Preclinical Detection of Non-catheter Related Late-onset Sepsis in Preterm Infants by Fecal Volatile Compounds Analysis

A Prospective, Multi-center Cohort Study

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Background: Late onset sepsis (LOS) in preterm infants is preceded by fecal volatile organic compound (VOC) alterations, suggesting an etiologic role of gut microbiota in LOS rather than being primarily caused by central venous catheters (CVC). To increase our knowledge about the involvement of the gut microbiota in LOS, we analyzed fecal samples from septic infants without a CVC.

Methods: In this prospective multicenter study, fecal samples were collected daily from all infants born at ≤30 weeks gestation. Fecal VOC profiles up to 3 days prior to sepsis onset from infants with non-catheter–related LOS were compared with profiles from non-septic controls by means of High-Field Asymmetric Waveform Ion Mobility Spectrometry.

Results: In total, 104 fecal samples were analyzed. Fecal VOC profiles allowed for discrimination between non-catheter–related LOS cases (n = 24) and matched controls (n = 25). Discriminative accuracy increased after focusing on center of origin (area under the curve, sensitivity, specificity; 0.95, 100%, 83%) and after focusing on LOS cases caused by *Staphylococcus epidermidis* (0.95, 100%, 78%), the most cultured pathogen (n = 11).

Conclusions: Fecal VOC profiles of preterm LOS infants without a CVC differed from matched controls underlining the increasing notion that aberrations in gut microbiota composition and activity may play a role in LOS etiology.

Key Words: neonatology, preterm, late-onset sepsis, volatile organic compounds, electronic nose

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In the preterm-born population, late-onset sepsis (LOS) continues to have a significant impact on morbidity and mortality, length of hospital admission and economic costs.[1] Extremely preterm infants (gestational age <28 weeks) have an increased infection risk due to a naive immune system and the almost universal requirement of invasive procedures such as intravascular catheter placement.[2] Although disruption of the skin by invasive procedures increases the risk of LOS development, bacterial translocation from the intestine may also be an important pathway initiating LOS in extremely preterm infants.[3] This is based on the various observations that microbial intestinal dysbiosis precedes clinical onset of LOS.[4–14] An immature intestinal epithelial barrier function,[15] low intestinal motility, scanty mucus flow and reduced enteric production of antimicrobial factors,[16] combined with microbial dysbiosis, may induce an inflammatory process affecting the mucosal integrity by loss of mucosal tight junctions.[17] An impaired intestinal integrity could provoke transmucosal translocation of gut microbes resulting in bacteremia and eventually LOS.[18,19] In recent studies, we have observed that fecal volatile organic compound (VOC) profiles from preterm infants developing LOS differed from the profiles from strictly matched controls, already up to 3 days prior to clinical onset.[20,21] Since fecal VOCs, referred to as the odorous fecal fingerprint, are considered to reflect microbiota composition, metabolic activity and the interaction between microbiota and the host,[22] fecal VOC composition analysis may be useful as an early, noninvasive diagnostic biomarker for LOS. These observed alterations in fecal VOC profiles increases the plausibility of the involvement of the intestinal microbiota in LOS pathogenesis. However, in these VOC studies, vast majority of LOS cases had a central venous catheter (CVC) during LOS development, and this variable should obviously be acknowledged. Although observed early alterations in fecal VOC profiles may reflect disturbance of gut microbiota composition, in infants with a central line-associated blood stream infection (CLABSI), these VOC differences may also result from a systemic inflammatory response rather than caused by alterations in microbiota composition. Therefore, to increase knowledge about the origin of fecal

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VOCs in preterm infants developing LOS, we investigated fecal VOC profiles of infants with non-CLABSI. Primary involvement of the gut in LOS pathophysiology may in future allow for the development of novel (microbiota-based) interventions aimed at the prevention of LOS in the preterm-born population.

MATERIALS AND METHODS

Subjects

Between October 2014 and August 2016, preterm infants born at a gestation of ≤30 weeks were recruited to participate in this prospective cohort study performed at 8 neonatal intensive care units (NICU) in the Netherlands and Belgium. Infants born with congenital gastrointestinal anomalies, receiving gastrointestinal surgery or developing either spontaneous intestinal perforation or necrotizing enterocolitis (Bells stage ≥IIA) within the first 28 days were excluded from further participation. In this ongoing study, fecal samples are collected daily from the diaper up to the 28th day postnatally. Probiotics were not administered routinely in any participating NICU. Written informed consent was obtained from all parents of included patients. This study was approved by local Institutional Review Boards of all participating medical centers.

