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ORIGINAL ARTICLE

Combinational approach using *in situ* hybridization targeting 23S ribosomal RNA genes and blood cultures for bacterial identification in patients with neutropenia and fever

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Abstract

Background: A new 23S ribosomal RNA genes-targeted *in situ* hybridization (ISH) probe to detect global bacterial genomic DNA (59 species from 35 genera; referred to as the GB probe) phagocytized in leukocytes was recently developed. This method provided early and direct evidence of bacterial infection with high sensitivity and specificity in spontaneous bacterial peritonitis ascites. However, the utility of this method in febrile neutropenia (FN) is unknown.

Methods: We prospectively evaluated the utility of the ISH approach using the GB probe and previously reported probes in patients with neutropenia and fever undergoing chemotherapy at our institution between June 2011 and July 2013. Blood samples for culture analysis and ISH tests were collected simultaneously at the onset of fever; the latter were performed repeatedly.

Results: Fifty febrile episodes were evaluated. In 24 episodes of fever of unknown origin and 15 episodes of local infection (all negative for blood cultures), ISH tests identified causal bacteria in 21% and 13% of cases, respectively, at the onset of fever. In seven sepsis cases (all positive for blood culture), positive ISH test results at fever onset were achieved in 71%; for two patients with neutrophil counts of 0/μl and 171/μl, respectively, negative results were obtained.

Conclusions: This new ISH approach could prove useful for early detection of bacteria in patients with neutropenia and blood culture-negative, with fever of unknown etiology after chemotherapy. Using this method in combination with blood culture, even in cases with extremely low neutrophil counts, might contribute to better management of FN.

Key words: febrile neutropenia; bacterial identification; *in situ* hybridization; 23S ribosomal RNA genes; blood cultures

Introduction

Despite recent advances in preventive intervention for infections, febrile neutropenia (FN) remains a common and important complication during intensive chemotherapy in patients with cancer, and particularly those with hematological disorders. Due to poor positive rates of the diagnostic gold standard (blood cultures with approximately 20 % [1]), a fever-driven approach using anti-pseudomonal β-lactam agents is widely accepted in the management of FN [2]. However, this method results in overuse of antibiotics, increased side effects or costs, and induction of drug-resistance in bacteria. Optimizing FN management requires the identification of causal bacteria, which remains very challenging.

Several available diagnostic tools such as the detection of serum biomarkers (e.g. procalcitonin (PCT), interleukin (IL)-6, and IL-8) [3–5], polymerase chain reaction (PCR) [6,7], or mass spectrometry [8,9] could improve the management of FN. Although PCR analysis and mass spectrometry have the advantage of obtaining direct evidence of bacteria, the former could be susceptible to contamination with bacterial DNA [10–14], whereas the latter demonstrates insufficient diagnostic accuracy. However, both methods might be useful for identifying causal bacteria from positive blood culture samples [7–9]. However, a strategy based on these tools has not been standardized for the management of FN.

Recently, Enomoto et al. reported a new *in situ* hybridization (ISH) method that detects global bacterial DNA (59 species of 35 genera) in leukocytes, and achieved positive results in 10 of 11 ascites samples from patients with spontaneous bacterial peritonitis (SBP); all other (40) ascites samples from non-SBP patients were negative, demonstrating high sensitivity (91%) and specificity (100%) [15]. Notably, ISH tests were positive in seven SBP patients with negative

culture results, and these tests were effective in samples with low concentrations of leukocytes (100/μl). In addition, ISH test results were obtained within one day, which is consistent with those observed for septic blood samples. These data suggest that this test could also be useful for obtaining direct evidence of bacteria in patients with FN. To our knowledge, the utility of this method in FN has not been investigated.

Therefore, we prospectively examined the utility of this new ISH method, together with previous methods [16–18], in patients with hematological disorders who developed neutropenia and fever after chemotherapy. We also evaluated if serum biomarkers such as PCT, IL-6, and IL-8 could provide additional insight for detection of bacterial infections.

Patients and methods

Study design

We conducted a prospective, single-center, observational study to investigate the utility of the ISH method, using the new global bacteria (GB) probe as well as previous ISH probes, (SA, SE, PA, EF and EK, Table 1) for detecting bacterial infections in patients with neutropenia and fever at our institution between June 2011 and July 2013. In addition, we assessed the utility of measuring serum PCT and 27 serum cytokines and chemokines (IL-1β, IL-1 receptor antagonist, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, Eotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, MIP-1β, PDGF-ββ, RANTES, TNF-α, and VEGF) in patients with available samples (Supplementary Table 1 and 2).

This study was approved by the Human Subjects Review Committee at Osaka City University and we obtained signed informed consent from all patients in agreement with the Declaration of Helsinki.

Study subjects and sample collection

The criteria for inclusion in the present study were as follows: patients who received chemotherapy, including a conditioning regimen for hematopoietic cell transplantation; patients who developed neutropenia and fever; patients who provided written informed consent. Neutropenia was defined as a neutrophil count of less than 500/μl or exceeding 500/μl with an expected decline to below 500/μl. Fever was defined as an axillary temperature of ≥ 37.5 °C based on a single record from previous reports [19,20]. Blood samples for culture analysis and ISH tests were collected simultaneously at the onset of fever using aseptic technique. To repeatedly follow the ISH tests and examine the kinetics of serum cytokines and chemokines, the protocol was modified in January 2012. After that date, we performed the ISH tests and analyses of serum cytokines and chemokines at the time of neutrophil recovery and completion of antibiotics, in addition to the time of fever onset. If the result of the last follow-up ISH test was positive, additional ISH tests were recommended. Serum levels of PCT were also measured.

Clinical evaluation and definitions for causes of febrile episodes

As a rule, two sets of blood cultures were taken at the onset of neutropenic fever. Evaluations were conducted including physical examinations, blood tests, cultures of samples from suspected sites of infection, and imaging including computed tomography. Clinical efficacy was defined as defervescence for at least 48 hours, which was also assessed at 72 hours, at day 7, and at the completion of intravenous antibiotic therapy, regardless of the addition of other antibiotics or antifungals, or changes in antibiotics used.

We defined the diagnostic categories of fever of unknown origin (FUO), local infection, and

sepsis according to the modified criteria applicable in patients with FN [19–21]. Bacteremia by coagulase negative staphylococcus (CNS) was identified after obtaining at least two isolations of CNS with identical antibiograms, taken from different sites [3]. Drug fever was defined as previously reported [24]. Classification of febrile episodes was reviewed by two independent investigators and a final diagnosis was determined.

Detection of bacterial DNA by ISH using the GB probe and previous ISH probes (SA, SE, PA, EF, and EK)

All bacteria have 23S ribosomal RNA (rRNA) genes; therefore, a novel cDNA probe using the 23S rRNA genes, "GB probe" was developed to detect genomic DNA of causal bacteria. The GB probe consisted of plural cDNA fragments corresponding to the 23S rRNA genes of various bacteria, and successfully detected the genomic DNA of 59 bacterial species, belonging to 35 genera, that were tested (however, the GB probe could have the potential to detect other bacterial species) [10,15]. All bacterial species detectable by SA, SE, PA, EF and EK probes are included in the GB probe (Table 1). The details of ISH methods for detection of bacterial DNA have been described previously [10,15]. Briefly, 10 ml of blood sample was aseptically drawn into a heparinized tube. Red blood cells were sedimented, and the supernatant (white blood cells) was collected. The white blood cells were then pelleted by centrifugation using phosphate buffered saline (PBS). Finally, the collected cell pellet was suspended at a concentration of 1–5 × 10^4 cells/μl in PBS and as a rule, 5–10 μl of the cellular suspension was smeared on the glass slides. Digoxigenin-labeled probes were used for hybridization, and positive (intra-cellular purple-brown) signals were detected with nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. All assessments were performed by MIROKU Medical Laboratory Co., Ltd.,

independent of our institution.

