

Proceeding

Development of Electronic-nose Technologies for Early Disease Detection based on Microbial Dysbiosis [†]

Alphus Dan Wilson ^{1,*}, Lisa Beth Forse ²

¹ Pathology Department, Southern Hardwoods Laboratory, Southern Research Station, USDA Forest Service, 432 Stoneville Road, Stoneville, MS 38776, USA; E-Mail: dwilson02@fs.fed.us

² Center for Bottomland Hardwoods Research, Southern Research Station, USDA Forest Service, 432 Stoneville Road, Stoneville, MS 38776, USA; E-Mail: lfwilson@fs.fed.us

* Correspondence: dwilson02@fs.fed.us; Tel.: +1-662-336-4809; Fax: +1-662-336-4829.

† Presented at the 5th International Electronic Conference on Sensors and Applications, 15–30 November 2018; Available online: <https://sciforum.net/conference/ecsa-5>.

Published: 15 October 2018

Abstract: A new frontier in clinical disease diagnostics was quietly launched by a series of recent discoveries of phenomena that make important connections between the metabolic activities of resident microbes and human diseases. Numerous studies have demonstrated that biochemical mechanisms leading to disease development involve not only pathogenesis, but also interactions between microbiota in the oral cavity, lungs, and gut; the microbial metabolites they produce, and the human immune system. Microbial dysbiosis (MD) or changes in commensal microbiota diversity and composition, often modulate disease development by at least two different mechanisms, including disease-induced dysbiosis and alterations in gut microbiota (GM) caused by abiotic and exogenous factors (diet, drug use, and environment). This paper summarizes recent evidence demonstrating how electronic-nose (e-nose) technologies with multi-sensor arrays and chemical-analysis capabilities could potentially be used for early diagnosis of certain diseases by identifying a new category of VOC-biomarker metabolites, called dysbiosis-related disease biomarkers (DRDBs), produced in specific locations of the body due to dysbiosis associated with specific diseases. Recent advances in e-nose technologies offer new tools for exploiting the common occurrence of MD for noninvasive early disease detection.

Keywords: bacterial dysbiosis; dysbiosis-related disease biomarkers; e-nose devices; microbiome composition; noninvasive early diagnosis; volatile organic compounds

1. Introduction

The significant roles that intracorporeal microbiomes (microbial communities) and gut microbiota (GM) play in human health (including effects on digestion, metabolism, immune system, and many other bodily functions), has long been recognized by healthcare providers [1,2]. Human metabolomic and gut metagenomic studies of intestinal microbiota have focused on the mechanisms of disease, the metabolic and regulatory interactions caused by changes in gut microbiome composition, and the associated production of abnormal bacterial metabolites that affect the human immune system. This research has led to a relatively recent homeostasis hypothesis that recognizes a bilateral interactive network of signaling pathways between the gastrointestinal tract (GIT) and the central nervous system (CNS) [3]. Significant alterations in the composition or diversity of microbiota,

known as microbial dysbiosis (MD), has been well established as a major factor affecting pathogenesis for certain human diseases.

This paper focuses on new ways in which e-nose devices could be used for the early diagnosis of MD-related diseases through the detection of abnormal volatile metabolites, produced and released as a result of dysbiosis, which occur in association with the early development of specific human diseases. Volatile metabolites that may potentially serve as early MD-biomarkers of disease, produced in the gut and other locations within the human body, are referred to here as dysbiosis-related disease biomarkers (DRDBs). An initial list of particular types of DRDB-metabolites that may be potentially detected with e-nose devices at early stages of pathogenesis for specific diseases are summarized. In addition, locations within different compartments of the body have been identified where DRDBs may be targeted for e-nose detection through acquisition and analysis of clinical samples. Furthermore, some ways in which DRDBs differ from conventional volatile disease biomarkers are described.

2. Electronic-nose Early Disease Detection

Electronic-nose (e-nose) devices of many types, based on a diverse range of operational mechanisms for chemical detection, have been developed and tested for the noninvasive early detection and diagnosis of a large number of plant, animal, and human diseases [4]. These specialized, but relatively simple gas-sensing instruments have the great capacity to effectively detect and discriminate between complex mixtures of volatile organic compounds (VOCs), including volatile disease biomarker metabolites, present in air samples (e.g. exhaled breath) or in headspace gases derived from solid or liquid clinical samples collected from healthy or diseased patients [5].

