Abstract
Non-β-hemolytic streptococci (NBHS) cause infective endocarditis (IE) and a short blood culture time to positivity (TTP) is associated with risk of IE in bacteremia with other pathogens. In this retrospective population-based cohort study, we investigate if TTP is associated to IE or mortality. Of 263 episodes with NBHS bacteremia, 28 represented IE and the median TTP did not differ significantly between episodes with IE (15 h) and non-IE (15 h) (p=0.51). TTP was similar among those who survived and those who died within 30 days. However, TTP significantly differed when comparing the different streptococcal groups (p<0.001).

Keywords Time to positivity · Bacteremia · Blood culture · Streptococcus · Infective endocarditis

Introduction
Streptococcal bloodstream infections are complicated by infective endocarditis (IE) in approximately 7% of cases [1]. Non-beta-hemolytic streptococci (NBHS) are common causative agents of IE [1, 2]. Especially the Streptococcus mutans group, the Streptococcus sanguinis group and the Streptococcus bovis group are associated with IE. In NBHS bacteremia heart valve disease, long duration of symptoms and community acquisition are also associated with increased risk of IE [1, 3].

Blood culture time to positivity (TTP) provides indirect information on the bacterial concentration in blood. However, TTP is affected by other factors such as the microbial growth rate and logistics around the blood sampling and transportation. Since IE is an intravascular infection, it has been speculated that high numbers of bacteria are present in the blood, and indeed, cases with Staphylococcus aureus or Enterococcus faecalis IE have shorter TTP compared to cases of bacteremia with these pathogens without IE [4–7] [8].

This study aims to examine whether TTP in NBHS bacteremia is associated with IE. Moreover, we aimed to examine if TTP in NBHS bacteremia is associated with mortality or other clinical and microbiological variables.

Methods
This is a retrospective observational study, comprising a cohort of 481 episodes of NBHS bacteremia in the province of Skåne, Sweden, from 1 January 2015 through 31 March 2016. A dataset of all patients with NBHS-bacteremia were obtained from the Laboratory for Clinical Microbiology and were reviewed according to a predefined protocol (Supplemental data). Patients from the five largest hospitals were included and these all had BACTEC FX blood culture systems (Becton Dickinson, Franklin Lakes, USA) where bottles were inserted directly (24/7). Positive bottles were transferred to the central laboratory and species determination was performed using MALDI-TOF MS (MALDI Biotyper Compass 4.1, with...
The NBHS were categorized into six groups; the
*S. bovis* group, the *S. sanguinis* group, the *S. salivarius* group,
the *S. mitis* group, the *S. anginosus* group, and the *S. mutans*
group [3]. The study was approved by the Regional Ethics
Committee (Reg nr. 2013/31).

Patients were excluded if all cultures were polymicrobial,
age <18 years, and if patient records were unavailable. TTP
was defined by the shortest TTP in the bottles with
monomicrobial growth of NBHS from a given patient. The
diagnosis of IE was based on the modified Duke criteria [9,
10] and only episodes fulfilling the definite criteria were
regarded as IE. Comorbidities were graded using the
Charlson comorbidity index [11]. Neutropenia was defined
as absolute neutrophil count < 0.5 × 10^9/L [3].

Consideration was given to infection site of acquisition, i.e.,
if infection was community, health care or nosocomial ac-
quired [12]. The focus infection was defined according to
Berge et al. [13] (for details, see Supplementary Data).

Statistics were based on NBHS-episodes (n=263) rather
than number of patients (n=253). Patients with confirmed IE
or absence of IE were compared using χ²-test, Fisher’s exact
test, or Mann-Whitney U test. Kruskal-Wallis test was per-
formed comparing TTP for individual species within the
NBHS group. Multivariable linear regression analysis was
performed analyzing TTP in relation to clinical characteristics.
Because of a skewness of TTP (Supplemental Figure 1A) and
the corresponding residuals for the regression (Supplemental
Figure 2A), the assumptions for linear regression were not
met. Hence, TTP was log-transformed (Supplemental
Figure 1B and 2B). A multivariable regression was performed
with ln TTP as outcome. The beta coefficients were then back-
transformed to the linear scale. Collinearity was assessed by
variance inflation factor (VIF) (Supplemental Table 1).

