

ORIGINAL ARTICLE

Oral bacterial DNA findings in pericardial fluid

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Background: We recently reported that large amounts of oral bacterial DNA can be found in thrombus aspirates of myocardial infarction patients. Some case reports describe bacterial findings in pericardial fluid, mostly done with conventional culturing and a few with PCR; in purulent pericarditis, nevertheless, bacterial PCR has not been used as a diagnostic method before.

Objective: To find out whether bacterial DNA can be measured in the pericardial fluid and if it correlates with pathologic–anatomic findings linked to cardiovascular diseases.

Methods: Twenty-two pericardial aspirates were collected aseptically prior to forensic autopsy at Tampere University Hospital during 2009–2010. Of the autopsies, 10 (45.5%) were free of coronary artery disease (CAD), 7 (31.8%) had mild and 5 (22.7%) had severe CAD. Bacterial DNA amounts were determined using real-time quantitative PCR with specific primers and probes for all bacterial strains associated with endodontic disease (*Streptococcus mitis* group, *Streptococcus anginosus* group, *Staphylococcus aureus*/*Staphylococcus epidermidis*, *Prevotella intermedia*, *Parvimonas micra*) and periodontal disease (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, *Fusobacterium nucleatus*, and *Dialister pneumosintes*).

Results: Of 22 cases, 14 (63.6%) were positive for endodontic and 8 (36.4%) for periodontal-disease-associated bacteria. Only one case was positive for bacterial culturing. There was a statistically significant association between the relative amount of bacterial DNA in the pericardial fluid and the severity of CAD ($p = 0.035$).

Conclusions: Oral bacterial DNA was detectable in pericardial fluid and an association between the severity of CAD and the total amount of bacterial DNA in pericardial fluid was found, suggesting that this kind of measurement might be useful for clinical purposes.

Keywords: oral; bacterial DNA; coronary artery disease; pericardium

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The pericardium has a complex role in the normal performance of the heart and pericardial disease is an important cause of morbidity in patients with cardiovascular disease (1). In clinical practice, both pericarditis and myocarditis may coexist because they share common etiologic agents, although in most cases they remain idiopathic. Myopericarditis caused by special inflammatory agents is less frequently reported when cardiotropic viruses are thought to be substantively prevalent, and bacteria or fungi uncommon (2). The diagnosis of pericardial inflammation frequently remains clinically challenging. The true incidence and prevalence

of pericarditis are difficult to measure, and according to autopsy studies pericarditis may be frequently subclinical (3).

It is well known that there is an association between cardiovascular diseases and dental infections. Many studies have shown that oral bacterial DNA can be found in atherosclerotic plaques but the role of these bacteria is still under debate (4–6). Recently, we found bacterial DNA, mainly from oral streptococci, in thrombi aspirated from the coronary arteries of patients who had had an acute myocardial infarction and had undergone percutaneous coronary intervention (7).

[†]These two authors have contributed equally to this work.

Table 1. Characteristics of the autopsy cases ($n = 22$)

Age at death, y, mean \pm SD	52.9 \pm 15.1
BMI, kg/m ² , mean \pm SD	27.9 \pm 6.41
Post-mortem time, h, mean \pm SD	88.4 \pm 30.88
Heart weight, g, mean \pm SD	499 \pm 141
Coronary artery disease (CAD)	
No CAD, n (%)	10 (45.5%)
Mild CAD, n (%)	7 (31.8%)
Serious CAD, n (%)	5 (22.7%)
Cardiovascular death (CVD), n (%)	8 (36.4%)
Myofibrosis (MF)	
No MF, n (%)	16 (72.7%)
Mild/disseminated MF, n (%)	4 (18.2%)
Extensive MF, n (%)	2 (9.1%)
Unstable plaque of the coronary arteries, n (%)	2 (9.1%)
Calcification of the coronary arteries, n (%)	9 (40.9%)

The aim of this study was to investigate whether oral bacterial DNA can be detected in the pericardial fluid using real-time quantitative polymerase chain reaction (RT-qPCR) and if there is a relationship with certain pathologic–anatomical (PAD) parameters associated with cardiovascular diseases. To the best of our knowledge, there are no previously published studies regarding the amount of bacterial DNA in the pericardial fluid.

Material and methods

Study cases and sample collection

The study material was composed of 22 pericardial aspirations (1 ml) collected from the Department of Forensic Medicine at The University of Tampere during 2009–2010. The samples were collected aseptically as described earlier (8) and clinical characteristics, demonstrated in Table 1, were obtained from post-mortem and microscopic reports.

