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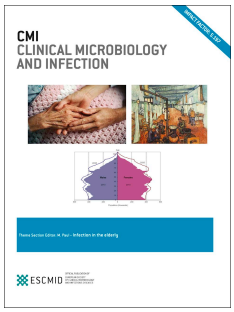

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The detection of microbial DNA but not cultured bacteria is associated with increased mortality in patients with suspected sepsis – a prospective multi-centre European observational study

Microbial DNA increases mortality in patients with sepsis

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Abstract

Objectives

Blood culture results inadequately stratify the mortality risk in critically ill patients with sepsis. We sought to establish the prognostic significance of the presence of microbial DNA in the bloodstream of patients hospitalised with suspected sepsis.

Methods

We analysed the data collected during the Rapid Diagnosis of Infections in the Critically Ill (RADICAL) study which compared a novel culture-independent polymerase chain reaction/electrospray ionization-mass spectrometry (PCR/ESI-MS) assay with standard microbiological testing. Patients were eligible for the study if they were having suspected sepsis and were either hospitalised or were referred to one of nine intensive care units from six European countries. Blood specimen for PCR/ESI-MS assay was taken along with initial blood culture taken for clinical indications.

Results

Of the 616 patients recruited to the RADICAL study, 439 patients had data on outcome, results of the blood culture and PCR/ESI-MS assay available for analysis. Positive blood culture and PCR/ESI-MSI result was found in 13% (56/439) and 40% (177/439) of patients respectively. Either a positive blood culture (p=0.01) or a positive PCR/ESI-MS (p=0.005) was associated with higher SOFA scores on enrolment to the study. There was no difference in 28 days mortality observed in patients who had either positive or negative blood cultures (35% versus 32%, p=0.74). However, in patients with a positive PCR/ESI-MS assay mortality was significantly higher in comparison to those with a negative result (42% versus 26%, p=0.001).

Conclusions

Presence of microbial DNA in patients with suspected sepsis might define a patient group at higher risk of death.

Key words: culture-independent; molecular detection; early-diagnosis; critically ill; infection; mortality

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Introduction

Sepsis is one of the major causes of worldwide mortality [1]. Within the intensive care unit (ICU) sepsis comprises one quarter of admissions yet accounts for almost half of all bed days [2]. Although decreasing, the mortality rate associated with sepsis remains far in excess of that observed for other ICU admission diagnoses [3,4]. Early identification and immediate treatment with appropriate antibiotic therapy is a central component of effective care of the septic patient [5-7]. However, traditional culture-based pathogen detection and identification methods are inherently slow, with up to 72 hours required to generate a complete result and fail to identify an organism in up to 40% of cases with severe sepsis [8]. Furthermore, even when organisms are detected by culture techniques in cases of suspected sepsis this approach fails to consistently identify a patient group with an increased mortality risk [9-11]. Our group has recently described the clinical performance of a novel technology involving polymerase chain reaction that is followed by electrospray ionization mass spectrometry (PCR/ESI-MS) in a multicentre observational study of patients with suspected sepsis referred to the ICU for further management (The RADICAL study) [12]. This technology is non-culture based and can detect the DNA of in excess of 800 relevant pathogens within approximately six hours. In the previous paper we reported that PCR/ESI-MS identified a relevant pathogen in the blood stream nearly four times more frequently than blood cultures in addition to having a 97% negative predictive value.

Data from the RADICAL study may offer important new information regarding the clinical significance of the detection of microbial DNA in the blood stream of patients referred for ICU treatment with a suspected infection. Here we describe an analysis of those patients recruited to the RADICAL study where matching data were available describing patient outcome, blood culture and PCR/ESI-MS findings. Our hypothesis was that the presence of microbial DNA in the bloodstream of patients with suspected sepsis may more effectively identify a cohort of patients at higher risk of death from sepsis, regardless of whether viable microbes were isolated from blood culture.

Methods

In this study we analysed the data from the observational multi-centre study Rapid Diagnosis of Infections in the Critically Ill (RADICAL). Detailed trial methods of the RADICAL study and results of the primary analysis were published previously [12]. The RADICAL study was conducted in nine intensive care units (ICUs) from six European countries. Written informed consent was sought and recorded from each participant or their legal representative. Research ethics approval was obtained in each participating centre and therefore the study has been conducted in accordance with the ethical standards of the Declaration of Helsinki and its following amendments.

