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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

Michael J. O'Dwyer, PhD, Malgorzata H. Starczewska, PhD, Jacques Schrenzel, MD, Kai Zacharowski, MD, David Ecker, PhD, Rangarajan Sampath, PhD, David Brealey, MD, Mervyn Singer, MD, Nicolas Libert, MD, Mark Wilks, PhD, Jean-Louis Vincent, MD

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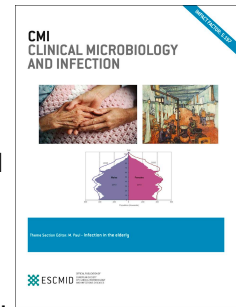
1   **The detection of microbial DNA but not cultured bacteria is associated with increased mortality in patients**

2   **with suspected sepsis – a prospective multi-centre European observational study**

3   Microbial DNA increases mortality in patients with sepsis

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5   Michael J. O'Dwyer, PhD*, Malgorzata H. Starczewska, PhD[1], Jacques Schrenzel, MD[2], Kai Zacharowski, MD[3],

6   David Ecker, PhD[4], Rangarajan Sampath, PhD[4], David Brealey, MD[5], Mervyn Singer, MD[5], Nicolas Libert, MD[6],

7   Mark Wilks, PhD[7], Jean-Louis Vincent, MD[8].

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9   *Corresponding author

10 Michael O'Dwyer

11 Department of Translational Medicine and Therapeutics, William Harvey Research Institute,

12 Barts and The London School of Medicine and Dentistry, Queen Mary University of London

13 Charterhouse Square, London EC1M 6BQ

14 email: m.odwyer@qmul.ac.uk

15

16 [1] Adult Critical Care Unit, Royal London Hospital, Barts Health NHS Trust, London, United Kingdom

17 [2] Genomic Research Laboratory, Department of Internal Medicine, Service of Infectious Diseases, University of

18 Geneva Hospitals, Geneva, Switzerland

19 [3] Klinik für Anästhesiologie, Intensivmedizin und Schmerztherapie, Universitätsklinikum Frankfurt, Frankfurt am

20 Main, Germany

21 [4] Ibis Biosciences, Abbott, Carlsbad, CA, USA

22 [5] Division of Critical Care, University College London Hospitals NIHR Biomedical Research Centre and

23 Bloomsbury Institute of Intensive Care Medicine, University College Hospital, London, United Kingdom

24 [6] Department of Anesthesiology and Critical Care, Val de Grâce Military Hospital, Paris, France

25 [7] Barts and The London School of Medicine and Dentistry, Queen Mary University of London and Barts Health

26 NHS Trust, London, United Kingdom

27 [8] Department of Intensive Care, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium

28

29   **Abstract**

30   **Objectives**

31   Blood culture results inadequately stratify the mortality risk in critically ill patients with sepsis. We sought to

32   establish the prognostic significance of the presence of microbial DNA in the bloodstream of patients hospitalised

33   with suspected sepsis.

34   **Methods**

35   We analysed the data collected during the Rapid Diagnosis of Infections in the Critically Ill (RADICAL) study

36   which compared a novel culture-independent polymerase chain reaction/electrospray ionization-mass spectrometry

37   (PCR/ESI-MS) assay with standard microbiological testing. Patients were eligible for the study if they were having

38   suspected sepsis and were either hospitalised or were referred to one of nine intensive care units from six European

39   countries. Blood specimen for PCR/ESI-MS assay was taken along with initial blood culture taken for clinical

40   indications.

41   **Results**

42   Of the 616 patients recruited to the RADICAL study, 439 patients had data on outcome, results of the blood culture

43   and PCR/ESI-MS assay available for analysis. Positive blood culture and PCR/ESI-MSI result was found in 13%

44   (56/439) and 40% (177/439) of patients respectively. Either a positive blood culture (p=0.01) or a positive PCR/ESI-

45   MS (p=0.005) was associated with higher SOFA scores on enrolment to the study. There was no difference in 28

46   days mortality observed in patients who had either positive or negative blood cultures (35% versus 32%, p=0.74).

