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FULL PAPER

The clinical impact of a multiplex real-time PCR system for microbiological diagnosis of sepsis: a mortality study

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SUMMARY

To evaluate the impact of the use of a multiplex-real time PCR-based assay (SeptiFast M-Grade®) on in-hospital mortality in ICU sepsis patients.

Demographic, clinical and microbiological data from ICU patients with suspected sepsis and available SeptiFast (SF) test results were gathered. The intervention group comprised patients in which SF indicated a clinical intervention; the non-intervention group included patients in whom SF result did not lead to any clinical intervention. The study looked at expected and observed in-hospital mortality rates in both intervention and non-intervention groups.

Two-hundred and fifty-five patients (121 patients in the intervention group and 134 patients in the non-intervention group) were included in the study. When comparing both groups, we found no significant differences in severity scores, either in estimated or observed mortalities. Older age, high APACHE II scores, and infections caused by Gram-negative pathogens and carbapenem-resistant enterobacteria were all associated with a higher risk of death in both groups. Overall, blood cultures and SF agreed in 75.3% of cases. Positivity rates were 22.0% for blood culture, 29.4% for SF, and 38.0% combined.

Though we did not find a correlation between SeptiFast-based intervention and changes in in-hospital mortality, SeptiFast improved positivity rates. The above improvement in microbiological diagnosis might be associated with fewer complications, lower hospitalization costs and presumably better long-term survival rates in sepsis patients.

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INTRODUCTION

Sepsis mortality is in the 30-50% range, though mortality rates have declined in recent years (Stevenson, Rubenstein, Radin, Wiener, & Walkey, 2014), (Bouza, López-Cuadrado, Saz-Parkinson, & Amate-Blanco, 2014). Annual incidences have, however, increased in several countries (221 in 2000 to 377/100000 in 2008 – USA data (Hall, Williams, DeFrances, & Golosinskiy, 2011) and 256 in 2007 to 335/100000 in 2013 – Germany data (Fleischmann *et al.*, 2016). It is interesting to note that recent sepsis coding changes have led, at least in the USA, to an overall higher sepsis incidence, which might contribute to an apparent decline in mortality (Gohil *et al.*, 2015). However, mortality rates for children with severe sepsis have declined in several countries (Thompson & Kissoon, 2014), (Hartman, Linde-Zwirble, Angus, & Watson, 2013). In England, 5.1% of deaths (general population) were found to be associated with sepsis (McPherson *et al.*, 2013).

Microbiological diagnosis is critical in sepsis management. Traditional microbiological techniques are valuable for antibiotic susceptibility testing (AST) and genotyping and may be complemented with newly introduced methods such as analytical (e.g., mass spectrometry) and molecular ones (Jordana-Lluch, Giménez, Quesada, Ausina, & Martró, 2014). Many studies have looked into the value of non-culture-based methods and biomarkers for the diagnosis of bloodstream infections – BSIs (Skvarc, Stubljar, Rogina, & Kaasch, 2013). Several reviews on molecular biology-based techniques have been published in recent years (Liesenfeld, Lehman, Hunfeld, & Kost, 2014) (Lebovitz & Burbelo, 2013) that highlight the potential value of these techniques in clinical sepsis settings.

The impact of non-culture-based techniques for the microbiological diagnosis of sepsis has been assessed (Skvarc *et al.*, 2013) (Ratzinger *et al.*, 2016) (Chang *et al.*, 2013) (Rodrigues *et al.*, 2019). The above techniques have a demonstrated value in the diagnosis of culture-negative BSIs, for instance in bacteraemia episodes caused by fastidious or slow-growing microorganisms and in patients with fungemia. Some studies identify the benefit of non-culture-based techniques in added positivity rates for microorganism identification when considering blood cultures and other techniques among a panel of available microbiological diagnostic methods. Overall sensitivities and specificities of commercially available molecular biology-based platforms are in general better than those of blood cultures alone (Makristathis *et al.*, 2018).

Key words:
Sepsis, APACHE II, real-time PCR.

