Circulating Bacterial-Derived DNA Fragments and Markers of Inflammation in Chronic Hemodialysis Patients

Maurizio Bossola,* Maurizio Sanguinetti,† Donata Scribano,‡ Cecilia Zuppi,‡ Stefania Giungi,* Giovanna Luciani,* Riccardo Torelli,† Brunella Posteraro,† Giovanni Fadda,† and Luigi Tazza*

*Istituto di Clinica Chirurgica, Servizio Emodialisi; †Istituto di Microbiologia; and ‡Istituto di Biochimica, Università Cattolica del Sacro Cuore, Roma, Italia

Background and objectives: Bacterial-derived DNA fragments (BDNAs) have been shown to be present in dialysis fluid, to pass through dialyzer membranes, and to induce IL-6 (IL-6) in mononuclear cells. The present study aimed at assessing the eventual presence of BDNAs in the blood of hemodialysis (HD) patients and if this is associated with markers of chronic inflammation.

Design, setting, participants, & measurements: Fifty-eight HD patients and 30 controls were included in the study. A blood sample was collected from a peripheral vein and from the central venous catheter (CVC) or the arteriovenous fistula (AVF) and examined for presence of BDNAs by 16S rRNA gene PCR amplification, bacterial growth, and measurement of C-reactive protein and IL-6. Thirty minutes after the start of HD, a sample of dialysis fluid was collected before the entry into and at the exit of the dialyzer and examined for presence of BDNAs.

Results: Controls had negative blood cultures and absence of blood BDNAs. All HD patients had negative blood cultures, but in 12 (20.7%), BDNAs were present in the whole blood. In five of the latter, BDNAs were also found in the dialysis fluid. C-reactive protein serum levels (mg/L) were significantly higher in patients with than in those without BDNAs. Likewise, IL-6 serum levels (pg/ml) were significantly higher in patients with BDNAs than in those without.

Conclusions: Circulating BDNAs are associated with higher levels of C-reactive protein and IL-6 in HD patients.


Chronic inflammation is highly prevalent in end-stage renal disease patients receiving maintenance hemodialysis, with approximately 30% to 50% of them exhibiting evidence of an inflammatory response (1–2).

Inflammation in dialysis patients may be related to processes associated with renal failure itself such as oxidative stress, may be dialysis related, or may be attributable to infectious causes (1–4). Among the dialysis-related causes of chronic inflammation, exposure of blood to bioincompatible dialysis membranes seems to play an important role. Bioincompatible membranes, such as cellulosic membranes, activate white blood cells and complement (1–2). Other investigators have suggested that even dialysis with biocompatible membranes may pose risks for activation of the acute-phase response (1–2).

The quality of water used to prepare the dialysis fluid may also contribute to inflammation (3–4). Mounting evidence suggests that the use of less-than-sterile dialysis fluid or back-leakage of lipopolysaccharide through the dialysis membranes can cause dialysis-related inflammation (3–4). Several groups recently prepared ultrapure, endotoxin-free water by membrane filtration of the dialysis fluid and observed reduced levels of cytokines (3–4), which suggests either that monocytes may be activated by endotoxin that remains on the dialysis fluid side of the membrane or that endotoxin can directly cross the dialysis membrane.

Recently, Schindler et al. (5) demonstrated that short bacterial-derived DNA fragments are present in clinically used fluids such as dialysis fluid, and that these fragments are of sufficiently small size to pass through dialyzer membranes. DNA fragments are thought to be derived from microorganisms inhabiting hemodialysis water and fluid (6). Most of these microorganisms that include potential pathogens might subsist in a “viable but not culturable” state or may need specific culture media (7). In addition, it has been shown that short bacterial-derived DNA fragments are able to induce IL-6 in human mononuclear cells (5) and that in vitro they promote the survival of inflammatory cells from patients with chronic kidney diseases, suggesting that this action may contribute to perpetuate inflammation in these patients (8). On these bases, it has been suggested that bacterial DNA fragments may be an overlooked factor contributing to inflammation in hemodialysis patients (5,8). However, there is no evidence in patients receiving chronic hemodialysis that circulating bacterial-derived DNA fragments, when present, are associated with enhanced inflammatory response (9). This is an important issue, because elucidating the association between bacterial-derived DNA

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Correspondence: Dr. Maurizio Bossola, Istituto di Clinica Chirurgica, Università Cattolica del Sacro Cuore, Largo A. Gemelli, 8-00168 Roma, Italia. Phone: +39-06-30155485; Fax: +39-06-30155491; E-mail: maubosso@tin.it

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fragments and markers of inflammation may facilitate the development of effective treatment strategies for chronic inflammation in such patients. The primary end-point of the present study was to assess whether bacterial-derived DNA fragments are present in the blood of end-stage renal disease patients on maintenance hemodialysis and to determine whether this eventual presence is associated with markers of chronic inflammation.