Infants were allocated to the LOS group when all 3 Vermont Oxford criteria[23] for LOS were met; (1) presence of at least 1 clinical sign of generalized infection; (2) isolation of a bacterial pathogen or a Coagulase-negative *Staphylococcus* (CoNS) from at least one blood culture obtained ≥ 72 hours after birth; and (3) administration of adequate intravenous antibiotic regimen directed to this specific pathogen for a minimum of 5 days. Septic infants with an umbilical venous catheter, umbilical arterial catheter and those with a peripherally inserted central catheter within 48 hours prior to the onset of LOS, thereby meeting the criteria for CLABSI,[24] were excluded. Infants without congenital gastrointestinal anomalies, not receiving gastrointestinal surgery or developing early-onset sepsis, LOS, spontaneous intestinal perforation or necrotizing enterocolitis during their first 28 postnatal days were eligible to be included as control infant. For a more detailed description, we refer to previously conducted studies and Methods, Supplemental Digital Content 1, http://links.lww.com/INF/D754.20,21.

Sample Selection

Fecal samples produced at 1 (t_{-1}), 2 (t_{-2}) and 3 (t_{-3}) days prior to clinical onset of LOS (t_0), were selected for VOC analysis, since previous studies demonstrated differences in fecal VOC profiles up to 3 days prior to LOS onset.[20,21] Each fecal sample from infants allocated to the LOS group was strictly matched to one fecal sample from a control, based on (1) center of birth, (2) gestational age, (3) birthweight, (4) postnatal age on the day of sample collection, (5) enteral feeding pattern (proportion breastmilk/formula) and (6) cumulative number of days exposed to administered antibiotics prior to t_0.

VOC Measurements

Fecal VOC analysis was performed by Field Asymmetric Ion Mobility Spectroscopy (Lonestar, Owlstone, Cambridge, England). In Lonestar, provided VOCs are first ionized by a NI-63 radiation source before being transported further using a carrier gas. Subsequently, these ionized molecules enter an asymmetric electrical field towards a detector plate while simultaneously passing 2 metal plates. The applied voltage (dispersion field) alternates between prolonged low electrical voltages and short high voltages, causing the ionized molecules to move in a ion-specific "tooth saw-like" pattern. Since these ions lose their electrical charge after touching one of the metal plates, a compensation voltage (CV) is applied, preventing ions to be left undetected by the detector plate.[25] In summary, ions in a complex gaseous mixture could be separated based on ion-specific mobility differences by stepwise altering the dispersion field and CV ultimately allowing for 52.224 data points.[26] For a more detailed description of a Field Asymmetric Ion Mobility Spectroscopy measurement, we refer to the Methods, Supplemental Digital Content 1, http://links.lww.com/INF/D754.

Data Analysis

All of the analysis was performed using R version 3.4.1. Independent *t* test and χ^2 test were applied for comparison of basic demographics and clinical characteristics between the 2 study groups. A *P*-value of <0.05 was considered statistically significant. The eNose data were analyzed using a previously developed pipeline.[21,27] In short, the Lonestar was configured to generate a 2D matrix of 52.224 data points per sample. Each sample was preprocessed by applying a 2D discrete wavelet transform (Daubechies D4) (*wavethresh* R package) to compress the data and extract subtle chemical signals. Once completed, a threshold was applied to the standard deviation of the features, removing those features that vary by only a very small amount (or not at all). A 10-fold cross-validation was then used, with 90% of the data as a training set and the remaining 10% as a test set. Within each fold, supervised feature selection and classification models were performed. For this, Wilcoxon rank sum test was used on the training set to calculate the *P*-value for each feature, then keeping only the features with the lowest *P*-value after applying Bonferroni correction. The selected features then used as an input to train the classification models. Finally, 5 different classification algorithms were used to produce the class predictions. They were Sparse Logistic Regression (*glmnet* R package), Gaussian Process Classifier (*kernlab* R package), Random Forest (*randomForest* R package), Support Vector Machine (*kernlab* R package), and Neural Network (*neuralnet* R packega). The receiver operating characteristic curves with corresponding sensitivity, specificity, positive predictive value, and negative predictive value were produced for each classification model. This was undertaken as it has been observed that one or more algorithm will be better suited to a given task and by evaluating a number of these algorithms allows for identification which is best for this task. Cronbach's alpha was calculated to demonstrate any consistency between the VOC profiles.

