Original Articles

Endoluminal colonization as a risk factor for coagulase-negative staphylococcal catheter-related bloodstream infections in haemodialysis patients

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Abstract

Background. Approximately 25% of haemodialysis (HD) patients use catheters as vascular access. Catheter-related bloodstream infections (CRBSI) are a major risk in this population. The objective of our study was to determine whether endoluminal catheter colonization (ECC) predicts CRBSI.

Methods. We followed up a cohort of HD patients in our institution who underwent HD with tunnelled cuffed central venous catheters (TCC) between December 2006 and June 2008. Colonization of the inner catheter lumen was assessed every 15 days immediately before HD by culture of blood–heparin mixture and the time to positivity (TTP) was recorded by the BacT/Alert automated system. CRBSI was confirmed by differential TTP (>2 h) between TCC and peripheral blood cultures.

Results. We studied 51 patients who required 64 TCC. The incidence of CRBSI was 1.65 episodes per 1000 catheter-days, with Staphylococcus epidermidis being the most common cause of infection (76.2%). ECC was more frequent in the CRBSI group than in the non-CRBSI group (100 vs 5.4%, P < 0.001). For S. epidermidis CRBSIs, the median time from ECC to CRBSI was 31.5 days (interquartile range, 27.0–79.0). The sensitivity, specificity and negative and positive predictive values of arterial lumen cultures for S. epidermidis CRBSIs were 100, 96.3, 92.3 and 100%, respectively, while for venous culture, these values were 92.3, 96.3, 92.3 and 96.3%, respectively. For predicting S. epidermidis CRBSI, endoluminal cultures with a TTP of ≤14 h had sensitivity and specificity of 52.1 and 97.7%, respectively.

Conclusions. This study shows that ECC may predict the risk of developing CRBSI. Surveillance cultures could, therefore, be used to triage individual HD patients who might benefit from specific intervention measures.

Keywords: bacteraemia; catheter colonization; catheter-related bloodstream infection; haemodialysis; Staphylococcus epidermidis

Introduction

Haemodialysis (HD) requires repeated reliable access to the systemic circulation, and arteriovenous fistulas or grafts are the preferred modes of vascular access. However, for a variety of reasons, ~25% of HD patients use catheters for this purpose and these patients are at highest risk of infection [1–4].

Among HD patients, infection is the most common cause of morbidity and the second most common cause of death [5,6], and bacteraemia accounts for more than 75% of deaths due to infection [7]. The incidence of catheter-related bloodstream infections (CRBSIs) in dialysis patients is reported to be 0.8–5.5 cases per 1000 catheter-days [8–16]. Gram-positive bacteria are responsible for most catheter-related infections and infection with coagulase-negative staphylococci (CoNS) and Staphylococcus aureus accounts for 40–81% of cases [8,17–20]. Although previously considered a mere saprophyte, in the last two decades, Staphylococcus epidermidis has emerged as an important nosocomial pathogen and the leading cause of foreign-body infection [21].

Bacterial colonization of the intraluminal surface of the catheter occurs in a high percentage of HD cases [3,22–24]. Endoluminal colonization is a step in the pathogenesis of CRBSI and precedes many symptoms of peripheral bacteraemia and sepsis [25,26]. The microorganisms adhere to the catheter material and form biofilms, allowing sustained infection and haematogenous dissemination [27].

Several studies of surveillance cultures of tunnelled cuffed catheter (TCC) exit sites in HD patients suggest that they are not useful in predicting subsequent catheter-related infections [28,29]. However, there is conflicting evidence regarding the frequency with which endoluminal colonization of TCC leads to bacteraemia [25,30].

Understanding the dynamics of endoluminal catheter colonization (ECC) in HD patients may be useful in pre-
dicting which patients are at risk of developing CRBSI and permit triage of those patients who could benefit from specific preventive strategies. We followed up the cohort of HD patients carrying TCC in our institution with the aim of determining whether regular microbiological surveillance of endoluminal colonization of these devices might be useful in predicting CRBSI. We also performed pulsed-field gel electrophoresis (PFGE) to ascertain if particular S. epidermidis clones were responsible for ECC and as well as their persistence on the inner catheter surface until the development of CRBSI.

