Time-to-positivity in patients with *Escherichia coli* bacteraemia


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ABSTRACT

The time from the start of incubation to a positive reading of blood cultures (time-to-positivity; TTP) is related to the concentration of bacteria in blood. Information concerning the correlation of TTP with clinical parameters, and its usefulness as a prognostic factor in patients with *Escherichia coli* bacteraemia, is limited. To investigate the relationship of TTP to clinical parameters, 459 cases of monomicrobial *E. coli* bloodstream infections from a single institution between 1997 and 2005 were reviewed. All cases involved patients who were not undergoing antibiotic treatment at the time of blood sampling. The in-hospital mortality rate was 6.3%. Median TTP was significantly shorter for patients who died than for those who survived (9.7 h, inter-quartile range 7.85–11.05 h vs. 11.2 h, inter-quartile range 10.1–11.4 h; p <0.001). Patients with TTP in the lowest quartile were more likely to be female, to have a non-urinary tract or an unknown origin of bacteraemia, to have severe sepsis or shock, and to subsequently die. In a multivariable Cox regression model, the hazard ratio for death from any cause for patients with a short TTP was 3.13 (95% CI 1.28–7.64; p 0.01). TTP in patients with *E. coli* bacteraemia provides prognostic information beyond that provided by the presence of haematological illness, a Charlson score‡, a non-urinary tract origin of bacteraemia, and the presence of severe sepsis or shock.

Keywords  Bacteraemia, blood cultures, *Escherichia coli*, mortality, prognosis, time-to-positivity

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INTRODUCTION

*Escherichia coli* is the most common cause of bloodstream infection involving Gram-negative bacteria [1–3]. Current knowledge concerning the primary site of infection, clinical presentation, epidemiology and prognostic factors of *E. coli* bloodstream infections is limited and mostly somewhat dated [4–9]. Such information is used in daily practice for predicting the origin of bacteraemia and the outcome of patients with *E. coli* bloodstream infection, but its sensitivity and specificity is limited and other prognostic parameters would be useful.

Bacterial blood concentration is associated with the prognosis for patients with bacteraemia caused by certain microorganisms [10–16], yet quantitative blood cultures are not performed routinely in clinical practice. The time from the start of incubation to a positive reading of blood cultures is related to the concentration of bacteria [17,18] and fungi [19] in blood. Recent data suggest that the time-to-positivity (TTP) of blood cultures may be associated with prognosis [9,20–22], although information for patients with *E. coli* bacteraemia concerning the correlation of TTP with clinical parameters is quite limited [9]. Accordingly, the present study investigated the TTP of *E. coli* bacteraemia and its relationship to clinical parameters and prognosis.

PATIENTS AND METHODS

Hospital setting and patients

The study was conducted at the Hospital Sierrallana, a 250-bed teaching hospital in Torrelavega, Spain, with c. 8000 admissions annually, which serves a population of 160 000...
inhabitants. Approximately 1800 blood cultures are performed annually at this hospital. Between January 1997 and December 2005, patients with *E. coli* bacteraemia were identified from the microbiology laboratory database and their charts were reviewed retrospectively with the aid of a standardised data collection form. Data collected included age, gender, previous surgery, antibiotic therapy, duration of hospitalisation before the onset of bacteraemia, presence of severe sepsis or shock, white blood cell count, haemoglobin concentration, plasma creatinine concentration, presence of co-morbidities (diabetes, chronic obstructive pulmonary disease, cirrhosis, previous stroke, cardiac failure, neoplasm, chronic renal failure, immunosuppression or haematological illness) and in-hospital mortality.

**Definitions**

A localised focus of *E. coli* infection was considered to be the source or primary focus of bacteraemia. Bacteraemia was considered to be nosocomial if it appeared ≥48 h after admission and no evidence of infection was present upon admission. Immunosuppression was defined as the presence of neutropenia, or infection with human immunodeficiency virus, or treatment with immunosuppressive agents. Co-morbidities were assessed using the Charlson co-morbidity score [23]. Sepsis, severe sepsis and septic shock were defined as proposed by Bone *et al.* [24]. Therapy was judged as adequate or inadequate, based on the in-vitro susceptibility of the isolated organism and whether the antibiotic treatment was initiated within 24 h of blood sampling.

