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Post-mortem detection of bacteremia using pairs of blood culture samples

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ABSTRACT

Aim: The objective of this study was to assess the utility of examining pairs of blood culture samples obtained from separate sites (both ventricles or the aorta and vena cava) for detecting bacteremia in the post-mortem setting.
Methods: Autopsy cases in which bacterial species were isolated from blood cultures were identified over a 4-year period. Ante-mortem and post-mortem records and the findings of pathological examinations were reviewed.
Results: Overall, 23 bacterial species were detected in 18 autopsy cases. *E. coli* was the most commonly detected species (5 cases, 27.8%), followed by *S. aureus* and *K. pneumoniae*, respectively. Seven of the detected bacterial species (3 cases, 16.7%) were obligate anaerobes (Clostridium spp. and Bacteroides spp.). Among the 3 cases involving obligate anaerobes, multiple bacterial species were detected in 2 cases. Clinically, 2 of the 18 patients in which bacteria were detected were treated for significant infections (urosepsis, pneumonia, and catheter-related bloodstream infection) before their deaths. Seven cases exhibited evidence of significant infection during the post-mortem pathological examination. The differences between the aerobic and anaerobic bacteria positivity rates of the single and paired blood culture samples were significant (aerobic: $p = 0.013$ and anaerobic: $p = 0.018$).
Conclusion: Analyzing pairs of blood culture samples obtained from separate sites is useful for detecting bacteremia during post-mortem examinations.

1. Introduction

Post-mortem microbiological detection has been discussed in several studies [1,2]. Heart blood and cerebrospinal fluid are the most useful substances for post-mortem bacteriological cultures [1,3]. Although tissue from organs such as the spleen has also been shown to be useful for post-mortem microbiological cultures, false-positive results caused by contamination have been obtained during lung tissue cultures [1,3–6]. In the clinical setting, several blood cultures are routinely obtained for detecting bacteremia [7,8]. More than 95% of episodes of bacteremia are detected when two or three blood culture samples are drawn [8,9]. The following points regarding the number of blood culture samples that should be obtained to detect bacteremia are important: i) one blood culture set is never sufficient for identifying or excluding bacteremia; ii) two blood culture sets are necessary and sufficient to exclude or identify bacteremia when the microorganism is not a common contaminant and the probability of bacteremia is low (5%) or moderate (20%), such as with pneumonia, intra-abdominal infections, or urinary tract infections; iii) three blood culture sets should be obtained to exclude bacteremia when the probability of bacteremia is high or continuous bacteremia is a consideration [10]. In addition, this approach helps physicians distinguish between clinically important infections and contamination since the proportion of positive blood cultures has been shown to be crucial for interpreting blood culture results [11]. In the post-mortem setting, although one study has reported an evaluation of the separate sampling of blood from the right and left cardiac ventricles [12], there have only been a few studies of the utility of obtaining pairs of blood culture samples for detecting true bacteremia or false-positive detection in the autopsy setting [1–6,13,14]. Therefore, we studied 31 autopsy cases to determine the utility of obtaining pairs of blood culture sets for detecting true bacteremia.

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2. Materials and methods

2.1. Study population

This study was carried out at the Department of Nihon University Itabashi Hospital from January 2012 to December 2015. We retrospectively investigated autopsy cases in which pairs of post-mortem blood culture samples were collected from two separate sites (both cardiac ventricles or the aorta and vena cava). The samples were collected from these locations because (i) these sites contain enough blood to allow the detection of microbiological organisms; (ii) this method allowed us to exclude skin-derived microorganisms; and (iii) when blood samples are collected from both cardiac ventricles, it is possible to take sufficient blood samples; i.e., 10–30 ml samples, which were recommended in previous articles [15–17]. Data regarding the patients' characteristics, prior antibiotic therapy, survival time after illness onset, the interval from death to sample storage, the interval from death to the autopsy, and the blood culture results were collected. This study was conducted with the approval of the ethics committee of Nihon University Hospital (clinical research No. RK-111111-4).

2.2. Post-mortem examinations

An autopsy examination was performed in each case using the standard en bloc autopsy technique [18]. Sections were obtained from each organ and tissue and processed in 20% neutral buffered formalin. Post-mortem examinations were performed clinical autopsy examination only.

