A new workflow for the microbiological diagnosis of febrile neutropenia in patients with a central venous catheter

Cécile Pautas1, Emilie Sbidian2†, Yosr Hicheri1,3†, Sylvie Bastuji-Garin2,4, Stéphane Bretagne2,5, Céline Corbel6, Laetitia Gregoire7, Sébastien Maury1,2, Lilia Merabet6, Catherine Cordonnier1,2 and Emmanuelle Cambau6,8,9*†

1APHP-Henri Mondor, Hematology Department, F-94010, Créteil, France; 2Université Paris Est, LIC EA4393, F-94010, Créteil, France; 3Université Paris Est, UFR de Médecine, F-94010, Créteil, France; 4APHP-Henri Mondor, Clinical Research and Public Health, F-94010, Créteil, France; 5APHP-Henri Mondor, Parasitology and Mycology Laboratory, F-94010, Créteil, France; 6APHP-Henri Mondor, Bacteriology Laboratory, F-94010, Créteil, France; 7APHP-Henri Mondor, Unité de Recherche Clinique, F-94010, Créteil, France; 8University Paris Diderot, EA3964, F-75018, Paris, France; 9APHP-Lariboisière, Bacteriology Laboratory, F-75010, Paris, France

*Corresponding author. Service de Bactériologie, Hôpital Lariboisière, 2 rue Ambroise Paré, 75 010 Paris, France. Tel: +33-1-49956554; Fax: +33-1-49958537; E-mail: emmanuelle.cambau@lrh.aphp.fr
†These authors contributed equally.

Received 12 June 2012; returned 5 September 2012; revised 19 October 2012; accepted 23 October 2012

Objectives: We aimed to improve the microbial diagnosis of first episodes of febrile neutropenia (FEFNs) since <30% of episodes are microbiologically documented. Consequently patients are usually treated by empirical antibiotic therapy.

Methods: A prospective study evaluated a new workflow combining: (i) one 40 mL blood culture (BC) sampled from the central venous catheter; (ii) immediate incubation in an automated BC system on the ward; (iii) direct detection of microbial DNA in blood; and (iv) identification and susceptibility testing using rapid methods performed directly on positive BC bottles. Patients were also sampled for the standard workflow with two BC sets incubated in the central laboratory and assessed by classical procedures.

Results: One hundred and twenty consecutive FEFNs were included (February 2008–March 2009). The new workflow was as sensitive as the standard workflow, with BC positivity rates of 30% (36/120) and 28% (34/120), respectively (McNemar's $\chi^2 = 0.67, P = 0.41$). Direct DNA detection was positive in nine episodes (7.5%) that were also positive in BC. The new workflow provided microbiological results significantly earlier than the standard workflow, with a shorter time to BC positivity (median 12 h 31 min, range 7 h 55 min–25 h 37 min versus median 13 h 01 min, range 9 h 31 min–43 h 33 min, $P = 0.004$) and shorter turnaround times for identification and susceptibility testing, with most of the results obtained <24 h after BC sampling. We retrospectively estimated that the new workflow would lead to earlier adequacy of antimicrobial therapy in 30% of documented cases.

Conclusions: Our new process improved the microbiological diagnosis in FEFNs. Cost effectiveness needs to be tested.

Keywords: blood culture, bacteraemia, rapid identification, susceptibility testing, CVC, neutropenic fever

Introduction

Patients with febrile neutropenia are urgently sampled for microbiological investigations and receive empirical broad-spectrum antibiotics.1,2 Since only one-third of first episodes of febrile neutropenia (FEFNs) are microbiologically documented, in the other cases treatment remains empirical and potentially inadequate due to multidrug resistance.3 This study aimed to improve FEFN documentation. In FEFNs, microbes are mainly isolated from blood; we therefore established a new workflow for blood culture (BC), including microbial DNA detection reported to increase the microbial documentation (Figure 1).4,5 This workflow was also designed to shorten the turnaround time (TAT) for identification and antibiotic susceptibility testing of the microorganism growing in BC, which is usually at least 48 h.6,7 We evaluated the performances of the new workflow versus our standard workflow with endpoints of positivity rate and TAT for final results.

