Microbiome in Critical Illness

Francesco Forfori

Department of Surgical,
Medical, Molecular Pathology
and Critical Care Medicine
University of Pisa
Pisa, Italy

francesco.forfori@unipi.it

Samuele Ferrari

Department of Surgical,
Medical, Molecular Pathology
and Critical Care Medicine
University of Pisa
Pisa, Italy

**samuele.ferrari.md@gmail.
com**

Alessandro Isirdi

Department of Surgical,
Medical, Molecular Pathology
and Critical Care Medicine
University of Pisa
Pisa, Italy

alessandroisirdi@gmail.com

Francesco Corradi

Department of Surgical,
Medical, Molecular Pathology
and Critical Care Medicine
University of Pisa
Pisa, Italy

francesco.corradi@unipi.it

Microbiome in Sepsis and COVID-19

Bacteria play a fundamental role in human life. Given the many roles of gut microbiota in critical illness and other pathological conditions, it is important to target therapeutical interventions to restore, preserve and enrich its composition.

Introduction

Bacteria play, for better or worse, a fundamental role in human life. As multidrug resistant bacterial infections are increasing in incidence and mortality, we often consider only the negative impact of bacteria on human life and forget the positive side of the "bacterial coin" - the microbiota. Commensal microbes are critical components that contribute to maintain and promote our health in a complex variety of ways. The gut microbiota is now regarded as an organ with roles in shaping our immunity, host defense and intestinal maturation and function (Moron et al. 2019).

Intestinal Epithelium and Commensal Flora

Intestinal mucosa is composed of epithelial cells closely joined together by tight junctions acting as a barrier to restrict substance passage between cells. Epithelial cells are anchored to a thin layer of connective tissue that hosts immune cells underneath which lies the muscolaris mucosae. Other mechanisms of intestinal defense include gut associated lymphatic tissue (GALT) and mesenteric lymph nodes, mucus production and commensal bacteria; together they compose the intestinal barrier (Assimakopoulos et al. 2018).

Microbiota functions are executed mainly through its composition: commensal microorganisms compete with opportunistic pathogens for adhesion sites and nutrients creating a first line of defense against bacterial translocation (Wang et al. 2019). Additionally, commensal microbes shape the mucosal immune system by regulating T cells expansion and differentiation, dendritic and macrophage activation and B cells produced IgA (Yamashiro 2017). T cells dependent IgA are induced in response to specific microbes in the gut and protect against lethal sepsis following intestinal barrier disruption. Their concentrations depend on a rich and diverse microbiota; in particular Proteobacteria resulted in increased IgA concentrations in murine models (Wilmore et al. 2018).

Alterations in gut microbiota composition may promote a selection of bacteria with the genetic capability to metabolise only specific molecule reducing microbiota

protective functions (Moron et al. 2019). Microbial fermentation is necessary both for nutrients uptake and immune system communication and modulation to pathogens. Short chain fatty acids (SCFA) act as mediators for epithelial cells in the gut: propionate, acetate and butyrate are energy sources for epithelial cells as well as modulators of cytokine production (Schirmer et al. 2016). An example is Clostridia, a well-represented commensal microbe, that regulates epithelial permeability to food antigens and, in response to butyrate, induces Treg cells differentiation suppressing inflammatory and allergic responses (Yamashiro 2017; Schirmer et al. 2016). In addition, bacteria-derived butyrate affects epithelial oxygen consumption and results in stabilisation of hypoxia-inducible factor (HIF), a transcription factor coordinating barrier protection (Kelly et al. 2015). All these aspects are essential to establish and maintain gut barrier integrity protecting from infection and regulating immune response.

Dysbiosis

Critically Ill Patients: Antibiotics and Sepsis

There are several reasons for microbiota changes: age, gender, diet and drugs as well as host conditions such as critical illness. Variations in compositions lead to a lack of diversity and richness creating a state of dysbiosis or *pathobiome* characterised by an increased pro-inflammatory profile and decreased protective factors as mucus layer, SCFAs, epithelial integrity

and permeability often associated with decreased nutrients absorption (Moron et al. 2019). On a cellular level macrophages of the lamina propria exposed to acute inflammatory stimuli, in the presence of butyrate, inhibit the synthesis of NF-κB induced pro-inflammatory mediator such as TNF-α, IL-6 IL-12 and increase expression of anti-inflammatory mediators and promote epithelial integrity (Parada et al. 2019). However, in the presence of inflammation, cellular mechanisms are reversed and a vicious cycle takes place: decreased SCFAs production, due to altered microbiota, leads to increased pro-inflammatory mediators and decreased anti-inflammatory mediators causing a decreased epithelial barrier integrity and further inflammation (Parada et al. 2019).