The effective application of e-nose technologies for early disease detection has been well established through numerous efficacy studies [4,6]. However, the use of e-nose instruments for early diagnosis of diseases based on the detection of dysbiosis-related VOC-metabolites is still in its infancy. Slow progress in the development of this new area of disease diagnostics is attributed to the lack of information on specific VOC-biomarker metabolite targets generated by MD associated with particular diseases. More research is needed to identify VOC-metabolite types produced by MD and precise locations in the body where DRDBs may be detected in clinical samples taken from diseased patients. Another limiting factor is that some e-nose instruments lack the capability of identifying individual chemical species, particularly disease biomarkers derived from clinical samples. The recent development of e-nose devices with chemical-analysis capabilities has enhanced detection and identification of disease biomarkers [6].

3. Associations of Dysbiosis to Disease

The gut-brain axis monitors bidirectional signals indicating the health-states of the body. Gut microbiota convey information through the GIT to the CNS that elicits a systemic response reflecting nutritional and energy states [3]. When microbial populations fall out of balance causing MD, messages sent to the brain propagate unhealthy signals manifested by low-grade inflammation, increased oxidative stress, unbalanced energy homeostasis and increases in cellular degeneration [7]. The production of gut dysbiosis-related VOC-metabolites may either be formed as a result or consequence of disease (disease-induced dysbiosis) or may actually be the determining factor that favors the initiation of disease development (dysbiosis-induced pathogenesis) due to inhibitory effects on host immunity. The originating causes of MD have not yet been thoroughly elucidated.

4. Dysbiosis-related Disease Biomarkers

The recent discovery of new potential sources of disease-related VOC-metabolites, produced directly by GM or indirectly induced in host metabolic pathways due to MD-related effects on pathogenesis, recently has been recognized as a new noninvasive or minimally-invasive diagnostic approach that could potentially be exploited for early disease detection [6]. The detection and identification of disease VOC-biomarker metabolites associated with MD, as specific indicators of

pathogenesis, should provide a new approach for noninvasive early disease detection using e-nose technologies. A significant number of putative disease biomarkers have been identified through metabolomic studies of individual diseases [5]. The discovery of a new category of potential disease biomarkers (DRDBs), due to GM activities related to MD, have yielded identities of additional VOC target analytes (VOC metabolites) for detection that could facilitate early disease diagnosis.

A variety of human diseases, occurring in different parts of the body, have been associated with MD. Many of these diseases are correlated with systemic inflammation of the human body, primarily caused by GM-related VOC-metabolites that enter the bloodstream as a result of leaky gut syndrome (LGS) [8]. Some examples of potential DBRBs associated with specific diseases in human organs are summarized in Table 1, along with the most probable clinical sample sources (from which these biomarkers may be detected), the GM bacterial taxa involved in MD, and the chemical processes or metabolic pathways by which these biomarkers are formed. All of the DBRBs indicated here are relatively low molecular weight VOCs that are from a wide diversity of chemical classes ranging from short-chain organic (fatty) acids, polyunsaturated omega-3 fatty acids, secondary heterocyclic bile-carboxylic acid derivatives, amino-acid derivatives, cresol derivatives, and amine oxides. The DBRB-category of disease biomarkers are somewhat different from conventional disease biomarkers associated with most human diseases, due to their MB origin, and some (e.g. bile acid derivatives) are from unique chemical classes associated with the IT and digestive system [5,9-11]. Another significant finding, realized from summarizations of clinical sample types and sources that contained DBRBs, was that the vast majority of sample types (sources) identified, among MD-related diseases studied, were found from blood plasma and fecal or intestinal samples. The development of methods for clinical sample collection, sample storage, and e-nose analysis methods correspondingly should be based on the particular sources and types of clinical samples collected for early disease detection.

Table 1. Volatile chemical biomarkers of disease associated with gut bacterial dysbiosis.

Disease ¹	Location/ Organs ²	Disease Biomarkers ³	Sample Source	Gut Microbes ⁴	Formation Mechanism	Ref.
ALS	Systemic	Low SCFA	fecal	BT	Low F/B ratio reduces SCFA	[12,13]
Autism	CNS	XA, QA	urine	Human production	Tryptophan catabolized	[14]
		I3AA, IL	fecal	BT, EB, SR	Tryptophan metab. by IF	[14]
CVD	Heart	TMAO	plasma	CM	Dietary choline to TMA, oxid. in liver	[15,16]
IBD	Intestine	SBA (DCA, LCA)	large intestine	RT, ER, BT	BA ex liver transformed by IF	[17-19]
LC	Liver	PUFA (EPA, AA, DHA)	plasma	CA, EB, LB (IF)	LC modifies fatty acid metab. of IF	[20]
PD	CNS	Pyruvate	plasma	LB	Primary metab. of glycolysis	[21,22]
		BMAA	plasma	CB	Excitotoxin metabolite	[23]
RFD/ELD/MS	Kidney	IS, PAS, PCS	plasma, urine	CS, RC, LS	Fermentation of amino acids in colon	[24]