47   However, in patients with a positive PCR/ESI-MS assay mortality was significantly higher in comparison to those

48   with a negative result (42% versus 26%, p=0.001).

49   **Conclusions**

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

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

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57 **Introduction**

58 Sepsis is one of the major causes of worldwide mortality [1]. Within the intensive care unit (ICU) sepsis comprises

59 one quarter of admissions yet accounts for almost half of all bed days [2]. Although decreasing, the mortality rate

60 associated with sepsis remains far in excess of that observed for other ICU admission diagnoses [3,4]. Early

61 identification and immediate treatment with appropriate antibiotic therapy is a central component of effective care of

62 the septic patient [5-7]. However, traditional culture-based pathogen detection and identification methods are

63 inherently slow, with up to 72 hours required to generate a complete result and fail to identify an organism in up to

64 40% of cases with severe sepsis [8]. Furthermore, even when organisms are detected by culture techniques in cases

65 of suspected sepsis this approach fails to consistently identify a patient group with an increased mortality risk [9-11].

66 Our group has recently described the clinical performance of a novel technology involving polymerase chain

67 reaction that is followed by electrospray ionization mass spectrometry (PCR/ESI-MS) in a multicentre observational

68 study of patients with suspected sepsis referred to the ICU for further management (The RADICAL study) [12].

69 This technology is non-culture based and can detect the DNA of in excess of 800 relevant pathogens within

70 approximately six hours. In the previous paper we reported that PCR/ESI-MS identified a relevant pathogen in the

71 blood stream nearly four times more frequently than blood cultures in addition to having a 97% negative predictive

72 value.

73 Data from the RADICAL study may offer important new information regarding the clinical significance of the

74 detection of microbial DNA in the blood stream of patients referred for ICU treatment with a suspected infection.

75 Here we describe an analysis of those patients recruited to the RADICAL study where matching data were available

76 describing patient outcome, blood culture and PCR/ESI-MS findings. Our hypothesis was that the presence of

77 microbial DNA in the bloodstream of patients with suspected sepsis may more effectively identify a cohort of

78 patients at higher risk of death from sepsis, regardless of whether viable microbes were isolated from blood culture.

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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 ($\geq$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°C or <35°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

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112   Blood specimens were collected when treating physicians requested blood cultures due to clinical suspicion of a

113   blood stream infection, pneumonia or an infection at a sterile site. Standard-of-care microbiology cultures were run

114   according to local policy in every institution. For PCR/ESI-MS assay, a sample of minimum 5 mL of whole blood

115   was taken from the same venepuncture as for blood culture testing into an Ethylene Diamine Tetra Acetic acid

116   (EDTA) tube. All samples were cooled to 4°C within 30 min from obtaining and stored at 4°C or frozen at -20°C

117   until further analysis. The technique of extraction of the genomic deoxyribonucleic acid (gDNA) from previously

118   collected blood specimens was published previously [12]. Eluates from the extraction were transferred into 16 wells

119   (30 µl per well) of a custom-mase PCR assay strip prefilled (25 µl per well) with 18 unique primer pairs and

120   concentrated PCR master mix. Details of the primer sequences, gene targets, and configuration have been published

121   elsewhere [12]. General PCR formulas and thermocycling conditions also have been published previously [12].

122   Potential contaminants were excluded from the analysis [12].

123   Blood culture results were available to treating clinicians according to the standard local protocols and the study

124   team did not influence the treatment delivered to the patient by the treating clinicians. The treating clinicians

125   remained unaware of the results of the PCR/ESI-MS assay.

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127   **Clinical data collection**

128   Clinical and demographic data were obtained on study enrolment. Patients were followed up for 28 days. Sequential

129   Organ Failure Assessment (SOFA) score was noted at enrolment to the study [13]. A quick SOFA (qSOFA) score

130   was obtained retrospectively based on the data available in the original dataset [14]. Vasopressors were defined as

131   either noradrenaline or vasopressin. Vital status at 28 days was recorded. Foci of infection were recorded as the

132   suspicion of the treating clinicians.