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Currently available molecular biology systems for detection and identification of microorganisms in suspected BSIs include real-time PCR platforms such as the LightCycler SeptiFast Mgrade Test – LC SeptiFast (Roche Diagnostics, Mannheim, Germany) which has been used for several years and tested in research and clinical scenarios (Chang *et al.*, 2013). The LC SeptiFast (SF) is a multiplex quantitative polymerase chain reaction-based assay that detects DNA sequences corresponding to a panel of 20 organisms prevalent in sepsis /BSIs. While the value of the Roche test is clear for the positivity of detection rates, only a few studies have looked at LC SeptiFast (SF) clinical value in terms of outcomes in groups of critically ill patients.

PATIENTS AND METHODS

Study design

We collected data from ICU patients with suspected sepsis admitted to a large referral hospital in Quito, Ecuador, through 12 months from April 2016 to April 2017. Selected patients had SeptiFast M Grade® (Roche Molecular Systems, Mannheim, Germany) testing from whole blood results available. The study collected clinical, microbiological and demographic data and uploaded them to a tailored database. The intervention group included patients in which the SF result led to a modification in antimicrobial therapy (start of antibiotic therapy, change of antibiotic scheme or antibiotic withdrawal). The non-intervention group included patients in whom the SF result did not lead to a change in antibiotic therapy. The study then retrospectively looked at estimated and observed in-hospital mortality rates in both groups. All patients provided written consent for the study. The hospital ethics committee approved the study and its protocols.

Severity scores

The APACHE II score (Knaus, Draper, Wagner, & Zimmerman, 1985) was chosen due to its demonstrated good correlation with expected mortality (Basile-Filho *et al.*, 2019) (Kądziołka, Świstek, Borowska, Tyszecki, & Serednicki, 2019). ICUs assess the APACHE II score on the patient's admission to the units. APACHE II data were available from medical charts and patients' logbooks.

Data collection

A database was built using Excel spreadsheets. Data were manually gathered from patients' logbooks as detailed above. Data clean-up and validation, as well as pivot tables and preliminary analysis, were performed with the built-in Excel tools. Patients who had neither blood culture nor SF results were excluded from the study.

Microbiological methods

Blood cultures: Blood cultures were drawn on attending physician's orders.

The hospital microbiology laboratory performed blood culturing according to CLSI standards. All microbiology procedures were carried out in the hospital lab and by experienced lab technicians.

LightCycler SeptiFast MGrade® Test: ICU physicians, following the unit's algorithm, ordered SF testing. The hospital's molecular biology laboratory carried out the SF tests under strict aseptic conditions and complying with the manufacturer's instructions.

Study endpoint and data collection

The in-hospital mortality rate was the dependent variable. The study retrospectively collected clinical and epidemiological data from medical charts and patients' data logs. The study end-point was the patient's condition at discharge.

Statistical analysis

Continuous values were shown as means ± standard deviations. Categorical values were expressed as counts and percentages. The T-test was used to compare continuous values and $\chi 2$ tests were used for categorical values. The level of agreement was measured using the kappa test. The relationship between treatment switch (intervention) after SF and hospital discharge was assessed using a logistic regression adjusted by potentially confounding factors, including age and sex.

RESULTS

Two hundred and fifty-five patients with suspected sepsis were included in the study. The mean age was 54.2 ± 17.5 years; 65.5% of patients were males. The intervention group included 121 patients in which SF determined any type of clinical intervention and the non-intervention group had 134 patients who continued with their original therapy. There was a statistical difference in hospitalization length, blood culture time and SF time between intervention and non-intervention groups (*Table 1*).

The APACHE II index provides a mortality estimation based on specific clinical and biochemical patient-characteristics. The study of concordance between mortality estimation and status at hospital discharge (dead or alive) showed a kappa index of 0.387 (95% CI: 0.273-0.502), equivalent to a moderate agreement, and a percentage agreement of 69.8% (95% CI: 63.7-75.3%).

Blood culture and SF level of agreement

Blood culture and SF tests agreed 75.3% (95% CI: 69.4-80.4) of the time; nevertheless, both tests showed a fair to moderate level agreement according to kappa (0.3575; 95% CI: 0.219-0.495). Also, six positive samples for blood culture and SF grew different bacteria in each test. Among patients with no-growth blood cultures, 16.1% had switched treatment due to positive SF results (*Table 2*).

