Diagnosis of Bacteremia in Febrile Neutropenic Episodes in Children With Cancer

Microbiologic and Molecular Approach

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Background: Bacterial isolation using conventional microbiologic techniques rarely surpasses 25% in children with clinical and laboratory findings indicative of an invasive bacterial infection. The aim of this study was to determine the role of real-time polymerase chain reaction (RT-PCR) from whole blood samples compared with automated blood cultures (BC) in detection of relevant microorganisms causing bacteremia in episodes of high-risk febrile neutropenia (HRFN) in children with cancer.

Methods: Children presenting with HRFN at 6 hospitals in Santiago, Chile, were invited to participate. Blood samples were obtained at admission for BC, and at admission and 24 hours for RT-PCR targeting DNA of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* causing bacteremia in children with HRFN.

Results: A total of 177 HRFN episodes were evaluated from May 2009 to August 2010, of which 29 (16.3%) had positive BC, 9 (5%) positive for 1 of the 3 selected bacterial species: 5 for *E. coli*, 3 for *S. aureus*, and 1 for *P. aeruginosa*. RT-PCR detected 39 bacteria in 36 episodes (20%): 14 *E. coli*, 20 *S. aureus*, and 5 *P. aeruginosa*. The sensitivity, specificity, and positive and negative predictive values of RT-PCR compared with BC were 56%, 80%, 13%, and 97%. The final clinical diagnosis was compatible with an invasive bacterial infection in 30/36 (83%) RT-PCR-positive episodes.

Conclusions: In our series, RT-PCR significantly improved detection of the most relevant bacteria associated with HRFN episodes. Large number of patients and close clinical monitoring, in addition to improved RT-PCR techniques will be required to fully recommend RT-PCR-based diagnosis for the routine workup of children with cancer, fever, and neutropenia.

Key Words: febrile neutropenia, bacteremia, molecular diagnosis, children with cancer

(*Pediatr Infect Dis J* 2011;30: 957–961)

In severely ill patients with life-threatening infections, selection of an appropriate antimicrobial therapy that covers the infecting microorganism is a critical factor for reducing mortality or microbiologic failure.[1–3] In this context, in children with cancer who have a febrile neutropenic episode obtaining an early etiologic diagnosis aids in targeting antimicrobial treatment, and thus for improving disease outcome.[4–6]

Bacterial isolation using conventional microbiologic techniques rarely exceeds 25% in children with clinical signs and laboratory findings, suggesting a high risk for an invasive bacterial infection (IBI).[7–9] Bacterial isolation increases to 60% in children with clinical sepsis and 80% among children who die with a sepsis-like syndrome.[10,11] It is noteworthy that in 40% of children clinically diagnosed with sepsis and in 75% of children with a high-risk febrile neutropenia (HRFN), a microorganism can not be detected by currently available conventional microbiologic techniques.[10,12]

Following 6 bacterial species cause more than 75% of IBI episodes in immunocompromised children worldwide: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, coagulase-negative *Staphylococcus*, *Staphylococcus aureus*, and viridans group *Streptococcus*.[12,13] The most common species identified in children with HRFN and sepsis are *E. coli*, *S. aureus*, and *P. aeruginosa*.[10]

The potential for a serious outcome of febrile neutropenic episodes has lead to an aggressive approach based on early empiric, broad-spectrum antimicrobials, which is fine-tuned throughout the clinical course of the episode based on clinical and laboratory evolution and culture results.[4] Unfortunately, the lack of a microbiologic diagnosis does not allow a target-specific antimicrobial regimen in most children with HRFN.

Studies performed in non-neutropenic patients with sepsis have shown that nucleic acid amplification tests, in addition to blood cultures (BC), can significantly improve microbiologic diagnosis of infection.[14,15] Only one small, recent report of nucleic acid amplification test used in children with FN is available. Sakaguchi et al[16] evaluated bacterial rRNA-targeted reverse transcription-PCR in 23 FN episodes in 13 pediatric patients; of these samples, bacteria were identified in 16 cases by rRNA-targeted reverse transcription-PCR (70%) and in 4 by BC (17%, $P <$

Accepted for publication June 20, 2011.