The analysis presented describes those patients recruited to RADICAL where study blood specimens were obtained simultaneously for both standard blood culture analysis and PCR/ES-MS analysis and outcome data for the patients were available.

Patients

Patients were enrolled to the RADICAL study between October 2013 and Jun 2014. Adult patients (≥18 yrs) were eligible for the study should they either 1. have a suspected or proven severe infection or sepsis and were either hospitalised or were referred for treatment to the ICU, or 2. had suspected or proven clinical diagnosis of pneumonia. To be eligible for enrolment into pneumonia group patients had to be intubated with an endotracheal tube and have proven or suspected clinical diagnosis of either severe community-acquired pneumonia (sCAP), healthcare-associated pneumonia (HAP/HCAP) or ventilator-associated pneumonia (VAP) defined by the presence of the following criteria: new infiltrates on chest radiograph plus temperature >38^{o}C or <35^{o}C, or increased production of sputum, or abnormal white blood cell count (>12 or <4 cells/mL^{3}). Alternatively, pneumonia could be diagnosed if the treating clinician was clinically suspecting pneumonia and was expecting the patient to remain intubated the next day. Exclusion criteria were: palliative intention of the treatment, death was deemed imminent or inevitable, the treating clinician was not committed to aggressive therapy or was predicting discharge of the patient from the ICU on the day of evaluation, or the next day, or the patient has been readmitted to ICU during same hospitalization.

Collection and processing of the specimen

Blood specimens were collected when treating physicians requested blood cultures due to clinical suspicion of a blood stream infection, pneumonia or an infection at a sterile site. Standard-of-care microbiology cultures were run according to local policy in every institution. For PCR/ESI-MS assay, a sample of minimum 5 mL of whole blood was taken from the same venepuncture as for blood culture testing into an Ethylene Diamine Tetra Acetic acid (EDTA) tube. All samples were cooled to 4°C within 30 min from obtaining and stored at 4°C or frozen at -20°C until further analysis. The technique of extraction of the genomic deoxyribonucleic acid (gDNA) from previously collected blood specimens was published previously [12]. Eluates from the extraction were transferred into 16 wells (30 µl per well) of a custom-mase PCR assay strip prefilled (25 µl per well) with 18 unique primer pairs and concentrated PCR master mix. Details of the primer sequences, gene targets, and configuration have been published elsewhere [12]. General PCR formulas and thermocycling conditions also have been published previously [12]. Potential contaminants were excluded from the analysis [12].

Blood culture results were available to treating clinicians according to the standard local protocols and the study team did not influence the treatment delivered to the patient by the treating clinicians. The treating clinicians remained unaware of the results of the PCR/ESI-MS assay.

Clinical data collection

Clinical and demographic data were obtained on study enrolment. Patients were followed up for 28 days. Sequential Organ Failure Assessment (SOFA) score was noted at enrolment to the study [13]. A quick SOFA (qSOFA) score was obtained retrospectively based on the data available in the original dataset [14]. Vasopressors were defined as either noradrenaline or vasopressin. Vital status at 28 days was recorded. Foci of infection were recorded as the suspicion of the treating clinicians.

Statistical analysis

Discrete variables are expressed as counts with percentages in parenthesis. Continuous variables were assessed for normality of distribution using the Shapiro-Wilk W test. Continuous variables that were non-normally distributed were described as median with interquartile range. All statistical tests are two-sided and a *p*-value of $p<0.05$ was considered significant. Differences in discrete variables were calculated with a chi-squared test and differences in

continuous variables assessed with a Wilcoxon Rank Sum Test. A McNemar test was used to compare paired categorical data.

A binary multiple logistical regression model was run where 28 day mortality was the dependent variable. All plausible demographic and clinical data were first assessed for an association with 28 day mortality in a series of univariable analyses. Variables with a p value <0.2 with 28 day mortality were then added to the multiple logistical regression model as independent variables. The model was developed with backward selection. The majority of variables, including our variables of interest, were dichotomous therefore precluding the need to test for linearity. We did not hypothesise any particular interactions in our model building process and our sample size was insufficient to test for multiple interactions. Model building is described more systematically in the legend of supplemental table 1. Data analysis was performed using the JMP (version 10) statistical software (SAS, Cary, NC, USA).

