Time to blood culture positivity an independent predictor of infective endocarditis and mortality in patients with Staphylococcus aureus bacteraemia

Soline Simeon, Vincent Le Moing, Sarah Tubiana, Xavier Duval, Damien Fournier, Jean-Philippe Lavigne, Marie-Line Erpelding, Claude-Alexandre Gustave, Stephanie Desage, Catherine Chirouze, et al.

Original article

Time to blood culture positivity: an independent predictor of infective endocarditis and mortality in patients with *Staphylococcus aureus* bacteraemia

Soline Siméon,[1] Vincent Le Moing,[2] Sarah Tubiana,[3-5] Xavier Duval, [3-5] Damien Fournier,[6] Jean-Philippe Lavigne,[7,8] Marie-Line Erpelding,[9] Claude-Alexandre Gustave,[10] Stephanie Desage,[11] Catherine Chirouze,[12] François Vandenesch,[10] Pierre Tattevin,[1,13] and the VIRSTA/AEPEI Study Group.

[1]*Maladies Infectieuses et Réanimation Médicale, Hôpital Pontchaillou, CHU Rennes, France;*

[2]*Maladies Infectieuses, CHU de Montpellier, France;*

[3]*INSERM CIC-1425, AP-HP, Hôpital Bichat Claude Bernard, Paris, France;*

[4]*INSERM, IAME, UMR 1137, Paris, France;*

[5]*Université Paris Diderot, IAME, UMR 1137, Sorbonne Paris Cité, Paris, France;*

[6]*Laboratoire de Bactériologie, CHRU Besançon, France;*

[7]*Service de Microbiologie, CHU Caremeau, Nîmes, France;*

[8]*INSERM, U1047, Université de Montpellier, Nîmes, France;*

[9]*INSERM, CHRU Nancy, Université de Lorraine, CIC-1433 Epidemiologie Clinique, Nancy, France;*

[10]*Centre National de Référence des Staphylocoques, Institut des Agents Infectieux, Hospices Civils de Lyon, Lyon, France;*

[11]*Laboratoire de bactériologie, Institut des Agents Infectieux, Hospices Civils de Lyon, Lyon, France;*

[12]*UMR CNRS 6249, Université de Bourgogne-Franche Comté – Service de maladies infectieuses et tropicales, CHRU de Besançon, France;*

[13]*INSERM U1230, Université Rennes-I, Rennes, France*

Corresponding author: Pierre Tattevin, Service des Maladies Infectieuses et de Réanimation Médicale, CHU Pontchaillou, 2 rue Henri Le Guilloux, 35033 Rennes Cedex, France. Tel +33 299289564 ; fax +33 299282452, E-mail address: pierre.tattevin@chu-rennes.fr

ABSTRACT

*Objectives***:** Time to blood culture positivity (TTP), a routinely available parameter in automated blood culture systems, may be a proxy for infectious burden in patients with bloodstream infections. We aimed to study the association between TTP and infective endocarditis (IE), or death, in patients with *Staphylococcus aureus* bacteraemia.

*Methods***:** VIRSTA is a multicenter prospective cohort study that included all adult patients with *S. aureus* bacteraemia in eight university hospitals in France (2009-2011). We analyzed data from four centers which collected data on TTP. Regression models were used to study the association between TTP and definite IE (Duke-Li criteria), and 30 day-mortality.

*Results***:** We included 587 patients with *S. aureus* bacteraemia: mean age was 65.3±16.3 years, 420/587 patients (71.6%) were male, 121/587 (20.6%) died, and 42/587 (7.2%) had definite IE. Median TTP of first positive blood culture was 13.7 h (interquartile range, 9.9-18). On multivariate analysis, 30-day mortality was associated with TTP≤13.7 h (74/295 (25.1%) vs 47/292 (16.1%), *P*=0.02), as well as old age, McCabe score, methicillin resistance, stroke, pneumonia, and C-Reactive Protein. TTP was also independently associated with IE, but with a U-shape curve: IE was more common in the first (TTP<10 h, 17/148, 11.5%), and the last (TTP≥18 h, 8/146, 5.5%) quartiles of TTP, *P*=0.002.

*Conclusions***:** TTP provides reliable information in patients with *S. aureus* bacteraemia, on the risk of IE, and prognosis, with short TTP being an independent predictor of death. This data readily available at no cost may be used to identify patients who require specific attention.

KEY WORDS: time to blood culture positivity; *Staphylococcus aureus*; bacteraemia; mortality; infective endocarditis

Introduction

Staphylococcus aureus bacteraemia (SAB) is a public health issue, with an incidence estimated at 15-40 per 100 000 person-years (1,2), and in-hospital mortality rates of 15-25% (3–5). Age, sepsis, comorbidities, methicillin-resistant *S. aureus* (MRSA), and inadequate antibiotic therapy are the main prognostic factors (3,6,7). In addition, *S. aureus* is the most prevalent microorganism causing infective endocarditis (IE) (8,9), a major complication of SAB (10,11).

Time to positivity of blood cultures (TTP) is defined as the time from the start of incubation to alert signal, in automated blood culture systems. TTP may provide useful information, since a short TTP may reflect high blood bacterial load, and/or enhanced virulence. This property has already been harnessed to diagnose catheter-related bloodstream infections based on differential TTP of paired blood cultures sampled from venipuncture and the catheter (12–14). However, this parameter, available at no cost with most automated blood culture systems, is rarely mentioned as a tool to adjust the management of patients with positive blood cultures.

A few studies evaluated the association between TTP and mortality in SAB (15–17), or IE (18), but they were limited by small sample size, retrospective and/or monocentre design, and found discordant results. We aimed to evaluate the association between TTP and i) IE, ii) mortality, in a prospective cohort of adult patients with SAB.

Methods

Population

The VIRSTA prospective cohort study included all consecutive adult patients with SAB between April 2009 and October 2011 in 8 tertiary-care university hospitals in France

(19,20). Trained research assistants collected clinical, biological, therapeutic data, and outcome. All patients, their relatives, or physicians, were contacted 12 weeks after the beginning of SAB to check if patients were alive, and the date of death in patients who died. The diagnosis of definite IE according to modified Duke criteria (21) was verified by a local adjudication committee including at least one cardiologist, one infectious diseases physician, and one bacteriologist. This ancillary study was restricted to the four VIRSTA sites where blood culture bottles were placed in automated blood culture instruments upon receipt in laboratory, 24/24 hours, 7/7 days, and TTP was routinely collected during the study period.

Definitions

SAB was defined as the isolation of *S. aureus* from at least one blood culture bottle sampled by venipuncture. For each patient, only TTP of the first set of positive blood cultures was retained for analysis. When both aerobic and anaerobic bottles were positive, we used the shortest TTP. SAB was classified as nosocomial, healthcare-associated, or community-onset using validated criteria (22). McCabe score classified patients in 4 categories (23): 1, no underlying disease; 2, non-fatal underlying disease; 3, ultimately fatal underlying disease (within 5 years); and 4, rapidly fatal underlying disease (within one year).

Microbiological methods

In each center, approximately 10 mL of blood was inoculated into aerobic and anaerobic bottles by nurses using a standardized sterile technique (24). The BACTEC® system (Becton Dickinson) was used in three sites, and BacT/Alert® (bioMérieux) in one. TTP was defined as the time from the start of incubation to alert signal, as previously (12–14). Antimicrobial susceptibility testing was performed per guidelines of the CASFM-EUCAST

(www.sfm-microbiologie.org). Strains isolated from patients with IE were centralized at the French National Reference Center for Staphylococci.