Materials and methods

Patients, setting and data collection of the study population

This study was approved by the ethics committee of our institution and all patients gave informed consent. The experimentation guidelines of our institution were followed in the conduct of the clinical research. Between December 2006 and June 2008, we enrolled patients older than 18 years of age with end-stage renal disease who underwent HD with a dialysis catheter (TCC) at the Hospital Universitario 12 de Octubre in Madrid and in a dialysis centre affiliated to the hospital. Infection control measures during insertion and care for catheters were in accordance with written in-house guidelines in both units [31]. Cefazolin 1 g was given intravenously at the time of insertion. We included patients that were carrying a TCC at the initiation of the study and who had no previous catheter-related infections, as well as those who underwent insertion of TCC during the study period. Patients were excluded if the catheter remained in place < 1 month. In cases in which the catheter was removed, the patients were re-entered into the study if a new TCC was inserted. Patients exited the study when their catheters were permanently removed. Nasal carriage of S. aureus was regularly investigated and topical mupirocin was prescribed if so affected. Demographic and clinical information were collected prospectively on all HD patients with TCC. All catheter exit sites were revised in each HD session. In addition, all clinical information regarding catheter-related infections were recorded, including CRBSI. Management of intravascular catheter-related infections was in accordance with published guidelines [32]. All CRBSI episodes were treated according to their aetiology with systemic antibiotics and antibiotic lock therapy during a period of at least 14 days.

Collection of study samples

Diagnosis of endoluminal catheter colonization. After each dialysis, each catheter lumen was filled with 2 mL of heparin (5000 IU/mL) prior to being locked. Colonization of the inner catheter lumen was assessed every 15 days immediately before HD as follows:

- Culture of blood-heparin mixture: the first 5 mL of the catheter fluid containing heparin (~2 mL) and blood (~3 mL) were separately aspirated from the arterial and venous lumens. These two samples were inoculated into aerobic culture bottles which were incubated for 5 days in an automated blood culture system (Bact/T-Alert; BioMérieux, Durham, NC). Time to positivity (TTP), defined as the time between the start of incubation and the start of the alert signal (as documented by the monitoring system), was recorded.
- Culture of heparin lock solution: in some instances, a syringe was used to remove ~0.5 mL of the heparin lock solution for culture. In these cases, the remaining fluid containing a mixture of blood and heparin mixture was also cultured. After collection of the catheter samples for culture, the patients were connected to their dialysis lines in the usual manner.

Diagnosis of CRBSI. These samples were taken when the physician responsible for the patient had the suspicion of catheter-related infection or when the catheter was considered colonized (see the ‘Definitions’ section). Blood cultures from two lumens of the TCC and a peripheral vein were obtained simultaneously. If the catheter was removed due to suspicion of infection, the catheter tip was also cultured.

Definitions

- For CoNS, ECC was defined when culture of two endoluminal samples collected on different days in a 1-month period were both positive for the same species based on phenotypic identification. In these cases, the date of processing of the first samples was considered as the date of colonization. For microorganisms other than CoNS, such as Gram-negative bacteria, S. aureus and yeasts, only a single positive culture was required for a patient to be considered colonized.
- CRBSI was defined as isolation of the same organism (identical biotype and susceptibility pattern) from both the TCC and peripheral blood cultures provided that the TTP from the catheter was ≥ 120 min less than that from peripheral blood (i.e. differential TTP ≥ 120 min) [33]. We considered patients to have had more than one episode of CRBSI if the first episode was successfully treated as evidenced by negative blood culture after treatment and there was an interval of >1 month between positive cultures.
- We considered patients to be in the non-CRBSI group if they had no signs or symptoms of catheter-related infection and both their central catheter and peripheral blood cultures were negative.

Microbiological procedures and molecular typing

All samples were submitted to the microbiology laboratory where they were processed according to routine laboratory methods. For culture of heparin lock solution, 50 μL of the solution were inoculated onto Columbia agar with 5% sheep blood and incubated aerobically for 48 h at 37°C. Samples of blood-heparin mixture and blood alone were processed using an automated blood culture system (Bact/T-Alert; BioMérieux, Durham, NC) which continuously monitors for microbial growth. The TTP was recorded. All isolates were subsequently identified to the species level and their antimicrobial susceptibilities were determined using conventional methods and the Wider System (Soria Melguizo, Madrid, Spain).