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Results

Of the 616 patients recruited to the primary study [12], temporally matching results of the blood culture and PCR/ESI –MS assay were available for 439 patients and matching assays and 28 day mortality data was available for 365 patients. Table 1 describes the patient demographics and their clinical characteristics. Positive blood culture and PCR/ESI-MSI result was found in 13% (56/439) and 40% (177/439) of patients respectively. Concordance between blood cultures and PCR/ESI-MS assay has been described elsewhere [12]. Patients with positive PCR/ESI-MS results were slightly older in comparison to those with a negative result (p=0.01, Table 1). Patients with either a positive blood culture or PCR / ESI-MS were more likely to have higher SOFA scores (p=0.01 and p=0.005, respectively) and require vasopressors (p=0.04 and p=0.02, respectively) on study enrolment but were less likely to have a pre-existing diagnosis of respiratory disease (p=0.03 and 0.04, respectively) than patients with negative test results (Table 1).

Critical Illness characteristics

The median length of stay in the ICU was 7 (4-14) days. Patients with positive PCR/ESI-MS result were ventilated for one extra day and remained shocked for two additional days (Table 2). The median number of days with antibiotic treatment was 7 (4-11) days and was not associated with the test result (Table 2). In patients with positive PCR/ESI-MS test result, the duration of antibiotics in patients whose blood culture result was positive was 6 (3-13) days compared to 8 days (4-13) (p=0.05) when the blood culture result was negative. In those patients that had a negative blood culture result, the duration of antibiotic therapy was similar between the patients with positive and negative PCR/ESI-MS results, respectively 8 (4-13) versus 7 (3-11), p=0.2.

Patients with negative PCR/ESI-MS result had a greater number of days alive and free of antibiotics than patients with a positive result (Table 2). In patients whose PCR/ESI-MS test result was positive the number of days alive and free of antibiotics to day 28 was not dependent on the blood culture result (3 days (0-21) versus 4 days (0-22), p=0.7). Those patients with negative blood culture results who also had a negative PCR/ESI-MS result had greater numbers of days alive and free of antibiotics to day 28 than those who had a negative blood culture and a positive PCR/ESI-MS result (17 days (1-23) versus 3 days (0-21), p=0.005).

Outcomes

Mortality rate at 28-day was 32% (118/365). Positive blood culture result was not associated with higher 28-day mortality (17/49 (35%) versus 101/316 (32%), p=0.7 for positive and negative blood cultures respectively). Conversely, 28-day mortality was significantly higher in patients with positive PCR/ESI-MS assay in comparison to those with negative PCR/ESI-MS result (62/147 (42%) versus 56/218 (26%), p=0.001 respectively). The odds ratio for 28-day mortality when the microbial DNA was detected by PCR/ESI-MS assay was 2.1 (95% CI 1.4-3.3).

In patients with negative blood culture results, a positive PCR/ESI-MS test result remained strongly associated with increased rates of death (45/103 (44%) vs. 56/213 (26%), p=0.003, odds ratio for 28-day mortality 2.2 (1.3-3.6), Figure 1). In keeping with the high negative predictive value of PCR/ESI-MS, only five patients (1.4%) had positive blood cultures despite a negative PCR/ESI-MS, all these patients survived, however due to small sample size statistical significant versus rates of death with positive blood cultures and positive PCR/ESI-MS was not achieved (p=0.15).

Univariable analyses demonstrated that increasing patient age (p<0.0001), a history of cancer (p=0.02), the presence of immune suppression (p=0.04) and a higher SOFA score on admission (p<0.0001) were associated with an higher risk of death at 28 days. None of: cardiovascular disease, respiratory disease, diabetes, chronic kidney disease, cirrhosis or smoking history were associated with 28-day mortality. In a multivariable logistical regression model, when the significant covariates were added to the model the presence of a positive PCR/ESI-MS result remained independently associated with 28-day mortality (Table 3 and supplemental table 1). When the blood culture result was also added to the model this was not independently associated with outcome but addition of the blood culture result as a covariate further strengthened the association between the PCR/ESI-MS result and 28 day mortality.

Organism specific outcomes

A full description of the organisms identified by both blood culture and PCR/ESI-MS techniques has been reported elsewhere [12]. In the cohort analysed for this study 35 patients had a Gram negative bacteria and 18 patients had a Gram positive bacteria isolated by blood culture. The 28 day mortality rate for the five most commonly isolated organisms by blood culture was: *Escherichia coli* 60% (6/15), *Staphylococcus aureus* 11% (1/9), *Klebsiella pneumoniae* 75% (3/4), *Pseudomonas aeruginosa* 50% (2/4), *Enterococcus faecium* 50% (1/2).

