CHEST

Blurred Lines on the Dysbiosis Spectrum

Pneumocystis Colonization vs Infection by Metagenomics

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Throughout history, the terms *colonization* and *invasion* have been used to describe the establishment of foreign settlements, yet they carry vastly different connotations. Formerly, colonization was viewed as a benign process of establishing new territories. However, this perspective has been thoroughly debunked, particularly in light of the experiences of indigenous populations who faced displacement, violence, and cultural erasure—precisely what the term *invasion* conveys.

A similar parallel can be drawn in the clinical management of lower respiratory tract (LRT) infections, where pathogen *colonization* and *infection* are traditionally seen as distinct clinical states requiring different treatments. Pathogen colonization is often perceived as an innocuous presence in the LRT, whereas infection is defined by host inflammatory responses and damage induced by proliferating pathogens.[1,2] Nonetheless, studies of the LRT microbiome have transformed our understanding of host-pathogen interactions.[3] The traditional dichotomy of *colonization* vs *infection* appears overly simplistic, failing to encompass the broad spectrum of microbiota deviations from health and homeostasis, collectively referred to as *dysbiosis*.[3]

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DOI: https://doi.org/10.1016/j.chest.2024.08.038

In this issue of *CHEST*, Jiang et al[4] explore the nuances of *Pneumocystis jirovecii* colonization (PJC) and its implications for pneumonia outcomes.[4] *P jirovecii* is an opportunistic fungal pathogen transmitted human-to-human via the airborne route, capable of causing severe pneumonia in immunocompromised hosts, particularly those infected with HIV.[5] Humans are considered the primary reservoir for *P jirovecii*, because the organism requires a living host to survive, and viable forms are unlikely to persist in the environment.[6,7] With the recent development of molecular detection methods for *P jirovecii* in LRT specimens, there is growing recognition that *P jirovecii* also may act as a colonizer of the LRT and has been implicated in airways disease pathogenesis, potentially through stimulating inflammation.[8,9] However, its role in pneumonia caused by other pathogens remains poorly defined.

To address this knowledge gap, Jiang et al[4] conducted a multicenter, retrospective study involving 1,787 patients with severe pneumonia.[4] These patients underwent testing of bronchoalveolar lavage fluid using clinical metagenomics for comprehensive screening of LRT microbiota through untargeted DNA sequencing. Their analysis revealed a positive detection rate of 9.2% (n = 160) for *P jirovecii* DNA among patients not infected with HIV. A consensus committee evaluated host factors, clinical characteristics, and beta-D-glucan testing to categorize these 160 patients into two groups: *P jirovecii* pneumonia (PJP, 60%, n = 95) and PJC (40%, n = 65). These groups were compared with the 1,577 patients who tested negative for *P jirovecii*, serving as pneumonia control patients (Fig 1).

Comparisons among the three groups—PJP, PJC, and pneumonia control patients—revealed significant differences in host-level factors, pathogen detection, and clinical outcomes, highlighting a continuum of host defense impairments and varying levels of illness severity. Patients with PJP and PJC exhibited a higher prevalence of immunosuppressive conditions and significantly lower lymphocyte counts.

Metagenomic analysis indicated that pneumonia control patients had higher detection rates of typical gram-negative pathogens, such as *Acinetobacter* and *Klebsiella* species. In contrast, patients with PJC showed a

higher prevalence of *Candida* species, whereas patients with PJP had higher prevalence of *Aspergillus* species. Furthermore, both patients with PJP and those with PJC had increased rates of detection of latent DNA viruses, such as cytomegalovirus and Epstein-Barr virus, compared with pneumonia control patients. These findings indicate a pattern of fungal and viral dysbiosis, characterized by increased detection of potentially co-colonizing fungal organisms and the recrudescence of latent viruses in *P jirovecii*-positive immunosuppressed hosts.

Notably, both patients with PJP and those with PJC experienced similarly poor outcomes, with a staggering 28-day mortality rate of 50%. The authors employed statistical adjustments and sensitivity analyses to account for clinical, biological, microbiota, and treatment differences between patients with PJC and pneumonia control patients. A statistically robust signal persisted, with patients with PJC exhibiting a 50% to 80% increased hazard of death compared with those who tested negative for *P jirovecii*.