**Blood cultures**

The common practice for blood cultures at Sierallana Hospital is to take 20 mL of venous blood and inoculate it in equal parts into one aerobic blood culture bottle (BacT/ALERT FA aerobic; bioMérieux, Durham, NC, USA) and one anaerobic blood culture bottle (BacT/ALERT FN; bioMérieux). Blood from a peripheral vein was sampled by nurses, three times at intervals of 30 min. On a 24-h basis, the blood culture bottles were sent to the microbiology laboratory, immediately loaded into the blood culture instrument (BacT/ALERT microbial detection system; bioMérieux), and cultured for 5 days. The BacT/ALERT system tests for CO₂ production and records the time interval between the addition of each blood culture bottle to the system and the detection of microbial growth (defined as TTP). No antibiotic removal device was used for the blood cultures of patients treated previously with antibiotics. When multiple cultures were positive, the shortest TTP was available for 459 patients and 1659 blood culture bottles were sent to the microbiology laboratory, immediately loaded into the blood culture instrument (BacT/ALERT FA aerobic; bioMérieux, Durham, NC, USA) and one anaerobic blood culture bottle (BacT/ALERT FN; bioMérieux). Blood from a peripheral vein was sampled by nurses, three times at intervals of 30 min. On a 24-h basis, the blood culture bottles were sent to the microbiology laboratory, immediately loaded into the blood culture instrument (BacT/ALERT microbial detection system; bioMérieux), and cultured for 5 days. The BacT/ALERT system tests for CO₂ production and records the time interval between the addition of each blood culture bottle to the system and the detection of microbial growth (defined as TTP). No antibiotic removal device was used for the blood cultures of patients treated previously with antibiotics. When multiple cultures were positive, the shortest TTP was selected for the analysis. Neither the volume of blood cultured nor the time interval between obtaining the blood for culture and incubation of the bottles was recorded.

**Statistical analysis**

Baseline characteristics of the study patients, grouped according to quartiles of TTP, are presented as percentages for dichotomous variables and as medians with inter-quartile ranges (IQRs) for continuous variables. Baseline characteristics were compared among quartiles using the chi-square test for discrete variables and the Wilcoxon or Kruskal–Wallis rank sum test for continuous variables, as appropriate. The non-parametric correlation (Spearman’s ρ coefficient) was used to analyse the association between two variables. A stepwise logistic regression analysis was used to control the effects of confounding variables. Survival curves were generated by means of Kaplan–Meier estimates, and differences in survival were compared using the log-rank test. To control for confounders, the multivariable Cox model was fitted. Inclusion of the variables in the model was determined according to their significance in the univariate analysis. Relative risks and 95% CIs were calculated as hazard ratios derived from the Cox proportional-hazards regression model, including variables associated with death in the univariate model. The level of significance was set at *p* < 0.05. Statistical analysis was performed using SPSS software for Windows, v.12 (SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Patients and blood cultures**

During the study period, *E. coli* bacteraemia was detected in 555 patients admitted to the hospital. Excluded from the study were 38 patients with polymicrobial bloodstream infections, 59 patients receiving treatment with antibiotics when blood was sampled, and two patients for whom TTP was not available; neither of the latter two patients had severe sepsis or septic shock or died. For the remaining 459 patients included in the analysis, the number of blood culture bottles inoculated for each episode of bacteraemia was six in 424 cases, five in two cases, four in 18 cases, three in one case and two in 14 cases.

Patients with *E. coli* bacteraemia were aged 15–98 years (median 77 years). Fifty (10.8%) patients had severe sepsis, and five (1.1%) had septic shock at presentation. Twenty-nine (6.3%) patients died during hospitalisation after 8.9 ± 8.6 days (range 0–29 days) following the onset of bacteraemia. A trend towards a lower incidence of severe sepsis or shock among male patients (9.4% vs. 14.2%, *p* 0.09) was detected. Demographic and clinical characteristics of these patients are listed in Table 1.

