Need for Procedural Details in Detection of Periodontopathic Bacterial DNA in the Atheromatous Plaque by PCR

We read with interest the recent paper published by Ishihara and colleagues (1) in which the authors detected the presence of DNA from periodontopathic bacteria in stenotic artery plaques and in samples obtained from periodontal pockets. This group has already published a report describing the detection of Treponema denticola in atherosclerotic lesions by using both PCR and polyclonal serum-based immunofluorescence assay techniques (2).

We agree with the conclusion of Ishihara et al. that these results, taken together with the large amount of already-published epidemiological, microbiological, and clinical data, give support to the hypothesis that periodontopathic bacteria could, in some way, play a role in the pathogenesis of the atheromatous plaque. Anyway, we feel that some additional details could add value to the data presented in this paper.

At first, the title of the paper refers to “carotid coronary artery plaque,” whereas in the text the authors wrote that “...we sought to detect...DNA from stenotic coronary artery plaques...” and no mention is made anywhere to vascular samples obtained from the carotids. This is in our opinion a point that need to be clarified, since the presence of bacteria could be different in the two vascular segments, and if no specimens from the carotids were studied, there is no reason to include this word in the title of the paper. In addition, in case the authors have been using only vascular fragments obtained from the coronary artery, it could be very useful to describe which segment of these arteries was studied and what have been both the clinical diagnosis and surgical procedures for the patients studied, since it is quite uncommon for the stenotic segment of the coronary artery to be removed during a bypass procedure with a vascular graft. Moreover, the addition of some details about the well-known risk factors for atherosclerosis, like cholesterol plasmatic level and smoking, for the patients that gave the vascular fragments used could increase the significance of these results. Another point that in our opinion needs to be clarified is the real significance of the negative controls used in this study. In fact, 2 clinically unrelated vascular samples are quite a disproportionate number when correlated to the 51 specimens obtained from the atherosclerotic patients. In addition, one of these two negative control vascular samples gave a positive result for the presence of Actinobacillus actinomycetemcomitans and for Campylobacter rectus, with the results of the dental examination of these two patients being totally negative and with, as far as we know, the etiology of the Kawasaki syndrome not being strictly related to any bacterial infection. These findings, which demonstrated the presence in the coronary artery of DNA from periodontopathic bacteria in a nonatherosclerotic and periodontally healthy patient, could suggest that these microorganisms are unrelated to the presence of atheromatous plaque. A last point that should benefit from additional details is the statistical analysis performed to assess the correlation between the rates of detection for DNA in periodontal and vascular specimens. In fact, it is quite difficult to understand why the correlation was statistically significant only in the case of detection of Porphyromonas gingivalis and C. rectus DNA and not for other bacteria, such as, for instance, T. denticola, which was detected in a very high percentage of samples (67.7 and 29.4% in subgingival and artery specimens, respectively, in patients showing four or more periodontal lesions). Further clarification of the above points would allow the reader to consider more deeply the results and conclusions of this interesting study, which could improve knowledge of the various factors that may have a role in atherogenesis.

REFERENCES

Authors’ Reply
In the comment, Sambri and colleagues pointed out that no mention is made anywhere to vascular samples obtained from the carotids. In our experiment, the specimens were taken from the coronary artery wall at the time of bypass grafting (1). We isolated the segment upstream from the coronary artery lesion during the bypass procedure with a vascular graft. We had not always prepared “atheromatous plaque” for each sample. Most of the samples were obviously degenerated, but they sometimes looked quite normal. However, these samples usually contained diffuse atherosclerosis lesions from stenotic artery plaque even when appearing normal. Furthermore, the sampled coronary vascular surface is too small to evaluate both the existence of microorganisms and the histological conditions. In this experiment, we used all of the specimens for...
detection of microorganisms because we intended to clarify the relationship between the existence of oral pathogens in periodontal pockets and stenotic plaque. In this context, the phrase “carotid coronary stenotic artery plaque” is correct.

We examined total cholesterol levels in plasma from 35 out of 51 patients and found that 6 patients’ levels were higher than the basal range for healthy adults. We also examined the smoking experience of 30 of 51 patients in the study. Present smokers (22.9%) belonged to the patient group possessing four or more periodontal pockets. The ratio of smoker and past smoker in patients with fewer than four periodontal pockets and for those harboring four or more periodontal pockets was 57.7 and 44.4%, respectively. The plasma cholesterol level was 196.2 ± 36.18 mg/dl. This value is in the range for normal patients. The levels for patients with fewer than four periodontal pockets and for those harboring four or more periodontal pockets were 194.6 ± 39.35 and 200.5 ± 26.79 mg/dl, respectively. No statistically significant difference was observed between the two groups. This indicated that the increase in the detection rate for periodontal pathogens in stenotic lesions is associated with the extent of periodontal lesions.

One of the comments questioned the significance of the control samples. It is true that we have to use a relatively large number of control samples, but samples from healthy sites cannot be obtained because of ethical considerations. In our experiment, we intended to compare the detection levels for periodontopathic bacteria from the coronary artery in patients with different periodontal conditions. We included the Kawasaki syndrome patients to show the low detection rate for periodontopathic bacteria as additional preliminary information because Kawasaki syndrome is not directly related to any bacterial species, and the patients usually do not suffer from periodontitis. We did not treat the data as an additional negative control. *A. actinomycetemcomitans* and *C. rectus* were detected from one patient, but neither was detected from the other patient. The number of bacterial species detected for Kawasaki syndrome patients was apparently low compared with that for the 51 stenosis patients. To verify the detection rates, we intended to obtain samples from patients with Kawasaki disease, but we could not utilize more patients in our experiment. Further analysis, increasing the sample size from patients with Kawasaki disease, is obviously required.

The results indicated that only *P. gingivalis* and *C. rectus* were related to detection in the oral cavity and coronary artery samples. Other microorganisms, such as *T. denticola*, were not correlated with detection in both the oral cavity and coronary artery. *T. denticola* is also detected in high numbers in stenotic lesions. The microflora of periodontal lesions is not the same in each lesion. In this experiment, we sampled two sites/patient but not from all periodontal lesions. It is possible that an individual sampled periodontal pocket does not harbor a specific microorganism. Increasing the number of sampling sites will be required to clarify these issues.

**REFERENCE**