Diagnostic value of blood culture independent molecular tests for the diagnosis of bloodstream infections in neutropenic patients with fever

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Objective

Bloodstream infections (BSI) and sepsis are a common cause of morbidity and death, especially in neutropenic patients. Neutropenic patients are at high risk of infectious complications, and due to the inadequate inflammatory response, sepsis is an important cause of death in this situation. Delay of appropriate antimicrobial therapy in neutropenic patients suffering from BSI is associated with a lower chance of survival. Therefore, these patients should be treated as medical emergencies. To combat the high mortality rate associated with BSI, timely administration of appropriate antimicrobial therapy is critical. Blood culture (BC) is considered the gold standard in BSI diagnostics, but can delay results due to the long incubation period. Molecular tests could shorten the time to pathogen identification and be a valuable complement to BC. **The aim of the present study was to evaluate the accuracy and suitability of three commercially available molecular tests (Hybcell Pathogens DNA xB, Micro-Dx™ and the T2 panels for Bacteria and Candida), for the rapid detection of BSI in neutropenic patients with fever using whole blood compared to conventional BCs and SeptiFast (**Roche**), a proven established test that was withdrawn from the market in 2019.**

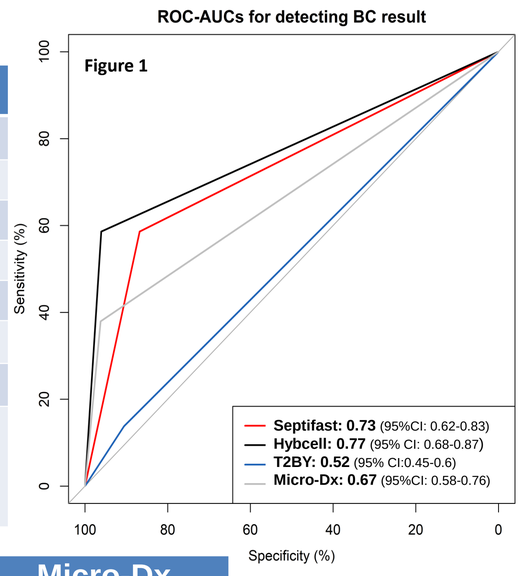
Patients and Methods

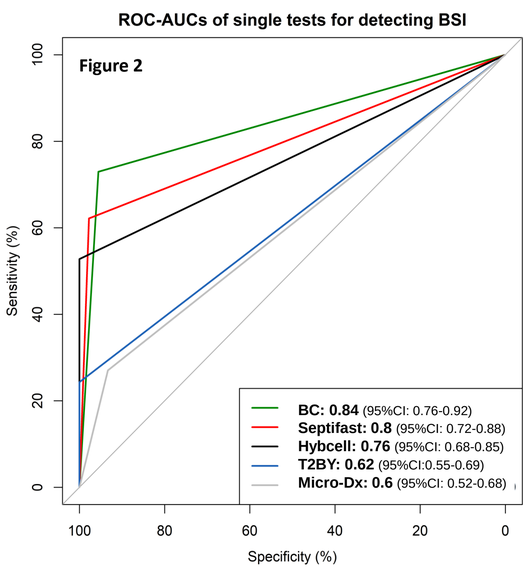
The study was conducted as a prospective observational study at the University Hospital of Vienna. The study protocol was approved by the ethical review boards of the Medical University of Vienna. Adult neutropenic (defined as absolute neutrophil count <500/µl) patients with an underlying haematological-oncological disease, a new febrile episode (measured ≥ 38°C) and a suspected BSI, were enrolled in the study. BSI was defined based on the adapted ECDC criteria and clinical data. Whole blood samples were collected by the same venipuncture as for routine blood cultures and the performance of the four molecular tests was assessed in comparison to BC and laboratory-confirmed BSI.
BC bottles were incubated for up to 7 days in a BacT/ALERT 3D analyser (bioMérieux, Marcy l'Etoile, France). If positive, Gram staining and the Sepsityper test (Bruker Daltonics, Germany) were performed directly from the positive BC bottle for species identification using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS , MALDI Biotyper, Bruker, Germany) followed by sub-culturing on solid culture media to confirm pathogen identification and perform antimicrobial sensitivity testing.
The samples for molecular testing were processed according to the instructions of the respective manufacturer:
SeptiFast Test M GRADE (Roche): With a turnaround time of about 5 hours, the test was able to detect the 20 most important pathogens or groups of pathogens. The test was well established in our laboratory (>2500 examinations/year) and was widely used in Europe until it was withdrawn from the market in 2019.
Micro-Dx™ (Molzym, Germany) is currently the only automated, commercially available, CE-IVD-labelled molecular test for broad-spectrum pathogen detection. The main advantage of this test is therefore its broad spectrum (>200 genera of bacteria). In the case of a positive PCR result the need for Sanger sequencing prolongs the time to result.
T2Biosystems offers 2 panels for the T2Dx instrument, the T2Bacteria and the T2Candida panel. The novel automated system uses T2 magnetic resonance (T2MR) for the detection of pathogens in whole blood samples. The Bacteria Panel can detect six bacterial targets, while the Candida Panel can detect the presence of the five more common Candida spp with a turnaround time of around 5 hours. Both panels are CE-IVD labelled and FDA approved.
Hybcell Pathogens DNA xB (Cube Dx, Austria) uses four separate PCR reactions (positive control, 16S rDNA, 28s rDNA, for the resistance markers vanA, vanB, mecA and mecC). PCR products were transferred to a microarray and amplicons were identified in the hyborg device by compact sequencing. Results were available as early as in three hours. The system can identify one panbacterial target, four bacterial genera and 28 bacterial species, and one panfungal target, two fungal genera and 13 fungal species.