**Time-to-positivity**

TTP was available for 459 patients and 1659 blood culture bottles. A correlation was found between the mean TTP of each series and the shortest TTP of the blood culture bottles of each patient (ρ 0.8, *p* < 0.0001). The median TTP was 11.2 h (range 1–115.2 h). In patients with more than one positive
bottle, the median maximal difference of the TTP among the blood culture bottles of each series was 1.1 h (range 0–145.3 h), and the median difference in TTP between aerobic and anaerobic blood culture bottles was 0 h (range 17 to 44.5 h). A significant negative correlation was found between the shortest TTP for each episode of bacteraemia and the number of positive blood culture bottles from each episode (\(\rho = 0.48, p < 0.0001\)) (Fig. 1).

Patients were divided into subgroups according to quartiles of the shortest TTP for each episode of bacteraemia. Patients with TTP in the lowest quartile were less likely to be male (OR 0.62; 95% CI 0.4–0.95), but were more likely to experience severe sepsis or shock (OR 5.333; 95% CI 2.97–9.59), to have a non-urinary tract origin of bacteraemia (OR 2.1; 95% CI 1.38–3.23), to experience an unknown origin of bacteraemia (OR 2.12; 95% CI 1.2–3.76), to require treatment in an intensive care unit (OR 4.85; 95% CI 1.93–12.18) or to die (OR 4.2; 95% CI 1.95–9.02).

Table 1 shows the baseline characteristics grouped according to quartiles of TTP. After adjusting for these variables in a logistic regression analysis, male gender was still associated with a TTP in the lowest quartile. Receiver operator curve analysis revealed that a TTP of 10.95 h was associated with the best sensitivity and specificity for predicting a urinary tract origin of the bacteraemia (61% and 67%, respectively).

### Relationship between TTP and mortality

The median TTP was significantly shorter for patients who died than for those who survived (9.7 h, IQR 7.85–11.05 h vs. 11.2 h, IQR 10.1–11.4 h; p < 0.001). Receiver operator curve analysis revealed that a TTP of 10.25 h was associated with the best sensitivity and specificity for predicting death (71% and 65.5%, respectively). A TTP value below this cut-off point was defined as ‘short TTP’. Univariate analysis revealed that an increased Charlson score, the presence of dementia,
the presence of any co-morbidity, a non-urinary tract origin of bacteraemia, a pulmonary origin of bacteraemia, the presence of severe sepsis or shock, decreased consciousness, an absence of fever and a short TTP were all associated with death (Table 2). Patients who survived with a short TTP tended to have a longer hospital stay following the episode of bacteraemia (13.59 ± 11.17 days vs. 10.61 ± 7.16 days, p 0.067).

A stratified univariate analysis showed that a short TTP was also associated with death among patients whose bacteraemia was of non-urinary tract origin (OR 4.24; 95% CI 1.57–11.46), or who were without severe sepsis or shock (OR 4.87; 95% CI 1.72–13.73), or who had a Charlson score ≤ 3 (OR 2.52; 95% CI 0.95–6.71).

In a Cox regression model, the unadjusted hazard ratio for the death of patients with a short TTP, compared with the remaining patients, was 4.36 (95% CI 2.03–9.37; p <0.0001) (Fig. 2). In a multivariable Cox regression model, variables associated with death were a short TTP, dementia, a Charlson score ≥ 3, the presence of severe sepsis or shock, and non-urinary tract origin of bacteraemia (Table 3).

