Longitudinal Evaluation of Aspartate Aminotransferase in the Crevicular Fluid of Implants with Bone Loss and Signs of Progressive Disease

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Aspartate aminotransferase (AST) has been shown to be a promising host marker for periodontal disease progression. The aim of the present study was to analyze AST in the crevicular fluid (CF) of implants exhibiting peri-implantitis and to evaluate the association between AST levels and progressive attachment loss. Twenty patients who had received a total of 42 endosseous cylindric titanium implants were examined. Radiographic assessment of preexisting bone loss and clinical measurements, including electronic attachment of probing, presence or absence of plaque, bleeding on probing, and AST analysis in CF, were performed on 2 occasions 6 months apart. During this study period 13 of 168 sites in 7 patients experienced further loss of attachment greater than or equal to 1.0 mm (median 1.7 mm; interquartile range 0.4 mm). Evaluation of a positive AST test (≥ 300 μIU) in site-specific diagnosis revealed low positive (8%) and high negative predictive values (92%), with a sensitivity of 15% and a specificity of 83%. These results indicate that, in contrast to periodontal disease, the assessment of AST in peri-implant crevicular fluid may be of limited value as a diagnostic and prognostic marker for peri-implant disease.
(Int J Oral Maxillofac Implants 1999;14:428–435)

Key words: aspartate aminotransferase, oral implant diagnosis, peri-implant crevicular fluid, peri-implantitis, peri-implant probing

Inflammatory reactions of peri-implant tissues with progressive loss of bone resulting from accumulation of bacterial deposits negatively affect the long-term prognosis of implant reconstruction.[1–5] It has also been stated that peri-implant bone loss may occur as a result of occlusal overload[6–10] and of prosthesis misfit.[11–13] Generally, it is accepted that after the first postsurgical year, an annual bone loss of less than 0.2 mm is acceptable.[14] Whereas peri-implant mucositis refers to reversible inflammatory reactions in the soft tissues without loss of attachment, peri-implantitis describes inflammatory reactions with loss of supporting bone.[14]

The early and reliable detection of progressive peri-implant attachment loss (disease activity) resulting from peri-implantitis is a prerequisite for treatment planning in diseased patients. However, traditional periodontal markers, ie, increased probing depths and the assessment of radiographic bone loss, are not suited for the detection of active disease or prediction of the progression of periodontitis or peri-implantitis because they allow only the documentation of the severity of the preexisting destruction. Bleeding on probing failed to be a useful marker of active disease in patients with periodontitis[15,16] and peri-implantitis,[17] whereas the absence of bleeding on probing was a useful clinical indicator of periodontal and peri-implant stability.[17,18]

There is a need for diagnostic tests, first to identify diseased sites of teeth and implants at an early and reversible stage, and second, to provide infor-

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mation regarding the risk of future ongoing disease.[19–21] Periodontal research has focused on the analysis of gingival crevicular fluid (CF) with the aim of identifying potential host markers and tissue breakdown products, which may permit identification of disease activity and allow a prognosis of future disease. This approach is based upon the concept that the metabolic or biochemical lesion precedes the clinical lesion, and identification of these early changes can identify the risk for disease progression prior to its occurrence.[22]

A number of markers, such as neutrophil-derived ß-glucuronidase or neutrophil elastase,[23,24] prostaglandin E_2,[25] and aspartate aminotransferase,[26] have been shown to indicate the risk of future active periodontal disease. Similar associations have not yet been reported in peri-implantitis. Aspartate aminotransferase (AST) is a cytoplasmatic enzyme, and its extracellular presence is an indicator of cell necrosis. Currently, it is one of the most promising host markers for periodontal disease, as demonstrated in periodontally derived cells[27] and in longitudinal studies in animals[28,29] and in humans.[26,30–35] However, no longitudinal studies have reported on the role of AST in the crevicular fluid of peri-implant tissues.