The 28 day mortality rate for the five most commonly isolated organisms by PCR / ESI-MS was: *E. coli* 43% (23/53), *S. aureus* 40% (8/20), *E. faecium* 11/17 (65%), *K. pneumoniae* 40% (4/10), and *Candida albicans* 56% (5/9).

There were four cases of *methicillin-resistant Staphylococcus aureus* in blood cultures and seven cases detected with PCR/ESI-MS. The four cases were concordant between the two groups. There was one case of vancomycin-resistant enterococci which was matched between blood culture and PCR/ESI-MS. No case of carbapenemase-producing organism was detected by either methodology.

There was no statistically significant difference between the mortality rates attributed to infection by any of the organisms, by whether the infection was Gram positive, Gram negative or fungal or by the presence of resistant organisms.

The most commonly identified source of infection was the respiratory tract in 157 (36%) cases. Intra-abdominal infection accounted for 81 (18%) cases, primary blood stream infections for 70 (16%) cases, urinary tract infection for 32 (7%) cases and the source was unknown in 27 (6%) of cases. There was no relation between the source of infection and 28-day mortality was identified.

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Discussion

The principal finding of this analysis is that mortality was greater amongst patients referred to an ICU team for treatment of suspected sepsis when microbial DNA was detected with the PCR/ESI-MS assay. In contrast to this finding, we found no difference in mortality rate between those patients with positive and negative blood culture. These findings might suggest that, apart from providing a more immediate microbiological diagnosis, PCR/ESI-MS may more effectively identify critically ill patients with active infection and hence an increased risk of death. We suggest that these data are consistent with a biologically important mechanism and describe a qualitatively different patient population with evidence of active infection that is missed using current microbiological diagnostics.

The patients analysed were typical of an ICU population with sepsis. Patients were predominantly male and a median age of 65 years and frequently possessed significant co-morbidities. On presentation, the septic illness was severe. The median SOFA score was seven and more than 50% of the patients were requiring immediate cardiovascular support and mechanical ventilation. More than half of the patients studied received a dose of antibiotic prior to study enrolment which likely reflects current guidelines recommending intravenous antibiotic treatment within the first hour following diagnosis of severe acute infection [7]. Prior antibiotic exposure is a key factor in the high incidence of culture-negative suspected sepsis and is also likely to interfere with the discriminant ability of blood culture in relation to patient outcome [15]. Consequently, blood culture does not consistently distinguish between non-survivors and survivors in patients with sepsis [8-11].

It is difficult to draw firm conclusions as to why patients with detectable microbial DNA in the PCR/ESI-MS assay had higher mortality rate. Although older, sicker patients were more likely to both have a positive PCR/ESI-MS assay and to subsequently die, the relationship between PCR/ESI-MS result and mortality remained following correction for these covariates. The key question that arises is whether the detection of microbial DNA is indicative of a pathogenic finding in and of itself or whether this is an epiphenomenon which reflects the overall disease burden in a manner different from acute illness scores. Microbial DNA certainly has the capacity to be inherently pathogenic. Unmethylated CpG dinucleoties, such as are found in microbial DNA, are known to be potent TLR9 agonists and binding can result in inflammatory cascades [16,17]. Microbial DNA is also a key component of biofilms, where it contributes to their structural stability and also plays an active role in the inhibition of antibiotics [18]. This may be particularly relevant in an ICU population where biofilms are frequently present on indwelling medical devices such as endotracheal tubes and venous catheters and where the presence of a biofilm may be a

factor in the failure to grow an organism using culture techniques. Alternatively, the presence of microbial DNA may indicate the presence of active infection which a poorly sensitive test such as blood culture fails to identify. We have previously reported that PCR/ESI-MS can readily identify fastidious and difficult to culture organisms [12]. It is also plausible that a positive PCR/ESI-MS result is merely an epiphenomenon of more severe disease and perhaps related to leakage of microbial contents from a porous gastrointestinal tract.

We did not demonstrate an association between any individual microbial species and subsequent outcome but this study is likely underpowered to detect any such an association. Furthermore, as the current PCR/ESI-MS technology detects only KPC, vanA, vanB and mecA as antibiotic resistance genes and these were detected at a very low frequency in our patients no definitive statement can be made regarding patient outcome in the presence of DNA from highly resistant organisms. Although the presence of multi-drug resistant organisms is likely to have a significant impact on determining patient outcome the relatively low incidence of culture positive sepsis in our patients limited further analysis of this association.