Materials and Methods
All patients affected by ESRD who had been receiving chronic hemodialysis for at least 6 mo at the Hemodialysis Unit of the Università Cattolica del Sacro Cuore of Rome, Italy were considered eligible for this study. Exclusion criteria were as follows: acute (defined by the presence of fever and/or leukocytosis) or chronic (hepatitis B and/or C) infection, sepsis, AIDS, periodontal disease, antibiotic administration in the previous 2 wk, and previous renal transplantation. Thirty healthy individuals were used as controls. The study was approved by the local ethic committee, and written informed consent was obtained from all patients before enrollment in the study.

In all controls, a blood sample was collected under sterile conditions. In all HD patients, two blood samples were collected, one from a peripheral vein and one from the central venous catheter (CVC) or the arteriovenous fistula (AVF), before the start of the dialysis session. All patients were studied at the beginning of the week.

In addition, in all patients, 30 min after the start of the hemodialysis session, a sample of dialysis fluid was collected from the dialysis machine before the entry into the dialyzer and at the exit of the dialyzer. All samples were immediately analyzed in the Department of Microbiology.

Hemodialysis Procedure
Patients were maintained on regular hemodialysis prescription, three times a week, for 4 h per session. All patients were treated with bicarbonate hemodialysis with low-flux membranes. All patients used low-flux polysulfone membranes. Dialyzer reuse was not practiced throughout the study. The blood flow ranged from 250 to 300 ml/min, with a dialysis rate flow of 500 ml/min. Most patients were taking recombinant human erythropoietin, antihypertensive medications (β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors), and other commonly used drugs such as phosphate and potassium binders, as well as vitamin D supplements. None of patients were receiving antibiotics, corticosteroids, statins, or cytotoxic drug at the time of the study.

Baseline Clinical Data
Baseline clinical data, including age, gender, underlying renal disease, hemodialysis regimen, duration on dialysis, number and duration of hospitalization within 12 mo before recruitment, number and type of comorbidities, and body mass index (BMI), were recorded.

DNA Extraction from Whole Blood and Dialysis Fluid
DNA from 200 μl aliquot of EDTA-treated whole blood and dialysis fluid was extracted using the EZ1 DNA tissue kit and BioRobot EZ1 with the EZ1 bacteria card (Qiagen), according to the manufacturer’s instructions. The EZ1 DNA Bacteria Card is a preprogrammed card containing protocols for extraction and purification of genomic DNA from clinical samples, swabs, biopsies, or bacterial cultures, including both Gram-positive and Gram-negative bacteria. Briefly, samples were pretreated by adding 200 μl of lysozyme (50 mg/ml), incubated at 37°C for 10 min, and further processed using the BioRobot EZ1 instrument. Finally, purified DNA was eluted in 50 μl of elution buffer, after which it was suitable for direct PCR use.

16S rRNA Gene Amplification
Universal primers used for PCR amplification of the bacterial 16S rRNA gene were p16SrRNA+ and p16SrRNA− (8–9), which are able to amplify DNA from either Gram positive or Gram negative bacteria. Aliquots of 20-μl DNA samples were used for amplification in a 50-μl PCR reaction mixture containing (final concentration) 67 mM Tris HCl (pH 8.8), 16 mM (NH4)2SO4, 200 μM dNTPs, 3.5 mM MgCl2, 25 pmol of each primer, and 1 U Taq polymerase (GoTaq DNA polymerase, Promega, Madison, WI), by using the following thermal conditions: initial denaturation of 95°C for 5 min; then 35 cycles of 95°C for 45 s, 53°C for 1 min, and 72°C for 1 min and 30 s; and a final extension step of 72°C for 10 min. The PCR products were visualized after agarose gel electrophoresis and staining with ethidium bromide. All DNA samples were tested at least twice before reporting. To avoid contamination risk, sample preparation, PCR amplification and electrophoresis were performed in different rooms. In each assay, negative and positive controls were run. For all of the samples tested, amplification of the human β-globin gene was performed to evaluate sample degradation and/or presence of inhibitors.