The aim of the present study was to analyze AST in the crevicular fluid of implants exhibiting mucositis and peri-implantitis, and to evaluate the association between AST levels and progressive peri-implant attachment loss.

Materials and Methods

Patients and Measurements. Twenty edentulous patients (age 69 ± 8 yrs) who had received a total of 42 endosseous cylindric titanium implants (IMZ, Friatec, Mannheim, Germany) in the interforaminal region were examined twice in a 6-month interval. The study protocol was approved by the Institutional Review Board. In 19 patients, a Dolder-type bar had been connected to 2 implants to support an overdenture; in 1 patient a similar bar had been connected to 4 implants. Implants had been loaded for 53 ± 13 months.

At the first examination, the following parameters were assessed at 4 sites around each implant:

1. Presence or absence of plaque (PL)
2. Aspartate aminotransferase (AST)
3. Probing depth (PD)
4. Probing attachment level (AL)
5. Bleeding on probing (BOP)

The percentage of radiographically existing bone loss was determined by an orthopantomogram on the mesial and distal aspect of each implant in relation to the total length of the implant cylinder.[36] All clinical measurements and the AST analysis were repeated at a second examination 6 months later.

Aspartate Aminotransferase Analysis in Peri-implant Crevicular Fluid. Following the assessment of presence or absence of plaque, test sites were dried and isolated and crevicular fluid samples were collected with absorbent paper strips (Xytronyx, San Diego, CA). Strips were placed at the orifice of the sulcus until mild resistance was detected and were left for 30 s beyond the detection of resistance. Samples were collected in 100 µL phosphate-buffered saline and were analyzed immediately after collection by a spectrophotometer (Analyzer 717, Hitachi, Tokyo, Japan) based on the method of Bergemeyer et al.[37] Precision controls were performed with triplicate samples of 800 µIU AST test serum (Precinorm, Boehringer, Germany) and 800 µIU test strips from a test kit (PerioGuard, Colgate, New York, NY). Samples were collected and sent immediately to the clinical laboratory. Examiners were unaware of the results at the time of examination.

Peri-implant Probing. Following crevicular fluid sampling, the Dolder bars were removed and probing measurements were performed by means of a controlled force electronic probe (PeriProbe, PD International, Malmö, Sweden). Modified probe inserts were used to reduce probing forces because peri-implant probing was painful.[17,36] The probing forces of the system varied from 0.35 N in 1 mm to 0.15 N in 10 mm pockets. Care was taken to introduce the probe parallel to the long axis of the implants. All measurements of probing depths were performed first and without removing the tip of the probe from the pocket; this was followed by measurements of the attachment level, with the top of the abutment serving as a fixed reference point. All measurements were performed in duplicate by the same examiner, who because of the nature of the probe, was unaware of the first recordings. The absence or presence of bleeding was assessed within 15 seconds of peri-implant probing.

Statistical Analysis. Analysis of duplicate baseline probing data revealed a high degree of reproducibility (r = .95), with a mean difference between 2 subsequent attachment level recordings of 0.1 mm and a standard deviation of 0.3 mm. Therefore, a minimum threshold of 1.0 mm (> 3 × SD) change in attachment during the following 6-month observation period was chosen to classify a site as experiencing gain or loss of attachment.

Table 1 Median and Interquartile Range of AST Level, Probing Depth, and Attachment Level in 20 Patients

	Attachment loss*		Stable attachment†		Attachment gain‡	
Test	Baseline	6 months	Baseline	6 months	Baseline	6 months
AST (μIU)	150/80	100/130	160/100	150/180	280/280	169/250
Probing depth (mm)	3.9/1.0	4.9/1.3	3.7/0.7	3.6/0.8	5.7/2.2	3.9/2.4
AST positive (% of sites§)	0/38	0/22	10/50	20/30	0/65	75/85
Attachment level (mm)	3.7/2.6	6.2/1.8	4.7/1.4	4.7/1.4	6.3/0.9	4.9/1.4
Difference in attachment level (mm)	–1.7/0.4		0.05/0.4		1.1/0.6	

There were no significant differences between the baseline and 6-month measurements or within the 3 probing attachment groups for any variable.
*n = 7 patients/13 sites; †n = 20 patients/144 sites; ‡n = 8 patients/11 sites; §AST ≥ 300 μIU.