This analysis is specific to one particular methodology of microbial DNA detection – PCR/ESI-MS. A numerous other technologies are available to detect microbial DNA. Two previous studies using other techniques did not suggest that the detection of microbial DNA was associated with an higher mortality although they did report an association between microbial DNA and a more severe acute illness [19,20]. This has led many investigators to question the relevance of microbial DNA in the bloodstream of a patient where viable microbes could not be cultured [21]. That our study describes a mortality difference may be partly explained by the diagnostic spectrum of the PCR/ESI-MS technology that is able to identify in excess of 800 microbes in a culture independent method in comparison to other PCR technology that usually limits detection to approximately 25 common pathogens and frequently requires enrichment via standard culture methodologies [19,22,23].

There are some limitations to the analyses presented here. During this study the PCR/ESI-MS result was not available to the treating clinicians and therefore could not influence treatment whereas the blood culture results were obtained as part of routine clinical care and results were available as normal. It is therefore plausible that patients with negative blood cultures and positive PCR/ESI-MS results may have had their antibiotic treatment ceased inappropriately early thereby affecting subsequent outcome. However, we found that the duration of antibiotic treatment was similar between those patients that had a positive PCR/ESI-MS result regardless of whether their blood culture result was positive or negative. Indeed, the duration of antibiotic treatment was similar amongst all

combinations of test results. In addition, given the limited resistance profiling of the current PCR/ESI-MS technology discussed earlier we were unable to comment on whether patients with a positive PCR/ESI-MS result received adequate antibiotic treatment during the study period. Finally, although each institute obtained blood cultures according to local protocols the lack of specific standardisation for this procedure could plausibly affect microbial yield and thus study results.

If replicated, these results could potentially alter management of the patients in the future. If the presence of microbial DNA represents a sub optimally treated infected process then specific antibiotic regimes may be suggested based on this test result. This approach would be greatly facilitated by the expanding the currently available panel of antibiotic resistance genes detected by PCR/ESI-MS technology. As the field of sepsis immunotherapy and personalised medicine rapidly expands PCR/ESI-MS may prove to have a role in identifying patients that would benefit from specific antagonism of TLR9 pathways or even from adjunctive immune stimulation [24-26]. Further mechanistic studies are required prior to suggesting more specific treatments.

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Conclusions

According to our best knowledge this is the first paper that reports that the presence of microbial DNA in the blood-stream of patients with suspected acute sepsis is associated with greater mortality. It is plausible that PCR/ESI-MS result may provide additional important information as regards the clinical trajectory of the patient with suspected sepsis above that garnered from the traditional blood culture results and from an assessment of the severity of illness. It is plausible that this assay could be used to direct specific adjunctive therapies to a high risk population with suspected sepsis.

Authors contributions

MW conceived the study. MOD, JS, KZ, DE, RS, DB, MS, NL, MW, JLV designed the study and contributed data. MOD, MHS and MW did the data analysis MOD, MHS, MW and JLV wrote the manuscript.

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Conflict of interests

Dr. O'Dwyer reports grants from Ibis Bioscences, during the duration of the study. Dr. Ecker reports funding from Ibis Biosciences Inc. an Abbott Company, during the conduct of the study and to be clear, I am an employee who works for the company that makes the technology that is the subject of the paper. Dr. Brealey reports personal fees from Abbott, outside the submitted work. Dr. Singer reports personal fees from Abbott, outside the submitted work. Dr. Wilks reports grants from Abbott during the conduct of the study. Dr. Starczewska, Prof. Zacharowski, Prof. Schrenzel, Dr. Sampath, Dr. Libert, Prof. Vincent have nothing to disclose.

