstrated a relationship between AST levels in the CF and the loss of periodontal attachment in both human and canine models.<sup>9–11</sup> The analysis of AST levels in the CF has the potential to facilitate the early diagnosis of periodontal and peri-implant tissue destruction.<sup>2–4</sup> However, to date, only a few studies have measured the presence of AST in the peri-implant sulcus fluid (PISF) and its relationship with implant health.<sup>3,12</sup>

The aim of the present study was to assess whether there is a relationship between AST levels and bleeding upon probing, gingival indices, or plaque indices in order to obtain a better understanding of probing measurements around osseointegrated oral implants. Our secondary aims were to establish the specificity, sensitivity, and positive or negative predictive value of the measurement with regard to bleeding upon probing.

## MATERIALS AND METHODS

### *Patients and study design*

Study subjects included 25 patients (9 men and 16 women) who received a total of 60 dental implants and were recruited consecutively as they arrived for their appointments. All subjects volunteered to participate in this study. The mean patient age was  $45.92 \pm 15.51$  years (range, 18–68 years; 95% confidence interval [CI], 39.51–52.32 years). The mean age of the male patients was  $52.66 \pm$

$13.39$  years (95% CI, 42.36–62.96 years), and the mean age of the female patients was  $42.12 \pm 15.7$  years (95% CI, 33.75–50.49 years).

Thirty-three of the implants were placed in women, and 27 were placed in men. Previous clinical and radiographic assessments revealed no systemic or local contraindications to participation. Complete medical and dental histories were taken, and a clinical examination was conducted by a single trained examiner. This study was conducted in accordance with the Helsinki Declaration of 1975 and as revised in 2002. All subjects provided informed consent to participate in the study.

All implants met the following criteria at the most recent checkup:

1. Functioned under a load for at least 12 months and underwent peri-implant maintenance at 6-month intervals
2. Met all of Albrektsson's criteria for success (Albrektsson et al<sup>13</sup>)
3. Supragingival plaque index  $\leq 2$  (Silness and L oe<sup>14</sup>)
4. Probing depth was no more than 3 mm at any site around the fixture
5. Showed no signs of peri-implantitis
6. Showed bone loss of less than 2 mm on either side of the fixture as measured by a comparison of an immediate postsurgical radiograph and another taken at the time of the inclusion

Exclusion criteria included awkwardness and smoking habits, poor oral hygiene and motivation (plaque index  $>2$ ), and gingival index (GI)  $>2$  (L oe and Silness<sup>15</sup>); bleeding upon probing  $>3$  (Saxer and Muhlemann<sup>16</sup>); severe bruxism or clenching habits; systemic disease (especially liver diseases), pregnancy, or long-term medical therapy or medication; and mobility by electronic device  $>3$  units (Periotest, Siemens AG, Bensheim, Germany).

We considered an implant healthy if it met all the criteria. According to Lindhe and

Meyle,<sup>17</sup> mucositis can be identified clinically by redness and swelling of the soft tissue, but bleeding upon probing is currently recognized as the important feature. Seventeen patients were identified as having mucositis, and 8 were considered healthy.

The Silness and Løe plaque index,<sup>14</sup> the Løe and Silness gingival index,<sup>15</sup> bleeding upon probing,<sup>16</sup> probing depth (at 6 points, 3 buccal and 3 lingual), mobility by electronic device, and AST were assessed upon examination.

### **Implants**

A single surgeon inserted all of the implants. All implants were placed using a nonsubmerged protocol and exhibited good primary stability at surgery. Abutments were connected 2 months after implant placement. The implants that were used were screw-shaped, sandblasted, and acid-etched (Alpha Bio, Implant System Ltd, Geseke, Germany).

### **Analysis level**

Two analysis levels were used: the level of the implant (when we considered implants as a unit) and the level of the patient (when we considered patients as a unit). For the second level, only 1 implant per patient was selected. This selection was carried out in order of the worst GI; if they had the same index, the second measure considered was the probing depth, and finally we considered mobility.

### **Radiographic bone-to-implant contact**

Digital periapical radiographs were taken using a long-cone paralleling and standardized method. The distance from the fixture-abutment junction to the first visible bone-to-implant contact, mesial and distal to the implant, was determined on the digitized intraoral radiographs using a computer program (Gesimag, Informatica Medica, Barcelona, Spain). No modifications

were made to the images. Measurements were made by an operator trained to read implant radiographs. The zero value was set at the fixture-abutment junction.

