Ann Clin Microbiol Vol. 16, No. 1, March, 2013
http://dx.doi.org/10.5145/ACM.2013.16.1.8
ISSN 2288-0585

A Multicentre Study about Pattern and Organisms Isolated in Follow-up Blood Cultures

Jeong Hwan Shin[1], Eui Chong Kim[2], Sunjoo Kim[3], Eun-Ha Koh[3], Dong-Hyun Lee[3], Sun-Hoi Koo[4], Ji-Hyun Cho[5], Jae-Seok Kim[6], Nam Hee Ryoo[7]

[1]Department of Laboratory Medicine, Inje University College of Medicine, Busan, [2]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, [3]Department of Laboratory Medicine, Gyeongsang National University School of Medicine, Jinju, [4]Department of Laboratory Medicine, Chungnam National University College of Medicine, Daejeon, [5]Department of Laboratory Medicine, Wonkwang University Medical School, Iksan, [6]Department of Laboratory Medicine, Hallym University College of Medicine, Chuncheon, [7]Department of Laboratory Medicine, Keimyung University School of Medicine, Daegu, Korea

Background: This study analysed patterns of requests for repeated blood cultures and the microorganisms isolated in follow-up cultures.
Methods: The frequencies and intervals of repeated blood cultures performed during January and February of 2010 at seven university-affiliated hospitals in Korea were evaluated. Results of microbiological cultures at follow-up were analysed with respect to pathogen replication, immune clearance, appearance of new pathogens, and skin contaminants.
Results: Among 3,072 patients who received repeated blood cultures, the average number of requests was 3.2. Of the 5,241 follow-up blood culture events recorded, durations of 1, 2, and 3 days between cultures were identified for 23.1%, 21.4%, and 15.0% of events, respectively. Relative to each initial culture, persistent pathogen growth in subsequent culture(s) accounted for 2.3% of events, whereas immune clearance was confirmed in 8.5% of events. Previously undetected pathogens were isolated in 5.2% of the follow-up cultures, the majority of which grew after an interval of six days. Skin contaminants were detected in 7.6% of the repeated cultures, and 76.1% of the follow-ups displayed no growth of microorganisms.
Conclusion: The most common numbers of repeat culture requests were two and three, and these were typically performed within three days of the initial culture. Among the follow-up cultures, new pathogens were identified in 5.2%, and the majority of this group likely presented for follow-up during a new disease episode. **(Ann Clin Microbiol 2013;16:8-12)**

Key Words: Bacteraemia, Follow-up study, Sepsis

INTRODUCTION

Appropriate blood culture procedures are essential for the accurate diagnosis of sepsis. Factors such as the number of blood cultures, the blood sample volume, and skin disinfection prior to puncture must be optimized to ensure reliable results. Although these factors have been studied extensively among single-occurrence blood cultures, few studies have examined quality control issues associated with follow-up blood cultures. According to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), repeat cultures are generally unnecessary [1]. Follow-up cultures are indicated for: (1) patients with infective endocarditis to assess therapy, and (2) patients with sepsis caused by *Staphylococcus aureus* to predict complicated *S. aureus* bacteraemia [2]. CLSI guidelines recommend against repeating blood cultures within 2-5 days because bacteria and fungi can persist for several days following antimicrobial therapy [1]. However, it is common for physicians to request follow-up blood cultures during this interval in potential bacteraemia cases. In this study, we investigated the pattern of requests for repeated blood cultures with regard to the microorganisms identified upon follow-up culture.

Received 11 July, 2012, Revised 17 August, 2012
Accepted 17 September, 2012
Correspondence: Sunjoo Kim, Department of Laboratory Medicine, Gyeongsang National University School of Medicine, 79 Gangnam-ro, Jinju 660-702, Korea. (Tel) 82-55-750-8239, (Fax) 82-55-762-2696, (E-mail) sjkim8239@hanmail.net

MATERIALS AND METHODS

1. Participating hospitals

Patient data were collected for all cases involving multiple blood culture requests during January and February 2010 at seven

university-affiliated hospitals throughout Korea: Daegu Dongsan Hospital, Daegu; Kangdong Sacred Heart Hospital, Seoul; Inje University Busan Paik Hospital, Busan; Wonkwang University Hospital, Iksan; Gyeongsang National University Hospital, Jinju; Chungnam National University Hospital, Daejeon; and Seoul National University Hospital, Seoul. The number of cases accessed per hospital ranged from 82 to 973.