Fecal VOC profiles from infants with non-CLABSI were compared with VOC profiles from strictly matched controls at 1 (t_{-1}), 2 (t_{-2}) and 3 (t_{-3}) days prior to clinical onset of the disease. To assess the potential of fecal volatiles as early biomarker for LOS in clinical practice, regardless of the number of days prior to clinical onset that the last fecal sample was produced, only the last fecal sample available prior to LOS onset was used from all cases in an additional analysis. Since each bacterial species produces a species-specific VOC profile,[28] VOCs deriving from fecal samples closest to t_0 from infants with LOS caused by similar species (CoNS and *Staphylococcus epidermidis*) were compared with controls. To limit the influence of center-specific differences in VOC outcome due to local protocols, such as differences in feeding pattern and in administered antibiotic types, VOC profiles of infants with and without LOS from the same center of origin were compared in an additional analysis by using the last produced samples before t_0.

RESULTS

Subjects

In total, 591 preterm infants were included during the study period, of whom 169 (28,6%) developed LOS within the first 28 postnatal days (Table, Supplemental Digital Content 2, http://links.lww.com/INF/D755). Ultimately, samples from 24 infants with

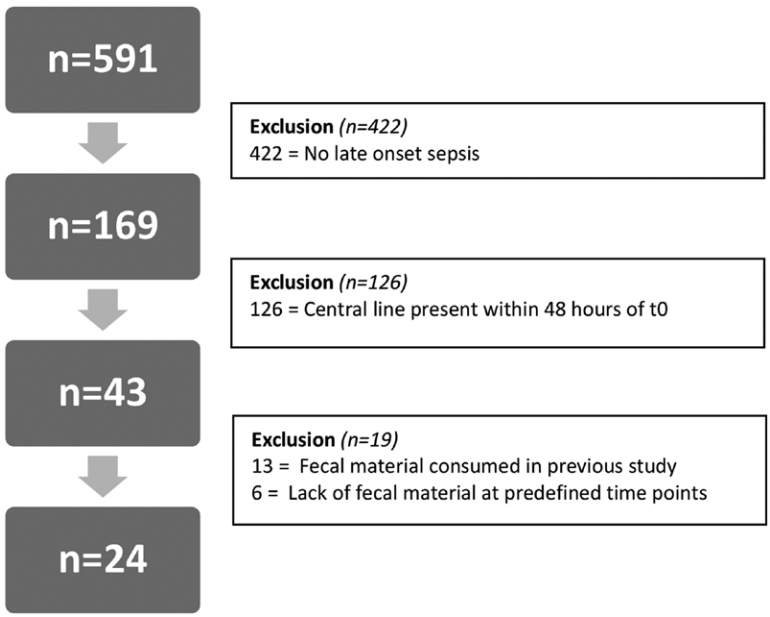

FIGURE 1. Flow-chart of participants in the study.

TABLE 1. Subject characteristics of patients with LOS and matched controls

Number (n)	LOS (24)	Control (25)	*P*
Sex			
Male [n(%)]	10 (42)	14 (56)	0.87
Birth weight [median (IQR)], *g*	1011 (446)	1060 (263)	0.87
Gestational age [median (IQR)], weeks + days (days)	27 + 6 (19)	28 + 0 (14)	0.92
Delivery mode [n(%)]			0.68
Vaginal delivery	13 (54)	15 (60)	
Cesarean section	11 (46)	10 (40)	
AB use before T_0 [n(%)]	22 (92)	24 (96)	
Days AB use before T_0[median (IQR)], days	4 (3.5)	4 (2)	0.34
Feeding pattern before T_0[median (%)]†	67	38	0.44
Totally formula fed [n(%)]	2 (8)	3 (12)	
Totally breastmilk fed [n(%)]	1 (4)	1 (4)	
Deceased [n(%)]	2 (8)	0 (0)	0.14
Age deceased (median, days)	20	NA	

AB, antibiotic; IQR, interquartile range; NA, not applicable.
†Cumulative percentage mother's milk relative to total amount of enteral feeding prior to t_0.