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Supported by Proyecto FONDECYT, Grant 1090194, Grant 11080113.

The authors have no conflicts of interest or funding to disclose.

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Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

ISSN: 0891-3668/11/3011-0957

DOI: 10.1097/INF.0b013e31822a37d7

0.001). More studies are needed in this population to support routine clinical use of molecular techniques, in addition to BC, for an optimal detection of bacterial pathogens.

The aim of this study was to determine the role of real-time polymerase chain reaction (RT-PCR) from whole blood samples compared with automated BC in the detection of 3 relevant microorganisms causing bacteremia in episodes of HRFN in children with cancer. Our hypothesis was that RT-PCR could significantly increase the likelihood of identifying a causal microorganism in this population.

PATIENTS AND METHODS

Overall Study Design

We designed a prospective, collaborative, multicenter study conducted between May 1, 2009 and August 31, 2010, in 6 large hospitals of Santiago, Chile, participating in the National Children's Program for Antineoplastic Drugs. Children and adolescents ≤18 years of age with cancer and chemotherapy, suffering an episode of severe neutropenia and fever were evaluated at admission by one of the investigators to determine their risk for IBI according to a standard protocol (mentioned in Definitions Section).[9,17] Patients at low risk for IBI or with a history of hematopoietic stem cell transplant were excluded from the study. Patients at high risk for IBI were invited to participate and were enrolled if the parents or legal guardians signed the informed consent; for children older than 11 years of age, a signed assent was also required. The study was approved by the Ethical Committees of each hospital. Children enrolled were treated in the 6 hospitals according to a standard protocol for evaluation and management of HRFN episodes.[18] For the purpose of this study, initial blood samples were obtained for automated BC at admission (hour 0) and for detection of bacterial DNA by RT-PCR (at admission and 24 hours).

Each child was monitored daily until episode resolution that is defined as a period of 48 hours without fever and an absolute neutrophil count (ANC) >500/mm^3. Blood samples available for detection of bacterial DNA were stored at −20°C for further analyses.

Clinical and Laboratory Evaluation

All patients were uniformly evaluated at admission, recording the following variables: (1) demographics: age, gender; (2) cancer characteristics: type of cancer, type of chemotherapy, start and end date of last cycle, presence of central venous catheter (CVC); (3) characteristics of the febrile episode: hours of fever prior to admission, signs and/or symptoms indicative of an infectious foci; (4) clinical characteristics: overall clinical status, axillary temperature, heart rate, respiratory rate, blood pressure; (5) laboratory procedures at the time of admission: white blood cell count, ANC, absolute monocyte count, platelet count, quantitative serum C-reactive protein (CRP), interleukin 8 (IL-8), chest x-ray, urine culture, BC (central and peripheral samples in children with CVC); and cultures from other locations (skin lesions, cerebrospinal fluid, bronchoalveolar fluid, stools), if clinically indicated.

Patient Treatment and Follow-up

All patients were treated empirically with a third-generation cephalosporin, an aminoglycoside, and an antistaphylococcal β-lactam antimicrobial based on bacteriologic findings from our previous studies in patients with HRFN.[9,17] Hematologic, biochemical, and culture tests were repeated at defined, previously standardized intervals.[18]

Laboratory Techniques

Standard Techniques for FN Episode Management

Hematologic and biochemical tests were performed at each hospital according to standard techniques. Quantitative CRP test was carried out using nephelometry according to a previously described protocol.[19] IL-8 was determined in serum through a commercial ELISA test (R&D) according to the manufacturer's instructions.[20] Serum samples for IL-8 quantification were stored at −80°C and were processed centrally at the L. Calvo Mackenna Hospital. BCs were analyzed at each hospital using the automated BC system (BacT/ALERT, bioMérieux, Inc, Durham, NC), according to laboratory-defined standard operating procedures. All hospitals obtained blood volumes in BacT vials with a blood-to-broth ratio of 1:5 to 1:10 adjusted to patient age, according to BacT/Alert technique recommendations.