2. Analysis of repeated blood cultures

The frequencies of repeated blood culture requests and the intervals between cultures were analysed for each hospital. For the analysis of repeat culture frequency, we recorded the total number of days with follow-up request(s) during the 2-month study period. Any blood sample cultured at least 1 day after the initial culture was defined as a follow-up culture. Blood cultures requested only once also were counted to determine the proportion of multi-day requests. Any number of blood culture sets requested for one patient within a single day was regarded as one request. The frequencies of repeat blood culture requests were classified from 2 to ≥7 times. Because 46.5% of repeat culture patients underwent more than two blood cultures, the number of cases differed from the number of follow-up culture events observed. Interval data were collected for the first six blood cultures from each patient; most blood cultures (93.3%) were repeated fewer than seven times.

3. Review of microbiological culture results

Microbiological culture results were analysed for each request and were classified as: (1) persistence (*i.e.*, re-identification of the initially cultured pathogen upon follow-up culture), (2) new pathogen isolation (*i.e.*, no growth in the initial culture with new growth during follow-up culture), (3) immune clearance (*i.e.*, growth of a pathogen in the initial culture but no growth during follow-up culture), (4) growth of skin contaminants, (5) or no growth. New and persistent microorganisms were analysed in respect to intervals. When multiple isolates were collected from the same patient, the first isolate or the most clinically significant microbial species was selected for analysis. Pathogen growth was defined as the growth of microorganisms other than skin contaminants in any one set. Skin contaminants were identified by the growth of known normal flora, such as coagulase-negative staphylococci (CoNS) including *Staphylococcus epidermidis*, viridans group streptococci (VGS), *Propionibacterium acnes*, *Bacillus* spp., *Corynebacterium* spp., or *Micrococcus* spp. in only one of the requested sets [1,3].

RESULTS

1. Frequencies and intervals of repeat blood cultures

During January and February of 2010, repeat blood cultures were ordered for 21.8% (3,072/14,101) of patients from the 7 hospitals (range 3.3-44.2%). A request for 2 total cultures was most common (53.5%); 3 cultures were requested for 20.1% of patients, 4 cultures for 10.1% of patients, 5 cultures for 5.5%, 6 cultures for 4.1%, and ≥7 for 6.7% of patients. Three patients received 19 orders each for blood cultures over the 2-month study period. Among cases with 2 or more total cultures, the mean number of repeat blood cultures was 3.2. Among the 5,241 follow-up culture events analysed, the frequencies of 1-, 2-, and 3-day intervals between requests was 23.1%, 21.4%, and 15.0% respectively. The frequency of an interval of ≥7 days was 17.7%.

2. Review of positive results

Initial and follow-up blood culture results are summarised in Table 1. Among 3,072 patients who submitted follow-up cultures, pathogen growth was observed in 16.0%. Persistent growth of previously identified pathogens accounted for 2.3% of cases. The occurrence of new pathogens during follow-up was confirmed for 5.2% of patients, and immune clearance of patho-

Table 1. Summary of initial and follow-up blood culture results (%) by hospital

Initial/follow-up culture	Hospitals							
	A (82)*	B (279)	C (334)	D (424)	E (447)	F (533)	G (973)	Total (3,072)
Growth†/growth	7.3	2.2	1.2	2.6	1.3	3.0	3.1	2.3 (70)
No growth/growth	14.6	5.0	4.5	2.1	4.5	4.7	6.8	5.2 (161)
Growth/no growth	12.2	7.2	4.8	5.7	11.0	6.0	11.3	8.5 (261)
Contaminants‡	13.4	11.8	2.1	5.0	7.4	8.6	8.3	7.6 (232)
No growth/no growth	52.5	73.8	87.4	84.6	75.8	77.7	70.5	76.4 (2,348)

*Number of patients; †Detection of pathogens; ‡Normal skin flora detected in only one set.

gens upon follow-up was observed for 8.5%. Contaminant growth was identified for 7.6% of patients, and no microbial growth was reported for 76.4% of cases.