To study the mechanisms behind short, and long TTP, we performed in-depth analysis of a subset of 13 isolates from IE patients with the shortest, and the longest quartiles of TTP. Firstly, we estimated the crude doubling time: each strain was adjusted to an OD600nm = 0.5, and 100 µL of a 1/100 dilution in BHI was inoculated in triplicate into 96-wells plates that were incubated for 24 hours at 37°C with continuous OD600nm monitoring (Tecan Infinite® 200 PRO). Doubling times were calculated from the exponential growth phase data by Growthrate® 2.1. Secondly, to mimic the conditions encountered in blood culture bottles and measure the TTP of a standardized inoculum, each strain was adjusted to an OD600nm = 2 in 0.9% NaCl solution, and this suspension was diluted to achieve a concentration of 60 CFU/mL of *S. aureus*. One mL of this suspension was added to aerobic and anaerobic blood culture bottles (bioMérieux, 3 pairs per strain), previously inoculated with 5 mL of whole human blood. Blood cultures bottles were then incubated in an automated blood culture instrument (Virtuo®, bioMérieux) in which TTP was recorded as in routine clinical laboratory conditions. Once detected positive, a Gram stain and a Maldi-Tof identification (Vitek MS, bioMérieux) were performed. Lastly, genotyping of isolates was performed with diagnostic DNA microarrays, *S. aureus* Genotyping kit 2.0 (Alere, Jena, Germany) (25). The assignment of isolates to clonal complexes (CCs) was performed by comparing hybridization profiles to multi-locus sequence typing (MLST) reference strains.

Statistical analysis

Non-normally distributed continuous variables were reported as median with interquartile range (IQR). Normally distributed continuous variables were reported as mean with standard deviation. Qualitative variables were expressed as number and proportions.

Continuous variables were compared using Student's *t*-test for normally distributed and the Wilcoxon-Mann-Whitney nonparametric test for non-normally distributed variables. Differences in proportions were compared by a chi-square or a Fisher's exact test, when appropriate. We performed a bivariate analysis to compare SAB with short (<median), and long (>median) TTP. A logistic regression model was developed to study factors associated with IE, excluding major Duke criteria in the final model. A Poisson regression model was used to assess factors associated with 30-day mortality. In regression models, continuous variables were modeled using fractional polynomial if relationship was nonlinear (26) . Risk was expressed as relative risk (RR) for Poisson regression, and odds ratio (OR) for logistic regression with 95% confidence intervals (CI).

First, regression analyses adjusted to age, sex and centre were undertaken to quantify the relationships between the explanatory covariates and the response variables 'IE' or 'dead at day 30'. Time to positivity, sex, age, centre and covariates with $P<0.2$ level in previous analyses were entered into logistic and Poisson multivariate regression models. Manual backward stepwise variable elimination was then performed to determine final multivariate models. We found no interaction between TTP and sex, and between TTP and methicillin-resistance in models. Goodness-of-fit was checked for each model by the Pearson, Hosmer-Lemeshow, and receiver operating characteristic curve tests. $P<0.05$ was considered as statistically significant in two-tailed. Analyses were performed using Stata software (version 12.0, Stata Corporation).

Ethics

VIRSTA study was approved by Sud-Méditerranée IV Protection to Persons Committee, and registered in the European Clinical Trials Database (EUDRACT) under the number 2008-A00680-55.

Results

Patient characteristics

Of the 2,008 patients enrolled in the VIRSTA cohort, data on TTP was prospectively collected in 587 (i.e., those admitted in the four sites where TTP was routinely collected during the study period, with immediate transfer of blood cultures samples to the laboratory for incubation, 24/24 hours, 7/7 days, see flowchart, web-only Supplementary Figure 1). Patients characteristics are summarized in Table 1. As compared to the rest of the VIRSTA cohort, these patients had a lower rate of IE: 42/587 (7.2%), vs 179/1421 (12.6%), $P<0.0001$, web-only Supplementary Table 1. Blood cultures were positive in both aerobic and anaerobic bottles for 423/587 patients (72.1%), only in aerobic bottles for 97/587 (16.5%), and only in anaerobic bottles for 67/587 (11.4%).

Factors associated with short TTP (Figure 1A)

The median TTP was 13.7 (IQR, 9.9-18) hours, with no significant difference in TTP distribution between the two automated blood culture systems. In univariate analyses, factors associated with short TTP (<13.7 h) were renal failure, immunodepression, McCabe score of 4, central venous catheter, IE, stroke, emboli, and MSSA (web-only, Supplementary Table 2). The median TTP was 13 (IQR, 9.5-18) hours for MSSA, and 15 (IQR, 10.6-18) hours for MRSA ($P=0.14$).

Association between TTP and IE (Figure 1B)

The relationship between TTP and IE was not linear, with a U-shape curve (Figure 2). Hence, we used fractional polynomials to describe the relationship between TTP, and IE. In univariate analysis, centre, community-acquired SAB, prosthetic valve, emboli, stroke,

osteitis, C reactive protein, and extreme quartiles of TTP (i.e. quartile 1, and 4), were associated with IE (Table 2). In multivariate analysis, centre, stroke, prosthetic valve, C reactive protein, TTP <10 h (quartile 1, OR 2.84 [1.33-6.10]), and TTP >18 h (quartile 4, OR 3.07 [0.99-9.45]) were independently associated with IE (Table 2). When we restricted the analyzes to the 393 patients who underwent echocardiography (either TTE, or TEE), we found similar findings, with a U-shape curve.

Factors associated with 30-day mortality

Old age, a McCabe score of 4, underlying liver disease, stroke, pneumonia, mitral vegetation, MRSA, and TTP <13.7 h were associated with mortality, whereas surgical site infection was associated with survival (Table 3). In multivariate analysis, TTP <13.7 h (RR 1.69, 95%CI 1.11-2.57), old age, McCabe score of 4, MRSA, stroke, pneumonia, and high C reactive protein were independently associated with 30-day mortality.

Microbiological study of selected strains originating from patients with IE and extreme TTP (quartiles 1, and 4)

Crude doubling time of *S. aureus* strains with short (<10 h, n=8) and long (>18 h, n=5) TTP were not different (Figure 3A). The two groups of isolates (short, and long TTP during the clinical study), had similar TTP in blood culture bottles inoculated with standardized inocula (P=0.9433, Figure 3B). All strains tested were methicillin susceptible, and no specific clonal complex was associated with either short or long TTP (Supplementary Table 2).

Discussion

In this prospective cohort of 587 patients with SAB, TTP of first positive blood culture was independently associated with IE, and 30-day mortality. Indeed, the prevalence of IE was

higher both in patients with short (quartile 1, <10 h), and long (quartile 4, >18 h) TTP, with a U-shape curve. Thirty-day mortality was 74/294 (25.2%) in patients with TTP <13.7 h (median), versus 47/292 (16.0%) in patients with TTP ≥13.7 h (*P*=0.03), and this association persisted after adjustment for other risk factors.

Significant differences were observed between patients enrolled in this ancillary study, and the rest of the VIRSTA cohort. However, our population was in line with most recent studies of SAB in adult patients, in terms of age (mean, 60-70 years), sex ratio (65-75% of males), major comorbidities (predominantly diabetes, cancer, and renal disease, >25% each), and outcome (30 day-mortality, 15-25%) (4,27). In addition, predictors of mortality were similar to those previously described: age, McCabe score, MRSA, pneumonia or C reactive protein (28,29). Of note, the automated blood culture systems in use during the study period, BACTEC®, and BacT/Alert®, are by far the most commonly used worldwide, and TTP distribution (median, 13.7 hours; IQR, 9.9-18) is in line with previous studies of TTP in SAB (15,16). This suggests that our findings may apply to most settings where automated blood culture systems are in use.