TABLE 2. Isolated pathogens [n(%)] from blood cultures in 24 sepsis patients without central line within 48 hours prior to clinical onset of LOS

Coagulase-negative *Staphylococcus* (CoNS)	15 (63)
Staphylococcus epidermidis	11 (46)
Staphylococcus warneri	1 (4)
Staphylococcus haemolyticus	1 (4)
Staphylococcus lugdunesis	1 (4)
Combination of >1 CoNS*	1 (4)
Escherichia coli	3 (13)
Staphylococcus aureus	2 (8)
Enterococcus faecalis	1 (4)
Streptococcus agalactiae	1 (4)
Klebsiella pneumoniae	1 (4)
Enterobacter aerogenes	1 (4)

**Staphylococcus epidermidis* and *Staphylococcus hominis*.

non-CLABSI could be strictly matched to controls and were selected for fecal VOC analysis (Fig. 1). Patient characteristics of these 24 LOS infants and their matched controls are depicted in Table 1. In fifteen infants (63%), a CoNS was isolated from the blood culture (Table 2).

Fecal Volatile Organic Compound Analysis

Per individual analysis, data of the best performing classification model is shown in Table 3, while in Table, Supplemental Digital Content 3, http://links.lww.com/INF/D756, a complete overview is given of the data generated by all 5 classification models. Up to 3 days prior to clinical sepsis onset, fecal VOC profiles differed between cases and controls, corresponding area under the curve (AUC) [±95% confidence interval], sensitivity and specificity at t_{-3} [0.78 (0.62–0.93), 0.82, 0.65], at t_{-2} [0.65 (0.46–0.85), 0.59, 0.82] and t_{-1} [0.78 (0.63–0.94), 0.61, 0.83]. Differences were statistically significant at t_{-3} (*P*-value = 0.002) and t_{-1} (*P*-value = 0.001), but not at t_{-2} (*P*-value = 0.061).

TABLE 3. Best available performance characteristics with corresponding AUC, sensitivity, specificity, positive and negative predictive value of fecal VOC analysis for the discrimination of late-onset sepsis and matched controls

Analysis	Sepsis samples† (n)	*P*	AUC (± 95% CI)	Sensitivity (± 95% CI)	Specificity (± 95% CI)	PPV	NPV
t-1	18	0.001	0.78 (0.63–0.94)	0.61 (0.36–0.83)	0.83 (0.59–0.96)	0.79	0.68
t-2	17	0.061	0.65 (0.46–0.85)	0.59 (0.33–0.82)	0.82 (0.57–0.96)	0.77	0.67
t-3	17	0.002	0.78 (0.62–0.93)	0.82 (0.57–0.96)	0.65 (0.38–0.86)	0.7	0.79
t-1 to t-3‡	24	0.012	0.69 (0.54–0.84)	0.58 (0.37–0.78)	0.75 (0.53–0.9)	0.7	0.64
CoNS	15	0.003	0.79 (0.62–0.95)	0.87 (0.6–0.98)	0.67 (0.38–0.88)	0.72	0.83
Staphylococcus epidermidis	12	0.0001	0.95 (0.86–1)	1 (0.74–1)	0.83 (0.52–0.98)	0.86	1
Center 1 with CoNS*	9	0.0001	0.95 (0.86–1)	1 (0.66–1)	0.78 (0.4–0.97)	0.82	1

* CoNS was isolated from blood culture in all subjects from Center 1.

† Corresponding number of fecal samples from controls were analyzed.

‡ For this analysis, only the last fecal sample produced prior to late-onset sepsis was used, *

AUC ± 95% CI, AUC with 95% confidence interval; NPV, negative predictive value; PPV, positive predictive value; S, *Staphylococcus*.