¹ Disease abbreviations: AD = Alzheimer's disease; ALS = Amyotrophic lateral sclerosis; CVD = Cardiovascular disease; ELD = Early kidney disease; LC = Liver cirrhosis; MS = multiple sclerosis; PD = Parkinson's disease; RFD = Renal function decline. ² Location abbreviation: CNS = central nervous system (brain and spinal cord) ³ Biomarker abbreviations: AA = arachidonic acid; BMAA = β -N-methyl amino-L-alanine; DCA = deoxycholic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; IS = indoxyl-sulfate; LCA = lithocholic acid; PAS = phenylacetylglutamine; PCS = p-cresyl-sulfate; PUFA = polyunsaturated fatty acids; TMAO = trimethylamine-N-oxide (amine oxide); SBA = secondary bile acids; SCFA = short-chain fatty acids (acetate, propionate, n-butyrate). ⁴ Gut microbial taxa abbreviations: BT = Bacteroidetes (phylum); CA = *Candida* spp. (fungi); CB = Cyanobacteria; CM = *Chryseomonas* spp.; CS = Christensenellaceae; EB = Enterobacteriaceae; ER = *Eubacterium rectale*; IF = intestinal microflora; LB = *Lactobacillus* spp.; LS = Lachnospiraceae; RC = Ruminococcaceae; RT = *Ruminococcus torques*; SR = Sutterellaceae.

5. Conclusions

The production of a newly-recognized category of VOC biomarker metabolites, referred to here as dysbiosis-related disease biomarkers produced by MD-associations with particular human diseases, has provided new targets for the noninvasive early detection of diseases using electronic-nose technologies. Our improved understanding of the specific identities and mechanisms of DRDB generation, along with knowledge of probable sites for detection within the body, offer means for improving the reliability and effectiveness of diagnostic methods for early disease detection in clinical practice. This new information could potentially provide important complementary information for confirmations of disease diagnoses based on conventional disease biomarkers, symptomology and other diagnostic tests. Early disease diagnoses allow opportunities for more effective preemptive treatments that significantly improve prognoses, minimize patient recovery time, and reduce total healthcare costs.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Carding, S.; Verbeke, K.; Vipond, D.T.; Corfe, B.M.; Owen, L.J. Dysbiosis of the gut microbiota in disease. *Microb. Ecol. Health Dis.* **2015**, *26*, 26191, doi: 10.3402/mehd.v26.26191
2. DeGruttola, A.K.; Low, D.; Mizoguchi, A.; Mizoguchi, E. Current understanding of dysbiosis in disease in human and animal models. *Inflamm. Bowel Dis.* **2016**, *22*, 1137–1150, doi: 10.1097/MIB.0000000000000750
3. Westfall, S.; Lomis, N.; Kahouli, I.; Dia, S.Y. Microbiome, probiotics and neurodegenerative diseases: deciphering the gut brain axis. *Cell. Mol. Life Sci.* **2017**, *74*, 3769–3787, doi: 10.1007/s00018-017-2550-9
4. Wilson, A.D. Applications of electronic-nose technologies for noninvasive early detection of plant, animal and human diseases. *Chemosensors* **2018**, *6*, e45, doi: 10.3390/chemosensors6040045
5. Wilson, A.D. Biomarker metabolite signatures pave the way for electronic-nose applications in early clinical disease diagnoses. *Curr. Metabolom.* **2017**, *5*, 90–101, doi: 10.2174/2213235X04666160728161251
6. Wilson, A.D. Application of electronic-nose technologies and VOC-biomarkers for the noninvasive early diagnosis of gastrointestinal diseases. *Sensors* **2018**, *18*, e2613, doi: 10.3390/s18082613
7. Noble, E.E.; Hsu, T.M.; Kanoski, S.E. Gut to brain dysbiosis: mechanisms linking western diet consumption, the microbiome, and cognitive impairment. *Front. Behav. Neurosci.* **2017**, *11*, e9, doi: 10.3389/fnbeh.2017.00009
8. Mu, Q.; Kirby, J.; Reilly, C.M.; Luo, X.M. Leaky gut as a danger signal for autoimmune diseases. *Front. Immunol.* **2017**, *8*, 598, doi: 10.3389/fimmu.2017.00598
9. Wilson, A.D.; Baietto, M. Advances in electronic-nose technologies developed for biomedical applications. *Sensors* **2011**, *11*, 1105–1176, doi: 10.3390/s110101105
10. Wilson, A.D. Advances in electronic-nose technologies for the detection of volatile biomarker metabolites in the human breath. *Metabolites* **2015**, *5*, 140–163, doi: 10.3390/metabo5010140
11. Wilson, A.D. Recent progress in the design and clinical development of electronic-nose technologies. *Nanobiosens. Dis. Diagn.* **2016**, *5*, 15–27.
12. Rowin, J.; Xia, Y.; Jung, B.; Sun, J. Gut inflammation and dysbiosis in human motor neuron disease. *Physiol. Rep.*, **2017**, *5*, e13443, doi: 10.14814/phy2.13443
13. Fang, X.; Wang, X.; Yang, S.; Meng, F.; Wang, X.; Wei, H.; Chen, T. Evaluation of the microbial diversity in amyotrophic lateral sclerosis using high-throughput sequencing. *Front. Microbiol.* **2016**, *7*, 1479, doi: 10.3389/fmicb.2016.01479
14. Gevi, F.; Zolla, L.; Gabriele, S.; Persico, A.M. Urinary metabolomics of young Italian autistic children supports abnormal tryptophan and purine metabolism. *Mol. Autism*, **2016**, *7*, 47, doi: 10.1186/s13229-016-0109-5
15. Bennett, B.J.; de Aguiar Vallim, T.Q.; Wang, Z.; Shih, D.M.; Meng, Y.; Gregory, J.; Allayee, H.; Lee, R.; Graham, M.; Croke, R.; Edwards, P.A.; Hazen, S.L.; Lusis, A.J. Trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metab.* **2013**, *17*, 49–60, doi: 10.1016/j.cmet.2012.12.011

