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Note

Time-to-positivity-based discrimination between *Enterobacteriaceae*, *Pseudomonas aeruginosa* and strictly anaerobic Gram-negative bacilli in aerobic and anaerobic blood culture vials

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ABSTRACT

Time-to-positivity (TTP) of first positive blood cultures growing Gram-negative bacilli (GNB) was investigated. When anaerobic vials were positive first, TTP ≤ 18 h differentiated *Enterobacteriaceae* from strict anaerobic Gram-negative bacilli (PPV 98.8%). When the aerobic ones were first, TTP ≤ 13 h differentiated *Enterobacteriaceae* from *Pseudomonas aeruginosa* and other GNB (PPV 80.8%).

Gram-negative bacilli (GNB) bloodstream infections are associated with high fatality rates (Leibovici et al., 1998). Poor outcome is associated with inadequate or delayed appropriate antibiotics therapy, and use of empirical broad-spectrum antibiotics is recommended (Leibovici et al., 1998; Garnacho-Montero et al., 2003). However, the struggle against the worldwide rise of bacterial resistance calls for a wise usage of these classes of antibiotics (Boucher et al., 2009). Early discrimination of *Enterobacteriaceae*, *Pseudomonas aeruginosa* and anaerobic GNB, which have different susceptibility to antibiotics, would allow a rapid implementation of an antibiotic specific regimen. Commonly used, automated vial incubators for blood cultures record the time elapsed between the introduction of vials into the incubator and positivity detection or time-to-positivity (TTP). In the present study, we estimated TTP diagnostic value to differentiate *Enterobacteriaceae*, *P. aeruginosa* and anaerobic GNB in patients with a blood culture vial, either an anaerobic or aerobic one, which was positive first with GNB upon Gram stain examination.

During two years, in our hospital, TTPs of all blood cultures (one Bactec Plus Aerobic/F and one Lytic/10 Anaerobic/F vial; Becton-Dickinson, Le Pont-De-Claix, France), first positive with GNB on direct smear examination, were analyzed. All vials were processed using the Bactec 9240 system (Becton–Dickinson). Vials with more than one microbial species were excluded. *Enterobacteriaceae*, *P. aeruginosa* or strict anaerobic Gram-negative bacilli (SAGNB) were identified using API identification kits (bioMérieux). TTPs, in hours without decimal digit, were aggregated in four time-interval groups with roughly the same number of positive bottles each. Bacterial identification was the gold standard. Clinical data were recorded. Aggregated and continuous TTPs were compared using χ^2 test and Student's *t*-test, respectively. Sensitivity, specificity, positive predictive value (PPV), and positive likelihood ratios (LR+) were calculated for each time interval, and the Receiver Operative Characteristic (ROC) curve was constructed to determine the best TTP cut-off value, as described (Sackett et al., 1991).

Overall, 607 consecutive GNB bloodstream episodes from 596 patients (mean age 67.1 years [range: 1–102]; sex ratio male/female = 0.55) were analyzed. Sources of bacteremia were urinary tract infections (35.3%, $n = 214$), intra-abdominal infections (16.1%, $n = 98$), pneumonia (7.4%, $n = 45$), central venous catheter related infections (4.3%, $n = 26$), skin and soft tissue infections (3.5%, $n = 21$), others (7.9%, $n = 48$), or not documented (25.5%, $n = 155$). The anaerobic vial was first positive in 394 (64.9%) patients and the aerobic in 213 (35.1%).

In patients with anaerobic vial positive first, *Enterobacteriaceae* were isolated in 343 (87.1%), SAGNB in 49 (12.4%) and other GNB in 2 (0.5%) (1 *Haemophilus influenzae* and 1 *Capnocytophaga* spp.) (Table 1). No

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Table 1
Likelihood ratio (LR+) and predictive positive values (PPV) for the presence of strictly anaerobic Gram-negative bacilli (SAGNB) vs *Enterobacteriaceae* and other GNB in anaerobic blood culture vials; and for the presence of *Enterobacteriaceae, P. aeruginosa* and other Gram-negative bacilli (GNB) in aerobic blood cultures vials.