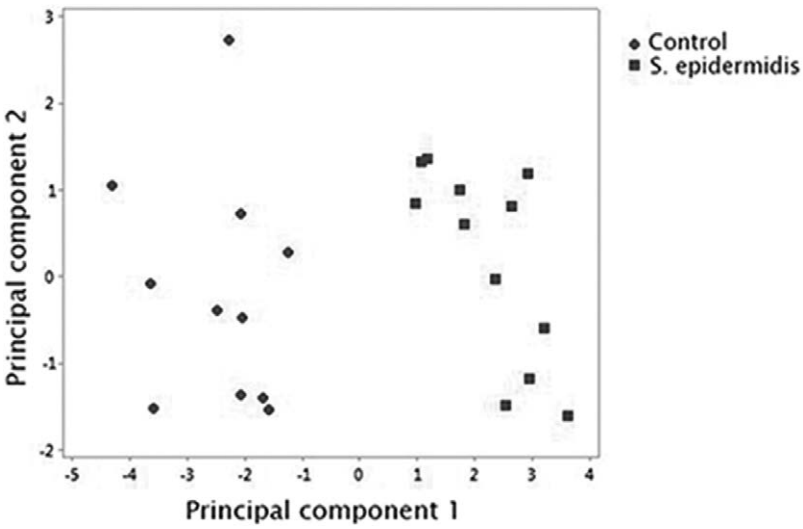

FIGURE 2. Principal component analysis plot depicting the last produced fecal sample prior to t_0 of cases with a *Staphylococcus epidermidis* as causative agent and their matched controls, showing the first 2 principal components, which hold the majority of variance in the samples.

Discriminative accuracy increased after selection of the last produced fecal sample prior to t_0 of LOS cases with a CoNS species as causative agent [0.79 (0.62–0.95), 0.87, 0.67]. This accuracy augmented even further after only selecting LOS cases caused by *S. epidermidis* [0.95 (0.86–1), 1, 0.83] (Table 3, Fig. 2). Fecal volatile profiles of LOS cases originating from the NICU with the largest group of LOS cases, irrespective of isolated pathogen (n = 9) (referred to as Center 1), could be statistically significantly discriminated from matched controls of the same center [0.95 (0.86–1),1, 0.78] (Table 3). Cronbach's alpha, a measure of internal consistency, was calculated to be 0.98 at each individual time-point, indicating a high degree of consistency.

DISCUSSION

In this study, we demonstrated that fecal VOC profiles allowed for the discrimination between preterm infants developing non-catheter–related LOS and matched controls, up to 3 days prior to clinical onset of LOS. Discriminative accuracy increased upon performing subgroup analysis on VOC profiles of LOS cases caused by the same bacterial species.

Two previous studies have attempted to discriminate LOS cases from controls by fecal VOC profiling.[20,21] In the first study, performed at 3 NICUs and including 36 LOS cases, affected subjects could be differentiated from controls at 3, 2 and 1 day prior to clinical sepsis onset with only modest accuracy; 58.8%, 72.7% and 64.3%, respectively.[20] The higher discriminative performance observed in the present study may potentially be explained by the exclusion of patients with an indwelling CVC. By excluding infants with a CLABSI, the number of LOS cases resulting from skin invasive procedures remained limited, simultaneously increasing the plausibility of a gut-derived sepsis. Hypothetically, uniform alterations in gut microbiota composition preceding LOS onset may have resulted in the increased discriminative accuracy observed in the current study. Another potential explanation is the use of a different eNose device. Whereas the currently applied eNose (Lonestar) generates 52.224 individual data points, the previously applied Cyranose320 only allows for 32 data points.[26,29]

In the second study, including 127 LOS cases from 9 different NICUs, highest discriminative accuracies were obtained in the subgroup analyses including LOS cases with the same bacterial species obtained from blood culture. In the subanalysis including cases with a *S. epidermidis* sepsis, fecal VOCs allowed for discrimination at day 1, 2 and 3 prior to LOS onset. Focusing on the fecal VOC profile of the last sample obtained prior LOS onset from all *S. epidermidis* cases resulted in a non-statistically significant discrimination between cases and controls with a corresponding AUC of 0.63, whereas the AUC obtained in the current study was 0.95.