In addition, another method for identification of attachment loss was employed.[38] The lower of the two 6-month recordings had to differ from the highest initial recording by more than 1 mm to qualify a site as having experienced attachment loss (gap method).

Since most data were not normally distributed, nonparametric statistical tests were performed. To perform a regression analysis on a patient basis, which allows change of attachment as a continuous variable, medians for AST baseline values and change of attachment were calculated. To analyze changes in AST levels, in peri-implant probing depths, and in attachment levels during the 6-month period, the data were subjected to analysis by the Wilcoxon signed rank test. The *P* values were adjusted according to Bonferroni, because 3 tests were performed on the same patient material. Differences between the 3 groups of attachment change were analyzed using the Friedman test. Since this analysis masks the site level, a patient-based but trichotomized analysis was performed at the same time. Based on the attachment change (Δ AT) during 6 months, the following 3 categories of sites were established: sites with loss in attachment (Δ AT ≤ –1.0 mm), sites with stable attachment (–1.0 < Δ AT < 1.0 mm), and sites with gain in attachment (Δ AT ≥ 1.0 mm). For each patient, medians based on this categorization were calculated.

Finally, the sensitivity, specificity, and positive and negative predictive values of the AST test (≥ 300 μIU) were calculated using contingency tables. The data were statistically analyzed, both on the level of the site and on the level of the implant experiencing changes in probing attachment. The worst site was chosen for classifying an implant as presenting with stable attachment or loss or gain of attachment. This approach was considered meaningful, since treatment is rendered on a site or implant basis but not on a patient basis.

Results

The radiographic examination revealed a mean peri-implant bone loss of 30% (range of 10 to 65%) related to the length of the implant. A relatively high number of sites harbored plaque (51%) and exhibited bleeding on probing (50%). Probing depths varied between 1 and 9 mm, with a mean of 3.9 mm. Approximately one third of the sites had probing depths greater than 4 mm. Nineteen percent of the implant sites displayed AST activities of 300 μIU or more, with a mean of 160 μIU and a range between 0 and 1200 μIU. To perform a regression analysis on a patient basis, which allows AT as a continuous variable, medians for AST baseline values and change in attachment were calculated. The Spearman rank correlation coefficient was 0.22, which was not significant. Since this analysis masks the site level, a patient-based but trichotomized analysis was performed at the same time, allowing separate consideration at the median of the sites that presented stability or loss or gain of attachment.

Thirteen of 168 sites in 7 patients experienced attachment loss during the 6-month observation period, with a mean loss of 1.7 mm (Table 1). Eleven sites in 8 patients presented with a gain in attachment (median 1.1 mm). At the first examination, sites with a subsequent gain in attachment demonstrated deeper pockets and higher AST activities than sites with subsequent loss of attachment (Table 1). Six months later, a higher percentage of sites that had experienced attachment gain showed an AST positive test result, compared to sites with attachment loss. Even though sites with subsequent attachment gain were characterized by high attachment level values, as compared to sites with subsequent attachment loss, these differences were not found to be statistically significant. Attachment gain was accompanied by a reduction in probing depths (Table 1).

Table 2 Evaluation of the Diagnostic Tests for Plaque, Bleeding on Probing, and AST ≥ 300 µIU at the Initial Examination for Identification of Progressive Peri-implant Attachment Loss

	Initial plaque		Initial bleeding on probing		Initial AST test positive	
Test	Site	Implant	Site	Implant	Site	Implant
Sensitivity	31	70	54	70	15	70
Specificity	47	18	58	15	83	52
Positive predictive value	5	21	9	20	8	30
Negative predictive value	88	67	94	63	92	85

Calculations were performed on a site-specific and implant-specific basis.