Molecular characterization of CoNS isolates was performed by PFGE following digestion of DNA extracts with Smal [34]. Migration of DNA fragments was normalized between different gels using a molecular weight standard (lambda ladder; New England Biolabs, Beverly, MA) that was run in two lanes on each gel. Computer-assisted analysis of PFGE was carried out using BioNumerics software (Applied Maths, Kortrijk, Belgium). A 1.8% tolerance was used for comparisons of DNA patterns.

Statistical analysis

The rate of CRBSI was calculated as a density of incidence and was reported per 1000 catheter-days. CRBSI and non-CRBSI groups were compared using a two-tailed chi-square test or Fischer’s exact test for categorical variables and the Mann–Whitney test for continuous variables. We compared the results of endoluminal cultures of CRBSI and non-CRBSI groups in order to analyse the clinical significance of the prevalence of samples in predicting CRBSI. We assessed the risk of CRBSI associated with positive culture results in each of the following scenarios: (i) any sample (at least one positive culture of any type of sample), (ii) arterial culture (at least one positive culture of an arterial lumen sample), (iii) venous culture (at least one positive culture of a venous lumen sample), (iv) paired cultures (positive cultures of arterial and venous lumen samples from the same intervention), (v) two consecutive samples (positive culture of two samples taken in different interventions) and (vi) culture of heparin lock solution. We calculated the sensitivity, specificity, positive and negative predictive values (PPV and NPV) and overall efficiency for prognosis of CRBSI for each scenario of positive test results obtained in the month prior to the date of clinical diagnosis. Statistical analyses were performed using the SPSS software package (SPSS) and Epidat 3.0 (Pan American Health Organization).

Results

Study population and characteristics of patients with CRBSI

During the 18-month study period, we enrolled 51 patients who required 64 TCC, of which 51 (79.7%) were...
Table 1. Characteristics of HD patients by CRBSI status during the study period

<table>
<thead>
<tr>
<th></th>
<th>CRBSI patients (n = 14)</th>
<th>Non-CRBSI patients (n = 37)</th>
<th>P-value</th>
<th>All patients (n = 51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)(^a)</td>
<td>54.6 ± 17.9</td>
<td>68.0 ± 14.6</td>
<td>0.093</td>
<td>65.71 ± 15.8</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>9 (64.3)</td>
<td>18 (48.6)</td>
<td>0.494</td>
<td>27 (52.9)</td>
</tr>
<tr>
<td>Causes of end-stage renal disease (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic nephropathies</td>
<td>7 (50)</td>
<td>11 (29.7)</td>
<td>0.181</td>
<td>18 (35.3)</td>
</tr>
<tr>
<td>Vascular nephropathies</td>
<td>0 (0)</td>
<td>8 (21.6)</td>
<td>–</td>
<td>8 (15.7)</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>2 (14.3)</td>
<td>3 (8.1)</td>
<td>0.512</td>
<td>5 (9.8)</td>
</tr>
<tr>
<td>Systemic</td>
<td>1 (7.1)</td>
<td>2 (5.4)</td>
<td>0.816</td>
<td>3 (5.9)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (14.3)</td>
<td>2 (5.4)</td>
<td>0.297</td>
<td>4 (7.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (14.3)</td>
<td>11 (29.7)</td>
<td>0.263</td>
<td>13 (25.5)</td>
</tr>
<tr>
<td>Prevalent TCC (%)</td>
<td>11 (78.6)</td>
<td>25 (67.6)</td>
<td>0.671</td>
<td>36 (70.6)</td>
</tr>
<tr>
<td>HD vintage (days)(^a)</td>
<td>1946.7 (1949.2)</td>
<td>1183.2 (1442.1)</td>
<td>0.070</td>
<td>1392.8 (1613.5)</td>
</tr>
<tr>
<td>Follow-up (days)(^b)</td>
<td>339.9 (137.60)</td>
<td>214.7 (120.87)</td>
<td>0.706</td>
<td>249.0 (136.5)</td>
</tr>
<tr>
<td>Number of catheters used during the study(^b)</td>
<td>19</td>
<td>45</td>
<td>–</td>
<td>64</td>
</tr>
<tr>
<td>Catheter site insertion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jugular vein</td>
<td>3</td>
<td>6</td>
<td>–</td>
<td>9</td>
</tr>
<tr>
<td>Subclavian vein</td>
<td>16</td>
<td>38</td>
<td>–</td>
<td>54</td>
</tr>
<tr>
<td>Femoral vein</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Number of colonized catheters (%)</td>
<td>14/19 (73.7)</td>
<td>2/45 (4.4)</td>
<td>&lt;0.001</td>
<td>16/64 (25.0)</td>
</tr>
<tr>
<td>Time from TCC insertion to ECC (days)(^c)</td>
<td>390.9 (388.8)</td>
<td>286.50 (135.1)</td>
<td>0.7195</td>
<td>377.9 (365.38)</td>
</tr>
<tr>
<td>Time from TCC insertion to CRBSI (days)(^c)</td>
<td>442.8 (378.8)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Deaths during follow-up(^c)</td>
<td>0</td>
<td>15 (40.5)</td>
<td>–</td>
<td>15 (29.4)</td>
</tr>
</tbody>
</table>