For identification purposes, amplicons were purified with the MinElute PCR purification kit (Qiagen) and sequenced on both strands with primers p16SrRNA+ or p16SrRNA− and the Big Dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) on an ABI Prism 3100 genetic analyzer (Applied Biosystems). Species were identified by searching databases using the BLAST sequence analysis tool (http://www.ncbi.nlm.nih.gov/BLAST/). The isolate was assigned to a species if it had ≥ 99% sequence homology to a sequence entry available at the GenBank database, and if the next species showed less than 95% homology over the whole length of the sequence.

In addition, to evaluate the length of DNA fragments present in the positive samples, these samples were amplified using primers 907r, 1175r, and 1371r (10), which are specific to the 584-bp region out looking the 798-bp fragment amplified by p16SrRNA+ and p16SrRNA− primers within the 16s rDNA gene.

Blood Cultures
Ten milliliters of blood from each patient was obtained and immediately inoculated into aerobic and anaerobic BacT/Alert bottles (BioMérieux; Marcy l’Etoile, France), that were processed by the BacT/Alert automated system for 7 d, after which time samples were recorded as negative by the instrument.

Dialysis Fluid Analysis
The levels of endotoxin in the treated water of the dialysis fluid were assessed by the limulus amebocyte lysate (LAL) method (11). Microbiological examination of the dialysis fluids was performed according to procedures described previously (6).

Biochemical Analyses
High-sensitivity C-reactive protein (hs-CRP) was measured by means of nephelometry (Department of Clinical Chemistry, Policlinico Agostino Gemelli, Catholic University, Rome). The limits of detection for serum hs-CRP was 0.2 mg/L. Plasma IL 6 was measured by a commercially available photometric enzyme-linked immunosorbent assay (ELISA) (Boehringer Mannheim, Mannheim, Germany).
Statistical Analyses

Statistical analysis was performed by Graphpad Prism (GraphPad Software, Inc. San Diego, CA). All data were expressed as mean ± SD. t test for unpaired data and Mann-Whitney test for parametric and nonparametric analyses, respectively, were used as appropriated. A P value < 0.05 was considered statistically significant.

Results

Of the 73 prevalent patients present in our hemodialysis service, 58 who met enrollment criteria agreed to participate in this study. Four patients were excluded because of previous renal transplantation and 11 because of chronic infection (three with hepatitis B and eight with hepatitis C). Thirty healthy individuals used as controls had normal kidney function. Kidney function was assessed through the Cockroft-Gault equation.

Baseline laboratory and clinical characteristics of the 58 hemodialysis patients included in the study are shown in Table 1. As shown, values of the variables are typical of ESRD patients. Blood cultures for the 58 patients, as well as those for the 30 controls, were all negative for bacterial growth.

Whole Blood and Bacterial DNA

All controls were negative for the presence of bacterial DNA in the whole blood. Interestingly, 12 (20.7%) of 58 patients had bacterial DNA in their whole blood samples obtained either from the peripheral vein or from the CVC or the AVF. Baseline characteristics of the 58 patients included in the study, stratified according to the presence or absence of bacterial DNA in whole blood, are shown in Table 2.

In particular, positive PCR results were obtained for the whole blood from seven (15.9%) of 44 patients with AVF and from 5 (35.7%) of 14 patients with CVC (P = 0.22). Sequence analysis of the obtained 798-bp amplicons showed that the bacterial DNAs detected in the whole blood samples of the seven patients with AVF were derived from Escherichia coli (three samples), Staphylococcus aureus (one sample), Pseudomonas aeruginosa (one sample), S. epidermidis (one sample), and Enterococcus faecalis (one sample), whereas DNAs of E. coli (two samples), Proteus mirabilis (one sample), E. faecalis (one sample), and S. haemolyticus (one sample) were found in the whole blood samples of the patients with CVC. The length of bacterial DNA fragments present in the 12 PCR-positive blood samples was assessed using primers that were able to amplify a 584-bp 16S rDNA region downstream of the DNA fragment corresponding to the 798-bp amplicon. This length ranged from 798 bp (approximately 526 kD) to 926 bp (approximately 611 kD).