Measurement of serum biomarkers

Serum levels of PCT and cytokines/chemokines were measured by an electrochemiluminescence immunoassay and the Bio-Plex Pro Cytokine Assay® system (Bio-Rad Laboratories, CA), respectively. Cut-off values of PCT and cytokines/chemokines, to determine positive results, were set at 0.5 ng/ml and those according to the manufacturer's instruction [3,4,25], respectively.

Statistical analysis

For analysis of cytokines/chemokines kinetics, a paired t-test (at onset vs. at neutrophil recovery, or at onset vs. at the end of therapy) was applied. To evaluate the diagnostic performance of each variable, we used receiver operating characteristic (ROC) curves. All *P* values and 95% CIs were determined by two-tailed tests, and a *P* value of less than 0.05 was considered statistically significant. All statistical analyses were performed using IBM® SPSS® Statistics, version 22.0 and Graph Pad Prism® version 5.02 (Graph Pad Software Inc., San Diego, CA, USA).

Results

In 34 patients, 51 febrile events were examined in the study. Of these, we excluded one non-neutropenic fever and thus 50 febrile episodes were evaluated. The details of study participants and febrile episodes are shown in Table 2. Of the final diagnostic categories, approximately half included FUO and approximately 40% included clinically probable infections

such as local infection. Local infections included six dental infections, three anal infections, one stomatitis, one pharyngitis, one colitis, one appendicitis, one cystitis, and one genital infection.

The results of the ISH tests, blood cultures, and serum PCT tests according to diagnostic category, are shown in Tables 3 and 4. The ISH tests were evaluated at the onset of fever in all 50 episodes. Those at neutrophil recovery were performed in 42 episodes and those at end of intravenous antibiotic therapy were performed in 21 episodes, because the timing of neutrophil recovery and the end of therapy were simultaneous in 21 episodes. Based on all 50 episodes, the positive ratio for this ISH test at fever onset was 36% (11/31) in the group with an absolute neutrophil count (ANC) less than 100/μL, 0% (0/12) in the group with an ANC of 100–500/μL, and 14% (1/7) in the group with an ANC of greater than 500/μL. In seven sepsis cases (all positive for blood cultures), positive results based on ISH and PCT tests at fever onset were observed in 71% and 43% of cases, respectively. In 15 cases of local infection, ISH and PCT positivity rates at fever onset were 13 % and 25%, respectively, and in 24 cases of FUO, ISH and PCT positivity rates at fever onset were 21% and 4%, respectively; all cases were negative for blood culture. For ISH tests performed at the end of therapy or for additional evaluations, positive results were obtained in approximately 20–50% of cases. Of these 12 cases with positive results, invasive procedures were performed in six cases, one to several days before the ISH tests, and included bone marrow aspiration (n = 4) and insertion of a central venous catheter (n = 2).

In 36 patients with available blood samples, we investigated the serum level kinetics of 27 cytokines and chemokines during follow-up of febrile episodes and calculated the area under the curve (AUC) by employing receiver operating characteristic (ROC) analyses for these values for the detection of sepsis or bacterial infection (Supplementary Table 1 and 2). With reference to an AUC of 0.7 [26], we chose five markers including IL-6 (AUC of 0.789 for sepsis; 0.594 for

bacterial infection), IL-8 (0.844; 0.651), MCP-1 (0.703; 0.600), MIP-1α (0.602; 0.702), and MIP-1β (0.898; 0.660) (Supplementary Table 2). Based on these analyses, we examined if these five markers could provide additional information, to results obtained from ISH tests, for diagnosing or monitoring bacterial infections in patients with local infection or FUO (Table 5). Information from both the ISH tests and kinetic analysis of IL-6 levels corresponded to clinical course in several patients (Table 5 and Figure). In addition, for each local infection (n = 11) and FUO (n = 17) groups, most of these biomarker levels (including 95% CI) of the patients with negative results based on the ISH tests at the onset of febrile episodes were higher than those of each patient with positive result (Table 5).

Discussion

In the present study, we demonstrated that the new ISH test that uses a GB probe provides early direct evidence of bacterial infections in patients with neutropenia who are blood culture-negative, with fever of unknown etiology after chemotherapy. In addition, we found that ISH tests, using both GB and previously reported probes, detected approximately 20% blood culture-undetectable bacteremia of the causes of FUO.

PCR-based analysis [6,7] or mass spectrometry [8,9] is useful for identifying causal bacteria from samples such as positive blood cultures. However, particularly for PCR, there are disadvantages such as contamination of bacterial DNA (e.g. Taq-polymerases or reagents for DNA extraction procedures) [10–14], indicating that these methods require further standardization. In addition, serum biomarkers including PCT and IL-6 might not be useful in patients with FN, compared to those in non-neutropenic sepsis patients, because the levels of these markers could trend lower in neutropenic patients [27], probably due to

chemotherapy-induced cytopenia. In fact, we observed that the levels of cytokines and chemokines such as IL-6 and IL-8 were elevated in patients with negative ISH test results at the onset of febrile episodes, compared to those in patients with positive results (Table 5). Therefore, detection of cytokines or chemokines as a single marker was not useful for detecting bacterial infections. However, the kinetics of serum IL-6 levels from the onset of the febrile episode to the end of therapy appeared to reflect infection activity in each individual case (Table 5 and Figure 1). Thus, the combined use of ISH methods and IL-6 determination could be more useful for FN management.

Several clinical studies have reported excellent diagnostic performance using ISH methods with high sensitivity and specificity [15,17,18]. Two previous clinical studies examined the utility of the ISH method for diagnosis of sepsis, and showed that the ISH method was four times more sensitive than blood culturing for the detection of causal bacteria [17,18]. In addition, these studies demonstrated that the ISH method and blood culturing identified the same causative bacteria. Recently, Enomoto et al. reported that this ISH method, with new and existing probes, could be used to obtain early direct evidence of causal bacteria from ascites samples with high sensitivity (91%) and specificity (100%) in patients with SBP (there were no false positive results in 40 non-SBP samples) [15]. This excellent specificity data, that is false positive rate of zero, could be explained by the fact that the ISH method detects the genomic DNA of bacteria phagocytized by neutrophils and macrophages and existing only in these cells.

In contrast, our data showed that the species of bacteria isolated from blood cultures did not necessarily correspond to the results of the ISH tests (Table 4). This suggests some important facts. Considering the previously mentioned excellent diagnostic performance of ISH tests, the positive ISH results in this study were most likely true positives. More specifically, the ISH tests

provided information on multiple bacterial infections in patients with FN (Table 4). It was previously reported that this ISH method was effective with low leukocyte counts (100/μl) [15,17,18]. However, two patients diagnosed with sepsis with neutrophil counts of 0/μl and 171/μl, showed negative results based on ISH tests (Table 4). Therefore, low neutrophil counts can decrease the sensitivity of ISH tests. In addition, the ability of this method to detect causal bacteria depends on the phagocytic activity of leukocytes [15]. Thus, inconsistent results might be caused by low neutrophil counts or decreased phagocytic activity of leukocytes because of chemotherapy. Another important point is that the diagnostic value of the ISH method needs to be assessed in the clinical context of the patient [28]; from this viewpoint, the results obtained herein seem reasonable (Table 4, Figure 1). Therefore, it should be considered that both blood culture and ISH test results could be true in patients with FN. However, the exact diagnostic performance including the rate of false positivity for the ISH test has not been established in FN. Further large-scale study is warranted to confirm the diagnostic utility of this ISH method in FN.

Previous studies reported other advantages for the ISH method based on existing probes as follows. The results were not affected by contamination or antibiotic treatment, because in contrast to the PCR method, this method does not amplify the amount of bacteria and detects bacterial DNA in the leukocytes after aseptic collection of leukocytes alone [15,17,18]. These advantages were also observed in the present study. In addition, the GB probe covers the majority of important bacterial strains in neutropenic patients, including *Staphylococcus aureus*, *Enterococcus* species, and *Pseudomonas aeruginosa* [2] (Table 1). Based on these observations, we postulate that the positive results observed in patients with FUO or local infection most likely were true positives, and the “negative” results were most likely true negatives. Therefore, the negative ISH result in local infection could provide the information that the infection does not

spread systemically, although that might be caused by a false negative or bacteria that are not covered by the GB probe.