\(^a\)Data are expressed as means ± SD.

\(^b\)In CRBSI patients, five catheters were removed (three because of persistent CRSBI and two due to other causes). In non-CRBSI patients, eight catheters were removed due to other causes than infection.

\(^c\)No patient died of CRBSI.

pre-existing and 13 (20.3%) were inserted during the course of the study period. Nineteen patients (37.3%) were dialysed at the hospital unit and 32 (62.7%) at the ambulatory unit. Median follow-up was 245.0 days (interquartile range, 110.0–314.5). During the period of the study, 15 patients (29.4%) died, none due to catheter-related infection. A summary of patient demographics and medical histories is shown in Table 1.

During the study period, 14 patients (27.4%) carrying 14 TCC had 21 CRBSI episodes (two patients had four episodes with one TCC, one had two episodes with one TCC and the rest had only one episode with one TCC). The incidence of CRBSI was 1.65 episodes per 1000 catheter-days. *S. epidermidis* was involved in 16 (76.2%) of cases. In 14 (66.7%) cases, *S. epidermidis* alone was isolated, while two cases (9.5%) involved mixed infections, one with *Enterococcus faecalis* and the other with *Stenotrophomonas maltophilia*. Of the remaining five cases, four were single infections involving *S. maltophilia* (two cases), *E. faecalis* and *Corynebacterium* spp. The remaining case of CRBSI was due to co-infection with *E. faecalis* and *Escherichia coli*. The demographic and clinical characteristics of the patients who presented with clinical episodes of CRBSI were similar to those of patients in the non-CRBSI group with regard to age, sex, centre of HD, cause of renal failure, catheter insertion site, days included in the study and days of HD. However, ECC was more frequent in CRBSIs than in the non-CRBSI group (73.7% vs 44.4%, P < 0.001) (Table 1). In three patients with a single CRBSI episode (two with *S. epidermidis* and one with *E. faecalis* and *E. coli*), catheters were removed due to persistent bacteremia after completing the treatment. Cultures of the tip showed that the microorganisms found were the same as those isolates from blood cultures and endoluminal samples.

**Dynamics of endoluminal catheter colonization in relation to CRBSI**

A total of 1521 endoluminal samples were obtained from the 51 patients. Of these, 1351 were blood–heparin mixtures: 654 from the arterial lumen, 654 from the venous lumen and 43 for which the lumen source was not recorded. The remaining 170 samples were of heparin lock solution and comprised 144 from the venous lumen and 26 from the arterial lumen. CoNS were the most common microbrial isolates and were recovered from 177 samples: 107 from 10 patients with 14 *S. epidermidis* CRBSIs, 54 from 36 patients of the non-CRBSI group and 16 from one patient who was removed from the study because of catheter dysfunction. Furthermore, in seven episodes of mixed and non-CoNS CRBSIs occurring in four patients, 34 endoluminal samples showed isolates other than CoNS.