Results

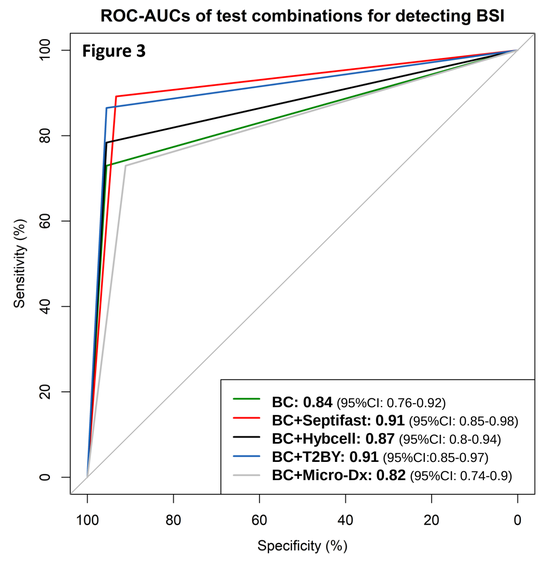
A total of 82 febrile neutropenic episodes occurred in 70 consecutive patients, all but three of whom received antimicrobial therapy. Thirty seven episodes (45.1%) were classified as BSI. At the onset of fever, 39 micro-organisms (8 Gram-negative and 31 Gram-positive bacteria; no fungi) were detected in 29/82 (35.4%) febrile episodes by BC. Contamination was found by BC in 2, by SF in 1, CubeDx in 1, T2 in 2 and Mollzym in 1 non-BSI cases. The comparison of the performance of the different tests with BC is shown in Table 1. The sensitivity of Hybcell Pathogens DNA and SeptiFast, with 59% each, was considerably higher than that of Micro-Dx and T2BY with 38% and 14%, respectively. Compared to BC, Hybcell Pathogens DNA showed the best overall performance with a ROC-AUC value of 0.77, followed by SeptiFast, Micro-Dx and T2BY (figure 1). Test performance and overall performance compared to BSI diagnosis are shown in table 2 and figure 2 respectively, while the added value of each test as an add-on to blood culture is shown in figure 3.

Tabel 1 Comparion to BC	Septifast		Hybcell		T2BY		Micro-Dx	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
BC=0	46	7	49	2	47	5	51	2
BC=1	12	17	12	17	24	4	18	11
Sensitivity	0.59 (0.39 - 0.76)		0.59 (0.39 - 0.76)		0.14 (0.04 - 0.33)		0.38 (0.21 - 0.58)	
Specificity	0.87 (0.75 - 0.95)		0.96 (0.87 - 1.00)		0.90 (0.79 - 0.97)		0.96 (0.87 - 1.00)	
NPV	0.79 (0.67 - 0.89)		0.80 (0.68 - 0.89)		0.66 (0.54 - 0.77)		0.74 (0.62 - 0.84)	
PPV	0.71 (0.49 - 0.87)		0.89 (0.67 - 0.99)		0.44 (0.14 - 0.79)		0.85 (0.55 - 0.98)	
Correctly classified proportion	0.77 (0.66 - 0.85)		0.82 (0.72 - 0.90)		0.64 (0.52 - 0.74)		0.76 (0.65 - 0.84)	





Tabel 2 Comparison to BSI diagnosis	Blood Culture		Septifast		Hybcell		T2BY		Micro-Dx	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
BSI=0	43	2	44	1	44	0	44	0	42	3
BSI=1	10	27	14	23	17	19	27	9	27	10
Sensitivity	0.73 (0.56- 0.86)		0.62 (0.45- 0.78)		0.53 (0.35- 0.70)		0.25 (0.12- 0.42)		0.27 (0.14- 0.44)	
Specificity	0.96 (0.85- 0.99)		0.98 (0.88- 1.00)		1.00 (0.92- 1.00)		1.00 (0.92- 1.00)		0.93 (0.82- 0.99)	
NPV	0.81 (0.68- 0.91)		0.76 (0.63- 0.86)		0.72 (0.59- 0.83)		0.62 (0.50- 0.73)		0.61 (0.48- 0.72)	
PPV	0.93 (0.77- 0.99)		0.96 (0.79- 1.00)		1.00 (0.82- 1.00)		1.00 (0.66- 1.00)		0.77 (0.46- 0.95)	
Correctly classified proportion	0.85 (0.76- 0.92)		0.82 (0.72-0.89)		0.79 (0.68- 0.87)		0.66 (0.55- 0.76)		0.63 (0.52- 0.74)	



Conclusion

Of the three molecular tests currently available, only Hybcell Pathogens DNA showed an acceptable performance in diagnosing BSI in neutropenic patients with fever.