Legal Medicine 24 (2017) 92–97

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

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

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ARTICLE INFO

Article history:
Received 24 July 2016
Received in revised form 15 November 2016
Accepted 17 December 2016
Available online 21 December 2016

Keywords:
Post-mortem
Pairs of blood culture samples
Bacteremia
Forensic/legal medicine

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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http://dx.doi.org/10.1016/j.legalmed.2016.12.009
1344-6223/

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-

Table 1
Characteristics of the cases involving post-mortem bacteremia.

Case No.	Age	Sex	Malignancy	Bacterial species (number of blood culture samples)	Blood culture samples		Detection of infectious source	
					Arterial samples	Venous samples	Clinical findings	Pathological diagnosis
1	72	F	Plasmacytoma	*Escherichia coli* (2)	P	P	Urosepsis	Pyelonephritis, pneumonia
2	75	F	ND	*E. coli* (2)	P	P	Urosepsis	Pyelonephritis
3	74	M	ND	*E. coli* (2)	P	P	ND	ND
4	71	F	ND	*Klebsiella pneumoniae* (2)	P	P	Pneumonia	Pneumonia
5	57	M	Bloodstream infection	*Staphylococcus aureus* (2)	P	P	Bloodstream infection	Bloodstream infection, neck abscess
6	77	M	Follicular lymphoma	*E. coli* (1)	P	P	Urosepsis	Pyelonephritis
7	78	M	Gallbladder cancer	*S. aureus* (1)	P	N	ND	ND
8	81	M	ND	Glucose non-fermenting Gram-negative rod (1)	P	N	ND	ND
9	67	F	Lung cancer	*S. epidermidis* (1), *Haemophilus influenzae* (1)	P	N	ND	ND
10	58	F	Ovarian cancer	*Pseudomonas aeruginosa* (1), *K. oxytoca* (1), *Morganella morganii* (1),	P	P	ND	Pneumonia
11	59	F	Ovarian cancer	*E. coli* (1), *K. pneumoniae* (1), *Citrobacter diversus* (1), *Streptococcus constellatus* (1), *Clostridium cadaveris* (1), *Clostridium ramosum* (1), *Clostridium tertium* (1)	P	P	ND	ND
12	70	F	Lung cancer	*S. epidermidis* (2), *Enterococcus raffinosus* (1)	P	P	ND	ND
13	23	F	Epithelioid sarcoma	*E. faecalis* (2), *S. parasanguinis* (1)	P	P	ND	ND
14	61	F	Breast cancer	*S. epidermidis* (1)	N	P	ND	ND
15	67	F	Ovarian cancer	*E. faecium* (1)	P	N	ND	ND
16	73	F	Uterine cancer	*Bacteroides fragilis* (2), *Clostridium innocuum* (1), *Eubacterium limosum* (1)	P	P	ND	ND
17	83	F	ND	Leuconostoc spp. (2)	P	N	ND	ND
18	68	M	Liposarcoma	*Bacteroides thetaiotaomicron* (1)	P	N	ND	Pneumonia
	Ave. 67.4	M: F = 6: 12	n = 12/18 (66.7%)	Non-obligate bacteria: 15/23 species (65.2%) Obligate anaerobes: 8/23 species (34.8%)	n = 17/18 (94.4%)	n = 12/18 (66.7%)	n = 5/18 (27.8%)	n = 7/18 (38.9%)

The examined characteristics included the case number, age, sex, malignancy, the bacterial species detected (the numbers in parentheses indicate the numbers of blood culture samples in which each microorganism was detected), the results of arterial and venous samples, information about the detection of the source of infection, clinical findings, and the pathological diagnosis.
*Abbreviations: N: negative, ND: not detected, P: positive

fermenting Gram-negative rod (the species name could not be determined) were detected, and *Haemophilus influenzae* was found in one case (4.3%).

E. coli was the most frequently detected species (5 cases, 27.8%; cases 1, 2, 3, 6, and 11), followed by *S. aureus* (2 cases, 11.1%; cases 5 and 7), *K. pneumoniae* (2 cases, 11.1%; cases 4 and 11), and *S. epidermidis* (3 cases, 16.7%; cases 9, 12, and 14). Four of the detected species were obligate anaerobes (4 cases, 22.2%; cases 11, 16, 17, and 18), and 7 were intestinal flora that rarely cause infections (7 cases, 38.9%; cases 11, 12, 13, 15, 16, 17, and 18).

3.2.2. Positivity of blood culture sampling sites

Positive arterial blood culture samples, e.g., from the left cardiac ventricle or aorta, were obtained in 17 cases (94.4%), and positive venous blood culture samples, e.g., from the right cardiac ventricle or vena cava, were collected in 12 cases (66.7%).

3.2.3. Positivity rates of single and paired blood culture samples

Post-mortem tests for bacteremia based on pairs of blood culture samples detected aerobes in a single blood culture sample in 10 cases, aerobes in paired blood culture samples in 16 cases, anaerobes in a single blood culture sample in 9 cases, and anaerobes in paired blood culture samples in 15 cases (Table 1). 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: $p = 0.018$).