The relationship between TTP and IE is poorly documented. In a cohort of 312 patients with SAB (18), TTP was shorter for IE (12.1±4.0 h, *P*=0.002), and catheter-related infections (15.1±5.3 h, *P*=0.004), and longer for bone (20.6±9.5 h, *P*=0.04), and respiratory tract infections (24.8±15.3 h, *P*=0.001). In our study, extreme quartiles (TTP ≤10 h, and TTP >18 h), were independently associated with higher risk of IE. We speculated that this biphasic behavior could reflect two unrelated biological phenomenon: i) SAB with high inoculum (hence, short TTP), could be at higher risk of IE; ii) once IE has developed, bacteria enter in a slow metabolic state within vegetations (30,31), which may be responsible for longer TTP when these bacteria seed the circulation and are inoculated in blood culture bottles. However, doubling time of isolates from IE patients with shortest, or longest TTP, were not different *in*

vitro, and blood culture bottles inoculated with a standardized quantity of *S. aureus* from these two groups had similar TTP. This suggests that differences in TTP reflect differences in the quantity of bacteria inoculated in blood culture bottles – and thus differences in bacterial concentration in circulating patient blood, rather than intrinsic characteristics of the isolates. This U-shape curve may also have reflected two pathophysiological models for IE: cases with shorter TTP, and thus high bacterial load, corresponding to rapidly progressive IE caused by virulent strains, whilst extended TTP could result from less virulent bacteria and/or better control by the host. However, IE cases with shortest (quartile 1), and longest (quartile 4) TTP were similar in terms of duration of symptoms before diagnosis, clinical presentation, and immunosuppression (data not shown). To our knowledge, increased risk of IE in patients with long TTP has never been reported. Hence, confirmatory studies on the association between TTP and IE are required.

We conducted a medline search to identify studies that investigated TTP in patients with SAB. We found 6 studies (15–18,32,33). TTP ≤12 h has been associated with increased in-hospital mortality (25.0%, versus 4.3% if TTP>12 h, *P*=0.006) (32), and increased 30-day mortality (OR 5.91, CI95% 1.80-19.44; *P*=0.003) (15), in two distinct cohorts of SAB. TTP ≤14 h was an independent predictor of SAB-related mortality in another study (18). Two recent studies did not confirm this association: a retrospective analysis of 684 adult and pediatric SAB (MSSA and MRSA), found that TTP >48 h was associated with higher 30-day mortality as compared to TTP <12 h (33). However, children and adults have different bacterial load during bacteremia (34), so that the merging of pediatric and adult population may not be appropriate. TTP was not associated with mortality in another cohort of 87 SAB (17). Interestingly, this latter study and another found that a TTP ratio of <1.5 between the first and the second positive blood culture in patients on appropriate antibacterial treatment was an independent risk factor for new infectious focus, and death (17,34). Accordingly,

failure to increase TTP by at least 50% in sequential blood cultures should prompt reinforcement of anti-staphylococcal regimen, and/or extraction of foreign devices or surgical intervention when appropriate.

Our study has limitations: only 587 patients of the VIRSTA cohort could be enrolled, with significant differences with the rest of the cohort. Among them, only 393 (67.0%) underwent echocardiography, of whom 42 fulfilled criteria for definite IE. Hence, analyses of risk factors for IE were limited by small sample size. Secondly, two different blood culture instruments were used during the study period. Finally, we did not monitor the volume of blood inoculated into blood culture bottles, although this parameter has a strong impact on pathogen detection, and TTP (24). Of note, short TTP implies that physicians in charge were informed earlier that their patients had SAB. Theoretically, this opportunity for earlier appropriate treatment may underestimate the prognostic value of short TTP. Strengths of our study include its design (prospective, multicenter, observational, with consecutive enrolment of all adult patients with SAB), which reduces the risk of selection bias. Many variables were prospectively collected, with a low rate of missing data, which enabled to consider a large range of potential confounding factors. To our knowledge, this is the largest study on TTP in SAB to date. Lastly, echocardiography was performed in 393/587 (67.0%) of SAB cases. Although a 100% rate would be preferrable, 67.0% is, to our knowledge, the highest rate of echocardiography across all observational SAB studies currently available.

In conclusion, results of our study advocate for the development of clinical use of TTP in the management of patients with SAB. Those with short TTP should be monitored more closely, and treated more aggressively, as they belong to a group at higher risk of early death. Echocardiography has a higher yield in this group.

Transparency declaration. All authors: No reported conflict of interest. The study was founded by the French Ministry of Health (Programme Hospitalier de Recherche Clinique 2008). French national network on IE/ AEPEI which participated in the study is supported by the Institut National de la Santé et de la Recherche Médicale (Inserm).

The VIRSTA/AEPEI Study Group:

Clinical centres: Besançon: Catherine Chirouze, Elodie Curlier, Cécile Descottes-Genon, Bruno Hoen, Isabelle Patry, Lucie Vettoretti. **Dijon:** Pascal Chavanet, Jean-Christophe Eicher, Sandrine Gohier-Treuvelot, Marie-Christine Greusard, Catherine Neuwirth, André Péchinot, Lionel Piroth. **Lyon:** Marie Célard, Catherine Cornu, François Delahaye, Malika Hadid, Pascale Rausch. **Montpellier:** Audrey Coma, Florence Galtier, Philippe Géraud, Hélène Jean-Pierre, Vincent Le Moing, Catherine Sportouch, Jacques Reynes. **Nancy:** Nejla Aissa, Thanh Doco-Lecompte, François Goehringer, Nathalie Keil, Lorraine Letranchant, Hepher Malela, Thierry May, Christine Selton-Suty. **Nîmes:** Nathalie Bedos, Jean-Philippe Lavigne, Catherine Lechiche, Albert Sotto. **Paris:** Xavier Duval, Emila Ilic Habensus, Bernard Iung, Catherine Leport, Pascale Longuet, Raymond Ruimy. **Rennes:** Eric Bellissant, Pierre-Yves Donnio, Fabienne Le Gac, Christian Michelet, Matthieu Revest, Pierre Tattevin, Elise Thebault.

Coordination and statistical analyses: François Alla, Pierre Braquet, Marie-Line Erpelding, Laetitia Minary, Sarah Tubiana.

Centre National de Référence des staphylocoques: Michèle Bès, Jérôme Etienne, Taissia Lelekov-Boissard, Anne Tristan, François Vandenesch.

Erasmus University Rotterdam: Alex Van Belkum, Fernando Rivadeneira, Willem Vanwamel.

Sponsor CHU de Montpellier: Sandrine Barbas, Christine Delonca, Virginie Sussmuth, Anne Verchère.

305 **Table 1. Study population (n=587)**

Variables	
Demographic characteristics	
Age (y)	65.3 ± 16.3
Male	420 (71.6)
Centre	
Besançon	106 (18.1)
Lyon	301 (51.3)
Nîmes	40 (6.8)
Paris-Bichat	140 (23.8)
Co-morbid conditions	
Renal failure	153 (26.1)
Diabetes mellitus	184 (31.4)
Heart failure	120 (20.4)
Intravenous drug use	17 (2.9)
Solid cancer	174 (29.6)
Immunocompromised status	123 (21.0)
Chronic liver disease	107 (18.2)
McCabe score (n=584)	
1	58 (9.9)
2	232 (39.7)
3	200 (34.2)
4	94 (16.1)
Presumed place of acquisition (n=563)	
Community-acquired	166 (29.5)
Healthcare-associated	93 (16.5)
Nosocomial	304 (54.0)
Site of infection	
Infective endocarditis	42 (7.2)
Osteitis (n=555)	62 (11.2)
Arthritis (n=554)	63 (11.4)
Pneumonia (n=540)	73 (13.5)
Skin and skin structure infection (n=555)	186 (33.5)
Urinary tract infection (n=554)	80 (14.4)
Catheter related-infection (n=565)	173 (30.6)
Devices	
Pacemaker	39 (6.6)
Prosthetic valve	38 (6.5)
Vascular grafts	43 (7.3)

Variables	
Prosthetic joint	73 (12.4)
Clinical features	
Fever	484 (82.4)
Heart valve disease (n=525)	125 (23.8)
Stroke (n=554)	18 (3.2)
Embolus	15 (2.6)
Intensive care unit	165 (28.1)
All-cause death	156 (26.6)
All-cause death at day 30	121 (20.6)
Biological parameters	
C reactive protein (mg/L) (n=534)	182 (104-284)
Time to positivity (hours)	13.7 (9.9-18)
MRSA (n=585)	112 (19.2)
Antibiotic therapy before first blood culture (n=577)	38 (6.5)
Echocardiographic data	
TTE	364 (62.0)
TEE	154 (26.2)
TTE and/or TEE	393 (67.0)
Vegetation (n=393)	31 (7.9)