All 21 CRBSI episodes were preceded by catheter colonization. The microorganism found in the catheter lock was the same as the one causing CRBSI. Overall, the median time between ECC and diagnosis of CRBSI was 28.0 days (interquartile range, 5.0–45.0). The median time from ECC to diagnosis of *S. epidermidis*-related CRBSIs (14 episodes) was 31.5 days (interquartile range, 27.0–79.0). For the group including mixed and non-CoNS CRBSIs (seven episodes), the median time was 2.0 days (interquartile range, 2.0–23.0). The difference between both groups was statistically significant (P = 0.032). Median TTP of endoluminal cultures was 14.5 h (interquartile range, 11.0–20.8), 9.6 h (interquartile range, 6.0–12.6) and
26.1 h (interquartile range, 20.5–37.3) in *S. epidermidis* CRBSI, mixed and non-CoNS CRBSI and non-CRBSI, respectively. The observed differences in TTP among the three groups were statistically significant (P < 0.05).

To investigate the dynamics of ECC, all endoluminal samples taken prior to CRBSI were analysed. Overall, the proportion of positive endoluminal samples in case of *S. epidermidis* CRBSI was 23.1% (107 out of 463), while for the non-CRBSI group, it was 5.9% (54 out of 920) (P < 0.001). We also analysed the proportion of positive endoluminal samples and TTP starting at the time of catheter insertion. This was accomplished by grouping the data into three periods: <180 days since catheter insertion, between 180 and 360 days and >360 days. The proportion of positive endoluminal samples was significantly higher in the *S. epidermidis* CRBSIs than in the non-CRBSI group for the three periods analysed (Figure 1, panel A). Furthermore, the proportion of positive endoluminal samples increased significantly from the second (16.5%) to the third period (29.6%) in the *S. epidermidis* CRBSIs (P = 0.009) but not in the non-CRBSI group (P > 0.05). The median TTP of endoluminal cultures was also lower in the *S. epidermidis* CRBSIs than in the non-CRBSI group for the second and third periods (P < 0.001) (Figure 1, panel B).

**Dynamics of ECC according to *S. epidermidis* genotype**

For microorganisms different from CoNS, we considered that having identical biochemical and similar antibiogram was enough to consider the isolates obtained from catheter colonization and from CRBSI episodes as an identical microorganism, without any need for molecular studies. For *S. epidermidis* CRBSIs, we considered the possibility of clonal diversity. Therefore, we analysed the isolates by PFGE in order to determine if the episodes of CRBSI were due to a single *S. epidermidis* clone which might have been the cause of persistent colonization of the internal surface of the catheter hub. A total of 91 available isolates of *S. epidermidis* from 12 patients were tested. These comprised 45 blood isolates (obtained from at least one lumen of a TCC and a peripheral vein) at the time of CRBSI and 46 endoluminal isolates obtained during the surveillance period prior to diagnosis of CRBSI. In each patient, all isolates obtained from the central and peripheral veins exhibited identical biotype and antimicrobial susceptibility patterns. The results of the PFGE analysis also confirmed that, in each patient, the genotype observed in blood and endoluminal cultures was the same (Figure 2, panel A). To investigate whether there was a clonal spread of *S. epidermidis* among HD patients, we also compared the PFGE patterns between patients. The isolates from each patient were shown to belong to different clonal types (Figure 2, panel B).

**Use of endoluminal samples to predict CRBSI**

In order to assess the validity of identifying ECC in the prediction of subsequent CRBSI, we analysed 14 patients who had a total of 21 episodes of CRBSI and 29 HD patients who showed no signs of CRBSI as demonstrated by negative TCC and peripheral blood cultures. For this analysis, we excluded eight patients only because TCC and peripheral blood cultures were not performed. Among the 14 patients with CRBSI, the median time from colonization to the development of CRBSI was 1 month. Consequently, for each patient, we analysed the results of the different cultures that were performed in the month prior to developing CRBSI. When we considered all endoluminal samples during this period as a single test, the validity values to predict CRBSI were: sensitivity, 95.2%; specificity, 86.2%; PPV, 83.3%; and NPV, 96.2%. The culture of blood–heparin mixture from arterial or venous lumens yielded similar va-
values for sensitivity (84.2 and 90.0%, respectively) and specificity (96.3 and 96.3%, respectively) (Table 2). Culture of heparin lock solution showed the highest sensitivity (100%) and specificity (100%).