Molecular Detection of Bacterial Microorganisms

DNA from 200 μL of whole blood was extracted by using the QIAamp DNA blood kit (Qiagen Inc., Valencia, CA). DNA was diluted in 200-μL distilled water and the DNA sample was used for pathogen detection by a commercial RT-PCR kit for *E. coli* (Primer Design), *S. aureus*, and *P. aeruginosa* (Applied Biosystem) according to manufacturer's instructions.[21–23] DNA for ATCC *E. coli* 25922, *S. aureus* 25923, and *P. aeruginosa* 27853 were used as positive controls. All RT-PCR detections were performed centrally at the Center for Molecular Studies, Hospital L. Calvo Mackenna, Department of Pediatrics, Faculty of Medicine, Universidad de Chile, by laboratory personnel blinded to the results of the BC and clinical diagnosis of the patients.

Definitions

(*a*) Neutropenia: ANC ≤500/mm^3; (*b*) fever: single axillary temperature ≥38.5°C or ≥38°C in 2 measurements separated by ≥1 hour; (*c*) HRFN: an FN episode with one of the following risk factors at the time of admission: (i) relapse of leukemia as cancer type, (ii) hypotension or (iii) quantitative CRP ≥90 mg/L or an FN episode with the following 2 factors at the time of admission: (iv) ≤7 days between the end of the last chemotherapy and the beginning of the fever and (v) platelet count ≤50,000/mm^3; (*d*) IBI: bacteremia or isolation of a microorganism from a normally sterile site or clinical signs suggestive of localized bacterial infection, with parenchyma involvement, with or without microbiologic isolation; (*e*) microbiologic and/or molecular confirmation of a bacterial infection: one or more of the following criteria: (i) demonstrated bacteremia: presence of one or more positive BC by automated BacT/Alert technique (except coagulase-negative *Staphylococcus* for which ≥2 positive BC were required) and/or positive RT-PCR for the detection of target genes for *E. coli*, *S. aureus*, and *P. aeruginosa*; (ii) positive microbiologic isolation in a normally sterile fluid; (*f*) sepsis: systemic inflammatory response syndrome accompanied by abnormal tissue perfusion, caused by a presumptive bacterial infection, with or without microbiologic or molecular confirmation.[24]

Statistical Analysis

Sample Size Calculation

Our current capacity of identifying a bacterial microorganism from a sterile site using automated BC in children with HRFN episodes is 20%.[17] Implementing the nucleic acid amplification test was expected to increase this capacity by at least 15% (minimum positivity expected was 35%). The number of HRFN episodes required to demonstrate this increased capacity with a confidence interval of 95% and a power of 80 was 120 episodes.

TABLE 1. Overall Admission Characteristics of 177 Episodes of High-risk Febrile Neutropenia (HRFN)

Admission Characteristics	HRFN Episodes (N = 177)
Median age in years (25%–75%)	8 (4–13)
Male (%)	104 (59)
Type of cancer (%)	
Leukemia/lymphoma	86 (48.6)
Solid tumor	44 (24.9)
Leukemia relapse	47 (26.5)
Use of central venous catheter (%)	147 (84.5)
Median hours of fever prior to admission (25%–75%)	2 (1–4)
Median temperature (°C) (25%–75%)	38.2 (38–38.5)
Median absolute neutrophil count (25%–75%)	45 (0–113)
Median absolute monocyte count (25%–75%)	0 (0–21)
Median C-reactive protein (mg/L) (25%–75%)	65 (30–125)
Median interleukin 8 (pg/mL) (25%–75%)	171 (50–425)

TABLE 2. Correlation Between Blood Cultures and RT-PCR for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* in 177 Episodes of High-risk Febrile Neutropenia

	Blood Culture Positive	Blood Culture Negative	Total
RT-PCR positive	5	34	39
RT-PCR negative	4	134	138
Total	9	168	177
Sensitivity	56%		
Specificity	80%		
Positive predictive value	13%		
Negative predictive value	97%		
Positive likelihood ratio	2.83 (95% CI: 1.46–5.47)		
Negative likelihood ratio	0.55 (95% CI: 0.27–1.15)		

TABLE 3. Clinical Diagnosis of 36 Episodes of High-risk Febrile Neutropenia With Positive RT-PCR for *E. coli*, *S. aureus*, and *P. aeruginosa*