Categorical variables are presented as numbers (%). Non-normally distributed quantitative variables are reported as median (quartile 1 - quartile 3). Normally distributed quantitative variables are presented as mean ± standard deviation. MRSA: Methicillin-resistant *Staphylococcus aureus*; TTE: transthoracic echocardiography; TEE: transesophageal echocardiography; Immunocompromised status: HIV, hematologic malignancy, immunosuppressive drugs

306

307 **Table 2. Risk factors for infective endocarditis in patients with *S. aureus* bacteraemia**

Variables	Definite IE (n=42)	Others (n=545)	Univariate analysis		Multivariate analysis (n=477)	
			OR (95% CI)	P	OR (95% CI)	P
Demographic characteristics						
Age, per year	64 (55-74)	67 (56-78)	0.98 (0.96–1.01)	0.10	0.99 (0.96–1.01)	0.26
Female	11 (26.2)	156 (28.6)	1.22 (0.82–1.84)	0.61	0.49 (0.18–1.29)	0.15
Centre				0.003		0.17
Lyon	14 (4.7)	287 (95.3)	1		1	
Besançon	4 (3.8)	102 (96.2)	0.81 (0.26–2.52)		0.54 (0.12–2.27)	
Nîmes	6 (15.0)	34 (85.0)	4.13 (1.46–11.68)		3.27 (0.81–13.23)	
Paris-Bichat	18 (12.9)	122 (87.1)	2.96 (1.42 – 6.15)		1.97 (0.69–5.67)	
Co-morbid conditions						
Diabete mellitus	11 (26.2)	173 (31.7)	0.84 (0.40–1.75)	0.64		
Renal failure	8 (19.0)	145 (26.6)	0.67 (0.30–1.51)	0.33		
Solid cancer	7 (16.7)	167 (30.6)	0.48 (0.20–1.12)	0.09		
Intravenous drug use	4 (9.5)	13 (2.4)	2.48 (0.72–8.52)	0.15		
Presumed place of acquisition (n=563)				0.01		0.02
Nosocomial	16 (38.1)	288 (52.8)	1		1	
Healthcare-associated	4 (9.5)	89 (16.3)	0.71 (0.23–2.21)		0.99 (0.26–3.77)	
Community	22 (52.4)	144 (26.4)	2.50 (1.24–5.04)		3.33 (1.31–8.48)	
Device						
Pacemaker	4 (6.4)	35 (9.5)	1.61 (0.50–5.14)	0.42		
Prosthetic valve	10 (23.8)	28 (5.1)	5.3 (2.25–12.49)	0.0001	7.39 (2.28–23.96)	0.001
Clinical features						
Fever	89 (73.6)	395 (84.8)	1.84 (0.62–5.46)	0.27		
Heart valve disease (n=525)	18 (43.9)	107 (22.1)	5.17 (2.43–11.03)	0.0001	4.24 (1.69–10.64)	0.002
Embolus	8 (19.0)	7 (1.3)	12.4 (3.77–40.9)	0.0001	5.50 (1.16–26.22)	0.03
Stroke (n=554)	9 (22.0)	9 (1.8)	11 (3.87–31.28)	0.0001	6.51 (1.56–27.09)	0.01
Osteitis	9 (22.0)	53 (10.3)	2.44 [1.06–5.60]	0.04		
All-cause death (n=586)	14 (33.3)	142 (26.1)	1.62 (0.80–3.27)	0.18		
Biological parameters						
TTP of first positive blood culture (h)				0.003		0.003
1.28–9.9	17 (40.5)	131 (24.0)	2.28 (1.23–3.97)		2.53 (1.22–5.25)	
10.0–13.7	11 (26.2)	136 (25.0)	1.23 (1.04–1.45)		1.22 (1.01–1.46)	
13.8–18.0	6 (14.3)	140 (25.7)	1		1	

Variables	Definite IE (n=42)	Others (n=545)	Univariate analysis		Multivariate analysis (n=477)	
			OR (95% CI)	P	OR (95% CI)	P
18.1–131.0	8 (19.0)	138 (25.3)	2.16 (0.81–5.74)		3.91 (1.26–12.11)	
MRSA (n=585)	3 (7.1)	109 (20.1)	0.30 (0.09–1.02)	0.05		
CRP (mg/L), per unit (n=534)	256 (150-302)	180 (102-282)	1.003 (1.00–1.01)	0.02		
Morphological data						
Echocardiography (TTE and/or TEE)	41 (97.6)	352 (64.6)	17.4 (2.32–130.34)	0.005	11.07 (1.28–95.28)	0.03

Categorical variables are presented as numbers (%). Non-normally distributed quantitative variables are reported as median (quartile 1 - quartile 3). Normally distributed quantitative variables are presented as mean ± standard deviation.

IE: infective endocarditis; OR: odds ratio; CI: confidence interval; TTP: time to positivity; MRSA: Methicillin resistant *Staphylococcus aureus*; CRP: C reactive protein.

308

309

310 **Table 3. Risk factors for 30-day mortality in patients with *S. aureus* bacteraemia**

Variables	**30-day mortality (n=121)**	**Survived (n=466)**	**Univariate analysis**		**Multivariate analysis (n=529)**	
			RR (95% CI)	*P*	RR (95% CI)	*P*
Demographic characteristics						
Age (y) per year	70.2 ± 14.8	63.6 ± 16.5	1.02 (1.01–1.04)	0.0001	1.02 (1.01–1.03)	0.02
Female	37 (36.3)	111 (29.3)	1.22 (0.82–1.84)	0.36	1.11 (0.72–2.68)	0.64
Centre				0.45		0.53
Lyon	61 (20.3)	240 (79.7)	1		1	
Besançon	19 (17.9)	87 (82.1)	0.89 (0.53–1.49)		0.92 (0.52–1.63)	
Nîmes	7 (17.5)	33 (82.5)	0.74 (0.34–1.62)		0.73 (0.33–1.65)	
Paris-Bichat	34 (24.3)	106 (75.7)	1.26 (0.83–1.92)		1.29 (0.79–2.11)	
Co-morbid conditions						
Diabete mellitus	41 (33.9)	143 (30.7)	0.99 (0.67–1.44)	0.94		
Renal failure	38 (31.4)	115 (24.7)	1.15 (0.78–1.70)	0.48		
Chronic liver disease	29 (24.0)	78 (16.7)	1.68 (1.09–2.60)	0.02		
Solid cancer	37 (30.6)	137 (29.4)	1.02 (0.69–1.50)	0.93		
McCabe score (n=584)						
1	8 (6.7)	50 (10.8)	1	0.0009		0.0009
2	36 (30.0)	196 (42.2)	0.69 (0.31–1.52)		0.83 (0.37–1.88)	
3	40 (33.3)	160 (34.5)	0.94 (0.43–2.08)		1.15 (0.52–2.54)	
4	36 (30.0)	58 (12.5)	1.93 (0.88–4.22)		2.34 (1.04–5.28)	
Clinical features						
Fever	89 (73.6)	395 (84.8)	0.67 (0.44–1.01)	0.06		
Stroke (n=554)	10 (9.1)	8 (1.8)	3.13 (1.60–6.14)	0.001	2.85 (1.36–5.96)	0.005
Embolus	3 (2.5)	12 (2.6)	0.98 (0.30–3.20)	0.97		
Mitral vegetation	4 (6.6)	6 (1.8)	2.77 [0.99-7.75]	0.05		
Source of infection						