Table 2. Primers and probes used

Primer and probe	Sequence (5' \rightarrow 3')	Reference
<i>Streptococcus</i> sp. mainly <i>S. mitis</i> group ^a		(8)
Forward	CCAGCAGCCGCGGTAATA	
Reverse	CCTGCGCTCGCTTTACG	
Probe	ACGCTCGGGACCTACG	
<i>S. mitis</i> and <i>S. oralis</i>		(9)
Forward	GCCATTGAAGCGGTTACTTTG	
Reverse	CATCCGACATTAACGCAAGTTC	
Probe	ATGATTGAGCGTGGAACGGTGGGT	
Streptococcal virulence factor gftG/gtfP ^b		(7)
Forward	TTTACCATGGATGAGCTCAAGCAA	
Reverse	TTGGAGAGCATGAGAGCATG	
Probe	ACGCAGTTCAATATCC	
<i>S. anginosus</i> group ^c		(7)
Forward	CCGTAGTGTTTGCTAGGTGAAA	
Reverse	CCAGAGACGTAGCTGTTTCGT	
Probe	CCGTAACGATTTCTCG	
<i>Staphylococcus aureus</i> and <i>S. epidermidis</i>		(8)
Forward	GCGTTTTTCACGTGGAATATC	
Reverse	AATCCAAAACACAAAACAAAGACAAGGT	
Probe	ACGTGCCATATTAATTTAC	
<i>Chlamydophila</i> (née <i>Chlamydia</i>) <i>pneumoniae</i>		(10)
Forward	GATCCGCTGCTGCAAACCTATACT	
Reverse	GTGAACCACTCTGCATCGTGTA	
Probe	TAGGCCGGGTTAGGTCTATCTACGGCAGT	
<i>Treponema denticola</i>		(11)
Forward	CCGAATGTGCTCATTTACATAAAGGT	
Reverse	GATACCCATCGTTGCCTTGGT	
Probe	ATGGGCCCGCGTCCCATTAGC	
<i>Prevotella intermedia</i>		(11)
Forward	TCCACCGATGAATCTTTGGTC	
Reverse	ATCCAACCTTCCCTCCACTC	
Probe	CGTCAGATGCCATATGTGGACAACATCG	

Table 2 (Continued)

Primer and probe	Sequence (5' → 3')	Reference
<i>Fusobacterium nucleatum</i>		(7)
Forward	AGGGTGAACGGCCACAAG	
Reverse	TCTCGGTCCATTGTCCAATATTCC	
Probe	ACACGGCCCTTACTCC	
<i>Aggregatibacter</i> (née <i>Actinobacillus</i>) <i>actinomycetemcomitans</i>		(12)
Forward	CAAGTGTGATTAGGTAGTTGGTGG G	
Reverse	CCTTCTCATCACCGAAAGAA	
Probe	ATCGCTAGCTGGTCTGAGAGGATGGCC	
<i>Dialister pneumosintes</i>		(12)
Forward	GAGGGGTTTGCGACTGATTA	
Reverse	CCGTCAGAC TTT CGTCCATT	
Probe	CACCAAGCCGACGATCAGTAGCCG	
<i>Porphyromonas gingivalis</i>		(12)
Forward	TGCAACTTGCCTTACAGAGGG	
Reverse	ACTCGTATCGCCCGTTATTC	
Probe	CACGAGCTGACGACA[A/G]CCATGCA	
<i>Parvimonas micra</i>		(12)
Forward	AAACGACGATTAATACCACATGAGAC	
Reverse	ACTGCTGCCTCCCGTAGGA	
Probe	TCAAAGATTTATCGGTGTAAGAAGGGCTCGC	
Universal ^d		(12)
Forward	TGGAGCATGTGGTTTAATTCGA	
Reverse	TGCGGGACTTAACCCAACA	
Probe	CACGAGCTGACGACA[A/G]CCATGCA	

^aRecognition of *S. mitis*-group (*S. mitis*, *S. oralis*, *S. gordonii*, *S. sanguinis*, *S. pneumonia*), *S. salivarius*, *S. thermophilus*, uncultured streptococci, *Lactobacillus lactis*.

^bRecognition of virulence factor for *S. sanguinis* (gftP) and *S. gordonii* (gftG).

^cRecognition of *S. anginosus*-group (*S. anginosus*, *S. milleri*, *S. constellatus*, *S. intermedius*).

^dRecognition of total amount of bacterial DNA.