Multivariable analysis and subsequent analysis were per-
formed in R Foundation for Statistical Computing, Vienna,
Austria version 3.6.2 with the packages haven, janitor, tidyr,
broom, ggplot2, and car.

**Results**

**Description of the cohort**

From a total of 481 episodes with NBHS bacteremia, 218
episodes were excluded; 35 episodes due to the fact that the
hospital did not hold a blood culture incubator, 153 due to
exclusively polymicrobial cultures, 57 due to age <18 years,
and 4 due to unobtainable records. Thus, there were 263 epi-
sodes in 253 patients that met the inclusion criteria of which
28 (11%) fulfilled modified Duke criteria for definite IE.
Sixty-three percent of patients had undergone any echocardi-
ography and 35% had been subjected to transesophageal
echocardiography. In Table 1, a description of the cases with
IE and non-IE is given.

**Time to positivity and IE**

TTP was compared between IE- and non-IE-episodes. Median
TTP for IE episodes was 15 h (IQR 12–19 h) and non-IE 15 h
(IQR 12–21 h) (p=0.51 for difference) (Table 1). There was no
association between TTP and IE in the univariate analysis and
there were few IE cases we, therefore, chose not to perform
multivariate regression with IE as outcome.

As sensitivity analyses, we also compared TTP between
patients with definite IE with those with IE rejected (excluding
patients with possible IE), between patients treated as IE and
those not treated as IE (irrespective of Dukes criteria), and
only among persons who underwent TEE (Supplemental
Figure 3). There were no significant differences in TTP be-
tween any of these groups.

**Time to positivity in relation to mortality**

The median TTP was 17 h (IQR 12–24) in episodes where the
patient died within 30 days (n=24) compared to 15 h (IQR 12–
21) in the 239 episodes where the patient survived (p=0.37 for
difference) (data not shown). There were no significant differ-
ces in TTP between survivors and non-survivors at hospital
discharge or at 6 months either.

**Other features possibly related to TTP**

As a secondary analysis, we investigated if other features such
as clinical and bacteriological variables were associated with a
particular TTP as the outcome. Significant differences were
observed in five characteristics: sex, site of acquisition, NBHS
group, other focal infection and neutropenia. However, after
multivariate regression analysis, the only variable revealing
statistical significance was streptococcal group where the
*S. mutans* and the *S. anginosus* group had a median TTP of
approximately 20 h compared to around 10 h for *S. bovis* and
*S. salivarius* (Table 2).

**Discussion**

We and others have observed a strong association between a
short TTP and IE in *S. aureus* [6] [4] [5] and *E. faecalis* bac-
teremia [8], indicating that if present, such differences would
be feasible to identify also for NBHS. NBHS are, in contrast to
*S. aureus* and *E. faecalis*, a heterogenous entity comprised of
many different species with different properties and propensi-
alties to cause IE [1, 3]. Not all that surprisingly, there were clear
differences when comparing TTP between different groups
within NBHS. These differences can be due to growth
characteristics of isolates. However, we cannot exclude that they could also reflect the biological properties of the types of infections that the different NHBS groups cause despite that we tried to control for this in the multivariable analysis.

This study indicates that TTP in NBHS bacteremia neither correlate with IE as defined by the Duke criteria nor with mortality and this is clearly different from the situation in S. aureus bacteremia. A reason for the NHBS bacteremia episodes with IE not displaying a shorter TTP could be that only few bacteria from the vegetation are released into the blood of the patient. However, it might also reflect that NBHS from an IE vegetation have a slower growth rate since it has been shown that streptococci inside vegetation with time develop a state of low metabolic activity [14].