We hypothesize that this difference can at least partly be attributed to the exclusion of central line-associated LOS cases in the current study. By excluding infants meeting the criteria for CLABSI, the number of LOS cases caused by a pathogen originating from skin-invasive procedures instead of intestinal bacterial translocation remained limited. In particularly bacteria belonging to the CoNS, including *S. epidermidis*, may extensively be found on the skin[30] and are often associated with CLABSI. In LOS cases where the pathogen originates from the skin and not from the intestines, fecal VOC profiles may not have been altered in a preclinical stage, hampering the ability to discriminate between cases and controls based on these profiles.

As demonstrated in previous studies, LOS onset by CoNS may be preceded by an intestinal increase in abundance of CoNS species.[9,12] This may presumably explain the high discriminative accuracy to differentiate between CoNS sepsis and control infants in the present study. However, in contrast to the current study, fecal VOCs did not allow for discrimination between cases with a CoNS sepsis and controls in the previous study.[21] We hypothesize that this apparent discrepancy may not only be explained by the exclusion of central line-associated LOS cases, but also by the presence of a more homogeneous group CoNS pathogens in the current study. In this study, *S. epidermidis* comprised 80% of the isolated CoNS pathogens, whereas they only accounted for 58% of the CoNS pathogens in the previous study. Different bacterial species produce a unique metabolic fingerprint and provoke a unique host-pathogen interaction.[28,31] The more homogenous group of CoNS LOS cases in this study may therefore have generated a more clustered VOC cloud and consequently higher discriminative accuracy to differentiate between CoNS cases and controls.

Since VOCs are produced during (patho)physiologic metabolic processes, CLABSI could hypothetically induce a systemic metabolic response, leading to (secondary) changes of fecal VOCs. Our observation that in septic preterm infants without a CVC, fecal VOCs differed from controls suggesting that, at least in this population, the gut microbiota may play a pivotal role in LOS pathogenesis, rather than being a secondary phenomenon. This concept has already been advocated by the findings of Lepainteur et al[7], who compared DNA from blood cultures with corresponding DNA of CVC in infants with CLABSI and concluded that CVC as origin of sepsis could be questioned in up to 70% of the cases.

Notably, 2 days prior to clinical LOS onset, fecal VOCs did not allow statistically significant discrimination between LOS and controls ($P = 0.06$). Possible explanations are the relatively small sample size and substantial differences in center of birth and clinical characteristics of LOS subjects. The influence of environmental factors on fecal VOC composition, including center of origin,[32] has been described previously, possibly resulting from differences in center-specific feeding patterns, medication usage and bacterial exposure. In contrast to chemical analytical techniques such as gas chromatography-mass spectrometry (GC-MS), an eNose such as Lonestar, with a sensing method mimicking the olfactory system, does not allow for identification of individual volatile compounds.[33] Confounding VOCs originating from non-avoidable and non-relevant environmental and sampling sources, like those deriving from antibiotics, may have significantly influenced eNose results.[32] Consequently, this may negatively influence discriminative accuracy. The influence of inter-center variation in VOC outcome is illustrated by the increased accuracy for sample classification in the sub-analysis including only infants born at 1 center.

It the present study, multiple prediction models were selected. Since performance of the various models demonstrate considerable variation, it is questioned whether a certain classifier model may have universal applicability in different populations.[21,34] Future studies should focus on assessing which model provides optimal accuracy in various clinical and research settings.

Strengths of this study are the prospective multicenter design with strict sampling and detailed data collection allowing the stringent matching of cases and controls based on clinical and demographic characteristics. In contrast, we also acknowledge several limitations, including the relatively small contribution of non-CLABSI to the total number of LOS cases in this cohort. Another limitation is the relatively small number of LOS cases caused by Gram-negative pathogens, although the proportion (about 20%) of total LOS was comparable with other studies.[2,35] Furthermore, other possible routes of LOS acquisition, like peripheral inserted infusions and invasive respiratory procedures, could not be corrected for, due to the limited sample size. Lastly, although current study outcomes increase the plausibility of the involvement of gut microbes in the etiology of LOS in preterm infants, concurrent analysis of the microbiota composition could add understanding on the detected differences in fecal VOCs.