DISCUSSION

The present study analysed the relationship between TTP and various clinical parameters among patients with E. coli bacteraemia. E. coli was found to grow faster in blood cultures from females, from those patients whose bacteraemia was of non-urinary tract or unknown origin, from cases of severe sepsis or shock, and from patients who died. More importantly, a short TTP was found to be an independent risk-factor for death. The study revealed that TTP provides prognostic information concerning mortality from all causes for patients with E. coli bacteraemia, independent of other prognostic clinical parameters, thus providing information beyond that supplied by clinical variables. Several previous studies have suggested that the concentration of bacteria in

Table 2. Variables revealed by univariate analysis to be associated with all-cause hospital mortality among patients with Escherichia coli bacteraemia

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Died n (%)</th>
<th>Survived n (%)</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>15 (51.7)</td>
<td>211 (49.1)</td>
<td>1.11 (0.52–2.36)</td>
<td>0.47</td>
</tr>
<tr>
<td>Age &gt;65 years</td>
<td>23 (79.3)</td>
<td>337 (78.4)</td>
<td>1.06 (0.42–2.67)</td>
<td>0.56</td>
</tr>
<tr>
<td>Nosocomial bacteraemia</td>
<td>2 (6.9)</td>
<td>48 (11.2)</td>
<td>0.59 (0.14–2.36)</td>
<td>0.37</td>
</tr>
<tr>
<td>Adequate empirical treatment</td>
<td>24 (82.8)</td>
<td>367 (85.5)</td>
<td>0.81 (0.3–2.21)</td>
<td>0.43</td>
</tr>
<tr>
<td>Co-morbidity</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Neoplasm</td>
<td>3 (10.3)</td>
<td>44 (10.2)</td>
<td>1.01 (0.29–3.48)</td>
<td>0.58</td>
</tr>
<tr>
<td>Dementia</td>
<td>7 (24.1)</td>
<td>33 (7.7)</td>
<td>3.83 (1.52–9.62)</td>
<td>0.008</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>3 (10.3)</td>
<td>15 (3.5)</td>
<td>3.19 (0.87–11.73)</td>
<td>0.1</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>3 (10.3)</td>
<td>12 (2.8)</td>
<td>4.02 (1.07–15.13)</td>
<td>0.06</td>
</tr>
<tr>
<td>Charlson index (mean ± SD)</td>
<td>1.86 ± 2.1</td>
<td>0.91 ± 1.58</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Origin of infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary tract</td>
<td>8 (27.6)</td>
<td>252 (58.6)</td>
<td>0.27 (0.12–0.62)</td>
<td>0.001</td>
</tr>
<tr>
<td>Biliary tract</td>
<td>4 (13.8)</td>
<td>87 (20.2)</td>
<td>0.63 (0.21–1.86)</td>
<td>0.28</td>
</tr>
<tr>
<td>Unknown</td>
<td>7 (24.1)</td>
<td>52 (12.1)</td>
<td>2.31 (0.94–5.68)</td>
<td>0.06</td>
</tr>
<tr>
<td>Intra-abdominal</td>
<td>3 (10.3)</td>
<td>22 (5.1)</td>
<td>2.14 (0.6–7.62)</td>
<td>0.2</td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>3 (10.3)</td>
<td>5 (1.2)</td>
<td>9.81 (2.22–43.31)</td>
<td>0.01</td>
</tr>
<tr>
<td>Manifestations of bacteraemia</td>
<td></td>
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<tr>
<td>Decreased consciousness</td>
<td>10 (34.5)</td>
<td>45 (10.5)</td>
<td>4.5 (1.97–10.28)</td>
<td>0.001</td>
</tr>
<tr>
<td>Severe sepsis or shock</td>
<td>13 (44.8)</td>
<td>42 (9.8)</td>
<td>7.51 (3.4–16.67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Short TTP</td>
<td>19 (65.5)</td>
<td>125 (29.1)</td>
<td>4.64 (2.1–10.25)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Time-to-positivity of <10.25 h.

