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1 ***Time-to-positivity in bloodstream infection is not a prognostic***  
2 ***marker for mortality: analysis of a prospective multicentre***  
3 ***randomised control trial.***

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23 **Abstract:**

24 **Objectives**

25 Time to positivity (TTP), calculated automatically in modern blood culture systems, is considered a  
26 proxy to microbial load and has been suggested a potential prognostic marker in bloodstream  
27 infection. In this large, multi-centre, prospectively collected cohort, our primary analysis aimed  
28 to quantify the relationship between TTP of monomicrobial blood cultures and mortality.

29 **Methods**

30 Data from a multi-centre randomised control trial (RAPIDO) in bloodstream infection was analysed.  
31 Bloodstream infections were classified into 13 groups/subgroups. The relationship between  
32 mortality and TTP was assessed by logistic regression, adjusted for site, organism, and clinical  
33 variables; and linear regression applied to examine the association between clinical variables and  
34 TTP. Robustness was assessed by sensitivity analysis.

35 **Results**

36 4,468 participants were included in RAPIDO. After exclusions, 3,462 were analysed, with the most  
37 common organisms being coagulase-negative staphylococci (1,072 patients) and *E.coli* (861  
38 patients). 785 (22.7%) patients died within 28 days. We find no relationship between TTP and  
39 mortality for all groups except for Streptococci (Odds ratio (OR) with each hour 0.98, 95% CI 0.96-  
40 1.00) and *Candida* (OR 1.03, 95%CI 1.00-1.05). There was large variability between organisms and  
41 sites in TTP. Fever (Geometric Mean Ratio GMR 0.95; 95% CI 0.92-0.99), age (GMR per ten years  
42 1.01, 95% CI 1.00 – 1.02), and neutrophilia were associated with TTP (GMR 1.03; 95% CI 1.02-1.04).

43 **Conclusions**

44 Time to positivity is not associated with mortality, except in *Candida* spp (longer times associated  
45 with worse outcomes), and possibly in Streptococci (shorter times associated with worse outcomes).  
46 There was large variation between median times across centres, limiting external validity.

47 **Introduction:**

48 Modern blood culture systems record detailed timing information about how long blood culture  
49 bottles are incubated for. This information, often known as time to positivity or TTP data, has been  
50 used for a wide variety of clinical indications.<sup>1-4</sup> The most clinically utilised use of TTP data is to  
51 identify central line associated bloodstream infections (CLABSIs) and differentiate them from other  
52 sources of bloodstream infection (BSI), with this diagnostic technique included in the Infectious  
53 Disease Society of America (IDSA) guidance on management of CLABSIs.<sup>5-7</sup>

54 Given TTP is associated with bacterial load and is easily measured, there is significant clinical and  
55 scientific interest in understanding the relationship between TTP and patient outcomes. Multiple  
56 prior studies have reported on the association of TTP and clinical outcome across multiple bacteria  
57 and fungi, with conflicting results.<sup>1-4,8-11</sup>

58 The multiple reasons cited for these conflicting results include: retrospectively recorded outcome  
59 data, heterogeneity of infective organisms, different blood culture systems across hospitals, and  
60 most importantly, a focus only on the time on the blood culture machine, ignoring the crucial  
61 information regarding how long the blood culture bottles were left before being placed on the  
62 incubating blood culture system.

63 In this study, we report clinical outcomes associated with time to positivity of blood cultures from a  
64 prospectively collected cohort of BSIs from the multicentre RAPIDO trial.

65 **Methods:**

66 For brevity, the methods are largely reported in the supplementary appendix, with an overview here

67 This study aimed to 1) quantify the association between TTP and clinical outcomes, and 2) identify  
68 clinical factors associated with TTP. All participants included were part of a large pragmatic  
69 randomised controlled trial (RAPIDO) of direct MALDI-TOF identification of adult blood cultures that  
70 ran across seven NHS laboratories in the UK, and was recently published in CMI.

71 For this analysis, all participants in RAPIDO who had monomicrobial blood cultures with clinically  
72 relevant and/or common pathogens were included (Flow chart in Figure 1, included organisms in  
73 table S1, excluded in Table S2). Microbial data (identification, timings) and clinical data  
74 (demographics, comorbidities, outcome) were extracted from the trial database.

75 For our primary analysis we estimated the association between 28 day mortality (our primary  
76 outcome) and time to positivity using logistic regression, as a univariate analysis. Recruiting site and  
77 infecting organism were included as fixed effects. For sensitivity analyses, we 1) replicated the  
78 analysis using time measure on the machine, rather than total time since blood culture taken 2)  
79 included relevant clinical comorbidities in a multivariable analysis, and 3) limited our analysis to  
80 those patients not on appropriate antimicrobial therapy at the time of blood culture collection. For  
81 our secondary analysis, we estimated the association between time to positivity and clinical  
82 variables with linear regression.