Main Clinical Diagnosis	No. Episodes With Indicated Clinical Diagnosis According to RT-PCR Bacterial Identification			
	E. coli	*S. aureus*	*Pseudomonas* sp	2 Species*
Pneumonia	1	5	0	1
Typhlitis	1	1	0	
Acute diarrhea	4	0	0	
Cellulitis	1	2	0	
CVC-related infection	0	2	0	
Sepsis	4	5	1	2
FUO	2	2	2	
Total	13	17	3	3

**S. aureus + P. aeruginosa*: 2 episodes, *S. aureus + E. coli*: 1 episode.
FUO indicates fever of unknown origin.

Analysis of Results

Descriptive noncontinuous variables were compared using χ^2 or Fisher exact test. For continuous variables, ANOVA or Kruskal-Wallis test was used or in cases with unequal variance, the Bartlett test. The significance level was set at $P < 0.05$. All statistical analyses were performed with the Sigma Stat 3.0 Program, Systat Software, CA.

RESULTS

Population Characteristics

A total of 309 episodes of FN were admitted to the participating hospitals during the 16-month study period of which 202 (65%) were HRFN episodes. Of these, 177 (88%) episodes occurring in 115 children were enrolled. The 25 HRFN episodes were not included in the study due to lack of consent (12) or failure of timely enrollment by study personnel (13). Median age of children was 8 years (interquartile range, 4–13 y), 59% were male patients, and 75.1% had a hematologic malignancy (Table 1).

Bacterial Detection by Blood Cultures and RT-PCR

One or more bacteria were isolated in a central or peripheral BC in 29 of the 177 HRFN episodes (16.3%), 9 (5%) of which corresponded to the 3 species selected: 5 to *E. coli*, 3 to *S. aureus*, and 1 to *P. aeruginosa*. RT-PCR was performed for the 3 selected species in blood samples collected at time 0 and 24 hours in all 177 episodes (total number of determinations: 1062). A total of 39 bacteria were amplified from 36 episodes for a 20% overall episode positivity by RT-PCR: 14 *E. coli*, 20 *S. aureus*, and 5 *P. aeruginosa*. In 3 episodes RT-PCR was positive for 2 species: *S. aureus* + *P. aeruginosa* (2) and S. *aureus* + *E. coli* (1) (Table 2; Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A902). Of the 39 positive PCR determinations, 12 were positive only at hour 0 (31%), 25 (64%) only at 24 hours, and 2 were positive at hour 0 and 24.

Three bacterial species under evaluation were cultured from 9 episodes using automated BC (5%) which increased to 36 episodes using DNA detection by RT-PCR (20%). Comparing with BC as gold standard for bacterial detection in blood, RT-PCR for *E. coli*, *S. aureus*, and *P. aeruginosa* had a sensitivity, specificity, and positive and negative predictive value of 56%, 80%, 13%, and 97%. The overall agreement between BC and RT-PCR, including positive-positive and negative-negative episodes was 79% (139/177). The positive and negative likelihood ratio was 2.83 (95% CI: 1.46–5.47) and 0.55 (95% CI: 0.27–1.15), respectively (Table 2).

The distribution of positive results by BC, RT-PCR, or both techniques for each of the 3 bacterial species evaluated is shown in Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A902. For 18 cases positive for *E. coli*, 4 of the 18 were detected only by BC, 13 of the 18 only by RT-PCR, and 1 of the 18 by both methods. For 20 *S. aureus* detected, all cases with BC positive were detected by RT-PCR (N = 3), and the other 17/20 (85%) were detected only by RT-PCR. For *P. aeruginosa*, 4 of the 5 cases were detected only by RT-PCR and 1 of the 5 by both methods.

Molecular Results and Clinical Diagnosis

Of the 36 HRFN episodes positive for 1 of the 3 selected bacteria by RT-PCR, 30 (83%) had a blinded clinical diagnosis compatible with IBI, of which 12 (40%) met the criteria for sepsis (Table 3). Of the 141 episodes with negative RT-PCR result, 19 (13%) had a clinical diagnosis of sepsis.