16. Koren, O.; Spor, A.; Felin, J.; Fåk, F.; Stombaugh, J.; Tremaroli, V.; Behre, C.J.; Knight, R.; Fagerberg, B.; Ley, R.E.; Bäckhed, F. Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proc. Natl. Acad. Sci. USA* **2011**, *108* (Suppl. 1), 4592–4598, doi: 10.1073/pnas.1011383107
17. Sagar, N.M.; Cree, I.A.; Covington, J.A.; Arasaradnam, R.P. The Interplay of the gut microbiome, bile acids, and volatile organic compounds. *Gastroenterol. Res. Practice* **2015**, *2015*, article ID 398585, doi: 10.1155/2015/398585
18. Malinen, E.; Krogius-Kurikka, L.; Lyra, A.; Nikkilä, J.; Jääskeläinen, A.; Rinttilä, T.; Vilpponen-Salmela, T.; Johannes von Wright, A.; Palva, A. Association of symptoms with gastrointestinal microbiota in irritable bowel syndrome. *World J. Gastroenterol.* **2010**, *16*, 4532–4540, doi: 10.3748/wjg.v16.i36.4532
19. Swidsinski, A.; Weber, J.; Loening-Baucke, V.; Hale, L.P.; Lochs, H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J. Clin. Microbiol.* **2005**, *43*, 3380–3389, doi: 10.3748/wjg.v11.i8.1131
20. Usami M.; Miyoshi, M.; Yamashita, H. Gut microbiota and host metabolism in liver cirrhosis. *World J. Gastroenterol.* **2015**, *21*, 11597–11608, doi: 10.3748/wjg.v21.i41.11597
21. Ahmed, S.S.; Santosh, W.; Kumar, S.; Christlet, H.T. Metabolic profiling of Parkinson's Disease: evidence of biomarker from gene expression analysis and rapid neural network detection. *J. Biomed. Sci.* **2009**, *16*, 63, doi: 10.1186/1423-0127-16-63
22. Hasegawa, S.; Goto, S.; Tsuji, H.; Okuno, T.; Asahara, T.; Nomoto, K.; Shibata, A.; Fujisawa, Y.; Minato, T.; Okamoto, A.; Ohno, K.; Hirayama, M. Intestinal dysbiosis and lowered serum lipopolysaccharide-binding protein in Parkinson's Disease. *PLoS ONE* **2015**, *10*, e0142164, doi: 10.1371/journal.pone.0142164
23. Nair, A.T.; Ramachandran, V.; Joghee, N.M.; Antony, S.; Ramalingam, G. Gut microbiota dysfunction as reliable non-invasive early diagnostic biomarkers in the pathophysiology of Parkinson's disease: A critical review. *J. Neurogastroenterol. Motility* **2018**, *24*, 30–42, doi: 10.5056/jnm17105
24. Barrios, C.; Beaumont, M.; Pallister, T.; Villar, J.; Goodrich, J.K.; Clark, A.; Pascual, J.; Ley, R.E.; Spector, T.D.; Bell, J.T.; Menni, C. Gut-microbiota-metabolite axis in early renal function decline. *PLoS ONE* **2015**, *10*, e0134311, doi: 10.13371/journal.pone.0134311