83

84

## 85 **Results:**

### 86 *Flow chart and baseline characteristics of included participants*

87 The RAPIDO trial included 4,468 participants. Of those, 4,104 had monomicrobial cultures in both  
88 bottle sets, and 4,037 had the same organism in each bottle. 384 cultures were excluded with rare  
89 organisms or known contaminants (list in Table S1). Finally, 191 participants were excluded with  
90 missing time to positivity data, leaving a final analysis population of 3,462 patients.

91 Figure 1 describes the flow throughout the study.

92 [FIGURE 1]

93 Table S2 in the supplementary appendix describes the baseline clinical characteristics for each  
94 subgroup of included bacteria, with Table 2 displaying the mortality for each subgroup, and Table 3  
95 describing the time to positivity of each organism stratified by mortality. Table S3 describes the time  
96 to positivity of each organism broken down by time on machine and time before machine.  
97 Importantly, this cohort was quite sick at baseline, with an overall mortality of 22.7%, and with a  
98 large number of patients frail (median Charlson's Comorbidity Score 3; IQR: 2-4) and sick (8.8%  
99 ventilated on day of blood culture sampling).

100 [TABLE 2]

101 The most common included group were Coagulase Negative Staphylococi (CoNS) isolates, with 1,072  
102 included patients, followed by *E.coli*, with 861 included patients. Mortality was highest in *Candida*  
103 spp (25/53, 47.2%), and in *Pseudomonas* spp (43/125, 34.4%). It was lowest in Group B Streptococci  
104 (4/45, 8.9%), Streptococci, other (24/162, 14.8%) and *Proteus* spp (11/65, 16.9%).

105 *Global relationship between TTP and mortality*

106 Table S4 describes the baseline demographics between cultures that were positive before and after  
107 24hrs. There were limited differences between groups, although fever was slightly more common in  
108 the TTP <24hrs group, as was organ transplantation and use of immunosuppressive drugs. Figure S1  
109 shows the raw mortality across the whole cohort by time-to-positivity, which shows no clear  
110 relationship, although mortality in the very few (47/3462) samples that grew in under 10 hours was  
111 higher (18/47, 38.3%) than any other time period. Additionally Figure 2 shows the total distribution  
112 of time to positivity within the whole cohort.

113 [Figure 2]

114 *Relationship between time-to-positivity and mortality in individual organism/group*

115 [TABLE 3]

116 Table 3 shows the median time-to-positivity for survivors and non-survivors for each  
117 organism/group. Time to positivity also varied greatly between organisms, as would be expected by  
118 microbial growth kinetics. The longest time-to-positivity was in *Candida* spp with a median total time  
119 of 45.3hrs (IQR 34.2, 69.9), and anaerobes (total time: 36.8hrs, IQR 31.7, 54.2). In contrast, the  
120 shortest time to positivity was in Group C/G Streptococci (total time: 15.7hrs, IQR 13.7, 21.0).

121 There was no clear relationship between median time to positivity and mortality. This is visualised in  
122 Figure 3, which displays the time to positivity against mortality for each group.

123 [Figure 3]

#### 124 *Logistic regression model*

125 In the logistic regression model, which adjusted for centre and organism alone there was no  
126 relationship between time-to-positivity and mortality in any organism except *Candida* spp, where  
127 there was a slight increase in mortality with increasing time-to-positivity (OR 1.03, 95% CI 1.00-1.05).  
128 This was in the opposite direction than would be expected, and should be interpreted with some  
129 caution given the low numbers (n = 53). All streptococci except pneumococci were combined for this  
130 model, due to low numbers of events in Group B streptococci. There was no evidence of an  
131 interaction between time-to-positivity and organism ( $p = 0.159$ ). These estimates are shown in  
132 Figure 4.

133 [Figure 4]

#### 134 *Sensitivity analyses*

135 In the subsequent model, we also included relevant clinical features as described in the methods.  
136 Again, this showed no clear evidence of a relationship between time-to-positivity and mortality in  
137 any organism group except *Candida* spp (Supplementary Figure S2). We also performed a sensitivity  
138 analysis adjusting for receipt of appropriate therapy on date of blood culture sampling and results  
139 were consistent with the primary analysis (Supplementary Figure S3). Unsurprisingly, the rate of

140 appropriate therapy differed by organism and by centre, but addition of this to the model made no  
141 difference to the primary outcome ((Supplementary table S5 and S6). Lowest rates of appropriate  
142 therapy were in *Candida* spp (51/53; 96.2% not appropriate), with the highest rates in Group A  
143 Streptococci (88/132; 71.7% on appropriate therapy).