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134   **Statistical analysis**

135   Discrete variables are expressed as counts with percentages in parenthesis. Continuous variables were assessed for

136   normality of distribution using the Shapiro-Wilk W test. Continuous variables that were non-normally distributed

137   were described as median with interquartile range. All statistical tests are two-sided and a p-value of $p<0.05$ was

138   considered significant. Differences in discrete variables were calculated with a chi-squared test and differences in

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

140   categorical data.

141   A binary multiple logistical regression model was run where 28 day mortality was the dependent variable. All

142   plausible demographic and clinical data were first assessed for an association with 28 day mortality in a series of

143   univariable analyses. Variables with a p value <0.2 with 28 day mortality were then added to the multiple logistical

144   regression model as independent variables. The model was developed with backward selection. The majority of

145   variables, including our variables of interest, were dichotomous therefore precluding the need to test for linearity.

146   We did not hypothesise any particular interactions in our model building process and our sample size was

147   insufficient to test for multiple interactions. Model building is described more systematically in the legend of

148   supplemental table 1. Data analysis was performed using the JMP (version 10) statistical software (SAS, Cary, NC,

149   USA).

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169 **Results**

170 Of the 616 patients recruited to the primary study [12], temporally matching results of the blood culture and

171 PCR/ESI –MS assay were available for 439 patients and matching assays and 28 day mortality data was available

172 for 365 patients. Table 1 describes the patient demographics and their clinical characteristics. Positive blood culture

173 and PCR/ESI-MSI result was found in 13% (56/439) and 40% (177/439) of patients respectively. Concordance

174 between blood cultures and PCR/ESI-MS assay has been described elsewhere [12]. Patients with positive PCR/ESI-

175 MS results were slightly older in comparison to those with a negative result (p=0.01, Table 1). Patients with either a

176 positive blood culture or PCR / ESI-MS were more likely to have higher SOFA scores (p=0.01 and p=0.005,

177 respectively) and require vasopressors (p=0.04 and p=0.02, respectively) on study enrolment but were less likely to

178 have a pre-existing diagnosis of respiratory disease (p=0.03 and 0.04, respectively) than patients with negative test

179 results (Table 1).

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181 **Critical Illness characteristics**

182 The median length of stay in the ICU was 7 (4-14) days. Patients with positive PCR/ESI-MS result were ventilated

183 for one extra day and remained shocked for two additional days (Table 2). The median number of days with

184 antibiotic treatment was 7 (4-11) days and was not associated with the test result (Table 2). In patients with positive

185 PCR/ESI-MS test result, the duration of antibiotics in patients whose blood culture result was positive was 6 (3-13)

186 days compared to 8 days (4-13) (p=0.05) when the blood culture result was negative. In those patients that had a

187 negative blood culture result, the duration of antibiotic therapy was similar between the patients with positive and

188 negative PCR/ESI-MS results, respectively 8 (4-13) versus 7 (3-11), p=0.2.

189 Patients with negative PCR/ESI-MS result had a greater number of days alive and free of antibiotics than patients

190 with a positive result (Table 2). In patients whose PCR/ESI-MS test result was positive the number of days alive and

191 free of antibiotics to day 28 was not dependent on the blood culture result (3 days (0-21) versus 4 days (0-22),

192 p=0.7). Those patients with negative blood culture results who also had a negative PCR/ESI-MS result had greater

193 numbers of days alive and free of antibiotics to day 28 than those who had a negative blood culture and a positive

194 PCR/ESI-MS result (17 days (1-23) versus 3 days (0-21), p=0.005).

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196 **Outcomes**

197 Mortality rate at 28-day was 32% (118/365). Positive blood culture result was not associated with higher 28-day

198 mortality (17/49 (35%) versus 101/316 (32%), p=0.7 for positive and negative blood cultures respectively).