A similar analysis was performed on the same group of patients to determine the ability to predict CoNS CRBSI (Table 3). For arterial endoluminal cultures, sensitivity and specificity were 100 and 96.3%, respectively, while for venous endoluminal cultures, these values were 92.3 and 96.3%, respectively. For heparin lock solution cultures, both sensitivity and specificity were 100%. We used endoluminal cultures with TTP ≤14 h to predict CoNS CRBSI. Sensitivity, specificity, PPV and NPV were 52.1% (95% CI, 38.3–65.5), 97.7% (95% CI, 88.2–99.6), 96.1% (95% CI, 81.1–99.3) and 65.1% (95% CI, 53.1–75.5), respectively.

Discussion

This study highlights the importance of the dynamics of catheter colonization in the prediction of CRBSI. During the period of this study, we estimated a CRBSI rate of 1.65 episodes per 1000 catheter-days, which is lower than the rate that has been reported elsewhere [8–18]. We observed that all HD patients who developed CRBSI had presented previously with ECC by the strain of microorganism that was ultimately responsible for their bloodstream infection. The aetiology of CRBSI caused by S. epidermidis involved colonization of the catheter with a single clonal population of the organism and subsequent development of CRBSI after a median interval of ~1 month. At the same time, we also observed that, in CRBSIs caused by microorganisms different from CoNS, the interval time between catheter colonization and CRBSI was significantly lower (median time, 2.0 days; interquartile range, 2.0–23.0). Based on endoluminal cultures, the interval between initial colonization and development of CRBSI may be related to the type of microorganism and to the time required to achieve a critical threshold of bacterial load. It is important to highlight that there was no ECC and CRBSI caused by S. aureus during our study. In all probability, the growth of S. aureus is too fast to be detected by regular screening cultures before the occurrence of CRBSI [35].

Although different techniques have been used to investigate catheter colonization [25,30,35–38], we wanted to use a readily accessible method to predict the risk of

Table 2. Utility of ECC culture to predict CRBSI

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any sample</td>
<td>95.2 (77.3–99.2)</td>
<td>86.2 (69.4–94.5)</td>
<td>83.3 (64.2–93.3)</td>
<td>96.2 (81.1–99.3)</td>
<td>90.0 (78.6–95.6)</td>
</tr>
<tr>
<td>Arterial lumen culture</td>
<td>84.2 (62.4–94.5)</td>
<td>96.3 (81.7–99.3)</td>
<td>94.1 (73.0–99.0)</td>
<td>89.7 (73.6–96.4)</td>
<td>91.30 (79.7–96.6)</td>
</tr>
<tr>
<td>Venous lumen culture</td>
<td>90.0 (70.0–97.2)</td>
<td>96.3 (81.7–99.3)</td>
<td>94.7 (75.4–99.1)</td>
<td>92.9 (77.4–98.0)</td>
<td>93.6 (82.8–97.8)</td>
</tr>
<tr>
<td>Paired arterial-venous lumens cultures</td>
<td>80.0 (58.4–92.0)</td>
<td>100 (88.0–100.0)</td>
<td>100 (80.6–100.0)</td>
<td>87.5 (71.9–95.0)</td>
<td>91.7 (80.4–96.2)</td>
</tr>
<tr>
<td>Two consecutive cultures</td>
<td>85.0 (64–94.8)</td>
<td>100 (86.7–100.0)</td>
<td>100.0 (81.6–100.0)</td>
<td>89.3 (72.8–96.3)</td>
<td>93.33 (82.1–97.7)</td>
</tr>
<tr>
<td>Heparin lock solution culture</td>
<td>100.0 (75.8–100.0)</td>
<td>100.0 (64.6–100.0)</td>
<td>100.0 (75.8–100.0)</td>
<td>100.0 (64.6–100.0)</td>
<td>100.0 (83.2–100)</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value; PLR. Values are percentages with 95% confidence intervals.