144 As a final sensitivity analysis, we analysed time-to-positivity calculated from the time on the  
145 machine, rather than as from time taken. In this model, time to positivity in both *Streptococcus*  
146 pneumoniae (OR 0.85, 95%CI 0.74-0.97) and other streptococci (OR 0.96, 95% CI 0.92-0.99) were  
147 statistically significant, although in the opposite direction to *Candida* spp, suggesting that increasing  
148 time-to-positivity is associated with increased survival in streptococci, but worsening mortality in  
149 *Candida* spp (Supplementary Figure S4).

#### 150 *Additional analyses on Candida spp*

151 Given the inverse relationship between mortality and TTP identified in *Candida* spp, we focused on  
152 this pathogen in more detail. Due to low numbers, we report a descriptive analysis only. Thirty-five  
153 of these blood cultures were identified as *Candida albicans*, with patient death in 46% (16/35). Ten  
154 were identified as *Candida glabrata*, with patient death in 40% (4/10). No other species was  
155 identified more than twice. Time to positivity was much greater in *Candida glabrata* (mean 87.9 hrs  
156 in patients that died, 51.1 hrs in patients that survived) than in *Candida albicans* (mean 46.8 hrs in  
157 patients that died, 41.8 hrs in patients that survived). Susceptibility data (where available) showed  
158 that 38/42 (90.4%) were susceptible to fluconazole.

#### 159 *Clinical and microbial features that are associated with time to positivity*

160 As a secondary outcome, we aimed to identify whether any clinical features are associated with time  
161 to positivity. We performed linear regression with time-to-positivity as the outcome variable, which  
162 was logged to improve model fit, with centre, organism, and clinical features as predictor variables.  
163 As such, the effect estimates should be interpreted as geometric mean ratios (GMR), rather than



164 odds ratios. GMRs should be interpreted on the multiplicative scale, not the additive scale, but the  
165 directions of association remain the same as odds ratio .

166 [Table 4]

167 Table 4 shows the output of this model. Unsurprisingly, organism group was strongly associated with  
168 time-to-positivity, with all organisms having a significant relationship with time-to-positivity  
169 compared to the reference group (coagulase-negative staphylococci). Centre also had a significant  
170 impact on time-to-positivity, with all centres except one showing a different time to positivity to the  
171 reference centre (Centre 3). In terms of clinical features, increasing age was associated with  
172 increasing time-to-positivity, as was increasing neutrophilia. However, the presence of fever had an  
173 opposite relationship, with fever associated with lower time to positivity.

174

#### 175 **Discussion:**

176 In this large, multi-centre, prospectively collected cohort of bloodstream infections with detailed  
177 timing information, we found no robust evidence of a relationship between mortality and time to  
178 positivity in Staphylococci (both coagulase negative and *S. aureus*), Pseudomonas, Enterococci,  
179 Bacteroides, and all of Enterobacterales. For *Candida* spp, we identified a relationship between  
180 increasing time to positivity and mortality, contrary to our expectations, although numbers were  
181 small. Conversely, in Streptococci, we found a more expected association between decreased time  
182 to positivity and mortality, although this was only identified in a sensitivity analysis, and not in the  
183 main results.

184 We did not find a clear relationship between any clinical variables except age, fever, and  
185 neutrophilia with time-to-positivity, suggesting in the case of fever and neutrophils the anticipated  
186 role of the organism load in driving the initial inflammatory response.

187 *Strengths and limitations*

188 This paper has the strength of the scale of prospective data collection from a large  
189 randomised control trial, and was largely complete..Notably, detailed information on timing both  
190 from sample collection and from time on machine were available, allowing us to take account of this  
191 potential source of heterogeneity.

192 However, as this was a pragmatic trial, we do not have detailed information on the clinical and  
193 laboratory processes at each site,) although all sites are UKAS accredited laboratories. Study centre  
194 had a significant impact on time to positivity, which was accounted for in our models, but has  
195 significance for external validity of previous single centre studies. We were unable to include time to  
196 effective treatment as a variable in our models; as this will strongly correlate (and is a collider with)  
197 time to positivity. However, 44% of the cohort were already on effective therapy at the time of the  
198 blood culture, and the evidence that delay in effective therapy is strongly associated with outcomes  
199 is weak, as shown by RAPIDO and other trials.<sup>12,14,15</sup> Although we controlled for time to appropriate  
200 therapy in our analyses, more detailed information on timings would allow a more nuanced  
201 understanding of the potential impact, and should be a focus of future research.