There are several limitations of the present study. First, we cannot accurately determine whether positive results based on the ISH method are indicative of active infection or are due to the detection of residual DNA of killed bacteria, especially when antibiotics are used. However, similar to blood cultures, we could assess active infection based on clinical signs or symptoms and inflammatory biomarkers including IL-6. Second, in blood culture-positive cases, positive ISH results were obtained in 71% of cases; two patients with neutrophil counts of 0/μl and 171/μl showed negative ISH results. This suggests that the ability of the ISH tests to detect causal bacteria might be diminished in cases of low leukocyte counts due to chemotherapy. However, the combined use of blood cultures and serum markers such PCT or IL-6 could compensate for this disadvantage. Third, the ISH tests showed positive results at or after the end of antibiotic therapy in more patients than expected. This suggests that the ISH test might not be useful for determining the appropriate endpoint for antibiotic therapy. However, this might be explained partly by detection of DNA from previously phagocytized and digested bacteria or blood culture-undetectable bacteremia by indigenous skin bacteria after invasive procedures such as bone marrow aspiration. Therefore, these positive results were most likely true positives, although these could have included false positives or results of contamination. A previous clinical trial demonstrated positive ISH results in patients with intravascular devices, and transient bacteremia was considered a possible explanation for this [17]. This disadvantage could be addressed by assessing clinical signs or symptoms and serum biomarkers such as pro-inflammatory cytokines. Conversely, the ability to obtain direct evidence of bacteria at the onset of FN is a strong point for this test. In addition, when a patient is in the recovery phase

from chemotherapy-induced neutropenia, and has a fever of unknown etiology, a negative ISH test result might prompt changes from intravenous broad-spectrum antibiotics to oral antibiotics including quinolones, or earlier cessation of antibiotics.

Taken together, this new ISH method provides more information on causal bacteria in patients with neutropenia and fever during chemotherapy than blood culture. Combined use of these tests and additional information obtained from serum biomarker levels including PCT and IL-6 might contribute to better management of FN. Future study is needed to validate the clinical utility of this combinational approach for FN.

Conflict of interest

AM and SI are employees of Fuso Pharmaceutical Industries. All other authors have no conflicts of interest to declare.

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Figure legends

Fig. 1. Clinical course and results of *in situ* hybridization (ISH) tests, blood cultures, and serum levels of interleukin-6 (IL-6) during follow-up of febrile episodes

Case 1

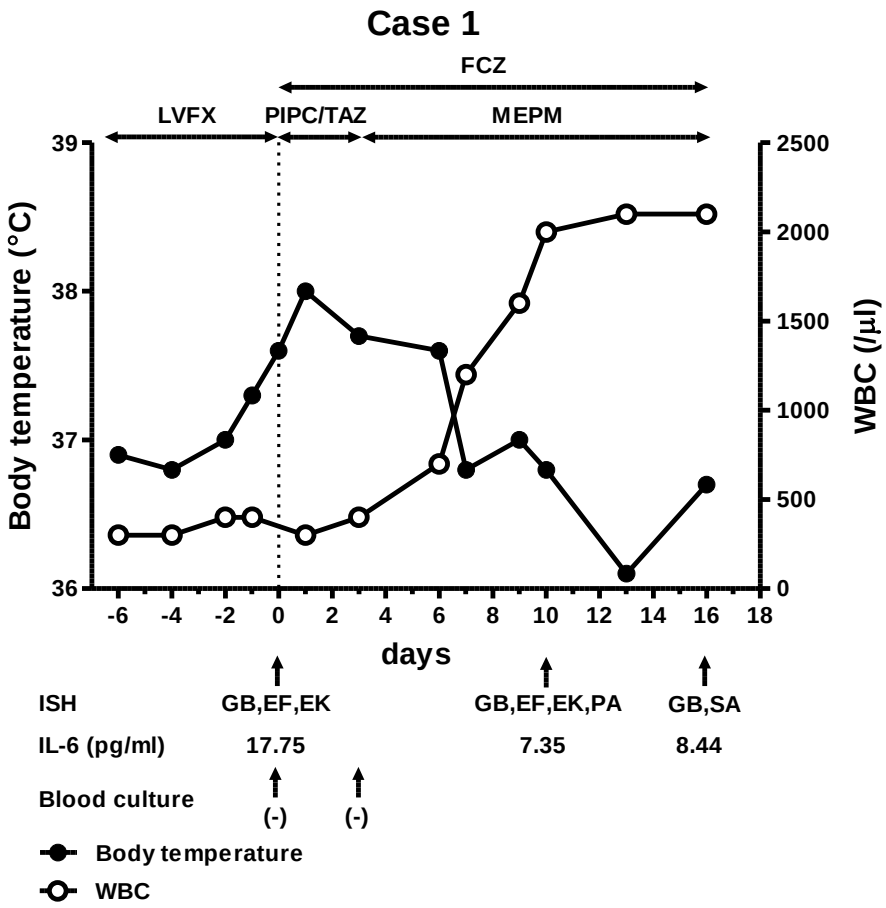
A case of local infection (dentalgia) (the identical case to Pt no.1 in Table 5)

Case 2

A case of fever of unknown origin (identical case to Pt no.3 in Table 5)

Abbreviations: CFPM, cefepime: FCZ, fluconazole; LVFX, levofloxacin; MEPM, meropenem; PIPC/TAZ, piperacillin/tazobactam; WBC, white blood cell

Figure


Case 1
FCZ
LVFX
PIPC/TAZ
MEPM
Body temperature (°C)
WBC (/μl)
days
ISH
GB,EF,EK
GB,EF,EK,PA
GB,SA
IL-6 (pg/ml)
17.75
7.35
8.44
Blood culture
(-)
(-)
Body temperature
WBC

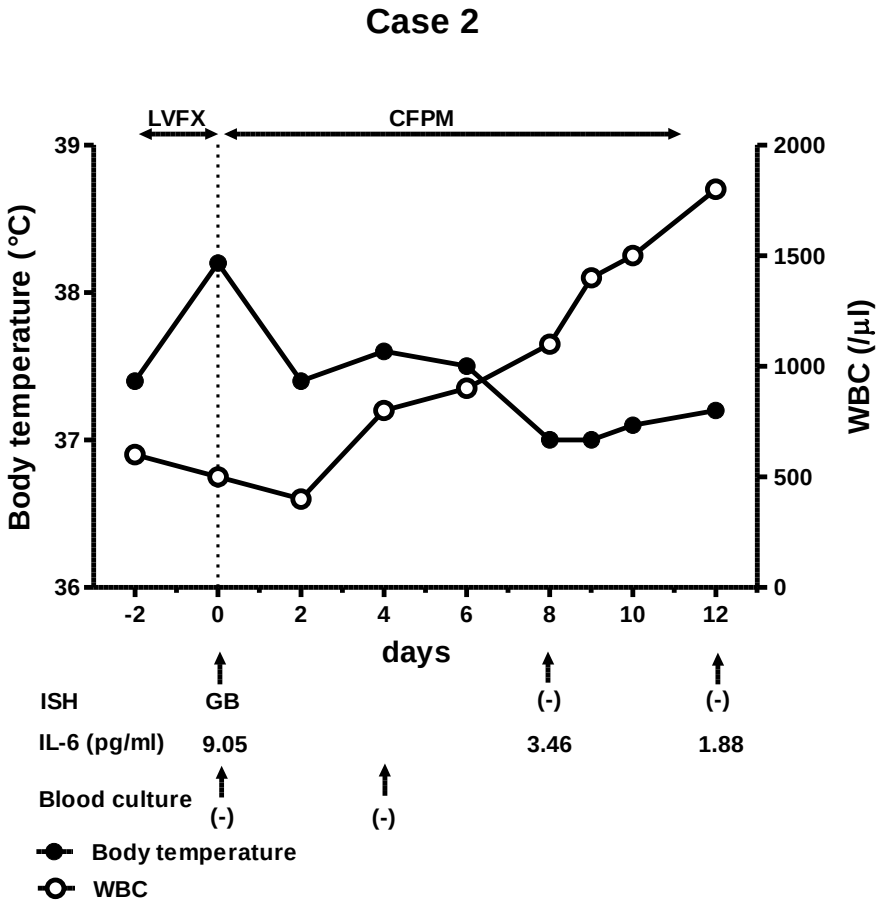

Case 2
LVFX
CFPM
Body temperature (°C)
WBC (/μl)
days
ISH
GB
(-)
(-)
IL-6 (pg/ml)
9.05
3.46
1.88
Blood culture
(-)
(-)
Body temperature
WBC