A reduction in intestinal barrier integrity is a high-risk factor for bacterial translocation and subsequent sepsis. Gut microbiota is not only a risk factor – when altered – for sepsis but has also been shown to modulate host response to sepsis in animal models (Adelman et al. 2020). It is quite common for patients, particularly in ICUs, to receive antibiotics and subsequently develop dysbiosis especially considering that hospitalisation alone is associated with gut microbiota alterations and consequent severe sepsis (Prescott et al. 2015). In critical care patients, within 48 hours of admission and throughout hospitalisation, microbial ecosystems of the mouth and skin, not just the gut, are flooded with antibacterial resistant pathogens with large personal and interpersonal variations in composition (Lankelma et al. 2017; McDonald et al. 2016). In a study patients that received antibiotics during hospital stay had a higher risk of developing sepsis within 90 days of discharge identifying third and fourth generation cephalosporines, fluoroquinolones, lincosamides, beta lactam/lactamase inhibitors, oral vancomycin and carbapenems as high risk and first or second generation cephalosporins, macrolide, tetracycline, metronidazole as low risk for developing

sepsis after discharge (Baggs et al. 2018). Risk factors for developing sepsis were not limited to the type of antibiotic administered but also included the overall number of antibiotic classes used and therapy duration (Baggs et al. 2018). Other drugs commonly used in ICUs such as proton pump inhibitors and opioids contribute to microbiome changes in different body sites creating, shortly after ICU admission, a loss in specificity and a subsequent constant decrease in colonisation resistance (Haak

> ❝❝ **the gut microbiota is now regarded as an organ with roles in shaping our immunity, host defense and intestinal maturation and function** ❞❞

et al. 2017; Yeh et al. 2016).

Mechanisms responsible for "a leaky gut" can both be a cause and a result of sepsis and are not only represented by dysbiosis but extend to incorporate hypoperfusion with tissue inflammation, increased permeability and bacterial translocation (Adelman et al. 2020). Microbial community structures, through opportunism, initiate and drive gut permeability; stress-induced intestinal permeability defects depend on microbial phenotype, though ligands and pathways involved in sepsis remain unknown (Alverdy et al. 2017). Additionally, altered gut flora has been proposed as a potential prognostic marker in patients with SIRS: obligate anaerobes decrease and increase in pathogenic microbes in the gut are associated with septic complications and mortality in SIRS (Shimizu et al. 2011).

Dysbiosis and COVID-19
Commensal microbes are also found in the lungs, but their growth is regulated by mucociliary clearance, surfactants, and lack of nutrients however, in case of injury

(i.e., large tidal volumes during mechanical ventilation, ARDS or pneumonia) inflammation causes protein rich fluid deposits in the alveoli providing a new energy source in addition to steep oxygen gradients favouring bacterial growth (Dickson 2016). For instance, catecholamines produced in response to activated innate immunity cells, combined with inflammatory cytokines, alter bacterial composition to favour *P. aeruginosa*, *S. pneumoniae* and *S. aureus* growth in the lungs (Dickson 2016).

Upper respiratory tract infections not only change lung microbiome but also impact gut microbiota. A cross sectional study on the effects of viral respiratory diseases on gut microbiome alterations showed that patients with H1N1 influenza and COVID-19, when compared to healthy controls, have decreased community richness and microbial diversity (Gu et al. 2020). Viral infections weaken the gut-lung axis by decreasing lung immunity, in terms of cell number and function, while simultaneously promoting gut dysbiosis (Sencio et al. 2021). When combined, these factors decrease SCFAs, TLR stimulation, barrier protection and antimicrobial peptides (AMPs) and increase inflammatory cytokines leading to uncontrolled pulmonary and enteric bacterial superinfection (Sencio et al. 2021).

However, this may not be the case when healthy microbiota is present and able to control SARS-CoV-2 lung infection by stimulating production of a large number of immune cells (Rajput et al. 2021). There is evidence suggesting a relationship, either in the form of 'gut lung axis' – where the gut microbiota is affecting the lungs – or in the form of immunomodulatory signals released by the gut microbiome (Rajput et al. 2021). After viral infection, immune cells in the airway, such as dendritic cells and macrophages, secrete cytokines to defend against pathogens (Mahooti et al. 2020). In probiotic-receiving subjects, high cytokine concentrations lead to immune cells migration from the gut to the lung

space through the gut–lung axis, resulting in rapid recruitment of activated T and B cells promoting upregulation of virus-specific immunoglobulins and cytokines; on the contrary, in the absence of activated immune cells, respiratory virus can cause severe lung damage due to lack of immediate immune response (Mahooti et al. 2020).

