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

Fergus Hamilton [1,2,4]

Rebecca Evans [3]

Peter Ghazal [4]

Alasdair MacGowan [1]

[1] Infection Sciences, Pathology, North Bristol NHS Trust, Bristol, UK

[2] Population Health Sciences, University of Bristol, Bristol, UK

[3] Bristol Trials Centre, Bristol Medical School, University of Bristol, Bristol, UK

[4] Project Sepsis, Cardiff University, Cardiff, UK

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Corresponding author:

Dr Fergus Hamilton

Infection Sciences, Pathology, North Bristol NHS Trust

Southmead Hospital

Westbury-on-Trym

BRISTOL

BS10 5NB

Tel: +44 (0) 117 414 6215

E-mail: Fergus.Hamilton@bristol.ac.uk

Abstract:

Objectives

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

Methods

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

Results

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

Conclusions

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

Introduction:

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

Given TTP is associated with bacterial load and is easily measured, there is significant clinical and scientific interest in understanding the relationship between TTP and patient outcomes. Multiple prior studies have reported on the association of TTP and clinical outcome across multiple bacteria and fungi, with conflicting results.[1–4,8–11]

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

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

Methods:

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

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

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

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

Results:

Flow chart and baseline characteristics of included participants

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

Figure 1 describes the flow throughout the study.

[FIGURE 1]

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

[TABLE 2]

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

Global relationship between TTP and mortality

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

[Figure 2]

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

[TABLE 3]

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

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

[Figure 3]

Logistic regression model

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

[Figure 4]

Sensitivity analyses

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

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

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

Additional analyses on Candida spp

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

Clinical and microbial features that are associated with time to positivity

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

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

[Table 4]

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

Discussion:

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

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

Strengths and limitations

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

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

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

Comparisons with previous literature

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

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

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

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

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

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

Implications for research

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

Implications for clinical practice

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

Conclusions

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

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Author contributions:

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

Conflict of Interest:

No authors have any relevant conflicts of interest.

References:

1. Cillóniz, C. *et al.* Time to blood culture positivity as a predictor of clinical outcomes and severity in adults with bacteremic pneumococcal pneumonia. *PLOS ONE* **12**, e0182436 (2017).
2. Chen, S.-Y. *et al.* Value of blood culture time to positivity in identifying complicated nontyphoidal Salmonella bacteremia. *Diagnostic Microbiology and Infectious Disease* **91**, 210–216 (2018).
3. Ning, Y., Hu, R., Yao, G. & Bo, S. Time to positivity of blood culture and its prognostic value in bloodstream infection. *Eur. J. Clin. Microbiol. Infect. Dis.* **35**, 619–624 (2016).
4. Lamy, B. Blood culture time-to-positivity: making use of the hidden information. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases* vol. 25 268–271 (2019).
5. Al-Juaid, A., Walkty, A., Embil, J., Crockett, M. & Karlowsky, J. Differential time to positivity: vascular catheter drawn cultures for the determination of catheter-related bloodstream infection. *Scand. J. Infect. Dis.* **44**, 721–725 (2012).
6. Mermel, L. A. *et al.* Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* **49**, 1–45 (2009).

7. Malgrange, V. B., Escande, M. C. & Theobald, S. Validity of earlier positivity of central venous blood cultures in comparison with peripheral blood cultures for diagnosing catheter-related bacteremia in cancer patients. *J. Clin. Microbiol.* **39**, 274–278 (2001).
8. Siméon, S. *et al.* Time to blood culture positivity: An independent predictor of infective endocarditis and mortality in patients with Staphylococcus aureus bacteraemia. *Clin. Microbiol. Infect.* **25**, 481–488 (2019).
9. Peralta, G., Rodríguez-Lera, M. J., Garrido, J. C., Ansorena, L. & Roiz, M. P. Time to positivity in blood cultures of adults with Streptococcus pneumoniae bacteremia. *BMC Infect. Dis.* **6**, 79 (2006).
10. Martín-Gutiérrez, G. *et al.* Time to positivity of blood cultures in patients with bloodstream infections: A useful prognostic tool. *Enferm. Infecc. Microbiol. Clin.* **35**, 638–644 (2017).
11. Liao, C.-H. *et al.* Correlation between time to positivity of blood cultures with clinical presentation and outcomes in patients with Klebsiella pneumoniae bacteraemia: prospective cohort study. *Clin. Microbiol. Infect.* **15**, 1119–1125 (2009).
12. MacGowan, A. *et al.* Impact of rapid microbial identification on clinical outcomes in bloodstream infection: the RAPIDO randomized trial. *Clin. Microbiol. Infect.* **26**, 1347–1354 (2020).
13. AMR (for R). https://msberends.github.io/AMR/.
14. Nadjm, B. *et al.* A randomised controlled trial of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDITOF-MS) versus conventional microbiological methods for identifying pathogens: Impact on optimal antimicrobial therapy of invasive bacterial and fungal infections in Vietnam. *J. Infect.* **78**, 454–460 (2019).
15. Alam, N. *et al.* Prehospital antibiotics in the ambulance for sepsis: a multicentre, open label, randomised trial. *Lancet Respir Med* **6**, 40–50 (2018).
16. Hackett, S. J. *et al.* Meningococcal bacterial DNA load at presentation correlates with disease severity. *Arch. Dis. Child.* **86**, 44–46 (2002).