202 Finally, while we are confident about findings in bacterial groups with a large number of patients,  
203 such as in *E.coli*, coagulase negative staphylococci, and *S. aureus*. For other groups, (e.g *Proteus* spp,  
204 Anaerobes, and Group B Streptococci), the numbers were relatively small and interpretation of  
205 these results should be more cautious.

#### 206 *Comparisons with previous literature*

207 These results are surprising, and largely inconsistent with the previous literature that has identified  
208 time to positivity as a potential independent biomarker of severity in multiple prior cohorts  
209 (reviewed in <sup>4</sup>), although our cohort is an order of magnitude larger in both scale and comprehensive  
210 data collection.

211 It is valuable to explore the reasons underlying our main finding of a lack of TTP and outcome.  
212 Firstly, it is important to note that time to positivity is a function of at least four factors: pathogen  
213 load in the bottle, pathogen growth kinetics, host factors, and laboratory/processing factors,  
214 although it is often simply thought of as a measure of microbial load. Most explanations for the  
215 association between mortality and time to positivity equate the increased mortality with an  
216 increased pathogen load, as is seen in evolutionary and ecological studies of infection, as the other  
217 factors are either fixed (growth kinetics), random (laboratory processing), or small (host factors).

218 There are therefore two broad explanations of our conflicting results: Firstly, pathogen load is simply  
219 not associated with outcome in clinical human infection, or that time to positivity is not reliable  
220 enough an indicator of pathogen load to be useful clinically. The first argument is plausible, although  
221 there is a wealth of data from non-culture based techniques (largely PCR) that has consistently  
222 associated higher microbial loads with worse outcomes in infection,<sup>16-25</sup> (reviewed in <sup>26</sup>)..

223 Despite this evidence, there is increasing recognition that survival from pathogens requires both  
224 resistance (host approaches that reduce pathogen loads) and tolerance (host approaches that  
225 improve survival independent of pathogens).<sup>27,28</sup> This is supported by the epidemiological evidence  
226 that patients with weakened immune systems, (e.g. transplant) do not, generally, have greatly  
227 increased mortality from severe infection<sup>29-31</sup>, and the evidence of benefit of steroids in infections  
228 like COVID-19.<sup>32</sup> It is therefore possible that microbial load is not that relevant to outcomes in a  
229 relatively elderly cohort with bloodstream infection.

230 The second explanation – that host and laboratory factors overpower the relevance of microbial load  
231 is perhaps more likely. Most prior studies focussed on single centre cohorts with a single pathogen,  
232 using a single laboratory. However, we found large differences in both time to the machine and time  
233 on the machine between centres for the same organisms, suggesting most variation was unrelated  
234 to the microbial load of the organism. Also, host factors appear to have some impact on time to  
235 positivity, suggesting that the case-mix within a hospital might also alter time to positivity. This has

236 significant implications for the external validity of time to positivity, suggesting that, even if time to  
237 positivity was associated with outcome, thresholds at one centre are very unlikely to be relevant at  
238 another centre.

#### 239 *Implications for research*

240 Future studies should focus on non-culture based techniques using an approach minimising external  
241 validation, and should aim to identify if the impact of pathogen load varies by organism.

#### 242 *Implications for clinical practice*

243 Time to positivity is not strongly associated with mortality and has limited external validity. Clinicians  
244 should be cautious in interpreting time to positivity data as a marker of severity. Studies should look  
245 at the impact of prior antimicrobial therapy on time to positivity and other microbial load markers.

#### 246 *Conclusions*

247 Time to positivity was not associated with mortality in a large, prospectively collected, multi-centre  
248 cohort, except in *Candida* spp (longer times associated with worse outcomes, caveated by small  
249 numbers), and possibly in Streptococci (shorter times associated with worse outcomes). There was  
250 large variation between median times across centres, limiting external validity.

251

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#### 255 Author contributions:

256 FH conceived of the idea, and performed some analyses. RE performed most of the analyses, and  
257 produced figures and graphs. PG provided writing assistance, drafting, and editing. AM provided the  
258 data, and assisted with writing and editing of the manuscript.

259 Conflict of Interest:

260 No authors have any relevant conflicts of interest.

261 References:

262

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345



## 346 Supplementary Methods:

347

### 348 *Primary aim*

349 The primary aim of this study was to identify and quantify the relationship between TTP of blood  
350 culture systems and 28-day mortality across a wide range of bacteria and fungi.

