Fecal volatile organic compound analysis and intestinal microbiota profiling in healthy and diseased infants

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General discussion and future perspectives

Parts of this general discussion has been published in:

Application of Fecal Volatile Organic Compound Analysis in Clinical Practice: Current State and Future Perspectives. el Manouni el Hassani S, Berkhout DJC, Bosch S, Benninga MA, de Boer NKH, de Meij TGJ. Chemosensors 2018, 6, 29.
Over the past decades, an increasing number of studies demonstrated early aberrations in gut microbiota composition to be associated with the development of a wide variety of clinical conditions, both early and later in life. In this thesis we focused on the gut microbiota composition (chapter 2) and derived volatile compounds (chapter 5-7, 9) associated with the development of sepsis and necrotizing enterocolitis (NEC). Based on the currently available literature, the identification of a NEC-associated gut microbiota composition is limited by inter-study variation in study protocol, variation in applied analytical techniques and the fact that majority of the available studies were single-center based and included a limited number of subjects, hampering the ability to make reliable comparisons between these studies. Therefore, in this thesis, gut microbiota composition of the largest cohort of NEC cases so far, included at nine neonatal intensive care units in the Netherlands and Belgium, were compared with the microbiota of matched control infants. In this study, the IS-pro technique was used, a validated DNA-based detection technique allowing for the provision of results within several hours, enforcing its potential as diagnostic tool in daily clinical practice\textsuperscript{1,2}. The relatively large sample size allowed us to stratify cases based on clinical stage (modified Bell’s staging criteria\textsuperscript{3}) and outcome. Based on the obtained results we concluded that infants with a severe phenotype of NEC harbor a distinct gut microbiota composition compared to matched infants without NEC and also with milder phenotypes. This compositional difference could already be observed in a preclinical phase, up to three days prior to onset. This observation is of clinical interest since cases with a severe phenotype of NEC exhibit a poor prognosis. Presumably, in these particular cases, timely intervention may have the most beneficial effect on clinical outcome and may reduce morbidity and mortality rates. Hypothetically, by manipulating this microbial composition in an early phase, for example by administering beneficial bacteria (probiotics) or targeted antibiotics, the onset of this microbiota-associated disease may even be prevented. However, prior to implementation of such therapeutic strategies and to allow for a more in-depth understanding of the etiological role of gut microbes in the onset of NEC and other clinical conditions, it is first key to determine the physiological variability characterizing ‘healthy gut’ state. In addition to determination of the boundaries of a healthy microbiota composition, intrinsic and extrinsic factors influencing this intestinal colonization process should receive research priority. Notably, insights in environmental factors influencing intestinal colonization process would eventually allow for the development of targeted interventions aimed at disease prevention. In line with this, we demonstrated that maternal antibiotic administration during a cesarean section has a significant influence on the initial intestinal colonization of the newborn (chapter 1). Potential health implications later in life resulting from these early in life alterations in gut microbiota composition should be investigated in future studies.

In addition to the number of studies focusing on the human microbiome, the volume of studies focusing on human VOCs has also increased exponentially over the past years. Simultaneously investigating both intestinal microbiota and fecal VOCs would provide
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valuable information concerning the exact pathophysiological mechanisms underlying the development of clinical conditions associated with early in life microbial aberrations. Whereas microbiota analysis provides insight in the presence and absence of specific bacterial species, VOCs provide additional information about the metabolic activity of both the host and present microbes. Moreover, since VOCs can be obtained in a non-invasive manner, they may also serve as an ideal diagnostic biomarker. However, in the currently available literature, the potential of fecal VOCs as diagnostic biomarker has almost exclusively been investigated in proof-of-principle studies. Consequently, an urgent need for the external validation of these results exists. Therefore we investigated the potential of fecal VOCs as early biomarker in several neonatal clinical conditions, associated with preclinical alterations in gut microbiota composition. In three different studies, using two different electronic nose (eNose) devices, we demonstrated that late-onset sepsis (LOS) could be detected preclinically by analyzing fecal VOC composition, underlining the hypothesis that LOS in preterm infants may have a gut origin. Interestingly, discriminative accuracy increased after focusing on cases with identical bacterial species isolated from the blood culture, underlining the potential of fecal VOCs to accurately detect sepsis and the causing pathogen already in a preclinical phase. In addition to LOS we demonstrated that fecal VOCs in infants developing severe bronchopulmonary dysplasia (BPD) could be discriminated from matched control infants not developing BPD (chapter 8). Although no microbial differences between BPD cases and controls could be detected, this early in life alteration in fecal VOC profile may be used as early biomarker in the future, providing clinicians the opportunity to timely intervene in its pathophysiological process. However, in this thesis, we also demonstrated that standardization of the applied methodology is crucial before VOCs could life up to its potential as early biomarker, applicable in daily clinical practice (chapter 4). Multiple environmental and sampling factors were demonstrated to have a significant influence on the detected fecal VOC profile. Development of international standardized protocols concerning the collection, storage, measurement and analysis of the samples increases the ability to make reliable inter-study comparisons.

In addition, identification of disease specific VOCs, using chemical analytical techniques, would eventually allow for the development of eNose sensors specifically designed to accurately detect specific clinical conditions in an early phase. Yet, as demonstrated in chapter 9, chemical analytical techniques may not always provide discriminating VOCs. In this particular chapter, fecal VOCs obtained by means of gas chromatography-mass spectrometry, did not allow for preclinical discrimination between NEC cases and controls. Interestingly, using an eNose device, we previously demonstrated that fecal VOCs allowed for preclinical discrimination between the two groups4. Therefore we hypothesized that a disease specific eNose could also be developed by collecting a large amount of healthy and diseased fecal VOC profiles. Connecting the eNose to a self-learning algorithm will optimize eNose accuracy with each new sample analyzed. Subsequently, newly obtained fecal VOC profiles are compared
with the profiles already available (VOC cloud), determining a possible diagnosis. By connecting an eNose to a self-learning algorithm this eNose accuracy is optimized with each new sample analyzed. The development of such bedside eNose devices could eventually be implemented in daily clinical practice supplementing the currently available diagnostic modalities, allowing for the accurate and early detection of specific clinical conditions. Eventually, this would provide the clinician opportunities to timely initiate therapeutic microbiota-based interventions aimed at prevention of disease.
REFERENCES