The positivity rate was 22.0% (56/255) for blood culture and 29.4% (75/255) for SF. The positivity rate in the group of patients with an identified bacterial species result was 57.7% (56/97) for the blood culture and 77.3% (75/97) for the SF test. The overall positivity was 38.0% (97/255), independent of the test used. With regard to the added positivity percentage, blood culture added 22.6% (22/97) over the SF test; however, SF added 42.3% (41/97) over blood cultures. When SF and blood culture were performed together positivity rates increased by 73.2%.

SF detected more than one bacterial species in a collected blood sample 28.9% of the time. These bacteria included (not exclusively) *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella aerogenes*, *Escherichia coli*, *Enterobacter cloacae*, *Serratia marcescens* and *Pseudomonas aeruginosa*. In samples where SF was negative and blood culture was positive, the most frequently isolated bacteria were Gram-positive (40.9% of cases), including *Staphylococcus epidermidis* and *Staphylococcus aureus*. (*Tables 3 and 4*)

The assessment of mortality estimation and switch of

Table 1 - *Baseline characteristics of the study patients on admission to the Intensive Care Unit.*

Characteristics	*Intervention Group N=121*	*Non-intervention Group N=134*	*p value***
Sex (%)			
Male	78 (64.5)	89 (66.4)	0.648
Female	43 (35.5)	45 (33.6)	
Age (years)			
Mean ± SD	54.9 ± 17.4	53.5 ±17.6	0.622
Interquartile range	17 - 92	17 - 90	
APACHE II score*			
Mean ± SD	22.8 ± 7.52	21.8 ± 9.08	0.321
Interquartile range	3 - 39	3 - 43	
Mortality Estimation (%)			
Mean ± SD	46 ± 23	44 ± 26	0.321
Interquartile range	4 - 90	4 - 94	
In-hospital length of stay (days)			
Mean ± SD	17.4 ± 14.1	8.7 ± 6.8	0.001
Interquartile range	1 - 66	1 - 40	
Blood Culture (days after admission)			
Mean ± SD	7 ± 9	3 ± 4	0.001
Interquartile range	0 - 52	0 - 26	
SF (days after admission)			
Mean ± SD	7 ± 9	3 ± 4	0.001
Interquartile range	0 - 52	0 - 26	
Hospital discharge			
Alive	61 (48.8)	81 (57.4)	0.158
Death	64 (51.2)	60 (42.6)	

*APACHE II denotes the Acute Physiology and Chronic Health Evaluation. Patients were assessed on the day of admission to the UCI. **P - values were determined with the use of the chi square test for categorical variables and the T-test for numerical variables.

Table 2 - *Level of agreement for blood cultures and SF test (n=255).*

Results	*Counts*	*Percent*	*Kappa index*	*P value*
BC- SF-	158	62.0	0.357	0.001
BC+ SF+	34	13.3		
BC+ SF-	22	8.6	95% CI: 0.219-0.495	
BC- SF+	41	16.1		

Abbreviations: BC, blood culture; +, positive (no growth); -, negative (growth) SF, Septi-fast; +, positive (amplification signal with Ct within accepted parameters); -, negative (no amplification signal or Ct outside accepted parameters).

Table 3 - *Microorganisms identified in blood cultures with growth and negative SeptiFast.*

*Bacterial species identified in blood culture (BC+,SF-)**		
Microorganism	*Counts*	*Percent*
Escherichia coli	4	18.2
Methicillin-resistant *Staphylococcus epidermidis*	4	18.2
Staphylococcus epidermidis	2	9.1
Methicillin-resistant *Staphylococcus aureus (MRSA)*	2	9.1
Staphylococcus aureus	1	4.5
Carbapenem-resistant *Acinetobacter baumannii (CRAB)*	1	4.5
Candida glabrata	1	4.5
Candida parapsilosis	1	4.5
Candida albicans	1	4.5
Eschericha coli ESBL	1	4.5
Klebsiella pneumoniae	1	4.5
Morganella morganii	1	4.5
Pseudomonas aeruginosa	1	4.5
Raoiltella ornithinolytica	1	4.5
Total	22	100

*BC= blood cultures, SF= SeptiFast.