For *E. coli*-associated episodes, 12 of the 14 (86%) had clinical diagnoses compatible with IBI, 4 of which distinctively

presented acute diarrhea, clinical finding was not present for *S. aureus*- or *P. aeruginosa*-positive cases. A majority of *S. aureus*-associated episodes, 17 of the 20 (77%), had clinical diagnoses compatible with infection by this microorganism: pneumonia, cellulitis, CVC-related infection and sepsis; for *P. aeruginosa*-associated episodes, 3 of the 5 had a clinical diagnosis of sepsis (Table 3).

DISCUSSION

Molecular detection methods for the bacterial pathogens in sterile fluids are proving to be both sensitive and specific with the additional advantage of rapid results. Nevertheless, their use in clinical practice remains limited due to the lack of standardization for most techniques and the lack of specific studies associating clinical findings to the molecular detection results.[25–27]

This study shows that for children with cancer admitted because of HRFN episodes, RT-PCR for the 3 most common invasive microorganisms significantly improves their identification compared with automated BC (5% to 20%).

In an adult population, Lamoth et al[28] using a commercially available multiplex RT-PCR increased the etiologic yield from 25% to 43% in 141 FN episodes. von Lilienfeld et al[29] reported an increase in positivity from 3% with BC to 15% with multiplex RT-PCR in 119 FN episodes occurring in adults during antimicrobial treatment. In a small pilot study in children, Nakamura et al[30] demonstrated, in 23 FN episodes, detection of a bacterial pathogen in 3 cases (13%) by BC and in 11 (48%) by multiplex RT-PCR.

The results of this study open new questions, leaving space for future studies. The blinded clinical diagnosis of 4 of the 14 children with a positive RT-PCR for *E. coli* was acute diarrhea, suggesting that bacterial detection in blood may represent bacterial translocation from the digestive tract, possibly at a bacterial load insufficient to be detected by BC but sufficient for molecular detection. On the other hand, PCR techniques have a general limitation in being too sensitive, and in the present study, the sensitivity was only 56%, due to a poor performance in detecting bacteremia due to *E. coli* (only 1 of 5 episodes). Although promising, RT-PCR for *E. coli* needs to improve this high false-negative rate.

The RT-PCR positivity increase at 24 hours compared with admission is intriguing, allowing the hypothesis that an increase of bacterial DNA in blood at 24 hours may be due to bacterial lysis associated with antimicrobial activity. Besides, the cohort of patients had a median time of fever prior to admission of only 2 hours, probably because of the high awareness level of the parents on their child's underlying disease. Bacterial load is probably low at the time of admission and increases during the following hours, allowing a greater detection of bacterial DNA by PCR at 24 hours.

Several limitations of this study need to be addressed. A positive bacterial DNA detection in whole blood does not necessarily indicate that it is the cause of the IBI. In our series, bacteria were correlated with a compatible clinical presentation in 83% of RT-PCR-positive cases, suggesting that in these cases, the identified microorganisms were true pathogens. In our study, we did not consider a control group (with neutropenia and cancer but no fever) that could help to clarify this point. We performed DNA amplification only for 3 bacterial pathogens; other pilot trials have used commercial PCR multiplex kits for bacterial and fungal species in patients with FN, with promising results, providing significant improvement in patients with negative BC obtained during antimicrobial therapy.[28,29,31]

We could not demonstrate an advantage of early etiologic diagnosis in clinical outcome, because the study was not designed to answer this question. This study supports further research avenues, including evaluation of the additional benefit of increasing the bacterial species suitable for amplification, early identification of resistance genes, and clinical evaluation of specific antimicrobial therapy targeting bacteria identified within 24 hours of admission in addition to other potential pathogen-specific strategies based on RT-PCR results.

The results of this study suggest the possibility to detect bacterial DNA directly from whole blood samples rather than from the BC vial of the patient. Although the latter provides more robust results, it is clinically less relevant. Our long-term objective is to detect bacterial DNA before the automated BC turn positive. Although the techniques of bacterial DNA amplification can increase the costs of care, it is possible that in the near future these studies could be cost effective and accessible at a lower cost.

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