Table 1. Fifty-nine bacterial strains targeted by the GB probe and seven by the previous probes for use in *in situ* hybridization

Probe	Genus	Species
GB	*Achromobacter*	*xylosoxidans*
	Acinetobacter	*calcoaceticus*
	Bacillus	*cereus*
	Bacteroides	*fragilis*
		ovatus
	Brevundimonas	*diminuta*
	Burkholderia	*cepacia*
	Citrobacter	*koseri*
	Clostridium	*perfringens*
	Corynebacterium	*diphtheriae*
		pseudodiphteritcum
		jeikeium
	Edwerdsiella	*tarda*
	Eggerthella	*lenta*
	Enterobacter	*cloacae*
		sakazakii
		aerogenes
		gergoviae
	Enterococcus	*faecalis*
		faecium
		avium
	Escherichia	*coli*
	Fusobacterium	*nucleatum*
		necrophorum
	Haemophilus	*influenzae*
	Hafnia	*alvei*
	Klebsiella	*pneumoniae*
		aerogenes
		oxytoca
	Kluyvera	*intermedia*
	Lactobacillus	*fermentum*
		acidophilus
	Micrococcus	*luteus*
	Morganella	*morganii*
	Pantoea	*agglomerans*

	Peptoniphilus	*asaccharolyticus*
	Porphyromonas	*asaccharolytica*
	Propionibacterium	*acnes*
	Proteus	*vulgaris*
		mirabilis
	Providencia	*rettgeri*
		alcalifaciens
		stuartii
	Pseudomonas	*aeruginosa*
		fluorescens
		putida
	Raoultella	*terrigena*
		planticola
	Salmonella	*enterica*
	Serratia	*marcescens*
		liquefaciens
	Staphylococcus	*aureus*
		epidermidis
	Stenotrophomonas	*maltophilia*
	Streptococcus	*pneumoniae*
		sanguinis
		pyogenes
		agalactiae
		salivarius
SA	*Staphylococcus*	*aureus*
SE	*Staphylococcus*	*epidermidis*
PA	*Pseudomonas*	*aeruginosa*
EF	*Enterococcus*	*faecalis*
EK	*Escherichia*	*coli*
	Enterobacter	*cloacae*
	Klebsiella	*pneumoniae*

Table 2. Patient characteristics and details of febrile episodes

Patients, n	34
Median age (range), years	42.5 (19–70)
Gender, n (%)	
male	18 (52.9)
female	16 (47.1)
Primary disease, n (%)	
Acute myeloid leukemia	11 (32.4)
Acute lymphoblastic leukemia	10 (29.4)
Non-Hodgkin lymphoma	8 (23.5)
Myelodysplastic syndrome	3 (8.8)
Others*	2 (5.9)
Febrile episode, n	50
Treatment, n (%)	
Chemotherapy	45 (90.0)
Autologous hematopoietic stem cell transplantation	1 (2.0)
Allogeneic hematopoietic stem cell transplantation	1 (2.0)
Others†	3 (6.0)
White blood cell count at enrollment (/μl), median (range)	974 (0–13500)
Neutrophil count at enrollment (/μl), median (range)	506 (0–13500)
Serum procalcitonin levels at onset of febrile neutropenia (mg/dl), median (range)	0.13 (0.00–10.38)
Serum creatinine at onset of febrile neutropenia (mg/dl), median (range)	0.59 (0.37–1.28)
Use of G-CSF, n (%)	
Yes	33 (66.0)
No	17 (34.0)
Oral quinolone prophylaxis	
Yes	40 (80.0)
No	10 (20.0)
Antifungal prophylaxis	
Fluconazole	33 (66.0)
Oral voriconazole or itraconazole oral solution	13 (26.0)
Others	1 (2.0)
None	3 (6.0)
Intravenous empirical or targeted antifungal therapy	
Yes	13 (26.0)
No	37 (74.0)
Duration of intravenous broad-spectrum antibiotic use (days), median	10 (4–51)

(range)	
Final diagnosis	
Fever of unknown origin	24 (45.3)
Local infection	15 (28.3)
Sepsis	7 (13.2)
Others‡	4 (7.5)
Response at 72 hours of intravenous antibiotic therapy, % (n/n)	40.0 (20/50)
Response at 7 days of intravenous antibiotic therapy	44.7 (17/38)
Response at end of intravenous antibiotic therapy	82.0 (41/50)

*Chronic myeloid leukemia blast crisis, n = 1; aplastic anemia, n = 1

†Cyclosporine plus anti-thymoglobulin, n = 1; imatinib, n = 1; none, n = 1

‡All patients were diagnosed as having drug-induced fever

Table 3. Number of positive cases based on *in situ* hybridization (ISH) method, blood cultures, or serum procalcitonin (PCT) test

	ISH at onset (n = 50)	Blood culture at onset (n = 50)	Serum PCT at onset (n = 46)†	ISH at neutrophil recovery (n = 42)	ISH at EOT (n = 21)	Additional ISH after EOT (n = 10)
Total (n = 50)	24 % (12/50)	14 % (7/50)	15 % (7/46)	26 % (11/42)	48 % (10/21)	20 % (2/10)
Sepsis (n = 7), % (n/n)	71 % (5/7)	100 % (7/7)	43 % (3/7)	67 % (4/6)	67 % (4/6)	0 % (0/1)
Local infection (n = 15), % (n/n)	13 % (2/15)	0 % (0/15)	25 % (3/12)	25 % (3/12)	50 % (2/4)	0 % (0/3)
Fever of unknown origin (n = 24), % (n/n)	21 % (5/24)	0 % (0/24)	4 % (1/23)	15 % (3/20)	22 % (2/9)	40 % (2/5)
Others‡ (n = 4), % (n/n)	0 % (0/4)	0 % (0/4)	0 % (0/4)	25 % (1/4)	100 % (2/2)	0 % (0/1)

*Abbreviations: EOT, end of intravenous antibiotic therapy

†The cut-off value of PCT to determine positivity was set at 0.5 ng/ml. Four cases were not included because information was absent.

‡ All patients were diagnosed as having drug-induced fever.