Table 4 - *Microorganisms identified with SeptiFast and with no growth in blood cultures.*

*Bacterial species identified by SeptiFast (BC- SF+)**		
Microorganisms	*Counts*	*Percent*
Klebsiella pneumoniae/ Klebsiella oxytoca	11	26.8
Staphylococcus aureus	11	26.8
Klebsiella pneumoniae/ Klebsiella oxytoca/ Staphylococcus aureus	3	7.3
Eschericha coli	3	7.3
Enterobacter cloacae/ Klebsiella aerogenes	2	4.9
Coagulase-negative Staphylococcus (CNS)	2	4.9
Serratia marcescens	2	4.9
Aspergillus fumigatus	1	2.4
Candida krusei/ Pseudomonas aeruginosa	1	2.4
Coagulase negative Staphylococcus/ *Enterobacter cloacae/ Klebsiella aerogenes/ Candida albicans*	1	2.4
Enterococcus faecium	1	2.4
Pseudomonas aeruginosa	1	2.4
Stenotrophomonas maltophilia	1	2.4
Streptococcus pneumoniae	1	2.4
Total	41	100

*BC= blood cultures, SF= SeptiFast.

therapy after the SF test did not show a statistically significant impact of therapy changes on hospital discharge condition (alive) [OR: 0.746; CI 95% 0.434-1.284; p = 0.22]. There was a tendency, however, for patients who changed treatment after SF (intervention group) and had higher predicted mortality to show higher survival rates (*Figure 1*). At hospital discharge, 50.4% of patients in the intervention group and 42.5% of patients in the non-intervention group had died. There was no statistical difference in status at hospital discharge.

Negative SF test results were found in 68.6% of patients who died, and 72.1% who did not. In samples from patients who died, the most common bacterial species identified by the following, by SF were *Staphylococcus aureus* and *Klebsiella pneumoniae*/*Klebsiella oxytoca*. Patients who died were 3.2 times as likely to have an infection caused by Gram-negative bacteria than patients who survived. Among patients with infections caused by Carbapenem-Resistant Enterobacteriaceae (CRE), 72.7% (8/11) died. The odds of having a CRE-associated infection for the death group was 5.3 times higher than for the survival (alive) group (*Table 5*). The higher the age and the mortality estimation, the greater the probability of dying regardless of the time of hospitalization, bacterial infection, bacterial phenotype, microbiological test and antibiotic treatment switch after the SF test. There was no influence of the time of sample collection and testing (blood culture or SF) during ICU stay on survival rates.

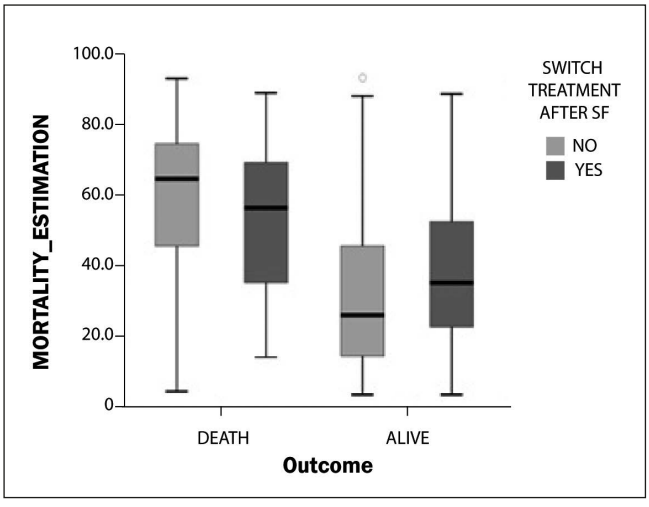

Figure 1 - *Mortality Estimation by group (antibiotic treatment switch after SF test result) across hospital discharge groups (outcome).*

DISCUSSION

Clinical microbiology aims at identifying the etiological agent of an infectious episode and at providing a pattern of antibiotic resistance/susceptibility for the clinical isolate. This diagnosis is crucial in BSIs, which has proved to be a major cause of hospital mortality worldwide. Although the multiplex PCR method does not allow determining bacterial resistance patterns, real-time PCR allows the identification of sepsis-causing bacteria in a fast, highly sensitive and specific manner, reducing the risk of contamination of the sample and enhancing the patient's treatment choices and, in turn, potentially improving patients' survival.