© The Author 2012. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com
Methods

This prospective non-interventional study included 120 episodes of FEFN. Longitudinal outcome and adequacy of antibiotic therapy were determined retrospectively since we aimed to test the performance of the new workflow blind to these factors. The study was approved by the local ethics committee (CPP of Ile-de-France IX (number 08-006) and registered at ClinicalTrials.gov (NCT00913042). All patients gave their written informed consent. Median age of patients (male/female ratio of 1.1) was 50 years (range 20–75 years). Neutropenia was due to chemotherapy (97.5%) for acute leukaemia or myelodysplastic syndrome (81%) or for lymphoid malignancy (16.5%) or others (2.5%) (aplastic anaemia in two cases and sickle-cell disease in one case). Patients had undergone stem cell transplantation in 28% of cases, either allogeneic (53% of transplants) or autologous (47%). They had received antimicrobial prophylaxis as non-absorbable gut decontamination (75%) or oral levofloxacin (17%).

In the new workflow, BC bottles were sampled in one drawing through the central venous catheter (CVC) in order to decrease the number of venous punctures and facilitate adequate filling of the bottles. They were introduced immediately into a BacT/ALERT 3D® incubator (bioMérieux, Marcy l’Etoile, France) implemented on the ward. Molecular identification was performed directly on BC broth without sub-culture using GenoType®BC tests (Hain Lifescience, Nehren, Germany). Detection of microbial DNA was performed using the LightCycler® SeptiFast (LC-SF) test (Roche Diagnostics, Switzerland). Antibiotic susceptibility (AS) was determined using VITEK® 2 (bioMérieux) cards inoculated directly with BC broth plus mecA detection for methicillin resistance in staphylococci using Xpert®BC SA (Cepheid, USA).

Pairwise analyses were performed using the McNemar test to compare documentation rates of both workflows and the Wilcoxon signed-rank test to compare delays. The kappa index with 95% confidence interval was calculated to estimate agreement between the two workflows. Qualitative variables were described as number (%). Quantitative variables were described as mean (SD) or median (range) as appropriate. All tests were two-tailed and P-values ≤0.05 were considered significant. No adjustments for multiple comparisons were performed. Data were analysed using STATA software version 8 and SE11 (Stata, College Station, TX).

Results and discussion

BC positivity rates were equal in the new (30%, 36/120) and standard (28%, 34/120) (P=0.41) workflows. This showed that immediate incubation did not increase the BC positivity rate in our current configuration where the laboratory was located in the same building as the ward. This is probably also due to comparable blood sample volumes (data not shown). We showed that a single draw from the CVC gave similar results to the usually recommended two sets of BCs including a venepuncture, contrary to what has been recently observed. There was no difference indeed in the positivity rate between BCs sampled by venepuncture or via the CVC (P=0.3), and the number of positive bottles per episode was similar in the two workflows, including episodes with coagulase-negative staphylococci (CoNS) or streptococci, which are often viewed as contaminants when present in only one BC bottle. Overall, there were 40 (33%) episodes documented with positive BCs, since four (new) and two (standard) episodes were positive by one workflow and negative with the other (kappa agreement of 0.88; 95% CI 0.79–0.97). Bacterial isolates (n=50 overall) were Gram-positive cocci in...
The TAT for BC positivity was significantly shorter with the new workflow (Table 1). This may be explained by a median transportation time that was significantly shorter (22 min versus 4 h 24 min) with the new workflow. Previous studies reported a delay of up to 3 days in reporting positivity when time to incubation was ≥24 h, such as in delocalized laboratories. Time to positivity was shorter in our study than that reported in the literature, especially for streptococci and CoNS, and this may be due to immediate incubation.