Table 4. Details of positive cases based on *in situ* hybridization (ISH) or blood culture at onset of febrile neutropenia

Pt no.	Age/ Sex	WBC counts (/μl)	Diagnosis	PCT (ng/ml)	ISH at onset	ISH at neutrophil recovery	ISH at EOT	Additional ISH after EOT	Response at 72 hours of therapy	Response at 7 days of therapy	Response at EOT
1	39/F	0	Sepsis (*Staphylococcus epidermidis*)	0.12	GB, EF, EK	GB	†GB	Negative	No	No	Yes
2	35/M	100	Sepsis (*Escherichia coli*)	0.52	GB, PA	GB	GB, EK	—	No	No	Yes
3	21/F	100	Sepsis (*Streptococcus intermedius*)	1.45	GB, SE	Negative	†GB, SA	—	No	No	Yes
4	48/M	100	Sepsis (*Streptococcus oralis*)	0.46	GB, EF, EK	GB, EF	Negative	—	No	No	Yes
5	21/F	0	Sepsis (*Streptococcus haemolyticus*)	0.17	GB, PA, EF	Negative	Negative	—	No	No	No
6	32/M	200	Sepsis (*Staphylococcus epidermidis*)	0.54	Negative	‡GB, EK	†GB, PA, EF, EK, SA, SE	—	No	Yes	Yes
7	40/F	300	Sepsis (*Streptococcus mitis*)	0.06	Negative	—	—	—	Yes	Yes	Yes
8	22/F	4600	Local infection (pudendal pain)	—	GB	Negative	—	—	No	Yes	Yes
9	20/F	400	Local infection (dentalgia)	—	GB, EF, EK	GB, EF, EK, PA	†GB, SA	—	No	Yes	Yes
10	65/M	500	FUO	0.10	GB	Negative	Negative	—	No	Yes	Yes
11	22/M	200	FUO	0.11	EK	Negative	Negative	—	Yes	No	Yes
12	29/M	400	FUO	0.09	GB, EF	Negative	—	—	Yes	NE	Yes
13	61/F	400	FUO	0.08	EK	Negative	—	—	Yes	Yes	Yes
14	49/M	100	FUO	1.65	GB, EK	—	—	—	No	No	Yes

*Abbreviations: EOT, end of intravenous antibiotic therapy; FUO, fever of unknown origin
†Invasive procedures were performed one to several days before ISH tests at EOT: central venous catheterization was performed in Pt no. 1 (3 days before the ISH test); bone marrow examination was performed in Pt no. 3 (1 day before), 6 (5 days before), and 9 (5 days before).
‡Pt no. 6 presented with anal pain from 12 days after onset of fever until two days before the ISH test during neutrophil recovery.

Table 5. Details of serum cytokine and chemokine kinetics according to positive or negative results based on *in situ* hybridization (ISH) tests at onset of febrile episodes in patients with local infection (n = 11) and fever of unknown origin (n = 17)

Pt no.	WBC counts (/μl); median (range)	Diagnosis	ISH at onset	PCT (ng/ml); median (range)		IL-6 (< 9.00 pg/ml); mean (95% CI)	IL-8 (< 116.00 pg/ml); mean (95% CI)	MCP-1 (< 48.00 pg/ml); mean (95% CI)	MIP-1α (< 2.00 pg/ml); mean (95% CI)	MIP-1β (< 47.00 pg/ml); mean (95% CI)
1	400	Local (dentalgia)	GB, EF, EK	NA	At onset	17.75	28.00	39.30	0.98	48.85
					At neutrophil recovery	7.35	27.00	20.81	2.72	76.40
					At EOT	8.44	24.29	37.98	4.38	78.76
2	4600	Local (pudendal pain)	GB	NA	At onset	4.85	0.62	17.25	8.98	40.19
					At neutrophil recovery	0.00	1.88	10.84	9.32	25.45
					At EOT	—	—	—	—	—
Negative ISH group of local infection (n = 9)†	200 (0–2400)	Local	—	0.15 (0.00–10.38)	At onset	55.12 (3.19–107.1)	73.51 (1.56–145.5)	282.9 (63.98–501.8)	4.83 (2.82–6.84)	144.3 (13.21–275.5)
					At neutrophil recovery	4.63 (1.13–8.14)	9.51 (2.63–16.40)	72.26 (1.92–142.6)	2.63 (0.86–4.40)	63.65 (41.29–86.02)
					At EOT	3.18 (0.75–5.61)	9.49 (2.62–16.36)	31.14 (14.53–47.75)	2.92 (1.06–4.77)	60.58 (37.13–84.03)
3	500	FUO	GB	0.10	At onset	9.05	12.86	22.52	1.90	153.62
					At neutrophil recovery	3.46	10.99	14.94	1.99	174.95
					At EOT	1.88	5.46	19.36	1.84	145.43
4	200	FUO	EK	0.11	At onset	7.85	26.21	47.37	2.14	33.20
					At neutrophil recovery	0.26	2.10	4.46	0.62	2.60

					At EOT	6.29	9.94	18.84	4.30	94.11
5	400	FUO	EK	0.08	At onset	21.67	26.23	44.28	1.39	25.20
					At neutrophil recovery	4.60	3.19	98.04	0.91	26.99
					At EOT	—	—	—	—	—
Negative ISH group of FUO (n = 14)†	450 (100–1200)	FUO	—	0.14 (0.05–0.35)	At onset	31.75 (14.61–48.90)	27.60 (15.15–40.04)	129.8 (75.09–184.5)	3.14 (1.33–4.95)	57.80 (40.27–75.32)
					At neutrophil recovery	12.41 (5.90–18.92)	12.71 (6.63–18.79)	55.86 (21.83–89.89)	4.02 (2.07–5.97)	68.52 (49.92–87.12)
					At EOT	9.87 (5.14–14.60)	11.42 (5.71–17.12)	47.43 (14.49–80.37)	4.74 (2.60–6.88)	72.89 (56.96–88.82)

*Abbreviations: EOT, end of intravenous antibiotic therapy; FUO, fever of unknown origin; IL, interleukin; MCP-1, monocyte chemoattractant-1; MIP-1α, macrophage inflammatory protein-1α

†Both groups included all patients with negative results based on ISH tests at onset of febrile episodes.

Supplementary Table 1. Kinetics of serum levels of 27 cytokines and chemokines during follow-up according to the diagnostic category of febrile episodes (n = 36)

		Diagnosis			
		Sepsis / septic shock n = 4	**Local infection n = 11**	**FUO n = 17**	**Others n = 4**
Cytokines and chemokines (normal reference range†, pg/ml)					
IL-1β (<0.70)	At onset of febrile episodes mean (range)	0.66 (0.21–0.98)	0.32 (0.00–0.71)	0.57 (0.02–1.92)	1.56 (0.37–2.72)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	0.82 (0.39–1.24) *P* = 0.310	0.41 (0.04–1.34) *P* = 0.404	0.64 (0.14–1.82) *P* = 0.606	0.82 (0.07–1.46) *P* = 0.114
	At EOT mean (range) *P* value (vs. at onset)	1.49 (0.48–2.27) *P* = 0.087	0.54 (0.04–1.78) *P* = 0.192	0.77 (0.14–1.82) *P* = 0.154	0.96 (0.07–1.78) *P* = 0.224
IL-1 receptor antagonist (<665.00)	At onset of febrile episodes mean (range)	69.90 (0.00–124.20)	43.47 (0.00–262.75)	60.14 (0.00–578.59)	130.55 (44.36–221.87)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	47.39 (3.21–90.25) *P* = 0.299	32.93 (0.00–92.33) *P* = 0.705	50.70 (0.00–255.88) *P* = 0.699	55.15 (0.00–81.90) *P* = 0.180
	At EOT mean (range) *P* value (vs. at onset)	77.84 (9.62–119.98) *P* = 0.651	38.73 (0.00–130.14) *P* = 0.872	58.51 (0.00–255.88) *P* = 0.946	58.95 (0.00–130.14) *P* = 0.178
IL-2 (<90.00)	At onset of febrile episodes mean (range)	4.58 (0.00–18.33)	1.20 (0.00–10.53)	0.87 (0.00–14.82)	3.13 (0.00–6.53)