Among the 70 patients who persistently yielded pathogens in the initial and follow-up cultures, *S. aureus* was most commonly detected (22.9%) followed by *Escherichia coli* (15.7%), *Klebsiella pneumoniae* and *Enterococcus faecalis* (both 10.0%), and *Candida* spp. (7.1%) (Table 2). Thirty-four (48.6%) and 14 (20.0%) patients harbouring persistent colonization submitted to repeated cultures 1 or 2 days, respectively, after the initial culture. Culture intervals exceeding 6 days occurred in only 5.7% of patients in this group.

Among 161 patients for whom microorganisms were detected only upon follow-up culture(s), *S. aureus* (17.4%) was most commonly identified followed by *Acinetobacter baumannii* (8.9%), *K. pneumoniae* (8.1%), *E. coli* (6.8%), and *Pseudomonas aeruginosa* and *C. albicans* (both 5.6%) (Table 3). Twenty-three (14.3%) and 31 (19.3%) patients in this subgroup received follow-up culture requests within 1 or 2 days, respectively. The proportions of newly isolated pathogens appearing within a 2-day follow-up interval were considerable, particularly for *E. coli, P. aeruginosa,* and *E. faecium.* Follow-up intervals often exceeded 6 days in this subgroup (40.4%).

Among 232 skin contaminants detected, *S. epidermidis* was the most common (41.8%) followed by other CoNS (37.9%), *Bacillus* spp. (5.6%), VGS (5.2%), *P. acnes* (3.9%), *Corynebacterium* spp. (3.5%), and *Micrococcus* spp. (2.2%).

DISCUSSION

In practice, blood cultures return a low positivity rate because the detection of bacteraemia is difficult. CLSI guidelines state that an optimal positivity range is 6-12% [1], meaning that approximately 90% of culture requests will return negative results. Typically, physicians order a blood culture when their patients have chills, fever, hypotension, shock, neutrophilia, leucopoenia, an increase of band forms, pneumonia, meningitis, cellulitis, and/or other serious infections [3]. However, specific standards regarding when to order blood cultures are unavailable.

Few published reports have investigated the patterns of follow-up blood cultures. Grace et al. [4] examined 139 hospitalised patients receiving antibiotic therapy who submitted to fol-

Table 2. Culture intervals for patients harboring persistently growing microorganisms

Microorganism	Interval days					
	1	2	3	4-6	7	Total (%)
Staphylococcus aureus	7	3	2	3	1	16 (22.9)
Eschericia coli	8	1	1	1		11 (15.7)
Klebsiella pneumoniae	4	1	1		1	7 (10.0)
Enterococcus faecalis	2	5				7 (10.0)
Candida spp.	1		2	2		5 (7.1)
Other Gram (+) bacteria	6	2	1	1	2	12 (17.1)
Other Gram (−) bacteria	6	2	4			12 (17.1)
Total (%)	34 (48.6)	14 (20.0)	11 (15.7)	7 (10.0)	4 (5.7)	70 (100)

Table 3. Intervals between blood cultures for cases in which pathogens were identified only upon follow-up culture

Microorganism	Interval days							
	1	2	3	4	5	6	7	Total (%)
Staphylococcus aureus	4	4	3	2	4	1	10	28 (17.4)
Acinetobacter baumannii	2	1	2	2	1	1	5	14 (8.9)
Klebsiella pneumoniae		2		1	1	1	8	13 (8.1)
Eschericia coli	3	1			1	2	4	11 (6.8)
Pseudomonas aeruginosa	1	4					4	9 (5.6)
Candida albicans		1	1			3	4	9 (5.6)
Enterococcus faecium	2	2	1				3	8 (5.0)
Other Gram (+) bacteria	7	8	3	5	3	1	13	38 (23.6)
Other Gram (−) bacteria	1	6	1	1	3		10	22 (13.7)
Non-albicans *Candida*	2			1			4	7 (4.3)
Total (%)	23 (14.3)	31 (19.3)	11 (6.8)	12 (7.5)	10 (6.2)	9 (5.6)	65 (40.4)	161 (100)

