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SHORT COMMUNICATION

Rapid Detection of Bloodstream Pathogens in Oncologic Patients with a FilmArray Multiplex PCR Assay: a Comparison with Culture Methods

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Abstract

The results of the FilmArray® Blood Culture Identification Panel (BCID) (BioFire Diagnostics) and the culture with susceptibility testing of 70 positive blood cultures from oncologic patients were compared. The multiplex PCR assay (BCID) identified 81 of the 83 isolates (97.6%), covered by the panel. The panel produced results in significantly shorter time than standard identification methods, when counted from receiving positive blood cultures bottles to the final results. It is an accurate method for the rapid identification of pathogens and resistance genes from blood culture in oncologic patients

Key words: bloodstream infection, FilmArray, multiplex PCR, oncologic patients

Sepsis in oncologic patients is a serious complication in the course of primary treatment. Surgery, often an extensive one, involves opening of the digestive tract, urinary tract, anastomosis of the colon, use of vascular lines, catheters in the bladder, parenteral nutrition, stay in the ICU, immunocompromised immune systems, and favours systemic infections (Encina *et al.* 2016; Alkhamis *et al.*, 2014; Smit *et al.*, 2016; Mahdi *et al.*, 2014). Generalized infections in cancer patients are burdened with high mortality; therefore, time is one of the important factors in their diagnosis and treatment (Namendys-Silva *et al.*, 2010; Rosolem *et al.,* 2012). Classic diagnosis of these infections, including identification, determination of antibiotic susceptibility and detection of resistance mechanisms of the cultured microorganisms, takes 2–5 days from the delivery of samples for microbiological examination. Due to the relatively long period of waiting for the test results, empiric therapy is implemented. The etiologic agent is often not grown, due to the sensitivity of the culture method. Empiric therapy carries the risk of not including the etiologic agent of the infection within its coverage, it can lead to overuse of antibiotics with a wide spectrum, prolongs hospitalization, increases the cost of treatment, and selects for multidrug-resistant strains in units of health care, which ultimately leads to increased mortality (Kumar, 2011).

In 2013, the Food and Drug Administration (FDA) issued a positive opinion on a FilmArray® Blood Culture Identification Panel (BCID) (BioFire Diagnostics, Salt Lake City, UT) for rapid identification of aerobic microorganisms in positive blood culture. The application of the multiplex PCR method in the diagnosis of bloodstream infections is designed to reduce the time for identification of the microorganisms grown from the positive samples to 24–48 hours, as pathogens are identified directly from a positive blood sample.

There have been several papers published on the usefulness of the test in diagnosis of sepsis in adults, children and patients undergoing organ transplantation in relation to conventional methods, but there are no papers defining the efficacy of the test in diagnosis of bloodstream infections in cancer patients, including patients after surgery (Blaschke *et al.*, 2012; Zheng *et al.*, 2014; Otlu *et al.*, 2015).

The aim of the study was to compare two methods of identification of microorganisms from positive blood cultures: the classical method – culture and the genetic method – multiplex PCR as well as the time from the receipt of positive samples to communicating the result of PCR and uploading the microbiological report into the hospital information system.

The examination involved 70 positive blood samples obtained in BacT/Alert 3D instrument, between

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Table I
Microorganism identified with BCID.

Organism Gram-negative	Organism Gram-positive	Yeast	Antibiotic resistance
Acinetobacter baumannii	*Enterococcus*	*Candida albicans*	*mecA*
Haemophilus influenzae	*Listeria monocytogenes*	*Candida glabrata*	*vanA/B*
Neisseria meningitidis	***Staphylococcus***	*Candida krusei*	bla_{KPC}
Pseudomonas aeruginosa	*Staphylococcus aureus*	*Candida parapsilosis*	
Enterobacteriaceae	***Streptococcus***	*Candida tropicalis*	
Enterobacter cloacae complex	*Streptococcus agalactiae*		
Escherichia coli	*Streptococcus pyogenes*		
Klebsiella pneumoniae	*Streptococcus pneumoniae*		
Klebsiella oxytoca			
Serratia marcescens			
Proteus sp.			