Variables	**30-day mortality (n=121)**	**Survived (n=466)**	**Univariate analysis**		**Multivariate analysis (n=529)**	
			RR (95% CI)	*P*	RR (95% CI)	*P*
Endocarditis	12 (9.9)	30 (6.4)	1.52 (0.83–2.78)	0.18		
Osteitis (n=555)	10 (9.0)	52 (11.7)	0.83 (0.43–1.60)	0.58		
Arthritis (n=554)	7 (6.4)	56 (12.6)	0.53 (0.25–1.15)	0.11		
SSSI (n=555)	30 (27.0)	156 (35.1)	0.73 (0.48–1.11)	0.14		
Pneumonia (n=540)	26 (23.6)	47 (10.9)	2.05 (1.31–3.2)	0.002	1.69 (1.01–2.85)	0.04
Surgical site	5 (4.1)	66 (14.2)	0.32 [0.13–0.78]	0.01		
Biological parameters						
TTP of first positive blood culture (h)				0.03		0.02
> 13.7	47 (38.8)	245 (52.6)	1		1	
≤ 13.7	74 (61.2)	221 (47.4)	1.51 (1.05–2.18)		1.69 (1.11–2.57)	
MRSA (n=585)	36 (30.0)	76 (16.3)	1.25 (1.02–1.53)	0.03	1.92 (1.20–3.07)	0.006
CRP (mg/L)				0.004		0.02
3 – 104	16 (12.1)	116 (87.9)	1		1	
105 – 182	21 (15.4)	115 (84.6)	1.18 (0.61–2.27)		1.20 (0.60–2.39)	
183 – 284	34 (25.6)	99 (74.4)	2.02 (1.11–3.68)		1.93 (1.02–3.65)	
285 – 927	31 (23.3)	102 (76.7)	1.87 (1.01–3.44)		1.92 (1.00–3.71)	
No data (n=53)	19 (35.8)	34 (64.2)	3.21 (1.63–6.32)		2.99 (1.42–6.30)	
Therapeutic parameters						
Antibiotic before blood culture (n=577)	8 (6.9)	30 (6.5)	1.03 (0.50–2.13)	0.94		
Appropriate empirical antibiotic	96 (79.3)	359 (77.0)	1.08 (0.69–1.69)	0.73		

Variables	30-day mortality (n=121)	Survived (n=466)	Univariate analysis		Multivariate analysis (n=529)	
			RR (95% CI)	*P*	RR (95% CI)	*P*

Categorical variables are presented as numbers (%). Non-normally distributed quantitative variables are reported as median (quartile 1 - quartile 3). Normally distributed quantitative variables are presented as mean ± standard deviation. RR: relative risk; CI: confidence interval; SSSI: Skin and skin structure infection; TTP: time to positivity; MRSA: Methicillin resistant *Staphylococcus aureus*; CRP: C reactive protein

311

312

313 **Figure Legends**

314 **Figure 1. Association between time to blood culture positivity, and i) infective**
315 **endocarditis (Figure 1A), and ii) 30 day-mortality (Figure 1B), in patients with**
316 ***Staphylococcus aureus* bacteraemia.**

317

318 **Figure 2. Association between time to blood culture positivity and infective endocarditis**
319 **in patients with *Staphylococcus aureus* bacteraemia: Second-degree fractional**
320 **polynomial adjusted for covariates.**

321 X axis : Time to blood culture positivity in hours adjusted for covariates

322 Y axis : Partial predictor + residual of infective endocarditis

323

324 **Figure 3. *In vitro* study of *Staphylococcus aureus* isolates originating from patients with**
325 **infective endocarditis.**

326 Strains originating from patients with short (first quartile), and long (fourth quartile) time-to-
327 positivity (TTP), referred to as 'early', and 'late' strains, were tested.

328 **Figure 3A.** Doubling time calculated from the exponential growth phase data of triplicate
329 cultures in BHI

330 **Figure 3B.** TTP in artificially inoculated aerobic and anaerobic blood cultures filled with
331 human blood, with standardized inoculum.

332

333 **Supplementary Figure 1**: flow chart

334 **Supplementary Table 1. Comparison of patients enrolled in this study, and the rest of**
335 **the VIRSTA cohort**

Variables	Study population (n=587)	Non-included patients (n=1421)	*P*-value
Demographic characteristics			
Age (years)	65.3 ± 16,3	65.0 ± 17.5	0.76
Male	420 (71.6)	875 (61.6)	0.0001
Co-morbid conditions			
Renal failure	153 (26.1)	408 (28.7)	0.23
Diabetes mellitus	184 (31.4)	383 (27.0)	0.05
Heart failure	120 (20.4)	400 (28.2)	0.0001
Intravenous drug use	17 (2.9)	46 (3.2)	0.69
Solid cancer	174 (29.6)	419 (29.5)	0.95
Immunocompromised status	123 (21.0)	229 (21.1)	0.50
Chronic liver disease	107 (18.2)	171 (12.0)	<0.0001
McCabe score			0.02
1	58 (9.9)	113 (8.0)	
2	232 (39.7)	483 (34.0)	
3	200 (34.2)	550 (38.7)	
4	94 (16.1)	274 (19.3)	
Presumed place of acquisition			
Community-acquired	166 (28.3)	355 (25.0)	0.13
Healthcare-associated	93 (15.8)	260 (18.3)	0.19
Nosocomial	304 (51.8)	771 (54.3)	0.31
Site of infection			
Infective endocarditis	42 (7.2)	179 (12.6)	<0.0001
Osteitis	62 (11.2)	149 (10.5)	0.67
Arthritis	63 (11.4)	104 (7.3)	4.10^{-3}
Pneumonia	73 (13.5)	141 (10.0)	0.02
Skin and skin structure infection	186 (33.5)	394 (27.8)	0.01

Variables	Study population (n=587)	Non-included patients (n=1421)	*P*-value
Urinary tract infection	80 (14.4)	132 (9.4)	0.001
Catheter related-infection	173 (30.6)	315 (22.3)	<0.0001
Device			
Pacemaker	39 (6.8)	105 (7.3)	0.71
Prosthetic valve	19 (3.2)	53 (3.7)	0.59
Vascular grafts	42 (7.4)	114 (7.9)	0.66
Prosthetic joint	70 (12.3)	214 (14.9)	0.69
Clinical features			
Fever	472 (82.7)	1302 (90.7)	<0.0001
Heart valve disease	125 (21.3)	240 (16.9)	0.02
Stroke	18 (3.2)	57 (4.0)	0.42
Embolus	15 (2.6)	48 (3.4)	0.34
Intensive care unit	165 (28.1)	449 (31.6)	0.25
All-cause death at day 30	121 (20.6)	291 (20.5)	0.95
Biological parameters			
C reactive protein (mg/L)	182 (104-284)	192 (115-280)	0.29
Time to positivity (hours)	13.7 (9.9 - 18)	-	-
MRSA	112 (19.2)	268 (18.9)	0.90

Categorical variables are presented as numbers (%). Non-normally distributed quantitative variables are reported as median (quartile 1 - quartile 3). Normally distributed quantitative variables are presented as mean ± standard deviation.

MRSA: Methicillin-resistant *Staphylococcus aureus.* Immunocompromised status: HIV, hematologic malignancy, immunosuppressive drugs

339 **Supplementary Table 2. Factors associated with time to blood cultures positivity (TTP) in**
340 ***Staphylococcus aureus* bacteraemia in univariate analysis**

Variables	TTP ≤ 13.7 h (n=295)	TTP > 13.7 h (n=292)	*P*-value
Demographic characteristics			
Age (years)	66.0 ± 15.7	64.6 ± 16.9	0.29
Male	205 (69.5)	215 (73.6)	0.27
Centre			0.70
Besançon	58 (19.7)	48 (16.4)	
Lyon	146 (49.5)	155 (53.1)	
Nîmes	19 (6.4)	21 (7.2)	
Paris-Bichat	72 (24.4)	68 (23.3)	
Co-morbid conditions			
Chronic liver desease	57 (19.3)	50 (17.1)	0.49
Diabetes mellitus	92 (31.2)	92 (31.5)	0.93
Renal failure	89 (30.5)	64 (22.4)	0.02
Intravenous drug use	11 (3.7)	6 (2.0)	0.23
Solid cancer	95 (32.2)	79 (27.0)	0.17
Immunodepression	78 (26.4)	45 (15.4)	0.001
McCabe score (n=584)			0.009
1	23 (7.8)	35 (12.1)	
2	104 (35.4)	128 (44.1)	
3	118 (40.1)	82 (28.3)	
4	49 (16.7)	45 (15.5)	
Device			
Central venous catheter (n=547)	30 (10.9)	8 (2.9)	0.0001
Vascular grafts	22 (7.4)	21 (7.2)	0.90
Prosthetic joint	44 (14.9)	29 (9.9)	0.07