199 Conversely, 28-day mortality was significantly higher in patients with positive PCR/ESI-MS assay in comparison to

200 those with negative PCR/ESI-MS result (62/147 (42%) versus 56/218 (26%), p=0.001 respectively). The odds ratio

201 for 28-day mortality when the microbial DNA was detected by PCR/ESI-MS assay was 2.1 (95% CI 1.4-3.3).

202 In patients with negative blood culture results, a positive PCR/ESI-MS test result remained strongly associated with

203 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),

204 Figure 1). In keeping with the high negative predictive value of PCR/ESI-MS, only five patients (1.4%) had positive

205 blood cultures despite a negative PCR/ESI-MS, all these patients survived, however due to small sample size

206 statistical significant versus rates of death with positive blood cultures and positive PCR/ESI-MS was not achieved

207 (p=0.15).

208 Univariable analyses demonstrated that increasing patient age (p<0.0001), a history of cancer (p=0.02), the presence

209 of immune suppression (p=0.04) and a higher SOFA score on admission (p<0.0001) were associated with an higher

210 risk of death at 28 days. None of: cardiovascular disease, respiratory disease, diabetes, chronic kidney disease,

211 cirrhosis or smoking history were associated with 28-day mortality. In a multivariable logistical regression model,

212 when the significant covariates were added to the model the presence of a positive PCR/ESI-MS result remained

213 independently associated with 28-day mortality (Table 3 and supplemental table 1). When the blood culture result

214 was also added to the model this was not independently associated with outcome but addition of the blood culture

215 result as a covariate further strengthened the association between the PCR/ESI-MS result and 28 day mortality.

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217 **Organism specific outcomes**

218 A full description of the organisms identified by both blood culture and PCR/ESI-MS techniques has been reported

219 elsewhere [12]. In the cohort analysed for this study 35 patients had a Gram negative bacteria and 18 patients had a

220 Gram positive bacteria isolated by blood culture. The 28 day mortality rate for the five most commonly isolated

221 organisms by blood culture was: *Escherichia coli* 60% (6/15), *Staphylococcus aureus* 11% (1/9), *Klebsiella*

222 *pneumoniae* 75% (3/4), *Pseudomonas aeruginosa* 50% (2/4), *Enterococcus faecium* 50% (1/2).

223 The 28 day mortality rate for the five most commonly isolated organisms by PCR / ESI-MS was: *E. coli* 43%

224 (23/53), *S. aureus* 40% (8/20), *E. faecium* 11/17 (65%), *K. pneumoniae* 40% (4/10), and *Candida albicans* 56%

225 (5/9).

226 There were four cases of *methicillin-resistant Staphylococcus aureus* in blood cultures and seven cases detected with

227 PCR/ESI-MS. The four cases were concordant between the two groups. There was one case of vancomycin-resistant

228 enterococci which was matched between blood culture and PCR/ESI-MS. No case of *c*arbapenemase-producing

229 organism was detected by either methodology.

230 There was no statistically significant difference between the mortality rates attributed to infection by any of the

231 organisms, by whether the infection was Gram positive, Gram negative or fungal or by the presence of resistant

232 organisms.

233 The most commonly identified source of infection was the respiratory tract in 157 (36%) cases. Intra-abdominal

234 infection accounted for 81 (18%) cases, primary blood stream infections for 70 (16%) cases, urinary tract infection

235 for 32 (7%) cases and the source was unknown in 27 (6%) of cases. There was no relation between the source of

236 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

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279   factor in the failure to grow an organism using culture techniques. Alternatively, the presence of microbial DNA

280   may indicate the presence of active infection which a poorly sensitive test such as blood culture fails to identify. We

281   have previously reported that PCR/ESI-MS can readily identify fastidious and difficult to culture organisms [12]. It

282   is also plausible that a positive PCR/ESI-MS result is merely an epiphenomenon of more severe disease and perhaps

283   related to leakage of microbial contents from a porous gastrointestinal tract.