Dialysis Fluid and Bacterial DNA

In all patients, the LAL test was < 0.25 EU/ml, and results of the bacterial count from the dialysis fluid were negative. For all of the 46 patients with negative PCR results in their whole blood samples, no bacterial DNA was found in their dialysis fluid samples. For five out of 12 patients with positive PCR results in their whole blood, bacterial DNA was also found in dialysis fluid samples. Sequence analysis of these amplicons showed that the bacterial DNAs detected in dialysis fluid and in whole blood were from the same bacterial species.

Whole Blood Bacterial DNA and Chronic Inflammation

Mean CRP serum levels (mg/L) were significantly higher in patients with circulating bacterial DNA (10.64 ± 4.73) than in those without circulating bacterial DNA (5.30 ± 4.77) (P = 0.0027). Similar results were obtained when the patients were stratified and analyzed according to the type of venous access. In patients with AVF, CRP levels were 5.2 ± 4.4 and 10 ± 5.1 (P = 0.016) in the absence or presence of circulating bacterial-derived DNA fragments, respectively. In patients with CVC, these levels were 2.1 ± 0.3 and 12.1 ± 3.9 (P = 0.0035), respectively (Table 3).

Likewise, IL-6 serum levels (pg/ml) were significantly higher in patients with circulating bacterial DNA (14.9 ± 18.4) than in those without circulating bacterial DNA (3.7 ± 4.3) (P = 0.0035). According to the type of venous access, IL-6 amounts for patients with AVF were 2.5 ± 1.6 and 8.1 ± 7.5 (P = 0.0015) in the absence or presence of circulating bacterial-derived DNA, respectively; for patients with CVC, these amounts were 10.6 ± 8.9 and 26.8 ± 16.8 (P = 0.29), respectively (Table 3).

The mean number of white blood cells (ng/ml) was similar in patients with (6052 ± 1568) and without (6871 ± 1982) circulating bacterial-derived DNA (P = 0.23) (Table 4).

Discussion

The present study shows that one fifth of patients receiving chronic hemodialysis in our unit have bacterial-derived DNA
fragments in the whole blood and that this presence is statistically significantly associated with higher levels of both serum CRP and IL-6. Interestingly, the presence of bacterial-derived DNA fragments in the blood is more frequent in patients with CVC than in those with AVF, although the difference is not statistically significant. To our knowledge, this is the first study to report a statistically significant association between the presence of circulating bacterial-derived DNA fragments and higher levels of inflammatory markers such as CRP and IL-6 in hemodialysis patients.

The 16S rRNA gene PCR amplification is considered an optimal tool for detection and identification of bacterial isolates (5,7,15–19,20). A significant number of studies have demonstrated that amplification of fragments of the 16S rRNA gene is both sensitive and specific for detection of DNA from all of the well-known species of bacteria (7,15–19,20). In addition, the

### Table 2. Baseline characteristics of the 58 patients included in the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bacterial DNA negative (n = 46)</th>
<th>Bacterial DNA positive (n = 12)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>61 ± 13.8</td>
<td>64.3 ± 10.6</td>
<td>0.27</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>19 (41.3)</td>
<td>4 (33.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>Primary cause of ESRD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypertension</td>
<td>14 (30.4)</td>
<td>3 (25)</td>
<td></td>
</tr>
<tr>
<td>glomerulonephritis</td>
<td>8 (17.3)</td>
<td>2 (16.6)</td>
<td></td>
</tr>
<tr>
<td>diabetes</td>
<td>10 (21.7)</td>
<td>3 (25)</td>
<td></td>
</tr>
<tr>
<td>interstitial nephritis</td>
<td>8 (17.3)</td>
<td>3 (25)</td>
<td></td>
</tr>
<tr>
<td>polycystic renal disease</td>
<td>3 (6.5)</td>
<td>1 (8.4)</td>
<td></td>
</tr>
<tr>
<td>others/unknown</td>
<td>3 (6.5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Duration of dialysis, years</td>
<td>5.6 ± 5.2</td>
<td>4.0 ± 3.2</td>
<td>0.31</td>
</tr>
<tr>
<td>Vascular access</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>arteriovenous fistula</td>
<td>39 (84.7)</td>
<td>7 (58.3)</td>
<td></td>
</tr>
<tr>
<td>central venous catheter</td>
<td>7 (15.3)</td>
<td>5 (41.7)</td>
<td>0.10</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>10.6 ± 2.4</td>
<td>10.2 ± 1.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Urea, mg/dl</td>
<td>85.3 ± 13.4</td>
<td>86.2 ± 12.8</td>
<td>0.65</td>
</tr>
<tr>
<td>Daily urine volume, ml</td>
<td>145 ± 374</td>
<td>129 ± 202</td>
<td>0.88</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25 ± 4.2</td>
<td>23.5 ± 4.3</td>
<td>0.29</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>4 (8.6)</td>
<td>1 (8.3)</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease, n (%)</td>
<td>10 (21.7)</td>
<td>3 (25)</td>
<td>0.88</td>
</tr>
<tr>
<td>Congestive heart failure, mild, n (%)</td>
<td>2 (4.34)</td>
<td>1 (8.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>Infections in the previous 2 months, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>Positive blood culture in the previous 2 mos, n (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>Drugs with potential anti-inflammatory action assumed during the study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sevelamer</td>
<td>36 (78.2%)</td>
<td>9 (75)</td>
<td>0.88</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>18 (39.1)</td>
<td>5 (41.6%)</td>
<td>0.86</td>
</tr>
<tr>
<td>statins</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD.