Future studies are needed to externally validate our findings, preferably in larger cohorts. Prior to implementation of eNose technology in clinical practice to predict LOS in an early stage, identification of LOS-specific and ideally species-specific VOCs, by techniques such as GC-MS, is required for the development of a tailor-made eNose. Chemical analytical techniques including GC-MS are relatively expensive, time-consuming and not applicable in daily clinical practice, but are inevitable for the development of a primed eNose for LOS-related VOCs, applicable in a clinical setting. Primed eNose sensors may allow for targeted VOC detection, not influenced by non-relevant environmental factors, resulting in an optimal discriminative accuracy under non-standardized conditions, consequently providing opportunities for development of interventions aimed at prevention of LOS. In addition, future studies combining both microbiota and VOC analysis may provide additional information about the exact pathophysiology underlying LOS in preterm infants.

In conclusion, we have shown that in preterm infants with LOS in absence of a CVC, fecal VOC profiles differed from matched controls, up to 3 days prior to clinical onset. Discriminative accuracy increased upon focusing on infants with LOS caused by the same pathogen. Our findings strengthens the increasing notion that the gut may be involved in LOS pathogenesis. Furthermore, early noninvasive selection of infants who will develop LOS could provide a window of opportunity for development of novel therapeutic strategies aimed at prevention of LOS.

REFERENCES

1. Erdei C, McAvoy LL, Gupta M, et al. Is zero central line-associated bloodstream infection rate sustainable? A 5-year perspective. *Pediatrics.* 2015;135:e1485–e1493.
2. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics.* 2002;110(2, pt 1):285–291.
3. Sherman MP. New concepts of microbial translocation in the neonatal intestine: mechanisms and prevention. *Clin Perinatol.* 2010;37:565–579.
4. Parm Ü, Metsvaht T, Sepp E, et al. Mucosal surveillance cultures in predicting Gram-negative late-onset sepsis in neonatal intensive care units. *J Hosp Infect.* 2011;78:327–332.
5. Madan JC, Salari RC, Saxena D, et al. Gut microbial colonisation in premature neonates predicts neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed.* 2012;97:F456–F462.
6. Stewart CJ, Marrs EC, Magorrian S, et al. The preterm gut microbiota: changes associated with necrotizing enterocolitis and infection. *Acta Paediatr.* 2012;101:1121–1127.
7. Lepainteur M, Desroches M, Bourrel AS, et al. Role of the central venous catheter in bloodstream infections caused by coagulase-negative staphylococci in very preterm neonates. *Pediatr Infect Dis J.* 2013;32:622–628.