3.2.4. Detection of the infectious source based on clinical findings and the pathological diagnosis

Clinically, 5 of the 18 patients were treated for a significant infectious disease (urosepsis, pneumonia, or catheter-related blood stream infections; cases 1, 2, 4, 5, and 6) before their deaths, but no bacteremia or sepsis was noted in the remaining 13 patients while they were alive. Only 7 of the patients exhibited evidence of an active infection during the post-mortem pathological examinations (cases 1, 2, 4, 5, 6, 10, and 18). Inflammatory cell infiltration into the kidneys, lungs, or soft tissue was seen in these cases, and these findings were compatible with the results of blood cultures and the patients' clinical and/or pathological findings in cases 1, 2, 4, 5, 6, and 10. Although *Bacteroides thetaiotaomicron* was detected in case 18, it was not considered to be the cause of the patient's pneumonia (Table 1).

4. Discussion

4.1. The usefulness of analyzing pairs of blood culture samples collected from separate sites for detecting bacteremia during post-mortem examinations

4.1.1. Usefulness of analyzing pairs of blood culture samples

This study is the first to investigate the usefulness of analyzing pairs of blood culture samples obtained from separate sites for detecting true bacteremia during post-mortem examinations. The number of blood culture samples is one of the most important fac-

tors affecting the detection of bacteremia [8]. For example, Washington [9] and Weinstein [20] have shown that more than 95% of episodes of bacteremia and fungemia are detected when two or three blood culture samples are drawn. Second, routinely obtaining more than one blood culture sample has the benefit of ensuring that adequate volumes of blood are cultured. Third, obtaining multiple samples helps physicians distinguish between clinically important and contaminating microorganisms [11]. Although routinely drawing more than 3 blood culture samples would be expensive and needlessly increase laboratory work, it is important to consider whether increased numbers of samples are required on a case by case basis.

4.1.2. Suggestion of diagnostic criteria for post-mortem bacteremia

We suggest that the following criteria should be used to diagnose bacteremia during post-mortem examinations using pairs of blood culture samples: 1) A single bacterial species that is likely to be the causative microorganism of the bacteremia is detected, 2) the detected species is a single non-obligate anaerobe, and 3) positive results are obtained during tests of paired blood culture samples. Bacteremia caused by several bacterial species (especially anaerobes) is rare (4.7%) in the United Sates [21]. Anaerobic bacteria are present in vast numbers as members of the normal bacterial flora in a variety of non-sterile bodily locations. Most infectious processes that involve anaerobic bacteria occur when the integrity of these normally colonized surfaces or mucosae is disrupted. Thus, the likelihood of anaerobic infection is directly related to the location of the infection [22].

4.1.3. Usefulness of blood culturing

Blood culturing is the most reliable method for detecting bacteremia in the clinical setting, as it can distinguish between true and pseudo-bacteremia and it can also be used to assess antimicrobial sensitivity [10]. Although multiplex polymerase chain reaction-based methods are more rapid, sensitive, and specific tools for detecting microorganisms than blood culturing, their use is limited and so clinicians still depend on established methods including blood culturing [23–27].

4.1.4. Differences in the interval from death to the autopsy

There was no difference in the length of the interval from death to the autopsy between the cases involving positive and negative blood culture samples. The mean time between death and the autopsy was 455.3 min (range: 130–1072 min) in the cases involving positive blood culture samples and 290.1 min (range: 98–11,126 min) in the cases involving negative blood culture samples. If we preserve bodies via refrigeration and perform autopsies as soon as possible, the time of the post-mortem might not influence bacterial growth [28].

4.1.5. Differences in the post-mortem blood culture sampling sites

In the clinical setting, venipuncture remains the method of choice for obtaining blood culture samples, and arterial blood cultures are not associated with higher diagnostic yields than venous blood cultures [29]. However, the volume of blood obtained for culture samples is more important for the detection of bloodstream infections. Most authors recommend that 10–30 ml of blood should be collected for culture samples in order to improve the diagnostic yield [15–17]. During post-mortem examinations, blood culture samples are obtained in a sterile manner as is the case in the clinical setting [7,8]. In addition, we are able to reduce the contamination during the puncturing procedure by acquiring blood samples from the surface of the heart or major vessels rather than by puncturing the skin. For these reasons, there are advantages to obtaining sufficient blood in a sterile manner from both ventricles or the aorta and vena cava rather than by puncturing the skin.

4.2. Distinguishing bacteremia from bacterial contamination

4.2.1. Analysis of post-mortem bacterial species

In this study, a single bacterial species was detected in pairs of blood culture samples in 12 cases. Of these cases, it was considered that those involving the following species were likely to be true cases of bacteremia: *Escherichia coli* (cases 1, 2, and 3), *Klebsiella pneumoniae* (case 4), and *Staphylococcus aureus* (case 5). It was also considered that bacteremia had probably developed in case 6 (*E. coli*), case 7 (*S. aureus*), and case 8 (glucose non-fermenting Gram-negative rod) because these cases met 2 of the 3 abovementioned criteria. A single species of Leuconostoc (case 17) and *B. thetaiotaomicron* (case 18) were also detected, but these patients could not be diagnosed with bacteremia because Leuconostoc spp. and *B. thetaiotaomicron* are obligate anaerobes and occur as part of the normal intestinal flora in humans. The pathological examination in case 17 did not obtain any evidence of an active infection or bacteria.