17. Sonthayanon, P. *et al.* Association of high Orientia tsutsugamushi DNA loads with disease of greater severity in adults with scrub typhus. *J. Clin. Microbiol.* **47**, 430–434 (2009).
18. Ziegler, I. *et al.* High nuc DNA load in whole blood is associated with sepsis, mortality and immune dysregulation in Staphylococcus aureus bacteraemia. *Infect. Dis.* **51**, 216–226 (2019).
19. Ho, Y.-C., Chang, S.-C., Lin, S.-R. & Wang, W.-K. High levels of mecA DNA detected by a quantitative real-time PCR assay are associated with mortality in patients with methicillin-resistant Staphylococcus aureus bacteremia. *J. Clin. Microbiol.* **47**, 1443–1451 (2009).
20. Darton, T. *et al.* Severity of meningococcal disease associated with genomic bacterial load. *Clin. Infect. Dis.* **48**, 587–594 (2009).
21. Ziegler, I., Lindström, S., Källgren, M., Strålin, K. & Mölling, P. 16S rDNA droplet digital PCR for monitoring bacterial DNAemia in bloodstream infections. *PLoS One* **14**, e0224656 (2019).
22. Chuang, Y.-C., Chang, S.-C. & Wang, W.-K. High and increasing Oxa-51 DNA load predict mortality in Acinetobacter baumannii bacteremia: implication for pathogenesis and evaluation of therapy. *PLoS One* **5**, e14133 (2010).
23. Ziegler, I., Josefson, P., Olcén, P., Mölling, P. & Strålin, K. Quantitative data from the SeptiFast real-time PCR is associated with disease severity in patients with sepsis. *BMC Infect. Dis.* **14**, 155 (2014).
24. Roine, I., Saukkoriipi, A., Leinonen, M., Peltola, H. & LatAm Meningitis Study Group. Microbial genome count in cerebrospinal fluid compared with clinical characteristics in pneumococcal and Haemophilus influenzae type b meningitis in children. *Diagn. Microbiol. Infect. Dis.* **63**, 16–23 (2009).
25. Guiducci, S. *et al.* Culture and Real-time Polymerase Chain reaction sensitivity in the diagnosis of invasive meningococcal disease: Does culture miss less severe cases? *PLoS One* **14**, e0212922 (2019).
26. Lisboa, T., Waterer, G. & Rello, J. We should be measuring genomic bacterial load and virulence factors. *Crit. Care Med.* **38**, S656-62 (2010).

27. Soares, M. P., Teixeira, L. & Moita, L. F. Disease tolerance and immunity in host protection against infection. *Nat. Rev. Immunol.* **17**, 83–96 (2017).
28. Medzhitov, R., Schneider, D. S. & Soares, M. P. Disease tolerance as a defense strategy. *Science* **335**, 936–941 (2012).
29. Chong, A. S. & Alegre, M.-L. Transplantation tolerance and its outcome during infections and inflammation. *Immunol. Rev.* **258**, 80–101 (2014).
30. Nielsen, L. H., Jensen-Fangel, S., Jespersen, B., Ostergaard, L. & Søgaard, O. S. Risk and prognosis of hospitalization for pneumonia among individuals with and without functioning renal transplants in Denmark: a population-based study. *Clin. Infect. Dis.* **55**, 679–686 (2012).
31. Kalil, A. C. *et al.* Is bacteremic sepsis associated with higher mortality in transplant recipients than in nontransplant patients? A matched case-control propensity-adjusted study. *Clin. Infect. Dis.* **60**, 216–222 (2015).
32. RECOVERY Collaborative Group *et al.* Dexamethasone in Hospitalized Patients with Covid-19. *N. Engl. J. Med.* **384**, 693–704 (2021).