284   We did not demonstrate an association between any individual microbial species and subsequent outcome but this

285   study is likely underpowered to detect any such an association. Furthermore, as the current PCR/ESI-MS technology

286   detects only KPC, vanA, vanB and mecA as antibiotic resistance genes and these were detected at a very low

287   frequency in our patients no definitive statement can be made regarding patient outcome in the presence of DNA

288   from highly resistant organisms. Although the presence of multi-drug resistant organisms is likely to have a

289   significant impact on determining patient outcome the relatively low incidence of culture positive sepsis in our

290   patients limited further analysis of this association.

291   This analysis is specific to one particular methodology of microbial DNA detection – PCR/ESI-MS. A numerous

292   other technologies are available to detect microbial DNA. Two previous studies using other techniques did not

293   suggest that the detection of microbial DNA was associated with an higher mortality although they did report an

294   association between microbial DNA and a more severe acute illness [19,20]. This has led many investigators to

295   question the relevance of microbial DNA in the bloodstream of a patient where viable microbes could not be

296   cultured [21]. That our study describes a mortality difference may be partly explained by the diagnostic spectrum of

297   the PCR/ESI-MS technology that is able to identify in excess of 800 microbes in a culture independent method in

298   comparison to other PCR technology that usually limits detection to approximately 25 common pathogens and

299   frequently requires enrichment via standard culture methodologies [19,22,23].

300   There are some limitations to the analyses presented here. During this study the PCR/ESI-MS result was not

301   available to the treating clinicians and therefore could not influence treatment whereas the blood culture results were

302   obtained as part of routine clinical care and results were available as normal. It is therefore plausible that patients

303   with negative blood cultures and positive PCR/ESI-MS results may have had their antibiotic treatment ceased

304   inappropriately early thereby affecting subsequent outcome. However, we found that the duration of antibiotic

305   treatment was similar between those patients that had a positive PCR/ESI-MS result regardless of whether their

306   blood culture result was positive or negative. Indeed, the duration of antibiotic treatment was similar amongst all

307 combinations of test results. In addition, given the limited resistance profiling of the current PCR/ESI-MS

308 technology discussed earlier we were unable to comment on whether patients with a positive PCR/ESI-MS result

309 received adequate antibiotic treatment during the study period. Finally, although each institute obtained blood

310 cultures according to local protocols the lack of specific standardisation for this procedure could plausibly affect

311 microbial yield and thus study results.

312 If replicated, these results could potentially alter management of the patients in the future. If the presence of

313 microbial DNA represents a sub optimally treated infected process then specific antibiotic regimes may be suggested

314 based on this test result. This approach would be greatly facilitated by the expanding the currently available panel of

315 antibiotic resistance genes detected by PCR/ESI-MS technology. As the field of sepsis immunotherapy and

316 personalised medicine rapidly expands PCR/ESI-MS may prove to have a role in identifying patients that would

317 benefit from specific antagonism of TLR9 pathways or even from adjunctive immune stimulation [24-26]. Further

318 mechanistic studies are required prior to suggesting more specific treatments.

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335 **Conclusions**

336 According to our best knowledge this is the first paper that reports that the presence of microbial DNA in the blood-

337 stream of patients with suspected acute sepsis is associated with greater mortality. It is plausible that PCR/ESI-MS

338 result may provide additional important information as regards the clinical trajectory of the patient with suspected

339 sepsis above that garnered from the traditional blood culture results and from an assessment of the severity of

340 illness. It is plausible that this assay could be used to direct specific adjunctive therapies to a high risk population

341 with suspected sepsis.

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343 **Authors contributions**
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345 MW conceived the study. MOD, JS, KZ, DE, RS, DB, MS, NL, MW, JLV designed the study and contributed data.