August 2015 and November 2016. Diagnostic materials were collected from 55 patients suffering from cancers, who were treated in clinical departments of Prof. F. Łukaszczyk Oncology Centre in Bydgoszcz. Among the patients from which blood was drawn, 40 patients (72.7%) were treated surgically, with different extensity of the surgical procedure, including pelvic exenteration in the case of advanced ovarian cancer, and 15 patients (27.3%) underwent conservative treatment. Positive aerobic blood cultures, confirmed by the microscopic preparation stained with the Gram method, underwent multiplex PCR analysis with the use of BCID. Microorganisms and resistance genes covered by BCID are presented in Table I. Positive blood cultures were also passaged on solid media and the isolated microorganisms were identified and analysed for antibiotic susceptibility with the use of VITEK 2 Compact and E-test® system (bioMérieux, USA). Due to the fact that there is a 12-hours shift system in the Microbiology Department, 13 test samples (18.6%) underwent genetic analysis in more than 8 hours after the signal from BacT/Alert 3D instrument was observed. Validation of the method did not confirm that the extension of bottles incubation time to 15 hours had a negative impact on the reliability of the results. As a gold standard, the culture method was used in the research. Quantitative data were developed with the use of U Mann-Whitney test, value $p < 0.05$ was acknowledged as statistically significant.

Eighty-nine isolates were cultured from 70 samples. In 55 (78.6%) cultures microbial growth in monoculture was obtained. The multiplex PCR assay revealed all bacterial species and types present in positive blood cultures bottles that have been included in the panel. However, for two isolates identified using BCID, despite the increase in cultivation time, the presence of the microorganism in the samples was not confirmed using multiplex PCR method; this concerned *Staphylococcus hominis* ssp. *hominis* present in monoculture and *Escherichia coli* present in mixed culture with three other microorganisms. In 6 (6.7%) cases, the microorganisms that are not covered by BCID were cultured: *Lactobacillus* spp., *Lactobacillus plantarum*, *Candida lusitaniae*, *Stenotrophomonas maltophilia*, *Acinetobacter lwoffii* and *Haemophilus parainfluenzae*. In one case, in a mixed culture (1.3%), *Haemophilus influenzae* was identified in PCR while *H. parainfluenzae* was cultured. Antibiotics resistance determined by detection of the resistance genes and by phenotypic methods showed good concordance. The presence of the *mecA* gene was confirmed in 30 strains of coagulase-negative staphylococci, and in two strains of *Staphylococcus aureus*. In one case (3.3%) a *mecA* gene was found, without confirmation of growth of methicillin-resistant strain in the culture. Among six enterococci identified, no strains with *vanA/B* gene were detected. Moreover, no Gram-negative bacteria with resistance to carbapenems resulting from the presence of the bla_{KPC} gene were cultured from the blood in the analysis period. Comparison of the results obtained using the multiplex PCR method (BCID) and the classical method is shown in Table II.

The average time of the positive samples detection in the BactAlert 3D system was 23.1 h (SD ± 14.2 h), with a period of time from detection of positive sample to start of BCID – 3.6 hours (SD ± 4.18 h). Information about the positive PCR results was submitted to a doctor within 4.9 hours (SD ± 4.2 h) while the report on the culture results was presented on average in 67.7 h (SD ± 22.9 h). Blood culture is the most commonly used microbiological method in the diagnosis of sepsis. In recent years, however, research is being conducted on the use of rapid, more sensitive tests for the detection of microorganisms directly from the blood. Rapid identification of microorganisms in sepsis is crucial for the selection of appropriate treatment. An adequate and early treatment significantly reduces mortality (Dellinger *et al.*, 2012). BCID con-

Table II
Comparison of blood culture and multiplex PCR (BCID) results.

