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EDITORIAL

Changes in lung microbiome do not explain the development of ventilator-associated pneumonia

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Not so long ago, pneumonia seemed such an easy disease to be explained: if a sufficient number of copies of a pathogen entered the lung, then an infection would ensue; for infection clearance, the host would need some help from antibiotics and that was it. Ventilator-associated pneumonia (VAP) was assumed to occur simply because we maintained an artificial "access road" towards the sterile lung. Over the past decade, we have learned that the lung is never sterile, even in healthy conditions. Thus, the following question arose: should we redefine pneumonia pathophysiology in ecological terms? [1].

In this issue of the journal, Emonet et al. present the results of an observational matched case–control study [2]. They performed metataxonomical analysis of bronchoalveolar lavage (BAL) fluid, endotracheal aspirates (ETA) and oropharyngeal swabs (OPS). The study included 18 cases and 36 matched controls and samples were collected at multiple time points per patient. The authors report a negative correlation between presence of bacilli at start of mechanical ventilation and pneumonia development. However, the predictive accuracy of any of the microbial indices was relatively low and the authors concluded that other approaches such as metagenomics or combinations with expression of human genes might be more suitable for VAP prediction. Last, they detected more difficult-to-culture organisms, such as mycoplasma species in patients with VAP. Let us put these results into perspective.

Several of the results that were observed by Emonet et al. are consistent with previous studies. Duration of mechanical ventilation is associated with a lower microbial diversity, irrespective of the use of antibiotics or development of pneumonia [3]. The lower diversity could be attributed to a decline of oropharyngeal bacteria such as *Streptococcus* species in BAL and ETA and an increase in *Pseudomonadales* and other Proteobacteria [3]. The current study confirms that these changes do not only occur in the lower airways, but also in the oropharynx, which is considered to be the main "island" leading to pulmonary colonization according to the adapted island model [1, 4, 5]. This ecological model suggests that any change in oropharyngeal flora may be associated with a change in pulmonary microbiome.

Previous studies suggested a larger change in the lung microbiome in patients who developed pneumonia than in those who did not [3, 6], but this was not confirmed in this study. Instead, the presence of bacilli in lower respiratory samples was found to be negatively predictive of VAP, days before the infection occurred. This difference disappeared at the moment of diagnosis. How can a lack of difference at the moment of diagnosis be explained? What does define VAP if it is not the composition of the lung bacterial community?

Metataxonomics analysis, the technology used for this study, relies on amplification of the 16s gene. This technique has limitations and some species are better differentiated than others. For example, it is notoriously difficult to differentiate between *Streptococcus* species based on 16s sequencing. However, this is insufficient to explain the lack of difference between patients with and without pneumonia in this study because the most prominent causative pathogens were Gram-negative gut

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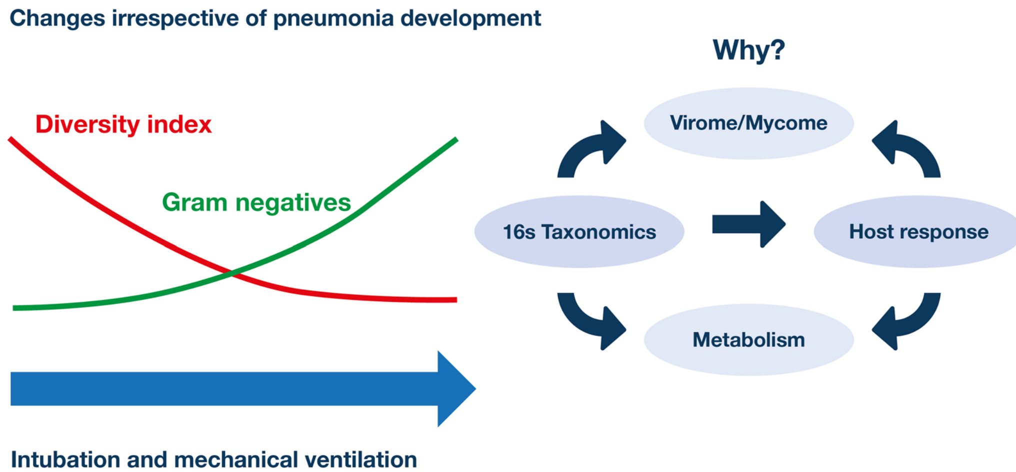

Fig. 1 The lung microbiome changes during mechanical ventilation irrespective of the development of ventilator-associated pneumonia

bacteria, typically well differentiated by the technique used in this study.

An alternative explanation for similarity in lung microbiome between patients with and without VAP might be that taxonomical analysis captures the static composition but not the activity and replication of the lung microbiome. Maybe the combination of taxonomical analysis with metabolomic and transcriptomic markers of pathogenic activity could provide better differentiation for VAP. A different approach could be done by including markers of host response, which may be as or more relevant to the VAP pathophysiology than the microbiome. Emonet et al. actually measured human DNA in the respiratory tract samples and found a much higher human DNA count with VAP, compared to patients without VAP, and it also showed the best discriminative power. Previous studies have also shown good discriminative power of markers of host response suggesting that it might be more about the response to the infection than the microbial composition itself [7, 8]. In fact, the host response may be among the most important determinants of survival in patients with sepsis, which is commonly caused by respiratory infections [9]. One more explanation for the findings of this study could be related to the lung virome and mycobiome, both of which interact with bacterial and host cells, but have been less investigated than the lung bacteriome.

