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Note

False-positive blood culture results in patients with hematologic malignancies☆

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ABSTRACT

Blood cultures are the most valuable tool when bacteremia is clinically suspected. Technical advances have led to the development of automated blood culture systems to detect bacterial infections. Usually positive signals in automated blood culture systems result from the proliferation of microorganisms. Cases are classified as false-positive when the automated blood culture system produces a positive signal but no microorganisms are detected on Gram-stained smears and no microorganism growth is observed in blood subcultures. False-positive blood culture results are very rare in patients with hematologic malignancies. Recently, we encountered four patients who had false-positive blood culture results. Two of the patients were diagnosed with acute leukemia, involving hyperleukocytosis and an excess of blasts. The other two patients were diagnosed with acute leukemia and diffuse large B cell lymphoma with leukocytopenia. Although hypercapnia or acidosis, apart from hyperleukocytosis, might also cause false-positive results, our cases clearly did not have these conditions. We should be aware of the possibility that false-positive blood culture results can occur in patients with leukocytopenia, as well as hyperleukocytosis. To understand the mechanisms responsible for the observed false-positive results, additional studies are needed after the accumulation of similar cases.

Bacteremia is one of the most dangerous complications affecting patients with hematologic malignancies who are treated with chemotherapy [1]. Although various biomarkers have been used to detect bacteremia, blood cultures are routinely used to diagnose bacteremia and are the most valuable tool when bacteremia is clinically suspected [2]. Technical advances have led to the development of automated blood culture systems, which has reduced the time required to detect bacterial infections. The BACTEC FX (Becton Dickinson, Inc.) is one such automated blood culture system. An increase in the concentration of carbon dioxide (CO_2) in culture media due to proliferating organisms causes a change in pH, resulting in the blood culture bottle being flagged as positive [3]. Usually, a positive signal indicates the presence of CO_2-producing microorganisms. However, the combination of a positive signal produced by the BACTEC FX, no microorganisms detected in Gram-stained smears, and no microorganism growth observed in blood subcultures is classified as false-positive blood culture results, which are different from blood culture sample contamination [4,5]. False-positive blood culture result are very rare in patients with hematologic malignancies. Recently, we encountered four patients with false-positive blood culture results.

The patients' profiles are shown in Table 1. Two patients were diagnosed with acute leukemia, involving hyperleukocytosis and an excess of blasts. The other two patients were diagnosed with acute leukemia and diffuse large B cell lymphoma (DLBCL), respectively. They had leukocytopenia due to disease progression or chemotherapy.

All of the patients had high fevers just before their blood culture samples were drawn (on the day of admission in all cases, except

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Table 1
Patient characteristics.

Patient #	1	2	3	4
Sex	M	M	M	F
Age (y)	26	42	78	35
Diagnosis	AML (M3)	AML (M1)	DLBCL	***Mixed leukemia
Blood culture taken	On admission	On admission	Day 9 after Tx[b]	On admission
Temperature (°C)	39.3	37.9	38.5	38.6
SpO_2 on room air (%)	98	98	98	99
Pulse rate (beats/minute)	107	83	89	88
Blood pressure (mmHg)	140/83	123/69	97/60	91/58
WBC count ($x10^6$/L)	83,990	167,800	120	1460
Blasts (%)	90.0[a]	96.0	0	0
Neutrophils (%)	2.0	2.0	6.7	1.0
Monocytes (%)	0	0	13.3	0
Lymphocytes (%)	4.0	1.0	76.7	99.0
Hb level (g/dL)	7.7	9.5	9.4	3.3
Platelet count ($\times 10^9$/L)	22	35	42	19
CRP level (mg/mL)	3.27	14.306	13.796	9.01
Antibiotics before blood culture	N	N	N	N
G-CSF treatment	N	N	Y	N
Duration of culture until positive result (h)	33	16	72	63

Abbreviations: M, male; F, female; AML, acute myeloid leukemia; DLBCL, diffuse large B cell lymphoma; Tx, treatment; CRP, C-reactive protein; SpO_2, blood oxygen saturation; WBC, white blood cell; Hb, hemoglobin; G-CSF, granulocyte-colony-stimulating factor; N, no; Y, yes.

[a] Blasts included 10% blasts and 80% promyelocytes.

[b] In case 3, blood cultures were drawn 9 days after chemotherapy (Tx) with R-CHOP (rituximab, cyclophosphamide, vincristine, and prednisolone). ***This case was diagnosed as mixed phenotype acute leukemia, B/myeloid, not otherwise specified.

case 3). They were not given antibiotic therapy before the samples were obtained. Blood culture samples were collected from different access points using two sets of culture bottles (BD BACTEC plus Aerobic/F and Anaerobic/F; BD) at the bedside, before being transported to a clinical laboratory. In the cases described here, the culture bottles were incubated in the BACTEC FX. The culture bottles were flagged as positive after incubation, which was suggestive of bacteremia. A positive signal was found in one of four bottles in every patient. Positive signals occurred in aerobic bottles (cases 1–3) and in an anaerobic bottle (case 4). To confirm the presence of microorganisms, Gram staining and subculturing were performed, but no microorganisms were found in the Gram-stained smears, and no microorganism growth was detected in the subcultures. These findings indicated that the blood culture results should be classified as false positives. In cases 1 and 2, marked hyperleukocytosis involving leukemic cells was observed. In cases 3 and 4, one the other hand, white blood cell (WBC) counts showed leukocytopenia, and the patient in case 3 had agranulocytosis, which is a high-risk factor for severe bacterial infections. All of our patients exhibited normal renal function and peripheral oxygen saturation (SpO_2) levels of 98–99% on room air without worsening of respiratory rate, indicating that neither obvious hypercapnia nor acidosis was present.