	At neutrophil recovery mean (range) *P* value (vs. at onset)	3.87 (0.00–15.46) *P* = 0.391	0.91 (0.00–9.99) *P* = 0.258	0.78 (0.00–8.05) *P* = 0.934	0.00 (0.00–0.00) *P* = 0.107
	At EOT mean (range) *P* value (vs. at onset)	4.30 (0.00–12.92) *P* = 0.889	1.01 (0.00–6.69) *P* = 0.756	0.83 (0.00–8.05) *P* = 0.967	1.10 (0.00–4.39) *P* = 0.372
IL-4 (<3.00)	At onset of febrile episodes mean (range)	1.29 (0.35–2.56)	0.53 (0.00–1.14)	0.80 (0.00–2.55)	2.70 (1.22–4.08)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	1.69 (0.66–2.51) *P* = 0.180	1.10 (0.27–1.98) *P* = 0.016	1.77 (0.08–4.58) *P* = 0.029	1.49 (0.46–2.41) *P* = 0.156
	At EOT mean (range) *P* value (vs. at onset)	2.43 (0.99–3.21) *P* = 0.113	1.38 (0.00–2.63) *P* = 0.015	2.02 (0.35–4.58) *P* = 0.004	1.78 (0.46–2.63) *P* = 0.304
IL-5 (<7.00)	At onset of febrile episodes mean (range)	2.35 (0.45–6.45)	0.70 (0.00–2.71)	3.50 (0.00–15.41)	2.78 (1.04–4.96)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	3.35 (0.48–6.99) *P* = 0.213	1.11 (0.08–2.77) *P* = 0.020	2.10 (0.08–5.09) *P* = 0.264	1.97 (0.75–3.45) *P* = 0.258
	At EOT mean (range) *P* value (vs. at onset)	4.23 (0.48–6.13) *P* = 0.207	1.23 (0.08–3.50) *P* = 0.023	2.81 (0.29–11.23) *P* = 0.617	2.12 (0.75–3.45) *P* = 0.283
IL-6 (<9.00)	At onset of febrile episodes mean (range)	155.33 (19.77–435.55)	47.16 (1.53–182.88)	28.42 (0.49–105.73)	34.58 (1.39–65.99)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	6.75 (1.67–13.38) *P* = 0.215	4.46 (0.00–13.32) *P* = 0.055	10.71 (0.26–37.96) *P* = 0.018	4.67 (0.96–7.35) *P* = 0.201
	At EOT mean (range) *P* value (vs. at onset)	7.26 (2.28–9.96) *P* = 0.213	3.37 (0.00–10.55) *P* = 0.048	8.88 (1.88–28.68) *P* = 0.009	4.86 (0.96–8.44) *P* = 0.205
IL-7 (<13.00)	At onset of febrile episodes mean (range)	4.08 (2.47–6.06)	13.69 (1.77–124.97)	13.14 (0.00–97.68)	13.65 (3.39–24.40)

	At neutrophil recovery mean (range) *P* value (vs. at onset)	5.40 (3.26–8.14) *P* = 0.165	12.66 (0.98–108.36) *P* = 0.549	20.01 (0.91–147.59) *P* = 0.081	4.61 (1.33–7.98) *P* = 0.088
	At EOT mean (range) *P* value (vs. at onset)	9.95 (3.68–14.39) *P* = 0.125	14.00 (0.47–108.36) *P* = 0.886	21.41 (1.01–147.59) *P* = 0.032	6.46 (1.33–12.83) *P* = 0.170
IL-8 (<116.00)	At onset of febrile episodes mean (range)	182.24 (22.70–457.62)	62.74 (0.62–296.47)	26.57 (7.05–91.30)	29.98 (10.45–49.74)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	9.49 (5.36–14.29) *P* = 0.167	10.41 (1.88–31.98) *P* = 0.059	11.42 (2.10–31.02) *P* = 0.018	11.53 (2.76–27.00) *P* = 0.086
	At EOT mean (range) *P* value (vs. at onset)	13.43 (7.21–17.00) *P* = 0.169	10.14 (1.88–31.98) *P* = 0.058	10.50 (2.69–31.02) *P* = 0.011	11.88 (2.76–24.29) *P* = 0.112
IL-9 (<500.00)	At onset of febrile episodes mean (range)	13.69 (1.72–27.98)	6.21 (0.00–21.89)	38.14 (0.00–561.42)	13.07 (0.72–24.10)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	6.48 (2.85–10.12) *P* = 0.166	6.75 (0.00–25.08) *P* = 0.745	8.52 (0.00–31.49) *P* = 0.383	5.72 (1.63–9.63) *P* = 0.104
	At EOT mean (range) *P* value (vs. at onset)	10.89 (3.01–17.07) *P* = 0.652	8.01 (0.00–25.08) *P* = 0.415	11.03 (0.37–31.49) *P* = 0.425	8.06 (3.67–14.00) *P* = 0.302
IL-10 (<2.00)	At onset of febrile episodes mean (range)	19.36 (0.67–48.8)	27.61 (0.47–202.29)	5.63 (0.00–13.10)	24.08 (14.03–40.79)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	4.93 (1.46–12.35) *P* = 0.277	8.92 (0.87–33.44) *P* = 0.332	14.58 (0.00–62.71) *P* = 0.058	8.08 (3.45–18.24) *P* = 0.166
	At EOT mean (range) *P* value (vs. at onset)	9.17 (0.89–14.34) *P* = 0.372	9.83 (0.89–30.78) *P* = 0.358	17.46 (0.76–62.71) *P* = 0.013	8.27 (3.69–11.29) *P* = 0.075
IL-12 (<6.00)	At onset of febrile episodes mean (range)	37.56 (2.35–129.00)	8.64 (0.30–18.29)	9.31 (0.00–26.37)	55.06 (11.67–92.46)

	At neutrophil recovery mean (range) *P* value (vs. at onset)	34.48 (3.39–104.42) *P* = 0.742	15.12 (0.65–51.65) *P* = 0.117	40.28 (0.01–242.45) *P* = 0.081	16.11 (7.86–21.26) *P* = 0.130
	At EOT mean (range) *P* value (vs. at onset)	46.66 (5.64–98.65) *P* = 0.587	22.59 (0.65–51.65) *P* = 0.036	48.82 (0.01–242.45) *P* = 0.025	22.08 (14.81–36.01) *P* = 0.081
IL-13 (<9.00)	At onset of febrile episodes mean (range)	0.62 (0.00–1.25)	0.99 (0.17–2.03)	1.72 (0.00–4.09)	6.47 (2.79–8.49)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	1.42 (0.24–2.57) *P* = 0.189	2.07 (0.24–4.61) *P* = 0.049	4.65 (0.17–17.65) *P* = 0.036	2.65 (1.07–4.28) *P* = 0.123
	At EOT mean (range) *P* value (vs. at onset)	3.45 (0.33–5.95) *P* = 0.087	2.73 (0.33–6.89) *P* = 0.029	5.70 (0.33–17.65) *P* = 0.004	3.56 (2.49–4.85) *P* = 0.193
IL-15 (<5.00)	At onset of febrile episodes mean (range)	4.18 (0.00–13.56)	0.60 (0.00–6.57)	0.50 (0.00–8.44)	23.00 (0.00–79.09)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	0.00 (0.00–0.00) *P* = 0.284	0.00 (0.00–0.00) *P* = 0.341	0.00 (0.00–0.00) *P* = 0.332	0.00 (0.00–0.00) *P* = 0.309
	At EOT mean (range) *P* value (vs. at onset)	0.00 (0.00–0.00) *P* = 0.284	0.24 (0.00–2.65) *P* = 0.604	0.00 (0.00–0.00) *P* = 0.332	0.66 (0.00–2.65) *P* = 0.327
IL-17 (<31.00)	At onset of febrile episodes mean (range)	18.19 (0.00–45.76)	13.29 (0.00–61.16)	9.00 (0.00–86.03)	27.66 (0.00–73.44)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	8.58 (0.00–30.49) *P* = 0.270	15.54 (0.00–81.23) *P* = 0.438	25.10 (0.00–222.88) *P* = 0.120	2.05 (0.00–8.19) *P* = 0.165
	At EOT mean (range) *P* value (vs. at onset)	29.44 (0.00–61.88) *P* = 0.513	19.59 (0.00–81.23) *P* = 0.123	32.51 (0.00–222.88) *P* = 0.029	9.12 (0.00–28.28) *P* = 0.335
Eotaxin (<39.00)	At onset of febrile episodes mean (range)	19.56 (0.00–58.92)	28.30 (0.00–93.30)	35.34 (0.00–171.23)	86.33 (52.26–151.73)