**Fig. 2.** Images of PFGE patterns of *S. epidermidis* from HD patients. (A) Image of PFGE patterns of *S. epidermidis* isolates obtained from a single representative patient during the study period. M, lambda molecular marker; 1–5, endoluminal samples; 6–8, blood cultures drawn by the arterial–venous branches of the catheter and from a peripheral vein sample. Date of isolation (month/day/year) of samples: 1 (01/16/2007), 2 (02/27/2007), 3 (03/15/2007), 4 (03/27/2007), 5 (04/03/2007), 6–8 (04/03/2007). This patient presented persistent ECC by the same clone of *S. epidermidis* and finally he developed CRSBI. (B) Dendrogram of the PFGE patterns of *S. epidermidis* isolates from blood culture in all episodes of CRBSI during the study period (one isolate per clinical episode). The dendrogram shows the lack of clonal dissemination of *S. epidermidis* in both HD units.
CRBSI with minimum risk to the patient. For that reason, we chose to use the first ~5 mL of catheter fluid that is typically discarded in the process of connecting the patient to the HD machine and which contains a mixture of heparin solution (~2 mL) and blood (~3 mL). In cases of ECC, the blood–heparin aspirate is expected to contain viable bacteria in suspension that have been sloughed off the biofilm surface coating the inner lining of the catheter. These samples can readily be inoculated in standard as aerobic blood culture bottles for automated processing and detection of bacterial growth. As has been reported previously, we hypothesized that the TTP of the culture would correlate with the initial bacterial load and could be useful in discriminating clinically significant infections [33].

This study shows that positive ECC may predict the risk of developing TCC-related bacteraemia. Over the study period, the proportion of positive endoluminal samples increased, and this increase was significantly higher in the patients that develop CRBSI (Figure 1A). Furthermore, positive endoluminal samples had a lower TTP in the S. epidermidis CRBSI group (14.5 h) than in the non-CRBSI group (26.1 h), suggesting that the inoculum in the catheter hub was much higher. In this study, CoNS endoluminal cultures with a low TTP was predictive of imminent CRBSI, offering potential in the future for prophylactic intervention based on monitoring of endoluminal cultures. Longer TTP is perhaps indicative of early phase ECC without concurrent or imminent bacteraemia.

Our results showed that cultures of blood–heparin mixture from the arterial or venous lumens or of heparin lock solution had a high sensitivity for the prediction of CRBSI. In particular, a TTP of ≤14 h had a very high PPV (96.1%) for CoNS CRBSI. These results may be very useful for the clinical management of HD patients. A possible strategy to apply to this group of patients is to monitor ECC by culture of blood–heparin TCC samples on a fortnightly basis. Asymptomatic patients with negative results would be candidates for confirmed surveillance culture, while those with positive results would be candidates for confirmation of colonization by a repeat culture and, upon confirmation, for preventive or therapeutic management. Knowledge of the antimicrobial resistance pattern of the colonizing agent would also enable specific intervention strategies to be tailored for individual patients. Thus, patients with ECC without CRBSI would be candidates to receive antibiotic lock therapy, and for those with CRBSI, antibiotic lock therapy should be used in conjunction with systemic antimicrobial therapy according to the recommendations for specific microorganisms [39]. This would enable early and targeted antimicrobial therapy, increasing the catheter salvage rate and reducing the development of bacterial drug resistance. On the other hand, we have estimated that the cost of the regular screening would not be extremely high because most clinical laboratories have an automated blood culture system. The main cost would be two culture bottles per intervention. Given that CRBSI is associated with a high morbidity, mortality and cost in HD patients, we think that the regular screening of ECC by culture of blood–heparin TCC samples might be cost-effective in reducing CRBSIs.