Table 2 presents the results of site-specific and implant-specific evaluation of the diagnostic tests for plaque presence, bleeding on probing, and AST. A positive AST test result possessed a low positive (8%) and a high negative predictive value (92%). The sensitivity to identification of sites with progressive loss of attachment with a positive test result was low (15%). The sensitivity to identification of nonprogressive sites with a negative test result was higher (83%).

Statistical calculation based on the gap method identified a higher number of sites (n = 24) with loss of attachment; however, differences in specificity and sensitivity of the AST test were small (data not shown).

Figure 1 demonstrates the variability of the AST test (≥ 300 µIU). In sites with initial probing depths of 3 to 6 mm (n = 108) and stable attachment (n = 93), a high number of positive AST test results were obtained (n = 79), whereas in sites with a loss in attachment (n = 10), most of the test results (n = 9) were negative.

Discussion

At present no longitudinal studies have investigated aspartate aminotransferase in the peri-implant sulcus. In a previous cross-sectional study, elevated AST levels were detected in patients with peri-implantitis by means of a diagnostic test kit.[39] Recently, Fiorellini et al[40] found significantly higher AST levels around implants with increased probing depths and bleeding on probing.

The aim of the present longitudinal study was to identify aspartate aminotransferase in the peri-implant crevicular fluid and to analyze the association between AST levels and peri-implant disease progression. A high number of patients with evidence of moderate to advanced preexisting peri-implant bone loss was included. By these inclusion criteria, it was anticipated that it would be possible to identify a relatively high portion of sites with ongoing loss of attachment.

Peri-implant attachment probing was used as the gold standard for identifying changes in attachment level. Electronic probes have been recommended for implant evaluation.[14,21,41] They allow measurements with high reproducibility,[36,42,43] and in the present study, the degree of reproducibility (r = .95) for duplicate attachment probings was superior to that found around natural teeth.[44] This finding could be explained by the ideal reference point for the sleeve of the PeriProbe tip on the top of the abutment and the cylindric, unthreaded form of the IMZ implant, which led to fewer probing errors.[17] The difference between 2 subsequent recordings was 0.1 mm ± 0.3 mm. Because of this error of the probe, a minimum threshold of 3 standard deviations of the mean difference of duplicate measurements was chosen to identify attachment changes. However, sites with attachment loss of less than 1 mm were not represented. This could have led to a number of apparently false positive tests for sites that undergo attachment loss greater than 1 mm (Fig 1).

In the present study, 13 (7.7%) of 168 sites displayed progressive loss of attachment, with a mean of –1.7 mm. The number of sites with ongoing disease appeared to be low. However, 7 of 20 patients were affected. This concurs with earlier findings[17] in the same study population, but in a different 6-month interval, in which 13 of 216 sites (6%) with progressive loss of attachment sites were identified in 7 of 28 patients.

At present, there is no consensus regarding the rate of progression of peri-implant destruction. It is not known whether destruction progresses continuously or in bursts and whether remissions are possible. A peri-implant attachment loss of 1 mm