There were no technical errors related to the sample handing, for example, 1) the correct amount of blood was injected into the bottles, 2) the time period between sample collection and incubation was appropriate, and 3) the BACTEC FX did not malfunction during the incubation procedure.

The frequency of false-positive results in blood cultures involving the BACTEC system is reported to range from 1.4% to 6.2%, while the frequency of false-negative results is reported to be 0.2% [6]. In our hospital, the frequency of false-positive blood culture results was 0.1% in the past 2 years. False-positive blood culture results were very rare not only in patients with hematologic malignancies but also in patients with other diseases.

More than two sets of blood culture bottles were recommended to increase the accuracy and precision of results. A positive result in one out of four bottles usually occurs in the case of contamination. We did not detect any microorganisms in gram staining and subculturing.

Administration of antibiotic therapy prior to the drawing of blood samples has been reported to decrease microorganism detection rates in blood cultures [7]. As mentioned above, our patients were not given antibiotic therapy before the samples were obtained. Eubacterial and panfungal polymerase chain reactions (PCR) can be used to confirm the presence of microorganisms [4,8]. Samples that produced false-positive results when tested with the BACTEC system have been found to produce negative results in these PCR assays [8]. In our experience, when false positives occur, the positive signals emerge earlier than in cases of true bacteremia, usually within a few hours. The median time for positive signals to emerge in cases of true bacteremia has been reported as 12.72 h, and has been reported as 40 h for candida species [9]. In our cases, patients with leukocytosis showed the emergence of positive signals earlier than patients with leukopenia. In cases 3 and 4, however, the positive signals appeared after 2–3 days of incubation. These results indicate that the mechanism underlying the positive signals might be different in patients with leukocytosis versus leukopenia.

Three cases of hematological malignancies have been reported in which false-positive blood culture results were obtained [10–12]. All of these patients were diagnosed with acute myeloid leukemia (AML; the types of AML were not mentioned) with an excess of blasts. In our cases, the types of hematologic malignancy differed among the cases (Table 1). Some reports have stated that hyperleukocytosis leads to increased CO_2 production in culture media, resulting in a pH change (acidosis) [10,12].

Besides hyperleukocytosis, hypercapnia or acidosis might also cause false-positive results. As reported previously [10–12], the marked proliferation of leukemic cells can produce an increase in the concentration of CO_2 in culture bottles, inducing a positive signal. In case 3, granulocyte-colony-stimulating factor (G-CSF) was administered daily from 5 days before the blood culture sampling because of a reduction in the patient's WBC count caused by the administration of R-CHOP (rituximab, cyclophosphamide, vincristine, and prednisolone) chemotherapy against DLBCL. At 4 days

after the blood culture sampling, the patient's WBC count had reached 5×10^9/L due to his recovery from bone marrow (BM) suppression. The immature cells in the BM were mobilized into the peripheral blood by G-CSF during the recovery from BM suppression. It is possible that immature cells were inadvertently included in the WBC count as monocytes. These immature cells in culture bottles could have been activated via the glycolytic pathway by hypoxia inducible factor (HIF) [13]. In this process, lactate would accumulate in the cells and be released into the extracellular media, which would induce a change of pH in the culture bottles, resulting in their being flagged as positive. In case 4, the patient's WBC count was low, but 99% of the WBCs were morphologically classified as lymphocytes. BM aspiration revealed a normocellular BM with a leukemic cell frequency of 59.3%. Although we most of the cells morphologically in the peripheral blood sample as lymphocytes in case 4 (Table 1), these cells were likely leukemic cells (we did not analyze the origin of these cells in the peripheral blood, but the patient was diagnosed as having mixed phenotypic acute leukemia, B/myeloid, not otherwise specified, in which leukemic cells may morphologically resemble lymphocytes). These cells might transiently proliferate, and then a change in the concentration of CO_2 or activation of HIF via the glycolytic pathway could lead to a release of lactate into the extracellular media [14], resulting in the blood culture bottle being flagged as positive. The speed of lactate release or CO_2 production in culture bottles might contribute to the time lag of emerging positive signals. Although we could not absolutely rule out latent hypercapnia or acidosis, our patients' status did not necessitate blood gas analysis when blood cultures were drawn.

Our experience indicated that we should be aware of the possibility that false-positive blood culture results may occur in the patients with leukocytopenia, as well as hyperleukocytosis. False-positive blood culture results can affect treatment and patient care. For example, unnecessary antibiotics might be administered. To understand the mechanisms responsible for the observed false-positive results, a further study is needed after the accumulation of similar cases, especially in patients with leukocytopenia.

Authors' contributions

YE, KK, and KI designed the research; TM, NT, and NA were responsible for the patient care; YE, KK, NW, YT, and AI analyzed the data; and YE, KK, and KI wrote the manuscript.

Conflicts of interest

The authors declare that no conflicts of interest exist.

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