### Table 3. C-reactive protein levels and IL-6 levels

<table>
<thead>
<tr>
<th>Group</th>
<th>C-reactive protein (mg/l)</th>
<th>IL-6 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacterial DNA negative</td>
<td>Bacterial DNA positive</td>
</tr>
<tr>
<td>Patients with AVF (n = 44)</td>
<td>5.2 ± 4.4</td>
<td>10 ± 5.1</td>
</tr>
<tr>
<td>Patients with CVC (n = 14)</td>
<td>2.1 ± 0.3</td>
<td>12.1 ± 3.9</td>
</tr>
<tr>
<td>Total (n = 58)</td>
<td>5.3 ± 4.7</td>
<td>10.6 ± 4.7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. AVF, arteriovenous fistula; CVC, central venous catheter.
method is reproducible and appears to be more accurate and sensitive than the phenotypic testing because it allows the detection of very small amounts of bacterial DNA regardless of microorganism viability, and it can work even when patients have been treated with antibiotics (7,15–19,20). 16S ribosomal DNA detection has been successfully used for diagnosis of CVC-associated bacterial infections (18), for the identification of bacteremia in surgical (13) and critically ill (17) patients, in patients at risk for infective endocarditis (19), and in the diagnosis of neonatal sepsis (16).

Bacterial-derived short DNA fragments, oligodeoxynucleotides of six to 20 nucleotides, are able to bind to Toll-like receptors and are stimulatory on immune cells (5). They induce natural killer cell activity and IFN-γ, TNF-α, and IL-6 release from mononuclear cells (5–6,20–22); they also display these same abilities when composed of five to six nucleotides (22).

Recently, the presence of bacterial-derived DNA fragments in the dialysis fluid and the passage through high-flux dialyzer membranes during sham hemodialysis have been clearly demonstrated (5). In addition, Navaro et al. have demonstrated that small fragments of bacterial DNA in vitro enhance cytokine production and promote the survival of inflammatory cells in patients with chronic kidney diseases, delaying apoptotic death of mononuclear cells, suggesting that this action may contribute to perpetuate inflammation in these patients (8). Taken together, these data and the results of the present study support the hypothesis that bacterial-derived DNA fragments may promote, at least in part, the inflammatory status commonly observed in chronic hemodialysis patients.

An other interesting finding of the present study is that because the blood samples were taken at the beginning of the week (thus after 2 d without dialysis) and before the start of dialysis, the sources of bacterial DNA more likely were endogenous, clinically silent foci present in HD patients. In addition, the percentage of patients with circulating bacterial DNA fragments tended to be higher in patients with CVCs than in patients with AVFs, although the difference was not statistically significant. This may be due to the small number of patients included in the study. However, these results may suggest that an alternative source of bacterial DNA may be the biofilm on the surface of the CVC. In the past few years, there has been growing recognition that a bacterial biofilm forms rapidly in the lumens of most indwelling CVCs, and this biofilm is the major source of catheter-related bacteremia (10,23–25). Treatment of catheter-related bacteremia with systemic antibiotics alone (without catheter removal) is relatively ineffective in eradicating the source of infection, presumably for the of persistence of the bacterial biofilm in the catheter lumen because systemic antibiotics achieve negligible antibiotic concentrations in the catheter lumen (26–29).