	At neutrophil recovery mean (range) *P* value (vs. at onset)	57.95 (2.25–92.77) *P* = 0.067	42.53 (18.52–121.42) *P* = 0.002	50.53 (0.00–152.82) *P* =0.208	62.85 (12.94–121.42) *P* = 0.417
	At EOT mean (range) *P* value (vs. at onset)	86.05 (49.26–142.46) *P* = 0.088	46.71 (10.40–158.79) *P* = 0.011	63.72 (7.02–152.82) *P* = 0.016	59.81 (12.94–158.79) *P* = 0.224
FGF basic (<55.00)	At onset of febrile episodes mean (range)	31.77 (0.00–59.32)	27.89 (0.00–76.93)	22.97 (0.00–121.73)	52.73 (0.00–93.83)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	35.08 (12.41–55.45) *P* = 0.802	32.49 (0.00–79.95) *P* = 0.414	32.22 (0.00–153.38) *P* = 0.156	34.72 (0.00–52.96) *P* = 0.203
	At EOT mean (range) *P* value (vs. at onset)	59.31 (21.53–92.07) *P* = 0.088	39.44 (0.00–79.95) *P* = 0.196	43.35 (0.00–153.38) *P* = 0.002	41.99 (0.00–66.42) *P* = 0.420
G-CSF (<1.50)	At onset of febrile episodes mean (range)	482.22 (43.10–1354.55)	692.37 (0.00–3485.95)	1124.57 (0.00–5366.01)	55.50 (2.31–135.23)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	44.26 (16.00–92.12) *P* = 0.242	467.41 (0.00–2564.71) *P* = 0.638	205.10 (0.00–1391.43) *P* = 0.008	14.85 (10.54–17.49) *P* = 0.272
	At EOT mean (range) *P* value (vs. at onset)	17.18 (3.60–27.55) *P* = 0.209	260.73 (0.00–2564.71) *P* = 0.355	87.03 (0.00–482.83) *P* = 0.010	14.66 (9.96–20.66) *P* = 0.264
GM-CSF (<122.00)	At onset of febrile episodes mean (range)	0.75 (0.00–0.30)	1.74 (0.00–17.20)	2.02 (0.00–20.77)	47.37 (0.00–159.75)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	0.88 (0.00–3.01) *P* = 0.301	0.78 (0.00–7.82) *P* = 0.604	3.36 (0.00–24.55) *P* = 0.576	0.83 (0.00–2.55) *P* = 0.310
	At EOT mean (range) *P* value (vs. at onset)	6.25 (0.00–17.05) *P* = 0.229	2.38 (0.00–10.22) *P* = 0.774	4.16 (0.00–24.55) *P* = 0.355	3.19 (0.00–10.22) *P* = 0.341
IFN-γ (<124.00)	At onset of febrile episodes mean (range)	30.98 (14.07–39.71)	8.24 (0.00–44.28)	22.72 (0.00–78.38)	70.48 (23.27–112.03)

	At neutrophil recovery mean (range) *P* value (vs. at onset)	40.85 (15.15–63.61) *P* = 0.291	17.94 (0.00–42.02) *P* = 0.190	40.71 (0.20–116.46) *P* = 0.098	43.46 (0.00–98.83) *P* = 0.270
	At EOT mean (range) *P* value (vs. at onset)	70.67 (23.43–98.72) *P* = 0.067	29.64 (0.00–77.48) *P* = 0.068	48.19 (1.62–116.46) *P* = 0.015	51.09 (0.00–98.83) *P* = 0.441
IP-10 (<637.00)	At onset of febrile episodes mean (range)	4237.89 (857.74–12420.88)	NE (255.75– OOR>)	NE (512.48– OOR>)	10295.80 (1762.24–26535.19)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	2232.25 (580.18–6487.64) *P* = 0.228	NE (580.18–OOR>) Not calculated	2857.92 (112.25–6735.49) Not calculated	NE (1154.97– OOR>) Not calculated
	At EOT mean (range) *P* value (vs. at onset)	2527.81 (545.84–6163.34) *P* = 0.645	NE (467.51–OOR>) Not calculated	NE (1603.34–OOR>) Not calculated	NE (467.51–OOR>) Not calculated
MCP-1 (<48.00)	At onset of febrile episodes mean (range)	218.95 (120.19–323.41)	236.58 (17.25–903.27)	113.61 (13.96–322.98)	250.81 (18.47–654.40)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	33.32 (10.33–71.25) *P* = 0.029	62.00 (8.10–251.78) *P* = 0.065	52.91 (4.46–240.56) *P* = 0.015	41.35 (16.40–73.62) *P* = 0.234
	At EOT mean (range) *P* value (vs. at onset)	15.13 (8.40–28.15) *P* = 0.034	29.92 (7.45–73.44) *P* = 0.032	47.07 (7.04–225.13) *P* = 0.012	43.48 (16.40–73.62) *P* = 0.241
MIP-1α (<2.00)	At onset of febrile episodes mean (range)	4.18 (0.93–5.89)	4.86 (0.98–9.44)	2.91 (0.00–11.36)	3.57 (0.73–6.11)
	At neutrophil recovery mean (range) *P* value (vs. at onset)	2.81 (1.73–3.46) *P* = 0.222	3.25 (0.65–9.32) *P* = 0.070	3.52 (0.62–12.61) *P* = 0.103	3.02 (1.23–4.43) *P* = 0.504
	At EOT mean (range) *P* value (vs. at onset)	4.49 (2.03–6.49) *P* = 0.744	3.63 (0.32–9.32) *P* = 0.234	4.32 (0.65–12.61) *P* = 0.004	3.94 (3.28–4.43) *P* = 0.741
PDGF-ββ (<3667.00)	At onset of febrile episodes mean (range)	199.38 (74.00–315.15)	150.66 (7.16–308.23)	223.62 (1.72–905.63)	3518.79 (339.63–11117.02)

	At neutrophil recovery mean (range)	303.66 (116.08–510.16)	523.74 (21.22–2111.39)	1129.45 (41.94–5370.62)	348.01 (134.25–585.62)
	P value (vs. at onset)	*P* = 0.161	*P* = 0.064	*P* = 0.038	*P* = 0.291
	At EOT mean (range)	1005.25 (41.52–2837.45)	695.07 (1.07–2111.39)	1418.98 (61.02–5370.62)	605.60 (134.25–1396.56)
	P value (vs. at onset)	*P* = 0.278	*P* = 0.018	*P* = 0.006	*P* = 0.342
MIP-1β (<47.00)	At onset of febrile episodes mean (range)	307.85 (102.81–626.84)	126.20 (17.06–557.60)	60.07 (22.01–153.62)	107.47 (51.42–199.88)
	At neutrophil recovery mean (range)	102.71 (66.43–134.04)	61.34 (23.64–105.44)	68.46 (2.60–174.95)	53.80 (26.86–76.40)
	P value (vs. at onset)	*P* = 0.195	*P* = 0.175	*P* = 0.43	*P* = 0.234
	At EOT mean (range)	136.31 (103.68–218.45)	59.04 (23.64–113.23)	75.70 (26.99–145.43)	62.96 (26.86–85.73)
	P value (vs. at onset)	*P* = 0.241	*P* = 0.159	*P* = 0.084	*P* = 0.360
RANTES (<2282.00)	At onset of febrile episodes mean (range)	1561.64 (552.01–2229.87)	NE (408.06– OOR>)	NE (490.45– OOR>)	NE (OOR>– OOR>)
	At neutrophil recovery mean (range)	NE (1090.28– OOR>)	NE (1090.28–OOR>)	NE (394.37– OOR>)	NE (3313.00–OOR>)
	P value (vs. at onset)	Not calculated	Not calculated	Not calculated	Not calculated
	At EOT mean (range)	NE (3622.71– OOR>)	NE (524.79–OOR>)	NE (425.96– OOR>)	NE (OOR>– OOR>)
	P value (vs. at onset)	Not calculated	Not calculated	Not calculated	Not calculated
TNF-α (<98.00)	At onset of febrile episodes mean (range)	17.83 (4.14–34.98)	6.66 (0.00–14.12)	23.31 (0.00–197.45)	29.88 (10.00–57.12)
	At neutrophil recovery mean (range)	22.65 (8.85–36.28)	11.97 (0.00–30.85)	17.06 (0.00–53.84)	18.91 (5.46–35.28)
	P value (vs. at onset)	*P* = 0.242	*P* = 0.092	*P* = 0.520	*P* = 0.108
	At EOT mean (range)	35.74 (14.24–51.56)	16.59 (0.00–46.96)	20.96 (1.38–53.84)	25.93 (5.46–46.96)
	P value (vs. at onset)	*P* = 0.132	*P* = 0.050	*P* = 0.808	*P* = 0.679
VEGF (<9.00)	At onset of febrile episodes mean (range)	20.79 (6.30–34.92)	13.16 (0.00–32.12)	15.88 (0.00–64.91)	299.48 (19.82–660.84)