low-up culture(s) within 72 hours. These authors reported that repeated culture yielded a new pathogen in only 1 patient. They recommended that physicians wait for the results of the initial blood culture before they order any additional cultures. These authors reported that 15-20% of patients harboured persistently growing pathogens throughout the 72-hour antibiotic treatment period. Staphylococci (76%) or streptococci (36%) were isolated significantly more often than Gram-negative bacilli (12%) during follow-up culture in their study [4]. Conversely, we detected persistent growth of the same pathogen in only 2.3% of patients in our study. Among the persistently growing bacteria, *S. aureus*, *E. coli, K. pneumoniae*, and *E. faecalis* were the most common pathogens we identified. Notably, follow-up intervals typically ranged from 1 to 3 days (84.3%) for patients harbouring persistent microorganisms, suggesting the same episode. This subgroup is unlikely to yield relevant clinical information for the management of sepsis patients [1].

The chance of a new disease episode caused by a different microbial species is low following the initiation of empirical antibiotic treatment. In our study, the isolation of a new pathogen during follow-up culture was observed in 5.2% of patients. This finding suggests either the occurrence of a new episode or a procedural failure to detect the infecting organism during the initial culture. This proportion might be regarded as the most meaningful cohort for the utilisation of follow-up cultures. A substantial proportion (40.4%) of patients in this group submitted to follow-up blood cultures after more than 6 days, suggesting a new disease episode.

A previous small-scale study reported that 48% (12/25 occasions) of cases exhibited immune clearance after 3 days of antibiotic treatment [5]. In contrast, pathogen clearance was confirmed in only 8.5% of patients in our large-scale study. Skin contaminants grew in 7.6% of our analysed cultures; this value is slightly higher than in previous reports [6] likely because all isolates were included regardless of follow-up interval. The CLSI guidelines suggest a $<3\%$ rate of skin contamination for blood cultures [1]. Among the skin contaminants we observed, *S. epidermidis* and other CoNS were predominant (79.7%). These results are consistent with those of previous reports [6].

From the time that a positive signal is observed in a blood culture, a minimum of 2 days are required to obtain the results of antibiotic susceptibility testing and to identify the organism. Physicians should wait at least 3-4 days to receive a complete report before requesting a follow-up blood culture. We determined that 59.5% of cultures were repeated within 3 days. The potential exists for a new episode of sepsis when a follow-up culture is requested after a longer time period (*e.g.*, 1 week). The proportion of follow-up cultures ordered after 6 days accounted for 17.7% of cases in our study. A majority of cultures (76.1%) showed no growth of microorganisms at any time. Many of these patients may not have been truly septic. Considering the substantial amount of blood drawn for culture, the cost of bottles, the time consumed by medical personnel, and the pain of needle insertion, physicians should be judicious about ordering follow-up blood cultures.

Patients with fungaemia may undergo repeat cultures to document clearance of the organism [3]. Persistent fungaemia may indicate a deep-seated infection that may require surgical intervention. However, our study confirmed fungaemia in only nine patients (0.3%) upon initial culture, supporting that this is a very rare event.

This study is associated with several limitations. The authors did not review patient medical records because too many subjects were included in the study. The reasons or clinical conditions leading to follow-up culture requests were beyond the scope of this study. We limited our analysis to the microbiological data from blood cultures that we retrieved via electronic medical records. Finally, the numbers of cases differed widely by hospital; this may have caused a bias.