2.3. Microbiological cultures

The blood culture samples were collected as soon as the chest cavity was opened. During post-mortem examinations, blood culture samples are obtained in a sterile manner as is the case in the clinical setting [7,8]. The heart and major vessel surfaces were decontaminated using cotton wool containing 70% alcohol. Then, a swabstick containing 10% povidone iodine was used to disinfect the proposed puncture site, before a needle was inserted, as described previously [8,19]. Pairs of blood culture samples were collected from separate sites (both cardiac ventricles or the aorta and vena cava). The blood culture samples were obtained using standard sterile autopsy procedures. A 20-ml syringe with a 5–10 gauge needle was introduced into the target cardiac ventricle or major vessel, and the blood was aspirated and injected into BACTEC™ PLUS anaerobic/F (product code 442023) and aerobic/F culture vials (product code: 442024), which were then placed in a Becton Dickinson BACTEC™ FX incubator. All of the samples were processed and identified in the same laboratory using standard methods. Cultures were considered negative if no growth appeared within 5–7 days. Gram staining was performed to identify any bacteria.

2.4. Statistical analysis

One-way ANOVA and multiple comparisons testing were used for all statistical analyses. The results are presented as mean and SD values. P-values of <0.05 were considered statistically significant.

3. Results

3.1. General comments

3.1.1. Background of the 31 patients in whom pairs of blood culture samples were obtained

From January 2012 to December 2015, 309 autopsies were performed, and pairs of blood culture samples were obtained in 31 of these cases. Information about the age, ante-mortem diagnoses, and main post-mortem diagnoses of the patients whose culture samples were found to contain bacteria are shown in the table. Among the 31 examined cases, positive results were obtained in 18 (58.1%), including 6 cases involving mixed bacterial growth (cases 9, 10, 11, 12, 13, and 16), whereas the blood cultures of the remaining 13 cases (41.9%, not shown) exhibited no growth. Of the 13 cases involving negative blood cultures, 6 patients (19.4%) were treated with antibiotics, and 7 (22.6%) were not.

3.1.2. Background information of the 18 patients with positive blood cultures

The mean age of the patients with positive blood cultures was 67.4 years (age range: from 23 to 83 years) (Table 1), and their gender ratio was as follows: M:F = 6:12. Eight of the patients with positive blood cultures had been treated with antibiotics, and 10 had not.

The patients had many comorbidities, some of which tended to predispose them to infections, e.g., 12 patients had malignancies, including 8 with cancer and 2 with hematological malignancies (plasmacytoma and follicular lymphoma) or sarcoma (epithelioid sarcoma and liposarcoma), respectively.

In the ante-mortem examinations, 3 (cases 1, 2, and 5) of the 31 patients were found to be positive for the same bacteria that were detected in their post-mortem blood culture samples and were diagnosed with ante-mortem bacteremia; *E. coli* were found in 2 cases (cases 1 and 2), and *S. aureus* was detected in one case (case 5). One patient (case 4) was diagnosed with enterococcal bacteremia before his death.

3.1.3. Time between death and the autopsy

The mean time between death and the autopsy was 455.3 min (range: 130–1072 min) in the cases involving positive blood cultures and 290.1 min (range: 98–11,126 min) in the cases involving negative blood cultures. There was no difference in the length of the interval from death to the autopsy between the cases involving positive and negative blood cultures ($p = 0.2$).

3.1.4. Information about the 13 patients with negative blood cultures

The 13 patients whose blood cultures were negative had a mean age of 70.0 years (age range: from 45 to 92 years), and their gender ratio was as follows: M:F = 9:4. Seven patients were treated with antibiotics, 6 patients were not, and 5 patients had cancer.

3.2. Patients whose blood cultures were found to contain bacteria during post-mortem examinations

3.2.1. Bacterial species detected in the cases involving post-mortem bacteremia

Overall, 23 bacterial species were detected in the 18 autopsy cases with positive blood culture samples (Table 1). Of these, 15 species (65.2%) were non-obligate anaerobes. In addition, 5 species (21.7%) were Enterobacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *Morganella morganii*, and *Citrobacter diversus*), two species (8.7%) were Staphylococcus spp., and 3 species (13.0%) were Enterococcus spp. In addition, two glucose non-fermenting species (8.7%), including *Pseudomonas aeruginosa*, and glucose non-