### **AST determination**

Two determinations of AST in the mucosal mesiobuccal sulcus were performed for each implant: before probing (AST1) and 15 minutes after probing (AST2).

The AST levels in the PISF was determined using a commercial AST test (Pocket-Watch, Sterio-Oss, Loma Linda, Calif) according to the manufacturer's recommendations. Briefly, each test site was air-dried and isolated with cotton rolls. The sampling strip was delicately placed into the mucosal pocket and left for 30 seconds; it was then placed into the corresponding plastic well containing buffer solution and the reagents added according to the instructions. The threshold used to denote a positive AST test was 1200  $\mu$ IU/mL.

### **Probing**

Probing was performed using a manual pressure-controlled (0.25 N) probe (TPS probe, Ivoclar, Schaan, Principality of Liechtenstein). Care was taken to introduce the probe parallel to the long axis of the implants. All probing depth measurements were performed first and without removing the tip of the probe from the pocket. This procedure was followed by measuring the attachment level with the top of the abutment serving as a fixed reference point. The probing depth of all implants was measured at 6 sites per implant: mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual. Only the site representing the deepest pocket of the implant was used for further analysis. The absence or presence of bleeding was assessed within 15 seconds of peri-implant probing. The implants with redness or swelling or that bled upon probing were considered test

	AST2 Negative	AST2 Positive	Total
<b>Implants as a unit</b>			
AST1 negative	37	6	43
AST1 positive	5	12	17
Total	42	18	60
<b>Patients as a unit</b>			
AST1 negative	15	1	16
AST1 positive	2	7	9
Total	17	8	25

\*AST indicates aspartate aminotransferase. Positivity is denoted by AST activity  $\geq 12\ 000\ \mu\text{U/mL}$ .

samples (mucositis), and implants without bleeding were considered controls. During the probing, the plaque and gingival indices were recorded and mobility tested using an electronic device.

### Statistical analysis

The worst value of each clinical variable in each implant or patient was used for analysis, which is based on the worst case. The variables recorded for each implant were analyzed by the chi-square test, *t* test, and analysis of variance. All statistical calculations were performed using a statistical software package for Windows (SPSS 15, SPSS Inc, Chicago, Ill).  $P < .05$  was considered significant.

## RESULTS

### Implant considered as a unit

Twenty-seven (45%) implants were placed in 9 men, and 33 (55%) implants were placed in 16 women. The mean implant plaque index was  $1.43 \pm 0.59$  (95% CI, 1.28–1.59); 3 implants had an index of 0 (5%), 28 had an index of 1 (46.7%), and 29 had an index of 2 (48.3%). The mean implant GI was  $1.48 \pm 0.56$  (95% CI, 1.34–1.63); 33 implants had an index of 1 (55%), and 27 had an index of 2 (45%). The mean probing depth for implants was  $1.75 \pm 0.72$  mm (95% CI, 1.56–1.94 mm). The mean mobility for implants, measured using the electronic device method, was

$-1.18 \pm 3.27$  units (95% CI,  $-2.03$  to  $-0.34$  units).

There was a significant difference between the AST levels measured at AST1 and AST2 ( $P < .000$ ; Table 1). There was also a significant difference at AST1 between the AST levels of implants that bled upon probing and those that did not ( $P = .02$ ; Table 2). There was a relationship between a positive AST1 measurement and the GI (above the threshold of  $1200\ \mu\text{U/mL}$ ); however, no significant relationships between the measurement at AST2 and bleeding upon probing were detected (Table 2). Furthermore, there was no significant relationship between the measurement at AST2 and the GI, and we found no correlation between the plaque index and the measurements at AST1 or AST2 ( $P > .05$ ).

Table 3 shows the sensitivity, specificity, and positive and negative predictive powers of the levels at AST1 and AST2 as markers of tissue damage, as determined by bleeding upon probing.

### Patient considered as a unit

The mean patient plaque index was  $1.4 \pm 0.64$  (95% CI, 1.13–1.66). Two patients had a plaque index of 0 (8%), 11 had an index of 1 (44%), and 12 had an index of 2 (48%). The mean patient GI was  $1.64 \pm 0.48$  (95% CI, 1.43–1.84). Nine patients had a GI of 1 (36%), and 16 had a GI of 2 (64%). The mean probing depth was  $2.04 \pm 0.73$  mm (95% CI,

TABLE 2

Relationships between bleeding upon probing and AST activity\*

	Bleeding Upon Probing	No Bleeding Upon Probing	Total
Implants as a unit			
AST1 negative	15	28	43
AST1 positive	12	5	17
Total	27	33	60
AST2 negative	16	26	42
AST2 positive	11	7	18
Total	27	33	60
Patients as a unit			
AST1 negative	9	7	16
AST1 positive	7	2	9
Total	16	9	25
AST2 negative	10	7	17
AST2 positive	6	2	8
Total	16	9	25

\*AST indicates aspartate aminotransferase. Positivity is denoted by AST activity  $\geq 12\ 000$   $\mu\text{IU/mL}$ .