Weaknesses of this study due to the retrospective design include risk of misclassifying IE episodes as non-IE due to underuse of echocardiography, a lack of information on time from blood culture to start of incubation, and on the actual blood volumes in culture flasks. Moreover, despite being relatively large, the number of episodes with a given NHBS group is low hampering the possibility to detect small differences in subgroups. A strength of the study is that all cultures were performed in hospitals having blood culture cabinets and thus similar times from the drawing of the blood until they enter the blood culture cabinet. This will likely decrease variability in TTP and would facilitate the detection of true differences.

### Table 1

| Characteristic | All cases (n=263) | IE (n=28) | Non-IE (n=235) | p value, IE vs. non-IE |
|---------------|------------------|----------|----------------|------------------------|
| Age, median (IQR) | 71 (59-81) | 73 (63-82) | 71 (59-81) | 0.34 |
| Sex | | | | 0.025 |
| Female | 108 (41) | 6 (21) | 102 (43) | |
| Male | 155 (59) | 22 (79) | 133 (57) | |
| Charlson score, median (IQR) | 2 (0–3) | 0 (0–1) | 2 (0–3) | <0.001 |
| Site of acquisition | | | | <0.001 |
| Community | 146 (56) | 25 (89) | 121 (51) | |
| Non-community | 117 (44) | 3 (11) | 114 (49) | |
| TTP, median hours (IQR) | 15 (12–21) | 15 (12–19) | 15 (12–21) | 0.51 |
| Echocardiography performed (TTE or TEE) | 167 (63) | 28 (100) | 139 (59) | < 0.00001 |
| TEE performed | 93 (35) | 26 (93) | 67 (29) | < 0.00001 |
| NBHS group | | | | |
| S. bovis | 19 (7.2) | 1 (3.6) | 18 (7.7) | |
| S. mutans | 7 (2.7) | 3 (11) | 4 (1.7) | |
| S. sanguinis | 32 (12) | 7 (25) | 25 (11) | |
| S. mitis | 105 (40) | 12 (43) | 93 (40) | |
| S. salivarius | 17 (6.5) | 3 (11) | 14 (6.0) | |
| S. anginosus | 83 (32) | 2 (7.1) | 81 (35) | |
| Neutropenia^2 | | | | 0.034 |
| Yes | 35 (13) | 0 (0.0) | 35 (15) | |
| No | 228 (87) | 28 (100) | 200 (85) | |
| Other focal infection | | | | 0.24 |
| Yes^3 | 61 (23) | 4 (14) | 57 (24) | |
| No | 202 (77) | 24 (86) | 178 (76) | |
| Death within 30 days^4 | | | | 0.088 |
| Yes | 24 (9.1) | 0 (0.0) | 24 (10) | |
| No | 239 (91) | 28 (100) | 211 (90) | |

Data is presented as numbers with percentages within parenthesis unless otherwise indicated. Statistical analyses were performed using Mann-Whitney U test or Fisher’s exact test. IQR, inter quartile range; TTE, transthoracic echocardiography; TEE, transeosophageal echocardiography; TTP, time to positivity. ^1 p value not possible to calculate due to few observations in some subgroups. ^2 Absolute neutrophil count < 0.5 × 10^9/L at the time positive blood culture was drawn. ^3 Including osteomyelitis, spondylodiscitis, gastroinstestinal infection, urogenital infection, airway infection, and soft tissue infection. ^4 From the time positive blood culture was drawn.
In conclusion, TTP varies between different subgroups of NBHS but it does not appear to provide clinically meaningful information in patients with NBHS bacteremia.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10096-021-04339-7.

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Author contribution DK, TS, and MR conceived and planned the study and assisted in this process. DK collected data and performed analyses. BN provided the cohort identification, collected data, and gave microbiology input. FK provided statistical expertise and performed data analysis and interpretation. MR and DK drafted the manuscript to which all authors gave input and provided final consent and wrote the manuscript to which all authors contributed and approved.

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Data availability Pseudonymized data will be made available upon reasonable request.

Declarations

Ethics approval The study was approved by the Regional Ethics Committee (Reg nr. 2013/31).