### 351 *Secondary aims*

352 To identify and quantify the relationship between clinical features and TTP of blood culture systems.  
353 Clinical features considered were: age, gender, on immunosuppressive drugs, any prior transplant,  
354 systemic corticosteroids, suspected hospital acquired infection, neutrophil count and fever on day of  
355 blood culture.

### 356 *Data source*

357 All participants included in this study were part of the RAPIDO trial, an NIHR funded randomised  
358 controlled trial on the impact of rapid identification of BSI organisms by matrix-assisted-laser-  
359 deionisation time of flight (MALDI-TOF) analysis across seven NHS laboratories in England and Wales,  
360 recently published in *CMI*.<sup>12</sup> Ethical approval for this study was granted by the National Research  
361 Ethics Committee South West.

362 Full details of the inclusion criteria are published with the original trial.<sup>12</sup> Briefly, all adult patients in  
363 the seven included NHS sites who had a blood sample culture positive for bacteria or fungi between  
364 July 2012 and August 2014 were potentially eligible for inclusion.

### 365 *Analysis population*

366 The analysis population included all RAPIDO participants with a monomicrobial infection  
367 (monomicrobial cultures in both bottle sets and the same organism in each bottle) excluding

368 cultures with rare organisms or known contaminants. Participants with missing time to flagged  
369 positive or time sample went on the machine missing were excluded from the analysis population.

#### 370 *Patient level data*

371 Information included from the trial in this study include: demographic details, hospital site, fever at  
372 presentation, Charlson Comorbidity Score (CCS), presence or absence of immunosuppression or  
373 organ transplantation, vasopressor requirement, receipt of corticosteroids, and initial blood  
374 pressure. Clinical outcomes recorded included: death (including time to death), and time to  
375 discharge.

#### 376 *Microbial data*

377 For each positive blood culture, the time taken from the patient, the time placed on the blood  
378 culture system, and the time taken of the blood culture system was recorded. The initial Gram stain  
379 and the final identification of the organism were recorded. Full technical details are recorded in the  
380 supplement of the original manuscript.<sup>12</sup> The timings of the first bottle (if both bottles flagged  
381 positive) was used, and the second bottle not analysed. As this trial was a multicentre trial, each  
382 laboratory had their own blood culture system and methodology for identification of organisms. The  
383 R package “AMR” was used to robustly identify and classify microorganisms based on the clinical  
384 report, ensuring consistency between sites.<sup>13</sup>

385 All organisms were identified according to local laboratory criteria, and were *a priori* classified into  
386 multiple groups depending on their clinical phenotype (Table S1).

387

388 Time to positivity was defined as the time from collection of the blood culture to time the sample  
389 was flagged positive, i.e. the total time from sample to machine positivity. Sensitivity analyses were  
390 performed using time on machine only (see below).

#### 391 *Statistical analyses*

392 Participant demographics were summarised by organism group. Continuous data are summarised  
393 using mean and standard deviation (or median and interquartile range (IQR) if distributions were  
394 skewed) and categorical data as number and percentage. A complete case analysis was performed.  
395 We estimated the association between time to positivity and 28-day mortality using multivariable  
396 logistic regression, adjusting for centre and organism as fixed effects. An interaction between  
397 organism and time to positivity was included to investigate whether the effect differed between  
398 organisms. For the secondary outcome, we estimated the effect of clinical features (age, gender,  
399 immunosuppressive drugs, any prior transplant, systemic corticosteroids, suspected hospital  
400 acquired infection and RAPIDO trial allocation) on time to positivity using linear regression.  
401 Assumptions underpinning the statistical models were checked using standard methods. If the  
402 assumptions were not satisfied, transformations or alternative methods were explored.

403 Subgroups of organisms with a small number of participants/events were combined to ensure  
404 estimation. Two sensitivity analyses were performed for the primary outcome (a) we replicated all  
405 analyses using time on machine only, rather than total time, as this is more likely to be consistent  
406 across centres, (b) adjusting for demographics and clinical variables and (c) adjusting for receipt of  
407 appropriate therapy on day on blood culture sampling, classified as clinically significant episode – on  
408 appropriate therapy on day of blood culture sampling, clinically significant episode – not on  
409 appropriate therapy on day of blood culture sampling and non-clinically significant episode –  
410 appropriate therapy not recorded.

411 Associations are reported as effect estimates with 95% confidence intervals (CI).

412 All analyses were performed using Stata version 16.0 (StataCorp), with initial processing of data in R  
413 4.0.0 (R foundation for Statistical Computing, Vienna).

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