Microorganism group	Culture result (no)	BCID result (no)
Gram-positive bacteria	*Staphylococcus hominis* ssp. *hominis* MR (9)	*Staphylococcus* (8) mecA (8)
	Staphylococcus hominis ssp. *hominis* MS (3)	*Staphylococcus* (3) mecA (1)[1]
	Staphylococcus epidermidis MR(16)	*Staphylococcus* mecA (15)[1]
	Staphylococcus epidermidis MS (4)	*Staphylococcus* (4)
	Staphylococcus haemolyticus MR (6)	*Staphylococcus* mecA (6)
	Staphylococcus capitis MS (2)	*Staphylococcus* (2)
	MSCNS (1)	*Staphylococcus* (1)
	Staphylococcus aureus MS (4)	*Staphylococcus; Staphylococcus aureus* (4)
	Staphylococcus aureus MR (2)	*Staphylococcus; Staphylococcus aureus* mecA (2)
	Enterococcus faecalis (4)	*Enterococcus* sp. (4)
	Enterococcus faecium (2)	*Enterococcus* sp. (2)
	Streptococcus salivarius (1)	*Streptococcus* sp. (1)
	Lactobacillus plantarum (1)	ND[2]
	Lactobacillus spp. (1)	ND[2]
Gram-negative bacteria	*Pseudomonas aeruginosa* (9)	*Pseudomonas aeruginosa* (9)
	Enterobacter cloacae (1)	Enterobacteriaceae*; Enterobacter cloacae* (1)
	Escherichia coli (9)	Enterobacteriaceae*; Escherichia coli* (8)
	Klebsiella pneumoniae ssp. *pneumoniae* (3)	Enterobacteriaceae*; Klebsiella pneumoniae* (3)
	Stenotrophomonas maltophilia (1)	ND[2]
	Proteus mirabilis (2)	Enterobacteriaceae*; Proteus* sp.(2)
	Acinetobacter lwoffii (1)	ND[2]
	Haemophilus parainfluenzae (1)	ND[2]
	ND	*Haemophilus influenzae*
Yeast	*Candida glabrata* (2)	*Candida glabrata* (2)
	Candida albicans (1)	*Candida albicans* (1)
	Candida parapsilosis (2)	*Candida parapsilosis* (2)
	Candida lusitaniae (1)	ND[2]

[1] in one sample two morphological different strains of *S. epidermidis*; [2] not detectable in BCID panel spectrum; ND – not detected; MR – methicillin resistant; MS – methicillin susceptible; MRCNS – methicillin resistant coagulase negative *Staphylococcus*

tains a wide panel of microorganisms: bacteria and fungi responsible for approx. 90.0% of bloodstream infections, detects the most common mechanisms of resistance in short time, and is an important tool in the surveillance of bloodstream infections (Blaschke *et al.*, 2012; Otlu *et al.*, 2015; Altun *et al.*, 2013.). In our study on cancer patients, BCID covered 93,3% of the microorganisms isolated from the blood. Similar results were obtained by Zheng *et al.* (2014) in a study of 166 positive blood cultures from 138 children, where BCID covered approximately 93.0% of cultured microorganisms. In most of the samples, approx. 97.0%, we obtained consistent identifications with both methods used: the genetic method and the culture method. In one case, we observed a growth of *S. hominis* ssp. *hominis* methicillin-resistant in a culture, without confirmation of presence of the microorganism with the multiplex PCR method. The similar results were obtained by Zheng *et al.* (2014); this microorganism was also not detected in one sample analysed. BCID did not detect the following microorganisms: *C. lusitaniae*, *S. maltophilia*, *A. lwoffii*, *L.* spp., *L. plantarum*, *H. parainfluenzae,* which do not fall within the spectrum of BCID. According to the manufacturer's instructions, the limitation of this method is that in mixed cultures BCID may not correctly identify all microorganisms. In our study, 15 blood cultures were positive for more than one species of bacteria. Blaschke *et al.* (2012) suggested that the uneven growth of two species in liquid culture could cause that despite the positive signal from the BacT/ALERT detection system one of the species may not be detected with the multiplex PCR. However, according to Altun *et al.* (2013), the lower limit of detection (LOD) in BCID, is generally sufficient to detect pathogens. According to the characteristics of BCID specified by the manufacturer, the density of bacteria in positive blood culture during the test was ~ 10^7–10^8 CFU/ml. In our study, usually Gram-positive cocci grown in mixed