Table 2 Clinical characteristics NBHS bacteremia episodes in relation to TTP

| Characteristic               | Episodes | TTP, median | TTP, mean | $p$ value univariate | $p$ value multivariate | Multiplication vs. reference level |
|-----------------------------|----------|-------------|-----------|----------------------|------------------------|-----------------------------------|
| All episodes                | 263 (100)| 15 (12–21)  | 18        | 0.080                | 0.92                   | 0.99                              |
| Age (years)                 |          |             |           |                      |                        |                                   |
| 18–70                       | 124 (47) | 14 (12–20)  | 18        |                      |                        |                                   |
| ≥ 71                        | 139 (53) | 16 (13–22)  | 18        |                      |                        |                                   |
| Sex                         |          |             |           |                      |                        |                                   |
| Male                        | 155 (59) | 16 (12–22)  | 19        | 0.044                | 0.15                   | 0.93                              |
| Female                      | 108 (41) | 15 (12–19)  | 17        |                      |                        |                                   |
| Charlson score              |          |             |           |                      |                        |                                   |
| 0–1                         | 130 (49) | 16 (13–22)  | 19        | 0.12                 | 0.80                   | 1.01                              |
| ≥ 2                         | 133 (51) | 15 (12–21)  | 18        |                      |                        |                                   |
| Site of acquisition         |          |             |           |                      |                        |                                   |
| Community                   | 146 (56) | 17 (13–23)  | 20        |                      |                        |                                   |
| Non-community               | 117 (44) | 14 (12–20)  | 17        |                      |                        |                                   |
| Infective endocarditis      |          |             |           |                      |                        |                                   |
| Yes                         | 28 (11)  | 15 (12–19)  | 17        |                      |                        |                                   |
| No                          | 235 (89) | 15 (12–21)  | 18        |                      |                        |                                   |
| NBHS group                  |          |             |           | < 0.001              |                        |                                   |
| S. bovis                    | 19 (7.2) | 10 (8.8–13) | 12        | 0.006                | 0.75                   |                                   |
| S. mutans                   | 7 (2.7)  | 22 (20–28)  | 26        |                      | 0.002                  | 1.66                              |
| S. sanguinis                 | 32 (12)  | 16 (12–19)  | 18        | 0.27                 | 1.10                   |                                   |
| S. mitis                    | 105 (40) | 14 (12–17)  | 15        |                      | 5                      |                                   |
| S. salivarius               | 17 (6.5) | 12 (11–13)  | 13        | 0.09                 | 0.83                   |                                   |
| S. anginosus                 | 83 (32)  | 21 (17–26)  | 24        |                      | < 0.001                | 1.45                              |
| Neutropenia¹                |          |             |           |                      |                        |                                   |
| Yes                         | 35 (13)  | 13 (11–16)  | 14        |                      | 0.001                  | 0.15                              |
| No                          | 228 (87) | 16 (13–22)  | 19        |                      |                        | 0.88                              |
| Other focal infection²      |          |             |           |                      |                        |                                   |
| Yes                         | 61 (23)  | 18 (13–24)  | 22        |                      | 0.005                  | 0.91                              |
| No                          | 202 (77) | 15 (12–20)  | 17        |                      |                        | 0.99                              |

“All cases” are presented as numbers (%). Time to positivity (TTP) is presented as median hours with interquartile range (IQR). ¹ Absolute neutrophil count < 0.5 × 10⁹/L at the time positive blood culture was drawn. ² Including osteomyelitis, spondylodiscitis, gastrointestinal infection, urogenital infection, airway infection, and soft tissue infection. ³ Univariate statistical analyses were performed using the Mann-Whitney U test or the Kruskal-Wallis test. ⁴ Statistical analysis performed via multivariate regression analysis. ⁵ S. mitis indicated as baseline NBHS group in statistical analysis.
Consent to participate  Not applicable.

Consent for publication  All authors gave consent to the publication of this manuscript.

Competing interests  The authors declare no competing interests.

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