The observation that in all healthy controls we did not find bacterial DNA strongly exclude the possibility that in HD patients with bacterial DNA in their whole blood, the dialysis needles were a source of bacterial DNA.

A further interesting finding of the present study is that at least 540-bp long DNA fragments may traverse the dialyzer membrane from the patient compartment to the dialysis fluid in vivo. In fact, when we collected dialysis fluid samples from the dialysis machine 30 min after the start of dialysis, we found DNA fragments in five out of 12 patients with circulating DNA fragments, and the sequence analysis of amplicons showed that the bacterial DNAs detected in dialysis fluid and in whole blood were from the same bacterial species. Interestingly, this occurred despite the fact that the LAL test was <0.25 EU/ml and that results of the bacterial count were negative. It has been suggested that DNA fragments derive from microorganisms inhabiting hemodialysis water and that most of these microorganisms that include potential pathogens might exist in a “viable but not culturable” state or may need specific culture media (7).

The data on the presence and the significance of bacterial DNA in the whole blood of dialysis patients are limited. Cazzavillan et al. (9) documented DNA fragments in the blood of four out 38 hemodialysis patients but did not find a significant association between their presence and CRP and IL-6 levels. Interestingly, such lack of difference was evident in both patients with and without evident causes of inflammation. Serwanska-Swietek et al. (30) were able to detect the presence of a DNA fragment of approximately 790 bp in length in all of the whole blood samples randomly selected from a group of 50 hemodialysis patients without signs of active infection but did not correlate this presence with markers of inflammation. With regard to other diseases, Sleigh et al. have shown that 16S rDNA PCR resulted positive in 14.8% of critically ill patients with negative blood cultures and was approximately twice as sensitive as blood culture in detecting bacteremia in patients who previously received antibiotics (17). Similar results were obtained by Ray et al. in a group of 156 patients from an intensive care unit diagnosed as clinically septic and requiring blood cultures. Interestingly, of the 29 patients that had blood culture negative but PCR positive, 23 (79%) were classified as having septic shock, and five had severe sepsis (29). Jordan and Durso

Table 4. Blood white cell levels (ng/ml)

<table>
<thead>
<tr>
<th>Group</th>
<th>Bacterial DNA negative (n = 46)</th>
<th>Bacterial DNA positive (n = 12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with AVF</td>
<td>6726 ± 1921</td>
<td>5682 ± 1121</td>
<td>0.18</td>
</tr>
<tr>
<td>(n = 44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients with CVC</td>
<td>7753 ± 2278</td>
<td>6608 ± 2144</td>
<td>0.44</td>
</tr>
<tr>
<td>(n = 14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n = 58)</td>
<td>6871 ± 1982</td>
<td>6052 ± 1568</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. AVF, arteriovenous fistula; CVC, central venous catheter.
have shown that the real-time PCR can augment culture-based methods for diagnosis of neonatal sepsis, especially in infants whose mothers have received intrapartum antibiotic prophylaxis. Interestingly, in this study, all 32 culture-negative samples were negative for the 16S rDNA target (16).

Confirmation of the present study results by future studies may raise the question whether to manage bacterial DNA detected in the whole blood of hemodialysis patients. To the best of our knowledge, there are limited data on this issue (9,30). In general, the presence of bacterial DNA in the whole blood is considered an expression of bacteraemia and suggests that the patient be considered for antibiotic therapy (16–19,31). Whether this also applies to hemodialysis patients remains to be clarified.

The limitation of the present study is that, because of the extreme sensitivity of the PCR technique, any contamination occurring during collection of the blood specimen or in the laboratory processing would lead to false positive results. Indeed, in the present study, sample preparation, PCR amplification, and electrophoresis were performed in different rooms to avoid contamination risk. In addition, in each assay, negative and positive controls were run. We performed amplifications without adding DNA samples, and the results were negative. These data suggest that external DNA contamination did not occur. For all of the samples tested, amplification of the human β-globin gene was performed to evaluate sample degradation and/or presence of inhibitors.

In conclusion, the present study shows that circulating bacterial-derived DNA fragments are commonly present in the blood of hemodialysis patients and are associated with higher levels of inflammatory markers such as CRP and IL-6.

Disclosures
None.

References