Bacterial analyses

Bacterial culturing from samples was performed in an accredited laboratory using standard protocols at the Department of Microbiology, Fimlab Laboratories Ltd, Pirkanmaa Hospital District, Tampere, Finland. Briefly, 10 µl aliquots of pericardial fluid were streaked on chocolate, 5% horse blood, MacConkey and fastidious anaerobe (FAA) agar plates, and into fastidious anaerobe broth (FAB) tubes. Plates were incubated at 35°C for 24–48 hours in aerobic (chocolate, blood, and MacConkey) and 48 hours in anaerobic (FAA) conditions. FAB tubes were incubated at 35°C for 10 days. Species identification was obtained by conventional identification methods (including gram staining, specific tests and API tests, and susceptibility testing) according to standard procedures. The amount of total bacterial DNA and the presence of candidate bacterial DNA for endodontic-disease-associated bacteria (*Streptococcus* sp. mainly *S. mitis* group, *S. mitis* & *S. oralis*, *S. sanguinis* & *S. gordonii*, *S. anginosus* group, *Staphylococcus aureus* & *Staphylococcus epidermidis*, *Parvimonas micra* and *Prevotella intermedia*)

and periodontal-disease-associated bacteria (*Porphyromonas gingivalis*, *Aggregatibacter* (née *Actinobacillus*) *actinomycetemcomitans*, *Fusobacterium nucleatum*, *Dialister pneumosintes*, and *Treponema denticola*) were determined using real-time quantitative PCR as earlier described (7, 8) (Table 2 and Supplementary file). Human DNA determined by the TaqMan RNase P Detection Reagents–kit (Applied Biosystems Ltd, Foster City, CA) were used as references and venous blood from a healthy person was used as a negative control. Total amounts of human and bacterial DNA were analyzed using a standard curve, for which a dilution series was made from both human and *Escherichia coli* DNA. One case found to be both culture and qPCR positive for streptococci was excluded from association analyses.

Ethics

The study was approved by the Ethics Committee of the Pirkanmaa Hospital District, Finland, and the National Supervisory Authority for Welfare and Health (VALVIRA), Finland.

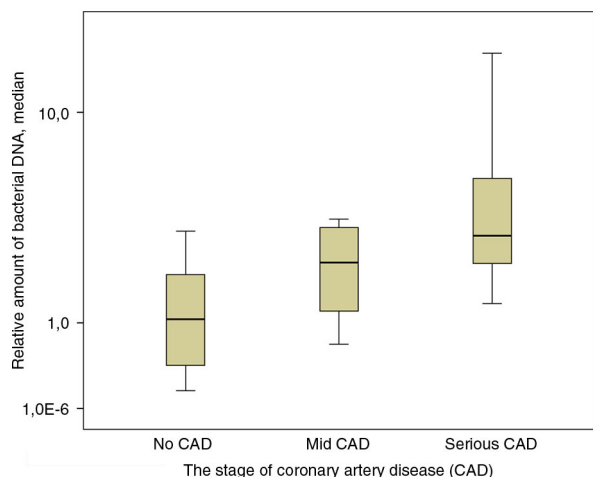


Fig. 1. Association between the stage of coronary artery disease and relative amount of bacterial DNA in pericardial fluid (post hoc ANOVA). The boxes indicate lower and upper quartiles and each central line is median. The upper end of each bar is maximum value and the lower end is minimum value.