This study confirms the usefulness of the APACHE II index as a predictor of mortality in patients admitted to the ICU with suspected sepsis. However, it emphasizes that the type of bacteria and its antibiotic susceptibility influence the outcome (status at hospital discharge) independent of the value of the APACHE II index on the admission diagnosis.

Our study hypothesized that the start of antibiotic therapy and changes of antibiotic schemes or antibiotic withdrawal based on SF test results were associated with both the in-hospital length of stay and patient status at hospital discharge. We found, indeed, a statistical difference in

Table 5 - *Clinical characteristics of SF-tested patients by condition at discharge.*

Variables	*Hospital discharge*				*p value**	*OR [IC95%]*
	Death (n=118)		*Alive (n=137)*			
	Counts	*%*	*Counts*	*%*		
Microorganism identified by SF						
Gram-positive bacteria	11	61.1	7	38.9	0.779	1.2 [0.4 - 3.5]
Gram-negative bacteria	21	65.6	11	34.4	0.009	3.2 [1.3 - 7.7]
Fungi	1	16.7	5	83.3	0.219	0.2 [0.02 - 2.3]
Negative SF	85	42.7	114	57.3	Ref	Ref
AST Profile (BC+ cases)						
ESBL resistance pattern	5	71.4	2	28.6	0.195	3.1 [0.6 - 17.7]
CRE pattern	8	72.7	3	27.3	0.034	5.3 [1.1 - 24.7]
MRSA pattern	5	55.6	4	44.4	0.847	1.2 [0.3 - 5.4]
Susceptible	14	60.9	9	39.1	0.206	1.9 [0.7 - 5.1]
No bacteria grown	86	42.0	119	58.0	Ref	Ref
SF test positive result	38	49.4	39	50.6	0.386	0.8 [0.4 - 1.4]
Switch Treatment after SF-test	64	51.2	61	48.8	0.272	1.4 [0.8 - 2.4]

*BC= blood cultures, SF= SeptiFast.

length of hospital stay among patients in the intervention group and those in the non-intervention group. Length of stay in the UCI, however, was almost the same for both groups at hospital discharge. We found that BSIs caused by Gram-negative bacteria, as well as infections caused by pathogens with antibiotic-resistant phenotypes, increase the odds of fatal outcomes. Importantly, the diagnosis of bacteraemia due to CRE species is associated with a higher risk of death. (Sabino *et al.*, 2019) (Satlin *et al.*, 2017) (Stewardson *et al.*, 2019)

We found no association between patient groups and status at hospital discharge. Factors such as the initial assessment of mortality risk by APACHE II scoring and age seem to be, however, useful predictors of in-hospital mortality in patients with both positive and negative blood cultures (Kądziołka *et al.*, 2019). Our hypothesis resonates with the previous study that concludes that mortality at hospital discharge and in-hospital length of stay depend on factors beyond initial medical assessment mortality risk.

The present study found a weak to moderate correlation between the results of blood cultures and those of the SF test. However, SF increased the overall pathogen detection rates, especially in BSIs caused by Gram-negative bacteria. A recent study found that the use of SF in a group of sepsis patients with grown blood cultures led to earlier therapy de-escalation in comparison to a BC-only controlled group (Rodrigues *et al.*, 2019). This implies a benefit of the multiplex PCR system for the microbiological diagnosis of sepsis with a potential reduction of the time of empirical antibiotic treatment duration (Makristathis *et al.*, 2018)

An impact on treatment and control of infections in patients with suspected sepsis, duration of hospitalization, medical expenses, and hospital discharge seems feasible when applying molecular diagnostics in groups of sepsis patients.

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