Dysbiosis was found to persist in COVID-19 patients from hospitalisation to recovery, and is characterised by decreased SCFAs producing commensals (*Eubacterium, Faecalibacterium, Roseburia*), and increased opportunistic pathogens (*Clostridium hathewayi, Actinomyces viscosus, Bacteroides nordii*) (Yeoh et al. 2021; Zuo et al. 2020). Disease severity and immune system dysfunction depend on dysbiosis as immunomodulatory commensals depletion contributes to severe forms of COVID-19 (Yeoh et al. 2021; Zuo et al. 2020). In fact, a possible explanation for COVID-19 related multi organ dysfunction is gut barrier disruption – favoured by old age, hypertension, diabetes and obesity – which causes SARS-CoV-2 to seep out of the gut and spread throughout the body causing severe inflammation due to a hyper immune response (Kim 2021). This exaggerated response is supported by altered tight junctions, apoptosis and pro-inflammatory signalling causing endogenous endotoxins passage to the circulatory system boosting pro-inflammatory activity via NF-κB pathways and Spike protein bound to LPS (Belančić 2020).

Therapeutic Approaches
Probiotics and Sepsis
Immune actions of probiotics mainly consist of inflammation response and modulation to pathological stimuli. Immune stimulation causes macrophages, dendritic cells, neutrophils and NK cells to increase their activity, as well as cytokines promoted Th1/Th17 polarisation in the gut mucosa (de Oliveira et al. 2021). On the other hand, anti-inflammatory functions are performed by certain probiotic strains, through dendritic cell modulation, and are capable of inducing regulatory T cells and IL-10, TGF-β production thus enhancing IgA secretion and gut barrier function (de Oliveira et al. 2021). A systematic review on the use of probiotics in critical illness found that the use of probiotics resulted in significant reduction in infection rates particularly in ventilation acquired pneumonia, and further subgroup analysis found the greatest improvement, in terms of infection outcomes, to be in critically ill patients (Manzanares et al. 2016). This may be because microbial fermentation products of a healthy gut – for example, bifidobacterial producing acetate – improve epithelial intestinal defense protecting against lethal infection (Fukuda et al. 2011). Moreover, in critical patients with end organ damage caused by sepsis, acetate was also found to ameliorate sepsis-induced acute kidney injury (AKI) by inhibiting NADPH oxidase signalling and restoring oxidative balance in T cells (Al-Harbi et al. 2018).

Probiotics and Respiratory Tract Infections
As mentioned before, microbiota plays a large role in protecting and modulating responses to respiratory pathogens. A possible therapeutic strategy may include oral administration of lactobacillus rhamnosus which has been shown to control immune response after viral infection by mobilising Th1 cells from the intestine to the respiratory tract to produce IFNγ and recruit local respiratory immune cells (Villena et al 2012). Its role is also supported by a RCT in which lactobacillus rhamnosus reduced rhinovirus infection rates in preterm infants (Luoto et al 2014). Although probiotics are better than placebo in reducing the number of acute episodes of upper respiratory tract infections evidence quality is low (Hao et al. 2015). At this time, unfortunately, there are no systematic reviews examining the effects of probiotics on COVID-19 patients, however, evidence collected by systematic reviews on critically ill patients, particularly those on mechanical ventilation, concluded that probiotics improve outcomes even if evidence was low in quality (Rozga et al 2021). Therefore, due to the lack of direct evidence in COVID-19 patients, the best resources to guide therapeutical approaches using probiotics come from comparable studies (Rozga et al 2021).

Conclusion
The concept of dysbiosis is specific to each person and it can be interpreted as a relative change in composition when compared to others in the community: loss of diversity, increased pathogenic and decreased beneficial bacteria (Bassetti et al 2020). Given the many roles of gut microbiota in critical illness (Dickson 2016), as well as other pathological conditions, we should target therapeutical interventions to restore, preserve and enrich its composition.

Conflict of Interest
None. ◼

References

Adelman MW, Woodworth MH, Langelier C et al. (2020) The gut microbiome's role in the development, maintenance, and outcomes of sepsis. Crit Care, 24(1):278.

Al-Harbi NO, Nadeem A, Ahmad SF et al. (2018) Short chain fatty acid, acetate ameliorates sepsis-induced acute kidney injury by inhibition of NADPH oxidase signaling in T cells. Int Immunopharmacol, 58:24-31.

Alverdy JC, Krezalek MA (2017) Collapse of the microbiome, emergence of the pathobiome and the immunopathology of sepsis. Crit Care Med, 45(2):337-347.

Assimakopoulos SF, Triantos C, Thomopoulos K et al. (2018) Gut-origin sepsis in the critically ill patient: pathophysiology and treatment. Infection, 46(6):751-760.

Baggs J, Jernigan JA, Halpin AL et al. (2018) Risk of Subsequent Sepsis within 90 Days of a Previous Hospital Stay by Type of Antibiotic Exposure. Clin Infect Dis Off Publ Infect Dis Soc Am, 66(7):1004-1012.

Belančić A (2020) Gut microbiome dysbiosis and endotoxemia - Additional pathophysiological explanation for increased COVID-19 severity in obesity. Obes Med, 20:100302.

For full references, please email editorial@icu-management.org or visit https://iii.hm/1c6e