Clinical features			
Fever	255 (86.4)	229 (78.4)	0.01
Heart valve disease (n=525)	63 (24.7)	62 (23.0)	0.64
Stroke (n=554)	17 (6.0)	1 (0.4)	0.0001
Emboli (n=551)	17 (6.1)	6 (0.7)	0.02
Site of infection			
Infective endocarditis	28 (9.5)	14 (4.8)	0.03
Osteitis (n=555)	31 (11.0)	31 (11.4)	0.87
Pneumonia (n=540)	30 (10.9)	43 (16.2)	0.08
Catheter related-infection (n=565)	109 (37.8)	64 (23.1)	0.0001
Biological parameters			
CRP (mg/L) (n=534)	187 (110-283)	179 (101-288)	0.66
MRSA (n=585)	42 (14.3)	70 (24.0)	0.003
Treatment data			
Antibiotics before first blood culture (n=577)	17 (5.9)	21 (7.3)	0.48
Morphological parameters			
Vegetations (n=393)	18 (9.1)	13 (6.7)	0.37

341 Categorical variables are presented as numbers (%). Non-normally distributed quantitative variables
342 are reported as median (quartile 1 - quartile 3). Normally distributed quantitative variables are
343 presented as mean ± standard deviation. CRP, C Reactive Protein.

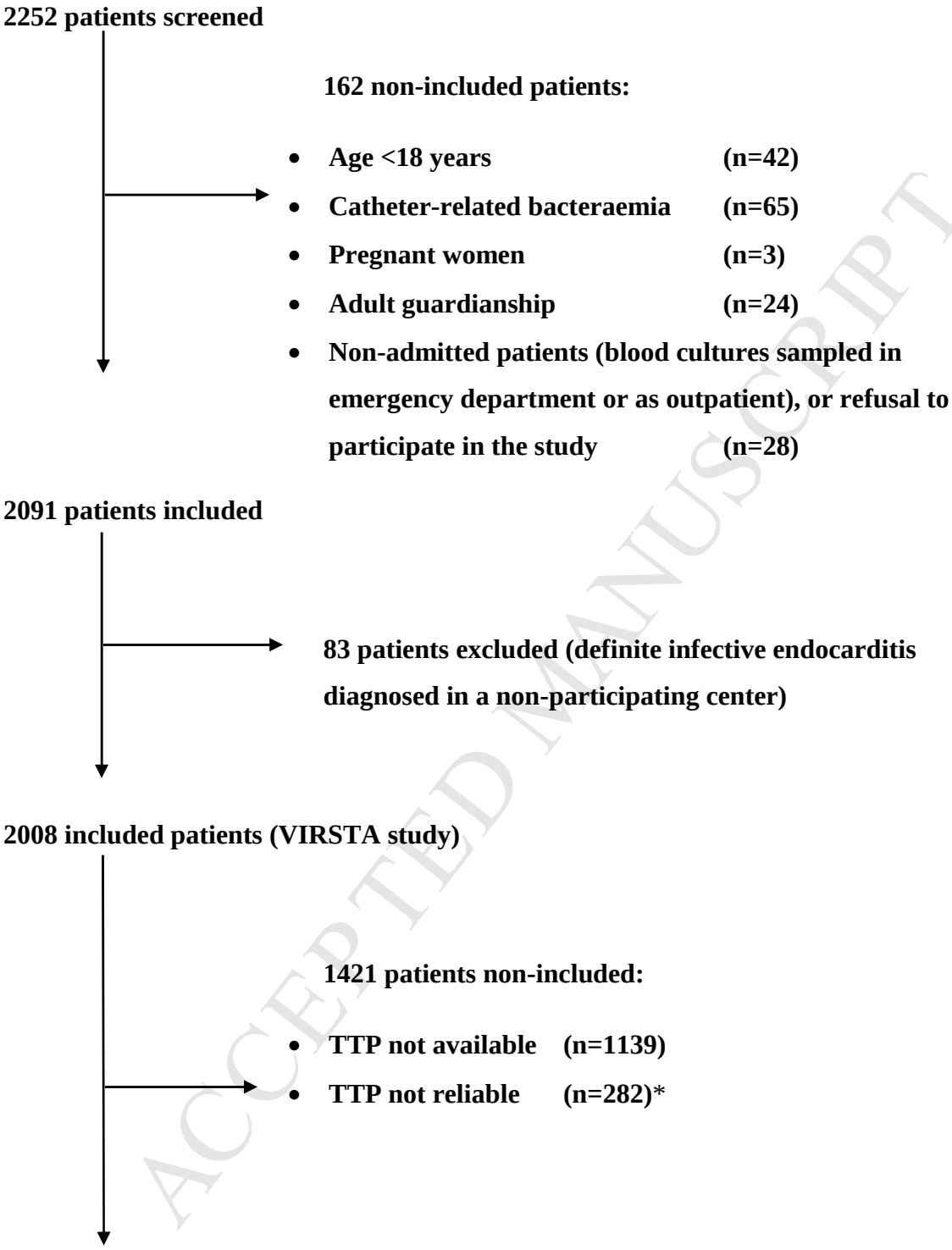
344

Supplementary Table 3. Clonal complex of the 13 isolates sampled from patients with infective endocarditis, and short (<10 h), or long (>18 h) time-to-positivity (TTP) of first blood culture

CC-MLST	TTP <10 h	TTP > 18 h
CC5	2	3
CC88	1	0
CC45	3	1
CC15	1	0
CC101	1	0
CC121	0	1
TOTAL	8	5

Note. All isolates were meticillin-susceptible *Staphylococcus aureus*

Supplementary Figure 1. Flow chart



* Time of incubation start not reliably collected

350

REFERENCES

1. Lyytikainen O, Ruotsalainen E, Jarvinen A, Valtonen V, Ruutu P. Trends and outcome of nosocomial and community-acquired bloodstream infections due to *Staphylococcus aureus* in Finland, 1995-2001. Eur J Clin Microbiol Infect Dis 2005;24(6):399–404.

2. Collignon P, Nimmo GR, Gottlieb T, Gosbell IB. *Staphylococcus aureus* bacteremia, Australia. Emerg Infect Dis 2005;11(4):554–61.

3. Braquet P, Alla F, Cornu C, Goehringer F, Piroth L, Chirouze C, et al. Factors associated with 12 week case-fatality in *Staphylococcus aureus* bacteraemia: a prospective cohort study. Clin Microbiol Infect 2016;22(11):948.e1-948.e7.

4. Tom S, Galbraith JC, Valiquette L, Jacobsson G, Collignon P, Schonheyder HC, et al. Case fatality ratio and mortality rate trends of community-onset *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect 2014;20(10):O630-632.

5. Madsen KM, Schonheyder HC, Kristensen B, Sorensen HT. Secular trends in incidence and mortality of bacteraemia in a Danish county. APMIS Acta Pathol Microbiol Immunol Scand 1999;107(3):346–52.

6. Mylotte JM, Tayara A. *Staphylococcus aureus* bacteremia: predictors of 30-day mortality in a large cohort. Clin Infect Dis 2000;31(5):1170–4.

7. Laupland KB, Church DL, Mucenski M, Sutherland LR, Davies HD. Population-based study of the epidemiology of and the risk factors for invasive *Staphylococcus aureus* infections. J Infect Dis 2003;187(9):1452–9.

8. Cresti A, Chiavarelli M, Scalese M, Nencioni C, Valentini S, Guerrini F, et al. Epidemiological and mortality trends in infective endocarditis, a 17-year population-based prospective study. Cardiovasc Diagn Ther 2017;7(1):27–35.