At neutrophil recovery mean (range) *P* value (vs. at onset)	18.37 (7.05–31.12) *P* = 0.790	23.73 (0.00–142.90) *P* = 0.395	102.20 (0.00–847.74) *P* = 0.122	19.75 (5.73–36.75) *P* = 0.139
At EOT mean (range) *P* value (vs. at onset)	47.65 (10.37–95.21) *P* = 0.217	42.55 (0.00–142.90) *P* = 0.087	117.13 (0.00–847.74) *P* = 0.067	38.98 (11.70–96.02) *P* = 0.113

*Abbreviations: EOT, end of intravenous antibiotic therapy; OOR>, out of range above; IL, interleukin; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; IFN-γ, interferon-γ; IP-10, interferon gamma inducible protein-10; MCP-1, monocyte chemoattractant-1; MIP-1α, macrophage inflammatory protein-1α; PDGF-ββ, platelet derived growth factor-ββ; RANTES, regulated on activation, normal T cell expressed and secreted; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

† The upper limit data are cited from the serum data of healthy controls (reference 25).

Supplementary Table 2. Diagnostic performance of serum levels of 27 cytokines and chemokines at onset of febrile episodes for sepsis or bacterial infections (n = 36)

	AUC (95%CI, *P*) **Sepsis vs. others**	**AUC (95%CI, *P*)** **†Bacterial infections vs. others**
Cytokines and chemokines (normal reference range‡, pg/ml)		
IL-1β (<0.70)	0.652 (95%CI 0.436–0.869, *P* = 0.326)	0.432 (95%CI 0.241–0.622, *P* = 0.490)
IL-1 receptor antagonist (<665.00)	0.586 (95%CI 0.280–0.892, *P* = 0.580)	0.489 (95%CI 0.297–0.681, *P* = 0.911)
IL-2 (<90.00)	0.555 (95%CI 0.219–0.890, *P* = 0.725)	0.511 (95%CI 0.316–0.706, *P* = 0.911)
IL-4 (<3.00)	0.656 (95%CI 0.406–0.907, *P* = 0.314)	0.438 (95%CI 0.246–0.630, *P* = 0.532)
IL-5 (<7.00)	0.531 (95%CI 0.244–0.819, *P* = 0.840)	0.294 (95%CI 0.116–0.472, *P* = 0.037)
IL-6 (<9.00)	0.789 (95%CI 0.591–0.987, *P* = 0.063)	0.594 (95%CI 0.395–0.792, *P* = 0.344)
IL-7 (<13.00)	0.648 (95%CI 0.462–0.835, *P* = 0.339)	0.543 (95%CI 0.349–0.737, *P* = 0.665)
IL-8 (<116.00)	0.844 (95%CI 0.618–1.00, *P* = 0.027)	0.651 (95%CI 0.447–0.855, *P* = 0.127)
IL-9 (<500.00)	0.680 (95%CI 0.432–0.928, *P* = 0.247)	0.517 (95%CI 0.325–0.710, *P* = 0.860)

IL-10 (<2.00)	0.641 (95%CI 0.300–0.981, *P* = 0.365)	0.548 (95%CI 0.344–0.751, *P* = 0.630)
IL-12 (<6.00)	0.563 (95%CI 0.237–0.888, *P* = 0.687)	0.479 (95%CI 0.285–0.674, *P* = 0.835)
IL-13 (<9.00)	0.250 (95%CI 0.070–0.430, *P* = 0.107)	0.289 (95%CI 0.116 –0.462, *P* = 0.033)
IL-15 (<5.00)	0.672 (95%CI 0.358–0.985, *P* = 0.268)	0.502 (95%CI 0.308–0.695, *P* = 0.987)
IL-17 (<31.00)	0.586 (95%CI 0.280–0.892, *P* = 0.580)	0.575 (95%CI 0.382–0.767, *P* = 0.451)
Eotaxin (<39.00)	0.313 (95%CI 0.00–0.630, *P* = 0.227)	0.367 (95%CI 0.181–0.552, *P* = 0.178)
FGF basic (<55.00)	0.559 (95%CI 0.279–0.839, *P* = 0.706)	0.573 (95%CI 0.383–0.763, *P* = 0.461)
G-CSF (<1.50)	0.508 (95%CI 0.283–0.733, *P* = 0.960)	0.471 (95%CI 0.279–0.663, *P* = 0.773)
GM-CSF (<122.00)	0.477 (95%CI 0.197–0.756, *P* = 0.880)	0.444 (95%CI 0.255–0.634, *P* = 0.574)
IFN-γ (<124.00)	0.664 (95%CI 0.494–0.835, *P* = 0.290)	0.359 (95%CI 0.179–0.538, *P* = 0.153)
IP-10 (<637.00)	0.539 (95%CI 0.242–0.836, *P* = 0.801)	0.438 (95%CI 0.235–0.641, *P* = 0.532)
MCP-1 (<48.00)	0.703 (95%CI 0.526–0.880, *P* = 0.191)	0.600 (95%CI 0.395–0.805, *P* = 0.312)

MIP-1α (<2.00)	0.602 (95%CI 0.288–0.915, *P* = 0.513)	0.702 (95%CI 0.519–0.884, *P* = 0.042)
PDGF-ββ (<3667.00)	0.531 (95%CI 0.312–0.751, *P* = 0.840)	0.416 (95%CI 0.227–0.605, *P* = 0.395)
MIP-1β (<47.00)	0.898 (95%CI 0.768–1.00, *P* = 0.010)	0.660 (95%CI 0.465–0.855, *P* = 0.105)
RANTES (<2282.00)	0.266 (95%CI 0.085–0.446, *P* = 0.131)	0.357 (95%CI 0.169–0.545, *P* = 0.149)
TNF-α (<98.00)	0.680 (95%CI 0.419–0.940, *P* = 0.247)	0.383 (95%CI 0.198–0.567, *P* = 0.235)
VEGF (<9.00)	0.594 (95%CI 0.364–0.824, *P* = 0.546)	0.443 (95%CI 0.253–0.633, *P* = 0.564)

*Abbreviations: AUC, area under the curve; IL, interleukin; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte-macrophage colony stimulating factor; IFN-γ, interferon-γ; IP-10, interferon gamma inducible protein-10; MCP-1, monocyte chemoattractant-1; MIP-1α, macrophage inflammatory protein-1α; PDGF-ββ, platelet derived growth factor-ββ; RANTES, regulated on activation, normal T cell expressed and secreted; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.
† Bacterial infections are defined as sepsis or local infections.
‡ The upper limit data are cited from the serum data of healthy controls (reference 25).