The strengths of this study include its large sample size, rapid turnaround of clinical metagenomics, and meticulous efforts to differentiate between PJP and PJC. However, the study has limitations. A central challenge lies in classifying PJP vs PJC without a definitive reference standard. Additionally, the investigators used clinically

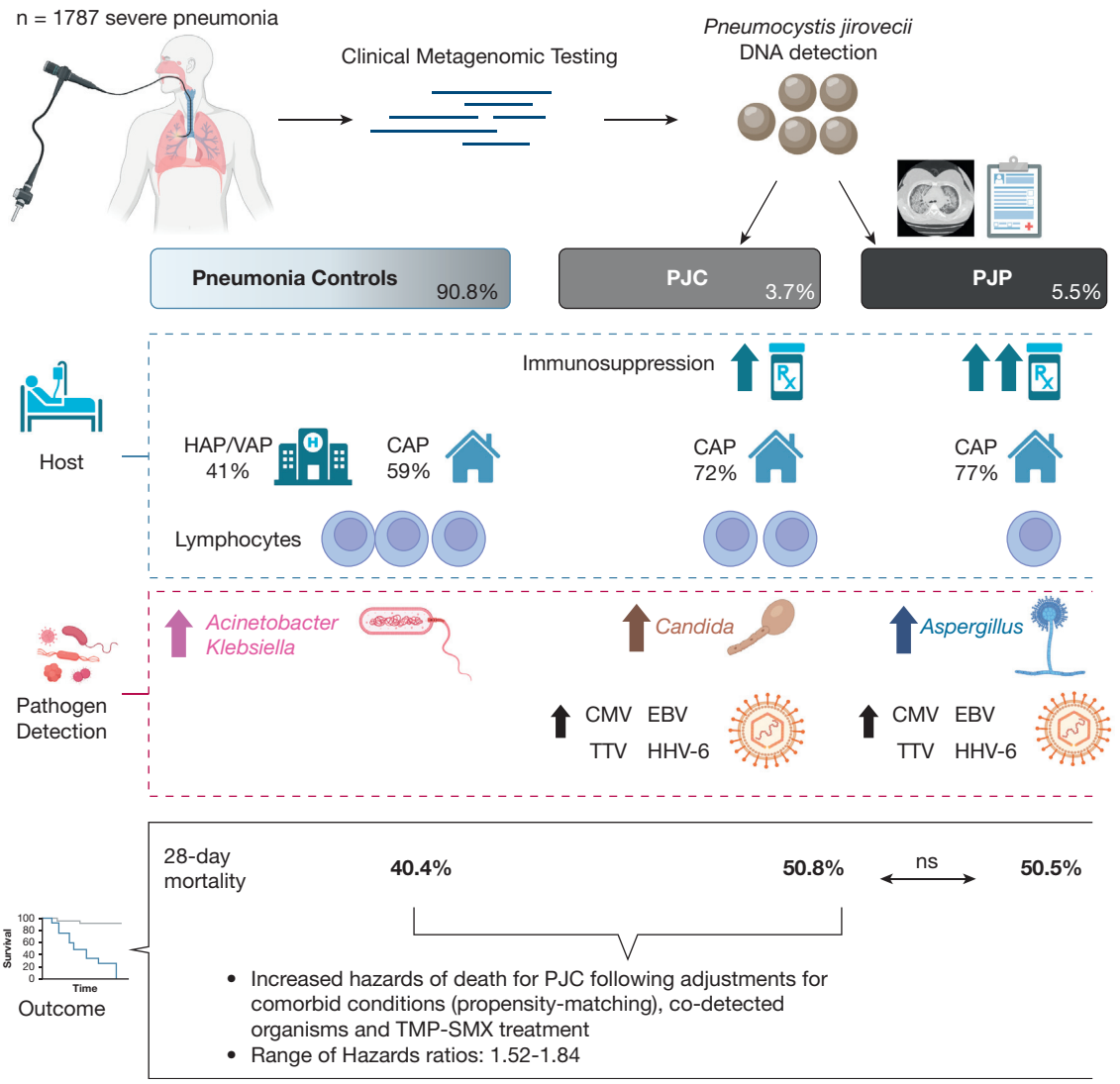

Figure 1 – *Major findings from the study by Jiang et al*[4] *comparing host factors, pathogen detection by metagenomics, and clinical outcomes between patients diagnosed as* Pneumocystis jirovecii *pneumonia (PJP),* Pneumocystis jirovecii *colonization (PJC), and pneumonia control patients who tested negative for* Pneumocystis jirovecii. *CAP = community-acquired pneumonia; CMV = cytomegalovirus; EBV = Ebstein-Barr virus; HAP = hospital-acquired pneumonia; HHV-6B = human herpes virus 6B; TMP-SMX = trimethoprim/sulfamethoxazole; TTV = torque teno virus; VAP = ventilator-associated pneumonia.*

available metagenomic testing, which is rarely accessible outside of China; elsewhere, *P jirovecii* molecular testing is typically performed using quantitative polymerase chain reaction (qPCR). Previous studies have shown similar detection rates for *P jirovecii* and high inter-platform agreement between qPCR and metagenomics,[10] suggesting the results may be generalizable to clinical settings with access to qPCR. Furthermore, the current study lacks a quantitative analysis of *P jirovecii* relative and absolute abundance, as well as an integration of microbiota profiles beyond categorical variable analysis for pathogen detection. Host response profiles were not integrated in diagnostic classifications or outcome predictions. Finally, the observational design of the study limits the ability to draw conclusions regarding the causal effects of PJC, or the response to trimethoprim-sulfamethoxazole treatment, which was used in one-half of patients with PJC.