Another finding from Emonet et al. is regarding the molecular detection of difficult-to-culture organisms, such as *Mycoplasma* spp. which could imply that standard laboratory techniques are missing these organisms. However, we conjecture this is unlikely due to two reasons. First, the simple DNA detection does not mean that a "pathogen" is present because DNA alone may represent normal flora, colonization, shedding, or dead organisms—none of this would per se indicate a pathogen causing an infection process like pneumonia. While sensitivity is unquestionably increased by molecular assays, this happens at the cost of specificity. Second, *Mycoplasma* spp. is a very rare cause of hospital-acquired pneumonia, even when molecular techniques are used in addition to standard assays [10].

Emonet et al. have elegantly shown that it is time to let go of any simplistic view of VAP pathogenesis. VAP is most certainly not about a single pathogen simply being present and thus causing pneumonia. VAP is a consequence of the disruption of the dynamic interface among the bacteriome, virome, mycobiome, host cells, and their metabolites (Fig. 1). Future lung microbiome studies should evaluate the bacterial, viral, and fungal milieu through metagenomics techniques in conjunction with transcriptomics and metabolomics to elucidate their interaction with the host.

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References

1. Dickson RP, Erb-Downward JR, Huffnagle GB, Martinez FJ, Huffnagle GB, Kollef MH, May AK, Brady JS, Romano-Keeler J, Drake WP, Norris PR, Jenkins JM, Isaacs RJ, Boczko EM, Toma I, Siegel MO, Keiser J, Yakovleva A, Kim A, Davenport L, Devaney J, Hoffman EP, Alsubail R, Crandall KA, Castro-Nallar E, Perez-Losada M, Hilton SK, Chawla LS, McCaffrey TA, Simon GL (2014) Towards an ecology of the lung: new conceptual models of pulmonary microbiology and pneumonia pathogenesis. Lancet Respir Med 147:238–246. https://doi.org/10.1128/JCM.01678-14
2. Emonet S, Lazarevic V, Leemann Refondini C, Gaïa N, Leo S, Girard M, Nocquet Boyer V, Wozniak H, Després L, Renzi G, Mostaguir K, Dupuis Lozeron E, Schrenzel J, Pugin J (2019) Identification of respiratory microbiota markers in ventilator-associated pneumonia. Intensive Care Med. https://doi.org/10.1007/s00134-019-05660-8
3. Zakharkina T, Martin-loeches I, Matamoros S, Povoa P, Torres A, Kastelijn JB, Hofstra JJ, De Wever B, De Jong M, Schultz MJ, Sterk PJ, Artigas A, Bos LD (2017) The dynamics of the pulmonary microbiome during mechanical ventilation in the intensive care unit and the association with occurrence of pneumonia. Thorax 72:1–8
4. Dickson RP, Erb-Downward JR, Freeman CM, McCloskey L, Beck JM, Huffnagle GB, Curtis JL (2015) Spatial variation in the healthy human lung microbiome and the adapted island model of lung biogeography. Ann Am Thorac Soc 12: 821–830 http://www.ncbi.nlm.nih.gov/pubmed/25803243
5. Roux D, Van Oort PM, Ricard J-D, Bos LDJ (2017) Airway microbiome research: a modern perspective on surveillance cultures? Ann Transl Med 5:1–7
6. de Steenhuijsen Piters WAA, Huijskens EGWW, Wyllie AL, Biesbroek G, Van Den Bergh MR, Veenhoven RH, Wang X, Trzciński K, Bonten MJ, Rossen JWA, Sanders EAMM, Bogaert D, Trzcinski K, Bonten MJ, Rossen WAJ, Sanders EAMM, Bogaert D (2015) Dysbiosis of upper respiratory tract microbiota in elderly pneumonia patients. ISME J 10:97
7. Morris AC, Kefala K, Wilkinson TS, Moncayo-Nieto OL, Dhaliwal K, Farrell L, Walsh TS, Mackenzie SJ, Swann DG, Andrews PJD, Anderson N, Govan JRW, Laurenson IF, Reid H, Davidson DJ, Haslett C, Sallenave JM, Simpson AJ (2010) Diagnostic importance of pulmonary interleukin-1β and interleukin-8 in ventilator-associated pneumonia. Thorax 65:201–207
8. Wilkinson TS, Conway Morris A, Kefala K, O'Kane CM, Moore NR, Booth NA, McAuley DF, Dhaliwal K, Walsh TS, Haslett C, Sallenave JMM, Simpson AJ (2012) Ventilator-associated pneumonia is characterized by excessive release of neutrophil proteases in the lung. Chest J 142:1425–1432
9. Kalil AC, Syed A, Rupp ME, Chambers H, Vargas L, Maskin A, Miles CD, Langnas A, Florescu DF (2015) Is bacteremic sepsis associated with higher mortality in transplant recipients than in nontransplant patients? a matched case-control propensity-adjusted study. Clin Infect Dis 60:216–222
10. Hagel S, Schmitt S, Kesselmeier M, Baier M, Welte T, Ewig S, Pletz MW (2019) M. pneumoniae and C. pneumoniae are no relevant pathogens in critically ill patients with hospital-acquired respiratory tract infections. Infection 47:471–474. https://doi.org/10.1007/s15010-019-01273-x