Molecular identification was obtained with the GenoType®BC test for 40/43 (93%) bacteria isolated from the BC of the new workflow after a median TAT of 5 h 45 min, including the polymicrobial cases, which cannot yet be processed with mass spectrometry. Rapid bacterial identification is therefore now possible within the same day thanks to molecular biology and mass spectrometry coupled or not with PCR. Antibiotic susceptibility results were obtained using VITEK® 2 for 9/10 (90%) Gram-negative bacteria and 8/33 (24%) Gram-positive bacteria after a median time of 9 h 45 min, ranging from 5 h 15 min (E. coli) to 18 h 15 min (S. maltophilia). Invalid results mostly concerned streptococci, as shown on subcultures. mecA was detected in 1 h 30 min for the 14 BCs positive with staphylococci. Rapid susceptibility testing is so far only available as a phenotypic automated microdilution assay in liquid medium and molecular detection of resistance genes. With the new workflow, species could be identified and methicillin resistance determined by molecular methods in a median time of 19 h from BC sampling (Table 1). Antibiotic susceptibility results were obtained in <24 h for staphylococci, enterococci and Gram-negative bacteria (Table 1).

The LC-SF test is a commercially available test for detecting microbial DNA in blood. In our study, its positivity rate was 7.5% and the nine LC-SF-positive episodes were also BC positive. The fact that LC-SF testing did not increase the microbial documentation of FEFNs, in contrast to previous studies, was probably related to the inclusion of patients at FEFN and naive to systemic antibiotics in our study. To explore why 27 episodes with positive BCs had a negative LC-SF, we compared the time to BC positivity of the 9 LC-SF-positive and 27 LC-SF-negative episodes [10 h 45 min (range 8 h–19 h 09 min) versus 13 h 09 min (range 7 h 55 min–25 h 32 min), P=0.0016]. The longer time of positivity of the LC-SF-negative episodes suggests a low bacterial inoculum, in accordance with the analytical sensitivity of the LC-SF (30–100 cfu/mL of blood).

Microbial documentation was obtained in four additional episodes from culture of urine (n=2), skin (n=1) and tracheal aspiration (n=1). Seventeen (14%) episodes were only clinically documented (six mucositis, seven skin infections, one CVC infection, three dental infections). Previous studies conducted on FEFNs in the same or in similar wards showed a similar distribution of 37% cases with microbiological documentation, 14% with only clinical documentation and 49% with fever of unknown origin. Severe sepsis and septic shock were observed in two and five cases, respectively. At the onset of FEFN, empirical antimicrobial treatment was prescribed.
according to international guidelines. Anti-infective treatment was broad-spectrum β-lactams for 119 cases (99.2%) in combination with vancomycin for 33 cases (27.5%), with aminoglycosides for 3 cases (2.5%) or with another antibiotic (fluoroquinolones, metronidazole) for 4 cases (3.3%). At least two antibiotics were prescribed in 36 cases (30%). The outcome of patients assessed 30 days after inclusion was alive and without neutropenia in 107 cases (89.2%), alive but still with neutropenia in 11 cases (9%) and died in two cases (1.7%). We determined the number of cases in which the treatment would have been modified if the results of the new work-flow had been known to the clinicians. In 13 out of the 36 positive cases (36%), the first-line regimen would have been changed either by adding vancomycin or amikacin or by replacing the first-line regimen with imipenem, because of results of methicillin-resistant CoNS, penicillin-resistant streptococci and enterococci, P. aeruginosa or E. cloacae producing extended spectrum β-lactamase. We verified that in each case this BC result was consistent with clinical signs of infection or absence of improvement with the empirical treatment, according to guidelines.

By combining new techniques for microbial detection in BC, we did not increase the microbiological documentation of FEFNs. However, we shortened the time to obtaining results, which could lead to earlier adequacy of therapy. Although it was not specific to FEFNs, a short time to microbiological notification has been significantly correlated with shorter hospital stay and lower hospital charges.

Acknowledgements
We are grateful to Jean-Pierre Marcel and Valérie Calas from bioMérieux, Marc Tordjeman from Biocentric and Rémé Rabeuf from Roche Diagnostics for providing equipment (Bact/ALERT, VITEK®2, Twincubator) or materials (LightCycler® SeptFast tests) for this study. We are also grateful to Laurent Raskine for 16S rDNA sequencing.

Funding
The work was supported by a clinical research grant from the Délégation à la Recherche Clinique et au Développement (DRRC) Ile-de-France (CRC 06 021).

Transparency declarations
None to declare.

References