Results for aerobic blood cultures vials						
Intervals of TTP (h)	Patients with first positive vials (%)		*P. aeruginosa*		Others	
	P. aeruginosa ($n = 62$)	Others ($n = 151$)	PPV	LR+	PPV	LR+
0–13	14 (22.6)	90 (59.6)	13.5	0.38	86.5	2.6
14–17	19 (30.6)	18 (11.9)	51.4	2.6	48.6	0.3
18–20	14 (22.5)	9 (5.9)	60.9	3.8	39.1	0.3
>20	15 (67.7)	34 (76.2)	30.6	1.1	69.4	0.9
Intervals of TTP (h)	Patients with first positive vials (%)		*Enterobacteriaceae*		Others	
	Enterobacteriaceae ($n = 124$)	Others ($n = 89$)	PPV	LR+	PPV	LR+
0–13	84 (67.7)	20 (13.2)	80.8	3.0	19.2	0.3
14–17	16 (12.9)	21 (13.9)	43.2	0.5	56.8	1.8
18–20	6 (4.8)	17 (11.2)	26.1	0.3	73.9	4.0
>20	18 (14.5)	31 (20.5)	36.7	0.4	63.3	2.4
Intervals of TTP (h)	Patients with first positive vials (%)		Other GNB		*P. aeruginosa* & *Enterobacteriaceae*	
	Other GNB ($n = 27$)	*P. aeruginosa* & *Enterobacteriaceae* ($n = 186$)	PPV	LR+	PPV	LR+
0–13	6 (25.0)	98 (52.7)	5.8	0.4	94.2	2.4
14–17	2 (7.4)	35 (18.8)	5.4	0.4	94.6	2.5
18–20	3 (11.1)	20 (10.7)	13.0	0.4	87.0	1.0
>20	16 (59.3)	33 (17.7)	32.7	3.3	67.3	0.3
Results for anaerobic blood culture vials						
Intervals of TTP (h)	Patients with first positive vials (%)		SAGNB		Others	
	SAGNB ($n = 49$)	Others ($n = 345$)	PPV	LR+	PPV	LR+
0–21	11 (22.4)	344 (99.7)	3.1	0.23	95.5	3.1
22–25	14 (28.6)	1 (0.0)	93.3	98.6	6.7	0.01
26–30	12 (24.5)	0 (0.0)	100.0	+∞	0.0	0.0
≥30	12 (24.5)	0 (0.0)	100.0	+∞	0.0	0.0

vial grew *P. aeruginosa*. Median TTP was 25 h [interquartile range of 22 h–30 h] for SAGNB and 9 h [8 h–10 h] for *Enterobacteriaceae* ($p < 0.001$). PPV for the presence of SAGNB increased from 3.1% for 0–21 h TTPs to 93.3% for the 22–25 h TTPs, and LR+ increased from 0.2 to 98.6, respectively (Table 1). The PPV for the presence of other GNB (large majority of *Enterobacteriaceae*) was 99.7% when TTP was ≤21 h. ROC curve analysis showed that a cut-off value of 18 h had the best combined sensitivity (98.8%) and specificity (95.9%) to discriminate *Enterobacteriaceae* from SAGNB; a TTP ≤ 18 h was predictive of *Enterobacteriaceae* with a PPV of 99.4%; and TTP > 18 h was predictive of SAGNB with a PPV of 92.4%. This result was true whatever the species of *Enterobacteriacae*.

In patients with aerobic vial positive first, *Enterobacteriaceae* were isolated in 124 (58.2%), *P. aeruginosa* in 62 (29.1%), and other GNB in 27 (12.7%) (*Acinetobacter* spp. n = 8, *Campylobacter* spp. n = 8, *Haemophilus* spp. n = 6, *Stenotrophomonas maltophilia* n = 4, *Brucella* spp. n = 1, *Burkholderia cepacia* n = 1 and *Flavimonas oryzihabitans* n = 1) (Table 1). Median TTP was 11 h [9 h–16 h] for *Enterobacteriaceae*, 17 h [14 h–20 h] for *P. aeruginosa* (p = NS), and 21 h [15 h–67 h] for other GNB in aerobic vials ($p < 0.01$ vs *Enterobacteriaceae*). TTP ≤ 13 h was associated with a PPV of 80.8% (LR+: 3.0) for *Enterobacteriaceae*, whereas TTP between 14 and 20 h was associated with a PPV ranging from 51.4% to 60.9% for *P. aeruginosa*, with LR+ of 2.6 and 3.8, respectively. Other GNB were more likely present when TTP was > 20 h (LR+: 3.3). A TTP cutoff value of 13 h had the best combined sensitivity (67.7%) and specificity (77.5%) to discriminate *Enterobacteriaceae* from other GNB (including *P. aeruginosa*). This result was true whatever the species of *Enterobacteriacae or* strict anaerobes. A decision making algorithm is displayed in Fig. 1.