1.73–2.34 mm). The mean mobility measured by the electronic device method was  $-0.24 \pm 3.16$  units (95% CI,  $-1.54$  to 1.06 units). There were no significant differences between men and women for these variables.

There was a significant difference between the AST levels measured at AST1 and AST2 ( $P < .001$ ; Table 1). There was no significant relationship between the AST levels at AST1 of patients who bled upon probing and those who did not ( $P = .401$ ; Table 2). There was no relationship between a positive value at AST1 and the GI (above

the threshold of 1200  $\mu\text{IU/mL}$ ). Furthermore, no significant relationship between the AST level at AST2 and bleeding upon probing were detected (Table 2), and we found no correlation between the plaque index and the AST levels at AST1 or AST2 ( $P > .05$ ).

Table 3 shows the sensitivity, specificity, and positive and negative predictive powers of the levels at AST1 and AST2 as markers of tissue damage, as determined by bleeding upon probing.

## DISCUSSION

### AST determination

We decided to use AST enzyme vs other cytokines (tumor necrosis factor- $\alpha$ , interleukin [IL]-1 $\beta$ , transforming growth factor- $\beta$ , IL-10) due to its being a more specific marker of tissue damage than previous inflammatory status.

On the other hand, we used a higher threshold of 1200  $\mu\text{IU/mL}$  based on a previous study that determined an appropriate cutoff using the receiver-operator characteristic curve generated by the manufacturer of the AST determination kit. A similar kit for measuring AST levels that has a threshold of 800  $\mu\text{IU/mL}$  (Perio Gard, Xytronyx Inc, San Diego, Calif) has been used to

TABLE 3

Sensitivity, specificity, and positive and negative predictive values of AST activity (threshold = 1200  $\mu\text{IU/mL}$ )\*

	>1200 $\mu\text{IU}$ AST1	>1200 $\mu\text{IU}$ AST2
Implants as a unit		
Sensitivity	44.44%	40.74%
Specificity	84.84%	78.78%
PPV	70.58%	61.11%
NPV	65.11%	61.90%
Patients as a unit		
Sensitivity	43.75%	37.5%
Specificity	77.77%	77.77%
PPV	77.77%	60%
NPV	43.75%	56.25%

\*AST indicates aspartate aminotransferase; PPV, positive predictive value; NPV, negative predictive value.

distinguish between active and inactive periodontal disease.<sup>2,18-21</sup>

### **Plaque**

No significant relationships were found between AST1 or AST2 and the plaque index. These results agree with those of Kamma et al<sup>22</sup> and might be explained by the fact that one of the inclusion criteria was a plaque index <2. In contrast, several authors have reported relationships between AST levels in the CF and the presence of specific microbiota.<sup>5,20,21,23-26</sup> However, it is difficult to draw conclusions about the relationships between specific pathogens and potentially destructive processes in specific sites at the time of sampling.

### **Probing**

We did not detect significant relationships between probing depth and AST1 or AST2. This finding was expected because one of the inclusion criteria was a probing depth ≤3 mm at any site around the fixture. We also did not detect significant relationships between mobility, as measured with the electronic device, and AST1 or AST2. This finding was also expected because another inclusion criterion was a lack of implant mobility.

In this study, we found significant differences between AST1 and AST2, with more implants showing positive values for AST2 than for AST1. There is well-known dramatic fluid production after a GCF strip is inserted, particularly after inserting a probe, and AST levels in this fluid are increased relative to baseline. The only potential explanation for the increase in AST is damage caused by probing the peri-implant sulcus in patients with mucositis. In these cases, penetrating the junctional epithelium with the tip of the probe may be easier.

Most studies have demonstrated that probe penetration is influenced by marginal health status, probing force, and probe type/

shape. The influence of most of these factors on probing around implants is insufficiently elucidated, although the influence of marginal health status and probing force has been documented.<sup>27,28</sup> However, it is still unknown why probing around implants seems more painful than around teeth.<sup>28-30</sup> We consider a plausible explanation to be that mucositis decreases the pain threshold.