9. Selton-Suty C, Celard M, Le Moing V, Doco-Lecompte T, Chirouze C, Iung B, et al. Preeminence of *Staphylococcus aureus* in infective endocarditis: a 1-year population-based survey. Clin Infect Dis 2012;54(9):1230–9.

10. Chang F-Y, MacDonald BB, Peacock JEJ, Musher DM, Triplett P, Mylotte JM, et al. A prospective multicenter study of *Staphylococcus aureus* bacteremia: incidence of endocarditis, risk factors for mortality, and clinical impact of methicillin resistance. Medicine (Baltimore) 2003;82(5):322–32.

11. Toyoda N, Chikwe J, Itagaki S, Gelijns AC, Adams DH, Egorova NN. Trends in Infective Endocarditis in California and New York State, 1998-2013. JAMA 2017;317(16):1652–60.

12. 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 2012;44(10):721–5.

13. Catton JA, Dobbins BM, Kite P, Wood JM, Eastwood K, Sugden S, et al. In situ diagnosis of intravascular catheter-related bloodstream infection: a comparison of quantitative

culture, differential time to positivity, and endoluminal brushing. Crit Care Med 2005;33(4):787–91.

14. Raad I, Hanna HA, Alakech B, Chatzinikolaou I, Johnson MM, Tarrand J. Differential time to positivity: a useful method for diagnosing catheter-related bloodstream infections. Ann Intern Med 2004;140(1):18–25.

15. Sowden D, Anstey C, Faddy M. Blood culture time to positivity as a predictor of mortality in community acquired methicillin-susceptible *Staphylococcus aureus* bacteremia. J Infect. 2008;56(4):295–6.

16. Kim J, Gregson DB, Ross T, Laupland KB. Time to blood culture positivity in *Staphylococcus aureus* bacteremia: association with 30-day mortality. J Infect 2010;61(3):197–204.

17. Hsu M-S, Huang Y-T, Hsu H-S, Liao C-H. Sequential time to positivity of blood cultures can be a predictor of prognosis of patients with persistent *Staphylococcus aureus* bacteraemia. Clin Microbiol Infect 2014 ;20(9):892–8.

18. Khatib R, Riederer K, Saeed S, Johnson LB, Fakih MG, Sharma M, et al. Time to positivity in *Staphylococcus aureus* bacteremia: possible correlation with the source and outcome of infection. Clin Infect Dis 2005;41(5):594–8.

19. Le Moing V, Alla F, Doco-Lecompte T, Delahaye F, Piroth L, Chirouze C, et al. *Staphylococcus aureus* Bloodstream Infection and Endocarditis--A Prospective Cohort Study. PloS One 2015;10(5):e0127385.

20. Tubiana S, Duval X, Alla F, Selton-Suty C, Tattevin P, Delahaye F, et al. The VIRSTA score, a prediction score to estimate risk of infective endocarditis and determine priority for echocardiography in patients with *Staphylococcus aureus* bacteremia. J Infect 2016;72(5):544–53.

21. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VGJ, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis 2000;30(4):633–8.

22. Friedman ND, Kaye KS, Stout JE, McGarry SA, Trivette SL, Briggs JP, et al. Health care--associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med 2002;137(10):791–7.

23. McCabe WR and Jackson JJ. Evidently the prognosis for some has changed. Arch Intern Med 1962;110:847–91.

24. Lamy B, Dargere S, Arendrup MC, Parienti J-J, Tattevin P. How to Optimize the Use of Blood Cultures for the Diagnosis of Bloodstream Infections? A State-of-the Art. Front Microbiol 2016;7:697.

25. Monecke S, Jatzwauk L, Weber S, Slickers P, Ehricht R. DNA microarray-based genotyping of methicillin-resistant *Staphylococcus aureus* strains from Eastern Saxony. Clin Microbiol Infect 2008;14(6):534–45.

26. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol 1999;28(5):964–74.

27. Bassetti M, Peghin M, Trecarichi EM, Carnelutti A, Righi E, Del Giacomo P, et al. Characteristics of *Staphylococcus aureus* Bacteraemia and Predictors of Early and Late Mortality. PloS One 2017;12(2):e0170236.

28. van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in *Staphylococcus aureus* Bacteremia. Clin Microbiol Rev 2012;25(2):362–86.

29. Molkanen T, Ruotsalainen E, Rintala EM, Jarvinen A. Predictive Value of C-Reactive Protein (CRP) in Identifying Fatal Outcome and Deep Infections in *Staphylococcus aureus* Bacteremia. PloS One 2016;11(5):e0155644.

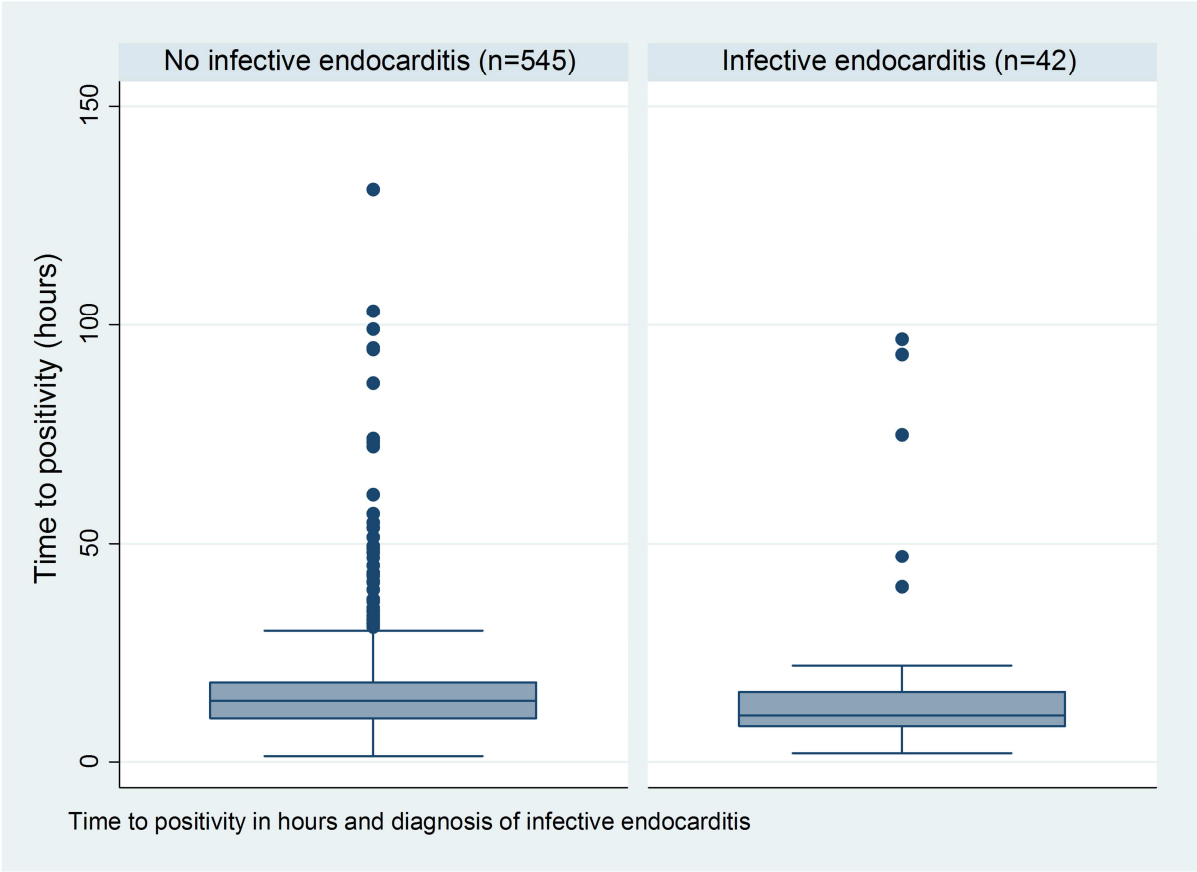
30. Durack DT, Beeson PB. Experimental bacterial endocarditis. II. Survival of a bacteria in endocardial vegetations. Br J Exp Pathol 1972;53(1):50–3.