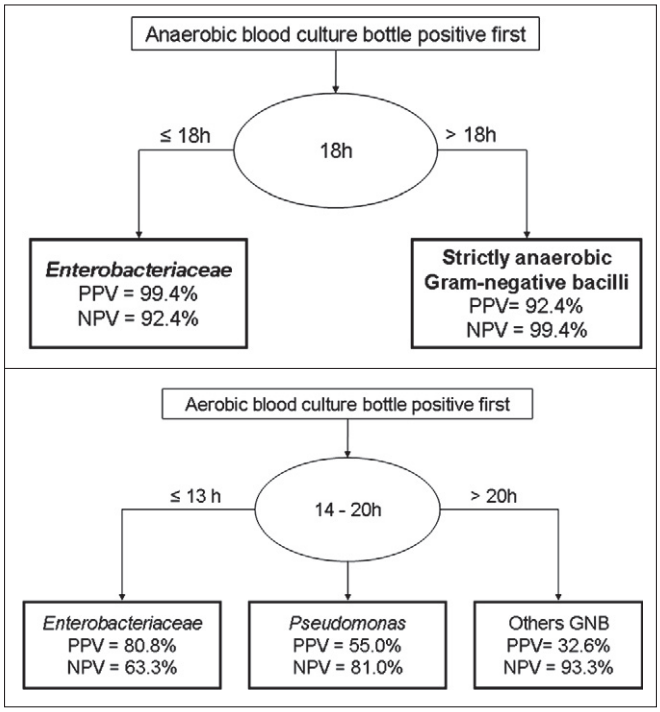

Fig. 1. Decision making algorithm based on TTP for the distinction of Gram-negative bacilli (GNB) in anaerobic and aerobic blood cultures vials.

We showed that TTP could help in discriminating types of GNB in patients with either an aerobic or anaerobic blood culture vial positive first with GNB upon Gram staining. Previous studies had described that *Enterobacteriaceae* grew faster than *P. aeruginosa*, SAGNB or other Gram-negative bacteria (Martinez et al., 2007; Passerini et al., 2009) but the diagnostic value of TTP had not been fully analyzed. We showed here that an 18-hour cut-off discriminated SAGNB from *Enterobacteriaceae* with a PPV as high as 99.4% in anaerobic vials. TTP analysis was less obvious when aerobic vials were positive first. However, TTP $\leq$13 h was associated with a PPV of 80.8% for the presence of *Enterobacteriaceae* but of only 13.5% for *P. aeruginosa*, a probability which increased to 60.9% for TTP of 17–20 h. This simple analysis may seem odd when techniques such as fluorescence, hybridization probes (Sogaard et al., 2007), real-time PCR (Cattoir et al.), and mass spectrometry (Kaleta et al.) are emerging for early and accurate diagnostic of bloodstream infections. Unfortunately, these methods are expensive, time consuming, and still not implemented in all laboratories. However, useful TTP analysis has limits. First, cut-off values calculated here with data from one type of automated blood culture system may vary slightly with others. Second, the volume of blood inoculated and the time before loading the vials were not recorded but variations occurred randomly and did not prevent significant patterns to emerge. Third, antibiotics at time of sampling were unknown but TTP has been presented independent of treatments (Liao et al., 2009; Passerini et al., 2009). In this context, our results offer a cost-free, fast and quantitative addition to Gram staining in order to differentiate *Enterobacteriaceae* from *Pseudomonas* or SAGNB in blood culture vials with GNB on direct smear examination and to choose the best empiric antibiotic treatments.

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