### **Gingival index**

There was a significant relationship between AST level at AST1 and the GI. These results agree with those of recent cross-sectional studies investigating AST levels around nonhealthy dental implants that found significantly higher AST levels around implants with increased probing depths and that bled upon probing.<sup>20,32</sup> Here, only implants with shallow crevices (<3 mm) were included, ruling out any peri-implantitis. Therefore, this finding could be explained by mucositis.

We encountered significant differences between implants and patients with respect to GI and bleeding upon probing. At the implant level, GI and bleeding upon probing were related to a positive AST1, but they were not connected at the level of the patient. This discrepancy can be explained by local hygienic conditions being more influential than implants. Therefore, implant position and personal hygienic care could play an important role, and when we considered patient as a unit, this particular situation may not be an important factor.

Many studies have shown that analyzing AST levels in the periodontal/peri-implant CF/PISF is useful for diagnosing unhealthy periodontal or peri-implant tissues; AST levels fall when periodontal health improves.<sup>4,33,34</sup> Nevertheless, Oringer et al<sup>4</sup> indicated that monitoring the progression of periodontal disease may be hindered by false-positive or -negative results, and that high AST levels are often found in tissues

that show no disease progression. Rühling et al<sup>12</sup> indicated that, unlike periodontitis, assessing AST in PISF might be of limited diagnostic and prognostic value for of peri-implant disease due to the weakness of peri-implant tissues.

However, Paolantonio et al<sup>3</sup> showed that AST levels are indicative of different peri-implant conditions according to well-defined clinical and radiographic criteria. The mean AST activity in healthy implants (HI) was  $0.26 \pm 0.16$  U/mL, in implants with mucositis  $0.38 \pm 0.27$  U/mL, and in implants affected by peri-implantitis  $0.62 \pm 0.29$  U/mL. Analysis of variance showed that the difference among HI, mucositis, and peri-implantitis was significant ( $P < .01$ ). Post hoc tests demonstrated that there was a significant difference in the AST activity between HI and peri-implantitis ( $t = 5.14$ ;  $P < .01$ ) and mucositis and peri-implantitis ( $t = 3.09$ ;  $P < .01$ ). No significant difference was found between HI and mucositis ( $t = 1.07$ ;  $P > .1$ ).

The results of the present study emphasize that probing measurements and bleeding upon probing in the evaluation of marginal tissues around implants are relevant only if the described influence of inflammation upon probe penetration is considered when the measurements are evaluated.

In this study, the sensitivity, specificity, and positive and negative predictive values of AST1 and AST2 were determined according to bleeding upon probing, even though bleeding upon probing might be a marker of inflammation or mucositis rather than tissue damage. On the whole, the determined values agreed with those of other studies.<sup>3,22</sup> Therefore, the AST test might be useful as an adjunct to traditional clinical parameters, although the validity of basing treatment decisions and implant prognosis on AST status cannot be determined from a cross-sectional study. In our opinion, the peri-implant probing depth does not confer an

increased risk of injury on implants and is a suitable measure for evaluating peri-implant health.

Our intention was not to determine whether AST levels around implants have any prognostic value for the long-term clinical success or failure of implants; we determined only the immediate effects of probing depth on implants with or without subclinical mucositis.

### CONCLUSIONS

Within the limits of this study, our results suggest that peri-implant crevicular fluid analysis can be further investigated in longitudinal studies as a suitable diagnostic strategy in the evolution of dental implants.

Peri-implant probing continues to be the simplest, quickest, and most economical method for assessing the state of the peri-implant mucosa, as long as probing forces less than 0.2 N are used. The detection of AST in the CF/PISF helps confirm clinical observations of periodontal or peri-implant disease. However, clinicians should use caution when applying AST levels for diagnosis because of the potential for false-positive results.

Probing does not trigger AST release at the threshold of 1200  $\mu$ IU in clinically healthy implants, demonstrating a lack of damage upon probing. However, implants that bleed on probing tend to release increased amounts of AST, which could be due to the ease with which inflamed peri-implant tissues are penetrated by the probe tip.

### ABBREVIATIONS

AST: aspartate aminotransferase  
 CF: crevicular fluid  
 CI: confidence interval  
 GI: gingival index  
 HI: healthy implant  
 NPV: negative predictive value

PISF: peri-implant sulcus fluid

PPV: positive predictive value

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