Despite these limitations, the study by Jiang et al[4] enhances our understanding of LRT ecology in severe pneumonia and challenges the perception of PJC as benign. Although PJC may not be causal for adverse prognosis, the similar outcomes to PJP underscore an immunosuppressed state characterized by fungal and viral dysbiosis. These findings align with evidence linking LRT bacterial dysbiosis,[11-13] high *Candida albicans* abundance,[12] and elevated circulating beta-D-glucan levels with poor outcomes in critical illness.[14] Although whether PJC serves as a precursor to PJP remains unclear, these data emphasize that detection of *P jirovecii* should not be overlooked. Even if it serves as an adverse predictor of outcomes rather than a direct treatment target, prospective longitudinal studies are needed to deepen our understanding of fungal and other organismal colonization in the LRT.

In summary, the study by Jiang et al[4] highlights the impact of PJC on severe pneumonia, demonstrating its association with higher mortality and a greater prevalence of immunosuppressive conditions. These findings underscore the urgent need for further research into the dynamics of LRT microbiota, to identify clinically relevant states of dysbiosis, ranging from low-level colonization to full-blown infection, and refine our molecular diagnostic tools for pneumonia. By drawing from the historical parallel and simplistic dichotomy of colonization vs invasion, we can develop a more nuanced understanding of the role of colonizing pathogens such as *P jirovecii* in the LRT. This deeper insight will ultimately improve our diagnostic and treatment strategies for pneumonia.

Funding/Support

G. D. K.: American Lung Association COVID-19 Respiratory Virus Award; University of Pittsburgh Department of Medicine KARAT Award; NIH R03 HL162655 and R01 HL1641677.

Financial/Nonfinancial Disclosures

The authors have reported to *CHEST* the following: G. D. K. has received research funding from Genentech, Inc and Pfizer, Inc, unrelated to this work. A. M. has received research funding from Pfizer, Inc, unrelated to this work.

Acknowledgments

Author contributions: G. D. K. conceptualized the study and wrote the first draft of the manuscript. A. M. critically reviewed and edited the manuscript, providing valuable input and revisions. Both authors approved the final version of the manuscript and are accountable for all aspects of the work.

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

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