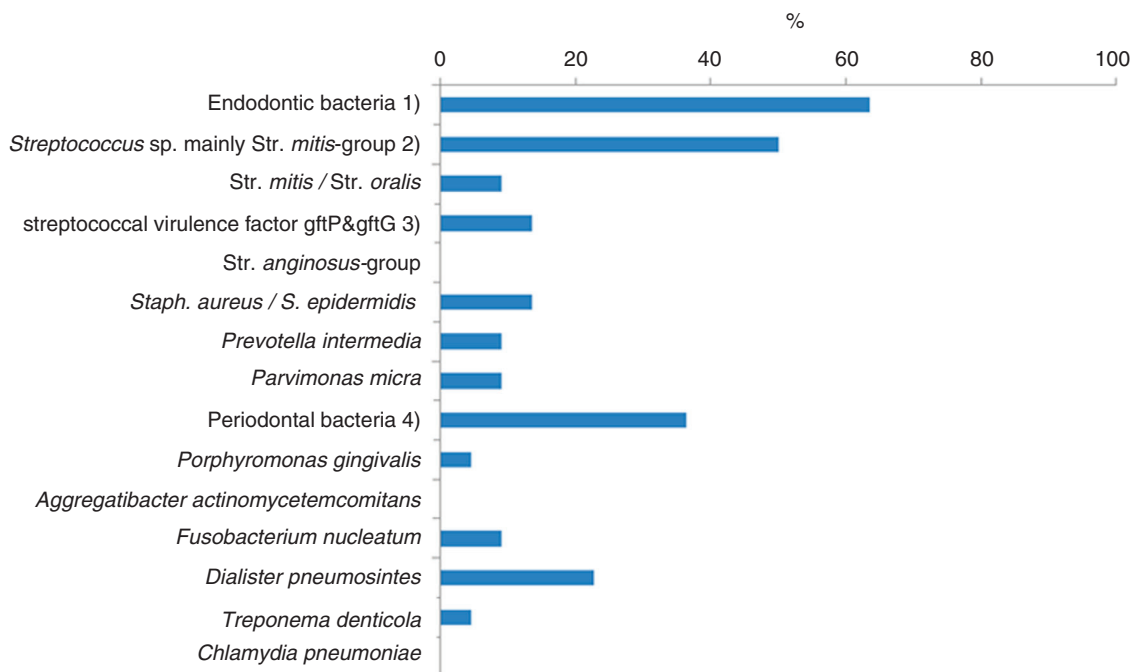
Statistical analyses

Frequencies of the variables were calculated and statistical analyses performed with PASW Statistical Software version 18 (SPSS Ltd, Quarry Bay, Hong Kong). Due to

the skewed distribution of the total amount of bacterial and human DNA, they were converted into logarithmic values. One-way ANOVA was used to calculate the associations between bacterial findings and clinical parameters.

Results and discussion

Figure 1 shows the relative amounts of total bacterial DNA measured in the pericardium compared to control peripheral blood, divided into three subgroups of coronary artery disease (CAD). There was a statistically significant association ($p = 0.035$, ANOVA) between the amount of bacterial DNA in the pericardial fluid and the stage of CAD. The standard curve shows that the median value for the total amount of bacterial DNA was highest in patients with serious disease (3.03 pg/ml, 25th–75th percentile, 1.83–5.11 pg/ml). There was no statistically significant relationship between other PAD parameters and the amount of bacterial DNA in pericardial samples. The samples with one or more measurements of bacterial DNA findings were determined as positive. Of the 22 cases, 14 (63.6%) were positive for endodontic and 8 (36.4%) for periodontal-disease-associated bacteria (Fig. 2). The most frequent bacterial DNA finding was from the *S. mitis* group and *Dialister pneumosintes*.



- 1) positive result from one or more measurements of *Streptococcus* sp. (mainly *Str. mitis*-group), *Str. mitis*, *Str. oralis*, gftP&gftG-streptococcal virulence factor, *Str. anginosus*-group, *Staphylococcus aureus*, *S. epidermidis*, *Parvimonas micra*, *Prevotella intermedia*
- 2) recognition of *Str. mitis*, *Str. oralis*, *Str. gordonii*, *Str. sanguinis*, *Str. pneumoniae*, *Str. salivarius*, *Str. thermophilus*, uncultured streptococci, *Lactobacillus lactis*
- 3) recognition of *Str. sanguinis* (gftP) and *Str. gordonii* (gftG)
- 4) recognition of *Str. anginosus*, *Str. intermedius*, and *Str. constellatus*
- 5) positive result from one or more measurements of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Dialister pneumosintes*, *Treponema denticola*

Fig. 2. Frequencies of bacterial-DNA-positive findings in pericardial fluid using specific primers and probes in RT-qPCR. Venous blood from a healthy subject was used as reference.

Bacteriological diagnosis is mainly based on conventional culturing. However, it is well known that some bacteria are poorly cultivable or non-cultivable, and inhibitory effects, like concurrent antibiotic therapy or the formation of a bacterial biofilm, may interfere with results (13). Many studies have already shown molecular methods to be dependable and practical in research use (7, 8, 13–15). Therefore, they have been proposed as an additional diagnostic tool, particularly when conventional bacterial culturing remains negative. By using different PCR methods, DNA from oral bacteria has been detected in atherosclerotic lesions (5, 16) and thrombus aspirates (7, 17). It is also known that oral bacteria such as streptococci have the capability to invade human heart endothelial cells *in vitro* (18). Similar concentrations of macromolecules have been measured in plasma as well as in pericardial fluid, suggesting active transport through the vascular wall (19). Since we have now found oral bacterial DNA in pericardial fluid, we suggest that in a similar manner, oral bacteria might be transported through the myocardium into the pericardial fluid.

This study material is limited and based on post-mortem samples. However, in our previous study we showed that the pericardial fluid is one of the most reliable post-mortem fluids for microbiological sampling (8). In terms of clinical application, more studies with antemortem material must be performed to evaluate the possible utility of pericardial fluid samples in patient care and diagnostics. Furthermore, the clinical use and relevance need to be debated after larger studies have been undertaken. Pericardial fluid aspirates are generally taken in cases where there is pericardial fluid effusion with an uncertain etiology. This is usually accompanied with endomyocardial biopsy (EMB), which highly increases the risk of complications (20). Nevertheless, analysis is usually restricted to histology, cytology, and conventional culturing (21). We believe that RT-qPCR for pericardial aspirates could be a sensible diagnostic tool and offer substantive additional value.

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Conflict of interest and funding

There is no conflict of interest in the present study for any of the authors.

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