8. Mai V, Torrazza RM, Ukhanova M, et al. Distortions in development of intestinal microbiota associated with late onset sepsis in preterm infants. *PLoS One.* 2013;8:e52876.
9. Soeorg H, Huik K, Parm U, et al. Genetic relatedness of coagulase-negative Staphylococci from gastrointestinal tract and blood of preterm neonates with late-onset sepsis. *Pediatr Infect Dis J.* 2013;32:389–393.
10. Stewart CJ, Marrs EC, Nelson A, et al. Development of the preterm gut microbiome in twins at risk of necrotising enterocolitis and sepsis. *PLoS One.* 2013;8:e73465.
11. Carl MA, Ndao IM, Springman AC, et al. Sepsis from the gut: the enteric habitat of bacteria that cause late-onset neonatal bloodstream infections. *Clin Infect Dis.* 2014;58:1211–1218.
12. Shaw AG, Sim K, Randell P, et al. Late-onset bloodstream infection and perturbed maturation of the gastrointestinal microbiota in premature infants. *PLoS One.* 2015;10:e0132923.
13. Taft DH, Ambalavanan N, Schibler KR, et al. Center variation in intestinal microbiota prior to late-onset sepsis in preterm infants. *PLoS One.* 2015;10:e0130604.
14. Cernada M, Bäuerl C, Serna E, et al. Sepsis in preterm infants causes alterations in mucosal gene expression and microbiota profiles compared to non-septic twins. *Sci Rep.* 2016;6:25497.
15. Lin PW, Nasr TR, Stoll BJ. Necrotizing enterocolitis: recent scientific advances in pathophysiology and prevention. *Semin Perinatol.* 2008;32:70–82.
16. Dollings MC, Brown L. An integrated review of intestinal microbiota in the very premature infant. *Neonatal Netw.* 2016;35:204–216.
17. Brooks HJ, McConnell MA, Broadbent RS. Microbes and the Inflammatory Response in Necrotising Enterocolitis, Preterm Birth, Offer Erez, IntechOpen. January 23, 2013. Available from: https://www.intechopen.com/books/preterm-birth/microbes-and-the-inflammatory-response-in-necrotising-enterocolitis. Accessed July 02, 2017.
18. Deitch EA. Gut lymph and lymphatics: a source of factors leading to organ injury and dysfunction. *Ann N Y Acad Sci.* 2010;1207(suppl 1):E103–E111.
19. Wiest R, Rath HC. Gastrointestinal disorders of the critically ill. Bacterial translocation in the gut. *Best Pract Res Clin Gastroenterol.* 2003;17:397–425.
20. Berkhout DJC, Niemarkt HJ, Buijck M, et al. Detection of sepsis in preterm infants by fecal volatile organic compounds analysis: a proof of principle study. *J Pediatr Gastroenterol Nutr.* 2017;65:e47–e52.
21. Berkhout DJC, van Keulen BJ, Niemarkt HJ, et al. Late-onset sepsis in preterm infants can be detected preclinically by fecal volatile organic compound analysis: a prospective, multicenter cohort study. *Clin Infect Dis.* 2019;68:70–77.
22. Garner CE, Smith S, de Lacy Costello B, et al. Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease. *FASEB J.* 2007;21:1675–1688.
23. *Manual of Operations: Part 2 Data Definitions & Infant Data Forms*. October 2015 ed. Vermont Oxford Network; 2016. Available from: https://vtoxford.zendesk.com/hc/en-us/articles/360013115393-2019-Manual-of-Operations-Part-2-Release-23-2-PDF-.
24. O'Grady NP, Alexander M, Burns LA, et al; Healthcare Infection Control Practices Advisory Committee. Guidelines for the prevention of intravascular catheter-related infections. *Am J Infect Control.* 2011;39(4, suppl 1):S1–S34.
25. van Gaal N, Lakenman R, Covington J, et al. Faecal volatile organic compounds analysis using field asymmetric ion mobility spectrometry: non-invasive diagnostics in paediatric inflammatory bowel disease. *J Breath Res.* 2017;12:016006.
26. Covington JA, van der Schee MP, Edge AS, et al. The application of FAIMS gas analysis in medical diagnostics. *Analyst.* 2015;140:6775–6781.
27. Arasaradnam RP, Westenbrink E, McFarlane MJ, et al. Differentiating coeliac disease from irritable bowel syndrome by urinary volatile organic compound analysis–a pilot study. *PLoS One.* 2014;9:e107312.
28. Bos LD, Sterk PJ, Schultz MJ. Volatile metabolites of pathogens: a systematic review. *PLoS Pathog.* 2013;9:e1003311.
29. Li J. The cyranose chemical vapor analyzer. *Sensors* 2000;17:56–61.
30. Bialkowska-Hobrzanska H, Jaskot D, Hammerberg O. Molecular characterization of the coagulase-negative staphylococcal surface flora of premature neonates. *J Gen Microbiol.* 1993;139:2939–2944.
31. Sohrabi M, Zhang L, Zhang K, et al. Volatile organic compounds as novel markers for the detection of bacterial infections. *Clin Microbial* 2014; 3:3.
32. Berkhout DJ, Benninga MA, van Stein RM, et al. Effects of sampling conditions and environmental factors on fecal volatile organic compound analysis by an electronic nose device. *Sensors*. 2016;16:1967.
33. Arasaradnam RP, Covington JA, Harmston C, et al. Review article: next generation diagnostic modalities in gastroenterology–gas phase volatile compound biomarker detection. *Aliment Pharmacol Ther.* 2014;39:780–789.
34. Ai L, Tian H, Chen Z, et al. Systematic evaluation of supervised classifiers for fecal microbiota-based prediction of colorectal cancer. *Oncotarget*. 2017;8:9546–9556.
35. Boghossian NS, Page GP, Bell EF, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Late-onset sepsis in very low birth weight infants from singleton and multiple-gestation births. *J Pediatr.* 2013;162:1120.e1–1124.e1.