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Table 1. Demographic and clinical features of the study population

	Total cohort (n=439)	BC+ve (n=56)	BC-ve (n=383)	p	PCR+ve (n=177)	PCR-ve (n=262)	p
Demographics							
Median age (years, median/IQR)	65(49-75)	64(48-71)	66(50-76)	0.2	66(54-78)	64(46-72)	0.01
Sex (male)	66%	69%	63%	0.2	64%	66%	0.9
Major comorbidities at baseline							
Hypertension	47%	54%	46%	0.3	49%	45%	0.5
Diabetes	24%	27%	23%	0.6	24%	23%	0.8
Cancer	29%	30%	29%	0.9	34%	26%	0.09
CKD	18%	23%	17%	0.3	18%	17%	0.8
Cirrhosis	8%	7%	9%	0.99	10%	8%	0.5
COPD or asthma	20%	9%	21%	0.03	15%	23%	0.04
Current smoker	15%	7%	16%	0.1	14%	15%	0.8
Immunosupressed	14%	20%	13%	0.2	16%	12%	0.2
Antimicrobial use							
Within 30 days prior to hospitalisation	11%	9%	12%	0.8	10%	12%	0.9
During hospitalisation but before enrolment	59%	57%	60%	0.8	58%	60%	0.6
Illness severity on study enrolment							
SOFA score on enrolment (median and IQR)	7 (4-11)	10 (6-12)	7 (4-11)	0.01	8 (5-11)	7 (4-10)	0.005
qSOFA score on enrolment (median and IQR)	1 (1-2)	1 (1-2)	1 (1-2)	0.2	1 (1-2)	1 (1-2)	0.1
Vasopressor use on enrolment	55%	68%	53%	0.04	62%	50%	0.02
Requirement for MV on enrolment	59%	54%	59%	0.5	66%	54%	0.02

A description of the demographic and clinical features of the patient population on enrolment in the study.

Abbreviations: BC+ve, positive blood culture; BC-ve, negative blood culture; PCR+ve, positive polymerase chain reaction / electrospray ionization-mass spectrometry; PCR-ve, negative polymerase chain reaction / electrospray ionization-mass spectrometry; Vasopressors were defined as either noradrenaline or vasopressin. IQR, inter quartile range; CKD, chronic kidney disease; COPD, chronic obstructive airways disease; SOFA, sequential organ failure assessment score; qSOFA, quick SOFA; IQR, interquartile range; MV, mechanical ventilation.

Table 2. Post-enrolment patient characteristics

	Total cohort	BC+ve	BC-ve	p	PCR+ve	PCR-ve	p
ICU LOS	7(4-14)	7(3-13)	7(4-14)	0.8	8(4-13)	7(4-14)	0.8
Hospital LOS	23(12-39)	23(10-48)	23(13-38)	0.8	22(12-41)	23(13-37)	0.9
Days of mechanical ventilation	2(0-8)	1(0-7)	2(0-8)	0.4	3(0-9)	2(0-7)	0.03
Days alive and free of MV to day 28	26(20-28)	27(21-28)	26(20-28)	0.4	26(19-28)	27(21-28)	0.03
Days on vasopressors	1(0-4)	2(0-5)	1(0-4)	0.08	2(0-5)	0(0-4)	0.007
Days alive and free of vasopressors to day 28	27(24-28)	26(23-28)	27(24-28)	0.07	26(24-28)	28(24-28)	0.01
Days on antibiotics	7(4-11)	6(3-13)	7(4-11)	0.2	7(3-13)	7(4-11)	0.9
Days free of A/B and alive up to day 28	10(0-22)	4(0-22)	12(0-22)	0.3	4(0-21)	17(1-23)	0.003

A description of the hospital stay and illness characteristics following enrolment in the study. Days alive and free of (MV/ vasopressors/ antibiotics) today 28 was calculated by adding the number of days up to and including day 28 that the patient was both free of the intervention and alive.

Abbreviations: BC+ve, positive blood culture; BC-ve, negative blood culture; PCR+ve, positive polymerase chain reaction / electrospray ionization-mass spectrometry; PCR-ve, negative polymerase chain reaction / electrospray ionization-mass spectrometry; ICU, intensive care unit; LOS, length of stay; MV, mechanical ventilation; A/B, antibiotics

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Table 3. Univariable and multivariable logistic regression analysis of 28-day mortality