31. Frehel C, Hellio R, Cremieux AC, Contrepois A, Bouvet A. Nutritionally variant streptococci develop ultrastructural abnormalities during experimental endocarditis. Microb Pathog 1988;4(4):247–55.

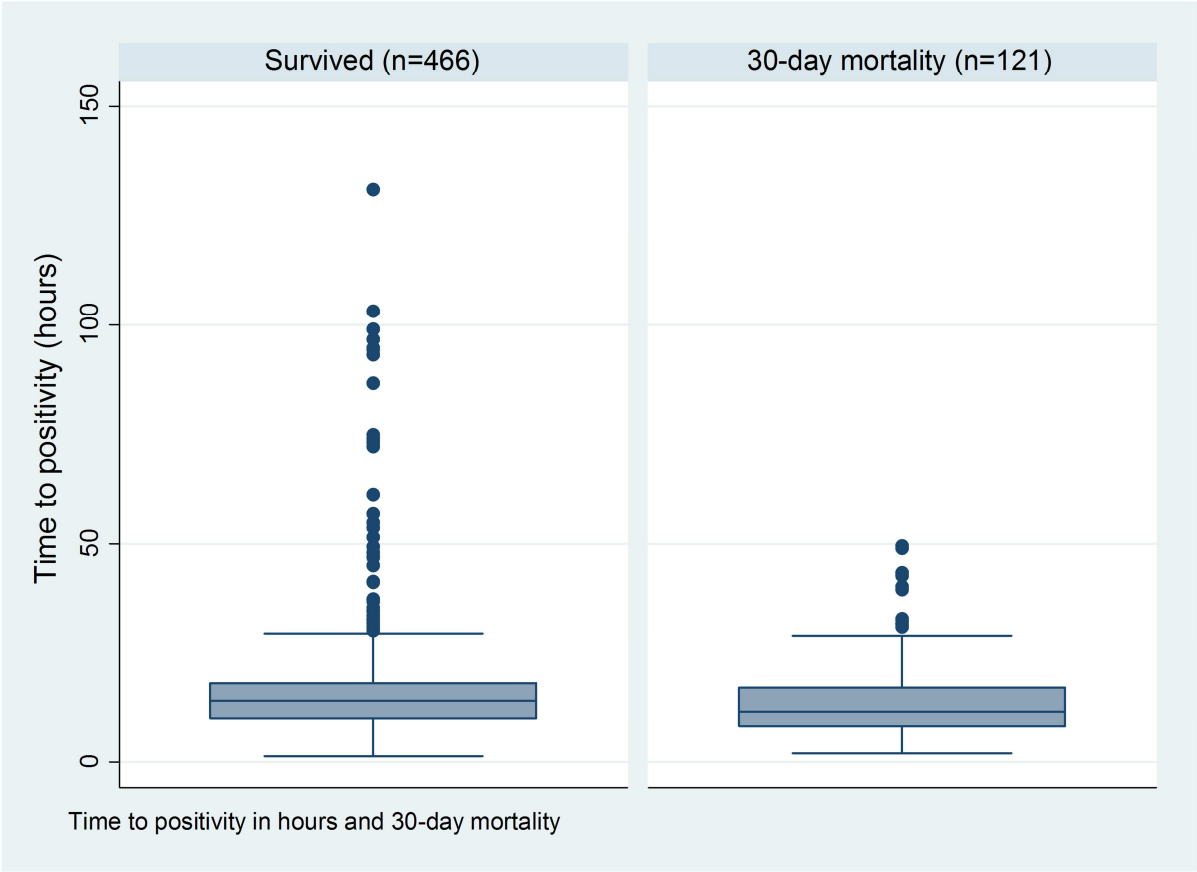
32. Marra AR, Edmond MB, Forbes BA, Wenzel RP, Bearman GML. Time to blood culture positivity as a predictor of clinical outcome of *Staphylococcus aureus* bloodstream infection. J Clin Microbiol 2006;44(4):1342–6.

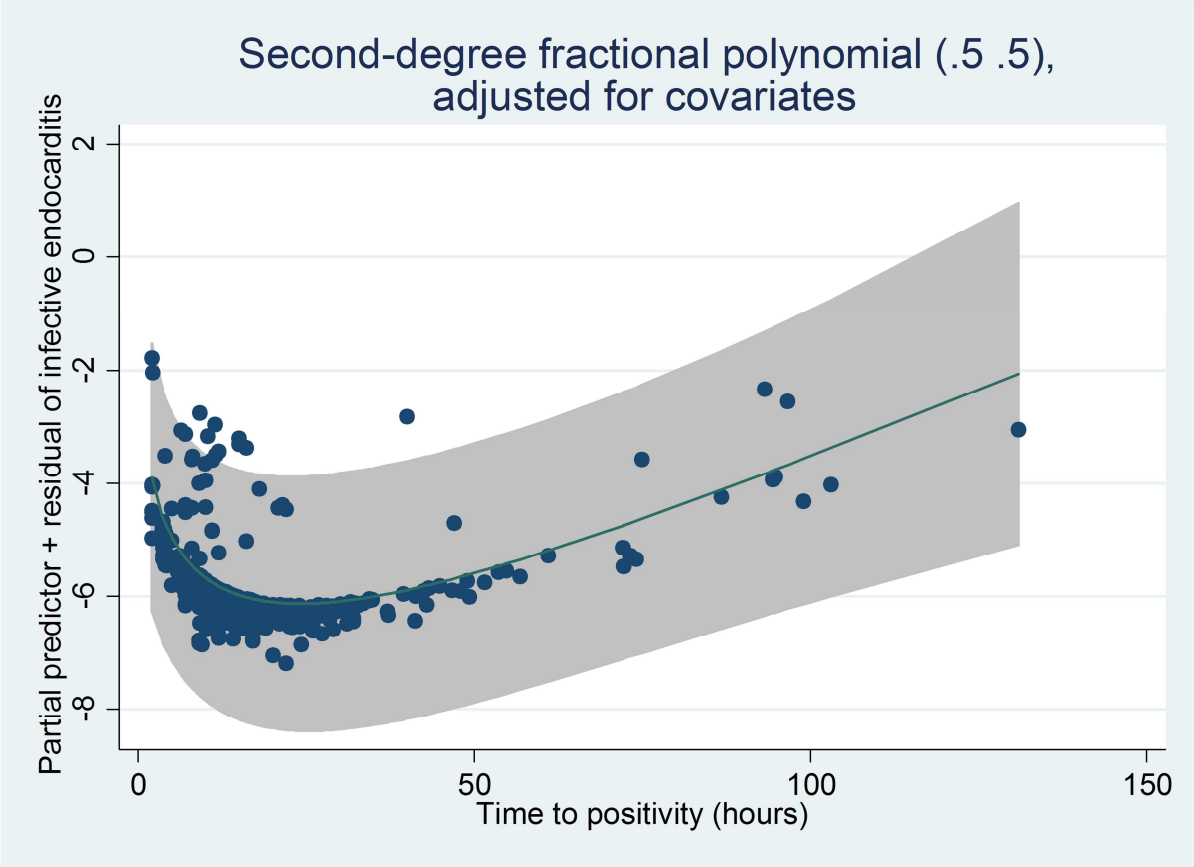
33. McGowan KL, Foster JA, Coffin SE. Outpatient pediatric blood cultures: time to positivity. Pediatrics 2000;106(2 Pt 1):251–5.

34. Choi SH, Chung JW. Time to positivity of follow-up blood cultures in patients with persistent *Staphylococcus aureus* bacteremia. Eur J Clin Microbiol Infect 2012;31(11):2963–7.



Time to positivity in hours and diagnosis of infective endocarditis


Survived (n=466)
30-day mortality (n=121)
Time to positivity (hours)
0
50
100
150
Time to positivity in hours and 30-day mortality


Second-degree fractional polynomial (.5 .5),
adjusted for covariates
Partial predictor + residual of infective endocarditis
2
0
-2
-4
-6
-8
0
50
100
150
Time to positivity (hours)