Fig. 2. Adjusted estimates of overall survival for patients with Escherichia coli bacteraemia, according to time-to-positivity (TTP). The survival estimates have been adjusted for the presence of a Charlson index ≥ 3, urinary tract or pulmonary origin of bacteraemia, the presence of severe sepsis or shock, and decreased consciousness (p 0.001 by the log-rank test for the overall comparison among the groups).
blood is related to outcome in patients with bacteraemia [10–16], and in-vitro studies have revealed a correlation between TTP and bacterial concentrations in blood [17–19]. TTP is automatically measured by continuously monitoring blood culture systems, and is considered to be a surrogate marker of bacterial concentrations in blood [18,25]. Moreover, the difference between the TTP of blood cultures drawn through a central venous catheter and that of those drawn from a peripheral vein is highly diagnostic of catheter-related bloodstream infection in patients with long-term catheters [18,25]. Recent studies have suggested that there is a relationship between TTP and outcome and the origin of bacteraemia in infections caused by Streptococcus pneumoniae [22], Staphylococcus aureus [20,21] and E. coli [9]. In these studies, TTP was related to the presence of severe sepsis or shock, the origin of bacteraemia, and outcome [9,20–22]. In view of these findings, TTP is an attractive candidate for an easy-to-measure laboratory prognostic factor for patients with bacteraemia.

Only one previous study has analysed TTP for patients with E. coli bacteraemia, and reported that a TTP of ≤7 h was associated with mortality; however, this study involved a relatively small sample, and failed to select a TTP cut-off value based on the best sensitivity and specificity for predicting death. In addition, >20% of the patients included were receiving antibiotic treatment when blood was sampled, which could have altered the TTP [9]. Despite its usefulness as a prognostic tool, TTP is somewhat limited in that it has a sub-optimal sensitivity in predicting death, and because factors other than the concentration of bacteria in blood can influence its value, including the precise microorganisms cultured [26–30], the automated blood culture system used [26–28], and the presence of antimicrobial agents in the blood [31,32], all of which can make the use of a standard cut-off TTP in clinical practice difficult.

The present data pose several questions. First, no satisfactory explanation exists for the relationship between gender and TTP. A second question concerns the relationship between TTP, urinary tract infection and outcome. Several studies have revealed that a urinary tract origin for patients with bacteraemia is associated with lower mortality [3,33]. A potential association between urinary tract infection and a lower concentration of bacteria in blood is supported by the results of the present study, which may explain, at least in part, the better outcome of these patients.

The present study had several limitations. Certain patient characteristics, e.g., the presence of neutropenia or immunosuppression, might also be important determinants of the severity of bacteraemia. The small number of patients with these conditions precluded a meaningful assessment of these potential variables. Additional studies are necessary to clarify the effect of the immune status on TTP. Another limitation is that neither the time between blood sampling and culture, nor the volume of blood drawn for culture, were recorded, although it is likely that differences in blood volume between culture bottles were random. The influence of these factors, as well as the effect of the system used for blood cultures, on TTP values complicates extrapolation of the results to other settings. It is also unclear whether a correction of TTP according to the blood volume in each culture bottle might improve the relationship of TTP to outcome. Nevertheless, the study supports the usefulness of TTP as a tool for prognosis and for defining the origin of bacteraemia in patients with E. coli bloodstream infection. Further studies are required to define its usefulness and the optimal cut-off points for bloodstream infections caused by other microorganisms.

### Table 3. Hazard ratios for death from any cause in the multivariate model

<table>
<thead>
<tr>
<th></th>
<th>Adjusted hazard ratios (95% CI)</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Dementia</td>
<td>2.88 (1.18–7.04)</td>
<td>0.021</td>
</tr>
<tr>
<td>Charlson index 23</td>
<td>5.61 (2.52–12.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>Non-urinary tract origin</td>
<td>3.31 (1.42–7.74)</td>
<td>0.006</td>
</tr>
<tr>
<td>Short TTP‡</td>
<td>2.98 (1.29–6.87)</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Relative risk and 95% CIs were calculated as hazard ratios derived from the Cox proportional-hazards regression model for overall mortality. Co-variates included in the initial model were the presence of dementia, a Charlson index ≥3, non-urinary tract origin of bacteraemia, pulmonary origin of bacteraemia, presence of severe sepsis or shock, decreased consciousness, and short time-to-positivity (TTP). Excluding, individually, the presence of dementia, the presence of severe sepsis or shock, and altered consciousness from the model did not change the prognostic value of TTP.

‡TTP of <10.25 h.

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