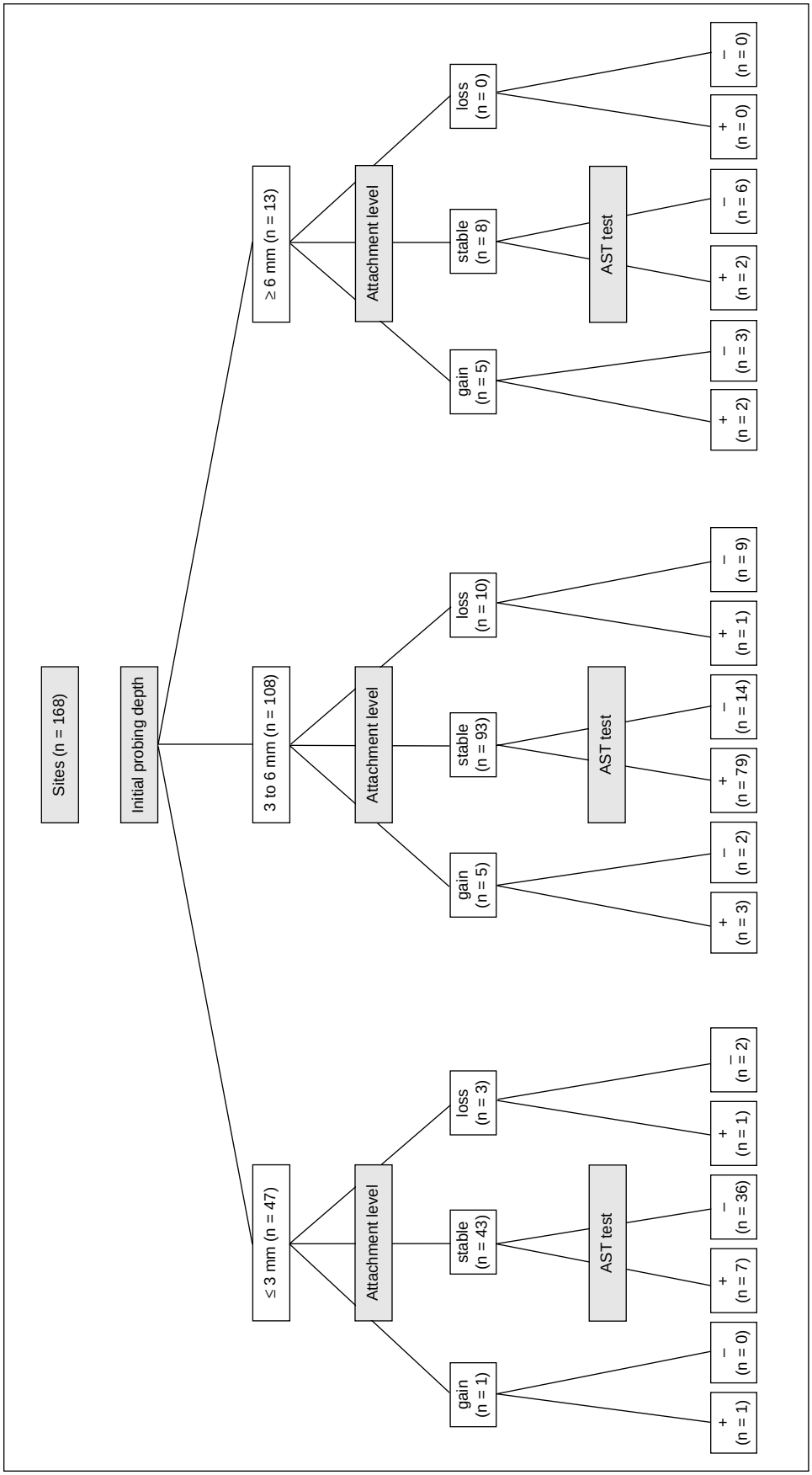

Fig 1 Diagram of features for 168 sites. Sites were divided according to initial probing depths and then subdivided into sites with gain, loss, or stable attachment. Below are the positive (≥ 300 µIU) and negative AST test results for each subset of sites.

or more within the short observation period of 6 months noted in this study should be regarded as critical, especially since an annual vertical bone loss of less than 0.2 mm following the implant's first year of loading is considered acceptable.[14] Nevertheless, it cannot be ruled out that the 6-month period of study may have taken place during an inactive period. In this case, the incidence of sites with attachment loss would have been underestimated.