4.2.2. Causative bacteria detected in blood culture samples in the clinical setting

Several studies have investigated the microorganisms that are most frequently isolated from blood samples in the clinical setting [30,31]. These studies found that the following species exhibited high frequencies: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, Enterococcus spp., and *Bacteroides fragilis*. Nine species out of the top 10 are non-obligate anaerobes or aerobes (*B. fragilis* is the exception). Thus, these bacteria are also the most likely causes of bacteremia in the post-motem setting.

The optimal methodology for blood culture examinations has also been investigated in the clinical setting [32]. Weinstein used a predictive model based on multiple variables for assessing the results of examinations of blood cultures obtained from living patients [8]. As a result, it was found that microorganism identity is an independent predictor of bacteremia. Specifically, it was demonstrated that the presence of certain microorganisms, including *S. aureus*, *Streptococcus pneumoniae*, *Escherichia coli* (cases 1, 2, 3, and 6), other enterobacteria (cases 4 and 10), and *Pseudomonas aeruginosa* (case 10, which involved multiple species), is nearly always (>90% cases) indicative of bacteremia. In contrast, coagulase-negative staphylococci (case 9, 12 and 14) were found to cause particular problems during the interpretation of the results of blood culture examinations, not only because they are so ubiquitous, but also because 12–15% of staphylococci blood isolates are pathogens rather than contaminants. Some organizations have suggested that the number of positive bottles in a culture set is a predictor of the clinical significance of an isolate. It is necessary to distinguish cases of bacteremia from cases of bacterial contamination. Bacterial contamination is most commonly associated with coagulase-negative staphylococci and corynebacteria. However, these bacteria, which are found in the normal skin flora, can also cause bacteremia in immunocompromised patients. Accordingly, it was considered likely that bacteremia had developed in case 9 (*Haemophilus influenzae* and *S. epidermidis*) because the detected bacterial species might not have been of intestinal origin.

4.3. Post-mortem microbiological detection

In this study, the cases were classified into four categories based on the kinds of bacterial species detected, the patients' clinical information, and the results of pathological examinations. In some

previous studies about the interpretation of post-mortem microbiological examinations, such analyses were included in the standard autopsy protocol [1,2,4,33]. There are various reasons why bacteria might be detected in culture samples: (i) True bacteremia: in bacteremia the bacteria invade and reach the target organs or fluid while the patient is alive (cases 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10). (ii) Agonal spread: this is a theoretical concept in which bacteria invade during the agonal process or during the period in which the circulation is artificially maintained by resuscitation. (iii) Post-mortem translocation: Bacteria can grow on the mucosal surface after death and migrate even after circulation has ceased, and such growth is likely to involve a mixture of bacterial species (cases 11, 12, 13, and 16) rather than a single isolate (cases 15, 17, and 18). (iv) Contamination: The contamination rate is influenced by the anatomical site, the skill of the clinician that collects the culture samples, and the methods used (case 14). Most contamination occurs from the fluid found in body cavities and on organ surfaces. Contaminated culture samples can often be recognized because they exhibit polymicrobial growth that reflects the source of the contamination. Some microorganisms are fragile and need to be stored and handled according to strict methods [34]. *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *H. influenza* are fragile bacteria and must be preserved and transported under the most ideal conditions possible. For this reason, case 9, in which *H. influenzae* was detected, probably involved true bacteremia.

On the other hand, it is difficult to define cases as bacteremia-negative even if the tested blood culture samples are negative because multiple factors can cause false-negatives; e.g., antibiotic treatment, inappropriate blood sampling, or a limited ability to detect bacteria in blood cultures. Thus, a negative blood culture result cannot be used to exclude true bacteremia. It is also important to identify cases in which the same bacteria are detected in both ante-mortem and post-mortem blood culture samples (e.g., cases 1, 2, 4, and 5).

Finally, 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: $p = 0.018$). These results suggest that the use of paired blood culture samples aids bacterial detection. After bacteria are detected, clinicians should consider whether the bacteria were detected because of true bacteremia or post-mortem pseudo-bacteremia, based on the patient's background, clinical course, and pathological findings, and the bacterial species detected.

5. Conclusion

In both the clinical and post-mortem settings, the results of blood culture examinations need to be interpreted carefully. It has been suggested that multiple anaerobes (usually intestinal microorganisms) are detected in most cases in which bacteria spread via agonal spread or post-mortem translocation. Another useful interpretive concept is the number of culture samples that are found to be positive *versus* the number obtained. If most or all blood culture samples are positive, regardless of the microorganism recovered, the probability that the microorganism is clinically important is high. Of course, it is up to physicians and anatomical pathologists to make a final judgment in each case, taking into account not only the patient's laboratory test results, but also the patient's clinical presentation and the findings of post-mortem examinations.

Competing interests

The authors declare that they have no competing interests.

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