The present multi-centre analysis of repeated blood culture requests confirmed that most follow-up cultures were ordered within 3 days and were repeated, on average, 3.3 times. Persistent growth was observed in 2.3% of cases, and the intervals between cultures were typically 1-2 days, suggesting that they corresponded to the same episode of sepsis. The most microbiologically relevant finding corresponded to patients who presented with a new pathogen upon repeat culture. This was observed in 5.2% of cases, and a majority of these seemed to stem from a new sepsis episode because the intervals between cultures often exceeded 6 days (40.4%). Follow-up culture was beneficial to detect a new episode of sepsis, but optimisations of extended between-culture intervals and overall frequencies of repeat cultures are warranted. Persistent growth of pathogens, suggested by CLSI guidelines, did not correspond to a substantial proportion of patients undergoing follow-up culture in our study.

ACKNOWLEDGMENTS

This work was supported by Grant from Inje University, 2009.

REFERENCES

1. Clinical and Laboratory Standards Institute. ed. Principles and Procedures for Blood Cultures; Approved Guideline. Document M47-A. Wayne, PA; Clinical and Laboratory Standards Institute, 2007.
2. Fowler VG Jr, Olsen MK, Corey GR, Woods CW, Cabell CH, Reller LB, et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. Arch Intern Med 2003;163:2066-72.
3. Mylotte JM and Tayara A. Blood cultures: clinical aspects and controversies. Eur J Clin Microbiol Infect Dis 2000;19:157-63.
4. Grace CJ, Lieberman J, Pierce K, Littenberg B. Usefulness of blood culture for hospitalized patients who are receiving antibiotic therapy. Clin Infect Dis 2001;32:1651-5.
5. Sarkar S, Bhagat I, Wiswell TE, Spitzer AR. Role of multiple site blood cultures to document the clearance of bacteremia in neonates. J Perinatol 2007;27:101-2.
6. Hall KK and Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev 2006;19:788-802.

=국문초록=

반복 혈액배양의 패턴과 미생물배양결과에 대한 다기관 연구

[1]인제대학교 의과대학 진단검사의학교실, [2]서울대학교 의과대학 진단검사의학교실, [3]경상대학교 의과대학 진단검사의학교실, [4]충남대학교 의과대학 진단검사의학교실, [5]원광대학교 의과대학 진단검사의학교실, [6]한림대학교 의과대학 진단검사의학교실, [7]계명대학교 의과대학 진단검사의학교실

신정환[1], 김의종[2], 김선주[3], 고은하[3], 이동현[3], 구선회[4], 조지현[5], 김재석[6], 류남희[7]

배경: 본 연구에서는 반복 혈액배양의 의뢰 패턴과 반복 혈액배양에서 검출되는 미생물 결과에 대해 분석하였다.

방법: 국내 7개 대학병원에서 2010년 1-2월 사이에 한 환자에서 반복해서 의뢰된 혈액배양을 대상으로 의뢰 빈도와 의뢰 간격을 조사하였다. 미생물 반복 배양 결과를 지속적 감염, 감염원 치료, 새로운 감염원 검출 및 피부 오염균으로 분류하였다.

결과: 연구기간 동안에 3,072명의 환자로부터 혈액배양이 중복 의뢰되었으며, 평균 중복 배양 횟수는 3.2회였다. 반복 배양된 5,241건을 조사한 결과 배양간격이 1일, 2일 및 3일인 것이 각각 23.1%, 21.4% 및 15.0%를 차지하였다. 초기검사와 후속검사에서 지속적으로 균이 분리된 것은 2.3%, 후속검사에서만 배양된 것이 5.2%, 초기에는 양성이었지만 후속검사에서 음성으로 전환된 것이 8.5%였다. 초기와 후속검사를 모두 포함하여 오염률은 7.6%였으며, 76.1%는 지속적으로 음성이었다.

결론: 다기관의 반복 혈액배양의 패턴을 분석하였는데 2-3회 반복하는 경우가 가장 많았고, 의뢰 간격은 3일 이내가 대부분이었다. 후속검사에서만 배양되는 것이 5.2%로서, 이들 대부분은 새로운 균혈증으로 판단되었다. [Ann Clin Microbiol 2013;16:8-12]

교신저자 : 김선주, 660-702, 경남 진주시 강남로 79
경상대학교 의과대학 진단검사의학교실
Tel: 055-750-8239, Fax: 055-762-2696
E-mail: sjkim8239@hanmail.net