cultures as follows: enterococci and coagulase-negative staphylococci, two species of coagulase-negative staphylococci as well as enterococci and yeast-like fungi. In these cases, we obtained full compliance of results in both methods. In mixed cultures of Gram-negative bacilli and Gram-positive cocci in one case, the system did not detect *E. coli*, despite longer culture time and identified *H. parainfluenzae*, which was not included in BCID. The oxacillin resistance determined by the presence of the *mecA* gene was correctly identified in both coagulase-negative strains of *Staphylococcus* and methicillin-resistant strains of *S. aureus*. Furthermore, in one case, the PCR system also detects the presence of the *mecA* gene, which could not be confirmed phenotypically. BCID correctly indicated negative results for *vanA* and *vanB* among enterococci. However, it should be noted that the system does not distinguish between *Enterococcus faecalis* and *Enterococcus faecium*, the most common species of enterococci. This is associated with the choice of antibiotic for treatment, as the majority of *E. faecium* is resistant to ampicillin. This is a certain limitation of the panel, especially in the case of sepsis after surgery in the abdominal cavity. MacVane *et al.* (2016) confirmed that BCID is useful in the diagnosis of sepsis caused by vancomycin-resistant strains of *Enterococcus* spp. The study included 68 patients with bacteremia caused by VRE. The authors showed statistically significant differences in the time to identify the microorganism by culture and genetic methods (47.7 h *versus* 18.2 h, $p < 0.001$), and statistically significant difference was also shown in the time required to evaluate the susceptibility to vancomycin and the time for the implementation of effective therapy ($p < 0.001$). The authors also pointed to a significant reduction in the cost of a patient's stay in hospital, when using a genetic method. Otlu *et al.* (2015) evaluated the usefulness of BCID in the diagnosis of sepsis in patients undergoing liver transplantation in order to shorten the time needed to obtain a result in relation to the classical methods, automatic Vitek II and mass spectrometry – Vitek MS system. These differences were significant, and the time to obtain the results was as follows: the classical method – 36.2 h (SD ± 19.2 h), automatic method Vitek II – 23.6 (SD ± 2.23 h) and Vitek MS system 19.5 h (SD ± 15.1 h). BCID identified pathogen within 65 to 100 minutes.

In our study, the difference in amount of time needed to inform the physician about the detection of the microorganism and its mechanisms of resistance using BCID compared to the time needed to obtain the same results by VITEK 2 Compact and E-test® was also statistically significant ($p < 0.05$). However, due to the 12-hours shift system, the time to transfer the result was longer than in the above-cited studies. Inglis *et al.* (2016) studied 149 blood cultures derived from 143 patients and evaluated the usefulness of BCID depending on the hospital referral level. The authors believe that BCID is particularly suitable for small laboratories in regional hospitals, dominated by the most common microorganisms. Our research confirms that the panel can also be used among cancer patients after surgery and conservative treatment of cancer.

In conclusion, BCID identified most of the microorganisms present in positive blood cultures in cancer patients, including patients undergoing abdominal surgery and pelvic exenteration. It can be a very useful tool in the surveillance of bloodstream infections providing information on the etiological agent and the basic mechanisms of microbial resistance. It should be noted, however, that the essential component of genetic diagnostics is culture, which remains the gold standard. Application of BCID speeds up the decision on the selection of appropriate treatment, because it significantly shortens the time to provide essential information to the doctor.

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