It is important to keep in mind that there are unique aspects of managing patients who are receiving HD through catheters and for whom catheter-related infection is suspected or proven [39]. In a position statement published recently by the European Renal Best Practice, the Infectious Diseases Society of America guidelines have been revised with the intention of focusing on the items which are relevant for nephrologists and amending them to HD conditions and/or for the European situation with regard to tunneled catheters [40,41]. Thus, an important aspect related to the diagnosis of CRBSI is the simultaneous sampling from peripheral vein and from catheter. Although in our study we recommend to obtain peripheral blood samples from vessels that were not intended for future use in creating a dialysis fistula, in many cases, it may not be possible to puncture a peripheral vein due to unavailability or because it is deemed desirable to preserve veins for future access creation. According to an ERPB recommendation, ‘if a hemodialysis catheter is not removed, blood cultures obtained during dialysis through the dialysis circuit linked to the catheter are a more realistic and practical method to isolate an organism related to catheter-associated infection than the dual-site approach including also a peripheral vein sample’ [40,41].

Although the data presented here are compelling, our study has some limitations. Firstly, the number of HD patients included in the study is small and many were enrolled after insertion of their central venous catheter. Other similar studies have also showed difficulty in recruiting a high number of patients [27,32]. Secondly, the original design of the study was meant to evaluate the validity of culture of heparin lock solution to detect ECC. However, due to technical and strategic difficulties in obtaining pure heparin lock solution, we were only able to include a small number of this type of samples. Although culture of heparin lock solution showed a high sensitivity.
and specificity, the number of these samples was very low. Thirdly, it is also important to mention that the validation study was performed in a subsection of the same population as the initial study. This type of validation is of lesser value than one performed in another population. Therefore, we believe that we have to be cautious in interpreting these results. Further research is necessary to clarify the validity of this type of samples.

Our findings indicate that TCC surveillance cultures, based on easily accessible samples, could be used to predict the risk of developing CRBSI. These surveillance cultures could, therefore, be used to triage individual HD patients who might benefit from specific intervention measures and help to avoid widespread administration of prophylactic antimicrobial therapy.

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Toll-like receptor expression in monocytes in patients with chronic kidney disease and haemodialysis: relation with inflammation

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Abstract
Background. Inflammation is one of the main contributors to atherosclerosis in haemodialysis (HD) patients. Activation of Toll-like receptors (TLRs) leads to inflammatory response. In this study, we aimed to evaluate the expression of TLRs on monocytes and relate their expression with inflammation in chronic kidney disease (CKD) and HD patients.

Methods. Thirty-four age- and gender-matched controls and stage 3–4 CKD patients and thirty-two HD patients were included in each study group. The effect of HD on the expression of Toll-like receptor-2 (TLR-2) and Toll-like receptor-4 (TLR-4) on CD14+ monocytes was determined at the beginning (baseline), during (120 min) and following (300 min and 24 h) HD and compared with control and stage 3–4 CKD groups. The HD procedure was performed by using low-flux polysulphone dialysers. In addition, serum IL-6 levels were evaluated in both groups at baseline and after a HD session.

Results. The percentage of CD14+ monocytes expressing TLR-2 were similar in all of the study groups, whereas the percentage of CD14+ monocytes expressing TLR-4 were significantly lower in both stage 3–4 CKD and HD patients at baseline than in controls. The mean fluorescence intensities (MFI) of TLR-2 were significantly lower in controls than in stage 3–4 CKD and HD patients at baseline. The MFI of TLR-4 was similar in all of the groups. The percentage of CD14+ monocytes expressing TLR-2 did not change during and after HD. The MFI of TLR-2 decreased at 120 min of HD compared with baseline (1837 ± 672 vs 1650 ± 578, P < 0.05), and recovered back to baseline values at 300 min and at 24 h post-HD. MFI of TLR-4 increased at 24 h compared with baseline (941 ± 294 vs 1087 ± 441, P < 0.05). Serum IL-6 levels correlated with MFI of TLR-2 and TLR-4 in stage 3–4 CKD patients and in HD patients at baseline and after HD in univariate analysis. Stepwise multiple regression analysis revealed that MFI of TLR-2 was an independent determinant of serum IL-6 concentrations in stage 3–4 CKD and in HD patients at baseline, at 300 min and at 24 h post-HD.

Conclusions. Our study demonstrates that TLR-2 is associated with the inflammatory response of non-dialysed and dialysed CKD patients.

Keywords: chronic kidney disease; haemodialysis; inflammation; Toll-like receptor