No relationship could be found between elevated levels of AST and progressive loss of attachment. On a patient basis, the Spearman rank correlation coefficient between change in attachment and AST levels at baseline was only 0.22. In the peri-implant sulcus, AST with a mean of 160 µIU and individual peak values up to 1200 µIU was measured at initial examination. However, no differences were detected between sites that remained stable and sites that experienced further loss of attachment (Table 1). Persson et al[33] examined 25 patients with periodontitis over 2 years. Fifteen sites displayed probing attachment loss of at least 2 mm. Median values for AST were greater for these active sites than for inactive sites. In sites with loss of attachment, AST activity was elevated on average by 725 µIU over inactive sites. Sites with severe gingival inflammation were associated with AST levels that were 600 µIU higher than those at sites with mild or no gingival inflammation. They concluded that a diagnostic test based on AST measurement might be possible. Considering the fact that most of the patients in the present investigation had already experienced moderate to advanced peri-implant destruction with present signs of inflammation, one would have expected higher AST levels.

Persson and Page[26] investigated the diagnostic and predictive characteristics of different threshold AST values for the detection of different patterns of periodontal disease. They calculated sensitivity, specificity, and the odds ratio for AST threshold levels at ≥ 600, ≥ 800, ≥ 1,000, and ≥ 1,200 µIU for each disease category and concluded that AST activities of 800 µIU or higher appeared to be associated with active periodontitis. This level is approximately 20 times greater than levels found in blood serum of periodontally healthy subjects and is 5 times higher than levels found in erythrocytes.[45] At present, there are no reports of AST thresholds to recognize peri-implant mucositis or peri-implantitis.

In the present study, statistical calculations were performed with AST thresholds increasing from 200 µIU up to 800 µIU without significant results. The variability of the 300 µIU AST test outcome is demonstrated in Fig 1.

The positive predictive value of a 300 µIU AST test (8%), as well as the respective values for bleeding on probing (9%) and plaque (5%), were not suited for the prediction of peri-implant attachment loss. However, the negative predictive value of the AST test for future attachment loss was 92%, utilizing the site as the unit of measure, and 90% in implant-specific analysis.

In patients with periodontitis, the 800-µIU AST threshold demonstrated a relatively high sensitivity of 93%, a specificity of 68%, and an odds ratio of 15.4 for attachment loss.[27] By contrast, in the present study, in patients with peri-implantitis, the sensitivity of an AST test (≥ 300 µIU) was very low (15% for site, 70% for implant), with a specificity of 83% (site) and 52% (implant). As recently suggested for periodontitis,[46] risk estimation and management should be performed at multiple levels: at the individual site, implant, and patient level.[47] However, the data cannot be accurately estimated on a per-patient basis with a total sample size of 20 patients. Therefore, in this study the data analysis was limited to sites and implants. In an earlier study evaluating a host response test for the prediction of peri-implant destruction on a site and implant basis, similar values were reported for sensitivity and specificity.[17]

Interestingly, 11 sites in 8 patients displayed a gain in attachment. There is clinical and histologic evidence that implant probing is closely related to bone height,[21,36,41,42,48] but in inflamed tissues the probe tip penetrates the base of the pocket.[41] Apparent probing attachment gain may therefore also result from reduced inflammation following therapy. In this investigation the suprastructure and abutments were removed, cleaned, and rescrewed, which could have had therapeutic effects. Thus, the significantly elevated AST levels in sites with a subsequent gain in attachment may reflect a higher degree of inflammation. The resolution of inflammation following therapy could have led to reduced probings and apparent clinical attachment gain. In fact, similar observations for AST were made following nonsurgical periodontal therapy.[49]

Conclusion

In the present study no relationship was found between AST levels and progressive peri-implant attachment loss. These results indicate that, in contrast to periodontal disease, the assessment of aspartate aminotransferase in crevicular fluid may be of limited value as a prognostic marker for peri-implant disease.

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