346 MOD, MHS and MW did the data analysis MOD, MHS, MW and JLV wrote the manuscript.

347

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350

351 **Conflict of interests**

352 Dr. O'Dwyer reports grants from Ibis Biosciences, during the duration of the study. Dr. Ecker reports funding from

353 Ibis Biosciences Inc. an Abbott Company, during the conduct of the study and to be clear, I am an employee who

354 works for the company that makes the technology that is the subject of the paper. Dr. Brealey reports personal fees

355 from Abbott, outside the submitted work. Dr. Singer reports personal fees from Abbott, outside the submitted work.

356 Dr. Wilks reports grants from Abbott during the conduct of the study. Dr. Starczewska, Prof. Zacharowski, Prof.

357 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	BC+ve	BC-ve	p	PCR+ve	PCR-ve	p
	(n=439)	(n=56)	(n=383)		(n=177)	(n=262)	
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.

433 **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

434 A description of the hospital stay and illness characteristics following enrolment in the study. Days alive and free of (MV/ vasopressors/

435 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

436 and alive.

437 Abbreviations: BC+ve, positive blood culture; BC-ve, negative blood culture; PCR+ve, positive polymerase chain reaction / electrospray

438 ionization-mass spectrometry; PCR-ve, negative polymerase chain reaction / electrospray ionization-mass spectrometry; ICU, intensive care unit;

439 LOS, length of stay; MV, mechanical ventilation; A/B, antibiotics

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451 **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)		

452 The C statistic for the whole model is 0.77. Abbreviations: OR, odds ratio; CI, confidence interval; SOFA, sequential organ failure assessment

453 score; PCR/ESI-MS, polymerase chain reaction/electrospray ionization-mass spectrometry; BC, blood culture

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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.
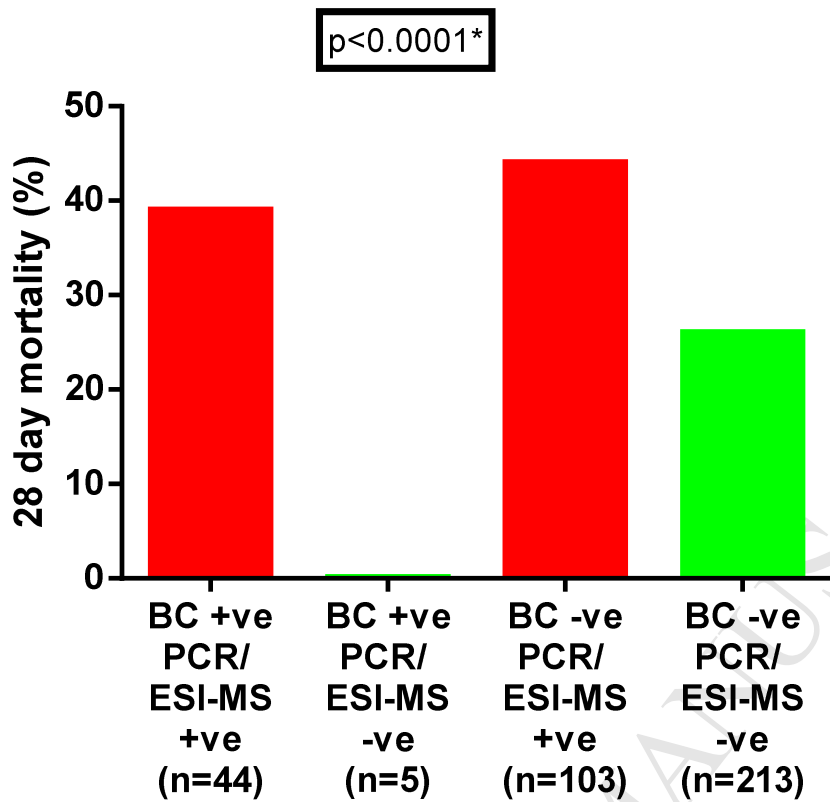
Amongst those patients that have a negative blood culture result those with a positive PCR/ESI-MS test result have a higher mortality. A McNemar's Test was performed on the non-surviving patients which indicated that the total number of positive tests for each method was statistically different (McNemar 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