Predictor	Univariable		Multivariable	
	p	OR (95% CI)	p	OR (95% CI)
Age (per year)	<0.0001	1.05 (1.03-1.07)	<0.0001	1.05 (1.03-1.07)
SOFA score (per unit)	<0.0001	1.15 (1.09-1.22)	<0.0001	1.15 (1.08-1.23)
History of cancer	0.02	1.8 (1.1-2.8)	0.02	1.8 (1.08-3.15)
Immune suppression	0.04	1.9 (1.1-3.6)	0.14	1.8 (0.8-3.7)
Positive PCR/ESI-MS	0.001	2.1 (1.4-3.3)	0.04	1.7 (1.01-2.82)
Positive BC	0.74	1.1 (0.6-2.1)		
Cardiovascular disease	0.5	1.3 (0.7-2.3)		
Respiratory disease	0.7	1.3 (0.67-2.0)		
Diabetes mellitus	0.5	1.2 (0.72-2.0)		
Chronic kidney disease	0.7	1.1 (0.6-2.0)		
Cirrhosis	0.6	1.4 (0.6-2.7)		
History of smoking	0.5	1.3 (0.7-2.7)		

The C statistic for the whole model is 0.77. Abbreviations: OR, odds ratio; CI, confidence interval; SOFA, sequential organ failure assessment score; PCR/ESI-MS, polymerase chain reaction/electrospray ionization-mass spectrometry; BC, blood culture

468 **Table 4. Organism specific outcomes**

Commonest organisms by blood culture	Mortality % (n)	Commonest organism by PCR/ESI-MS	Mortality %(n)
1 *Escherichia coli*	60% (6/15)	1 *Escherichia coli*	43% (23/53)
2 *Staphylococcus aureus*	11% (1/9)	2 *Staphylococcus aureus*	40% (8/20)
3 *Klebsiella pneumoniae*	75% (3/4)	3 *Enterococcus faecium*	65% (11/17)
4 *Pseudomonas aeruginosa*	50% (2/4)	4 *Klebsiella pneumoniae*	40% (4/10)
5 *Enterococcus faecium*	50% (1/2)	5 *Candida albicans*	56% (5/9)

469 28-day organism specific mortality for five most commonly isolated organisms by blood culture and by PCR/ESI-MS.

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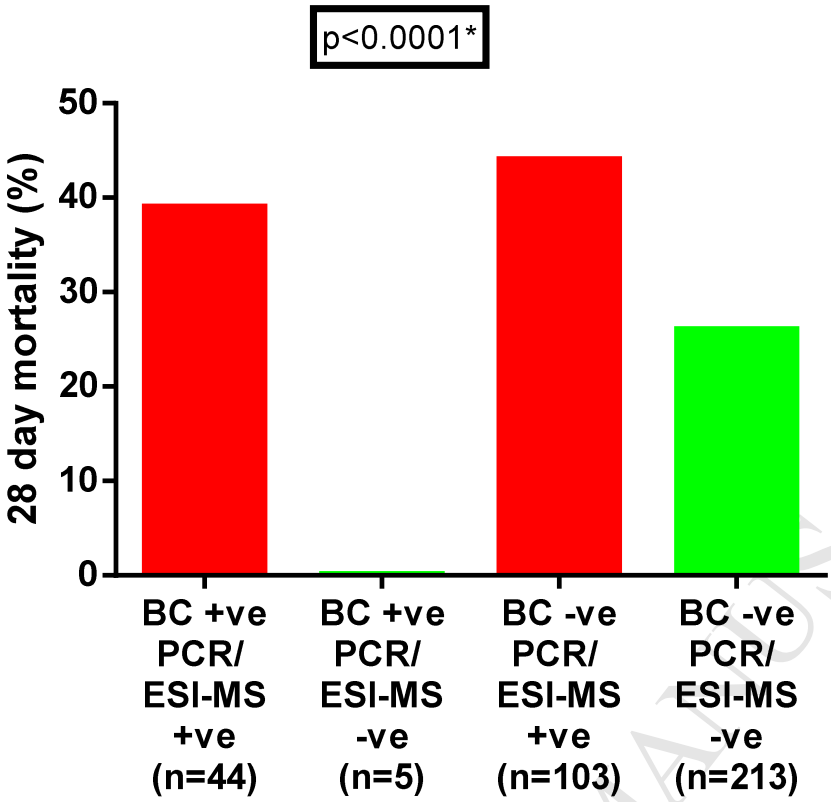
Figure 1. 28-day mortality.



Amongst those patients that have a negative blood culture result those with a positive PCR/ESI-MS test result have a higher mortality. A McNemar's Test was performed on the non-surviving patients which indicated that the total number of positive tests for each method was statistically different (McNemar test statistic = 45, degree of freedom = 1 and $p<0.0001$).

BC, blood culture. PCR/ESI-MS, Polymerase chain reaction followed by electrospray ionisation-mass spectrometry

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