University of Louisville Journal of Respiratory Infections

OPINION PIECE

A Research Framework for Evaluating Next Generation Sequencing in Community-Acquired Pneumonia

Ashley Wilde[1], PharmD; Elena A. Swingler[1], PharmD; Sarah E. Moore[1], PharmD; Brian C. Bohn[1], PharmD; Matthew Song[1], PharmD; Ruth Carrico[1] PhD; Julio Ramirez[1], MD*

[1]Norton Infectious Disease Institute, Norton Healthcare, Louisville, KY, USA

*j.ramirez@louisville.edu

Recommended Citation: Wilde A, Swingler EA, Moore SE, et al. A research framework for evaluating next generation sequencing in community-acquired pneumonia. Univ Louisville J Respir Infect **2021**; 5(1):Article 32.

Introduction

Rapid diagnostic technologies are revolutionizing the clinical microbiology laboratory. Next generation sequencing (NGS) is poised to be the next powerful tool in standard clinical laboratories building on the widespread adoption of multiplex polymerase chain reaction (PCR) panels and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) technology.[1] NGS can provide a quantitative analysis of all non-human DNA or RNA in a sample without requiring growth on a traditional medium. This improves the diagnostic yield of infections that are difficult to culture due to biofilm production, such as prosthetic joint infections.[2] As these technologies become faster and cheaper, research efforts are urgently needed to guide clinicians to wider applications of NGS, including use in non-sterile sites, such as lower and upper respiratory tract samples. The diagnostic utility of NGS of respiratory samples has already been noted in cases of pneumonia caused by pathogens that are difficult to identify through conventional testing.[3, 4] However, the use of NGS as a diagnostic tool in community-acquired pneumonia (CAP) remains to be elucidated. The characterization of the respiratory microbiome in clinical practice may improve the diagnosis and therefore the treatment of CAP. However, without adequate research, using NGS in patients with suspected CAP may unnecessarily accelerate antimicrobial prescribing simply by providing the names of all commensal organisms present in a respiratory sample.

A clinical diagnosis of CAP has limited utility in differentiating bacterial versus non-bacterial causes of infections. Clinical respiratory cultures more often than not fail to yield a definitive causative pathogen. Biomarkers, such as procalcitonin, have been studied heavily in the management of CAP but have limitations, such as low levels during early course of infections and false negative results.[5] The need for additional objective criteria to assist in the diagnosis of CAP is therefore needed. In this opinion piece, we present a research framework that can be used to evaluate the role of NGS in hospitalized patients with a clinical diagnosis of CAP.

Research framework to study NGS in CAP

In our opinion, prospective clinical trials can be performed by randomizing patients with a clinical suspicion of CAP into two groups (**Figure 1**). The NGS group would have their NGS results shared and discussed with the treatment team to guide anti-infective therapy. The standard of care (SOC) group would have their NGS results blinded to the treatment team, and therefore, treatment would be based on standard of care.

In patients with a working diagnosis of CAP, biomarkers indicative of bacterial infection (e.g., procalcitonin) can be evaluated together with NGS. A proposed research framework for exploring NGS and biomarkers in hospitalized patients with a working diagnosis of CAP is outlined in **Figure 2**.

This proposed research framework will facilitate four possible clinical scenarios. Several questions are proposed below to guide clinical research based on the four clinical settings.

Scenario #1

In Scenario #1, a patient would have a working diagnosis of CAP, with biomarkers and NGS results suggestive of bacterial pneumonia. In these patients, NGS may demonstrate a clinical benefit by identifying an organism that is not adequately treated by the empiric regimen and not isolated from standard clinical cul-

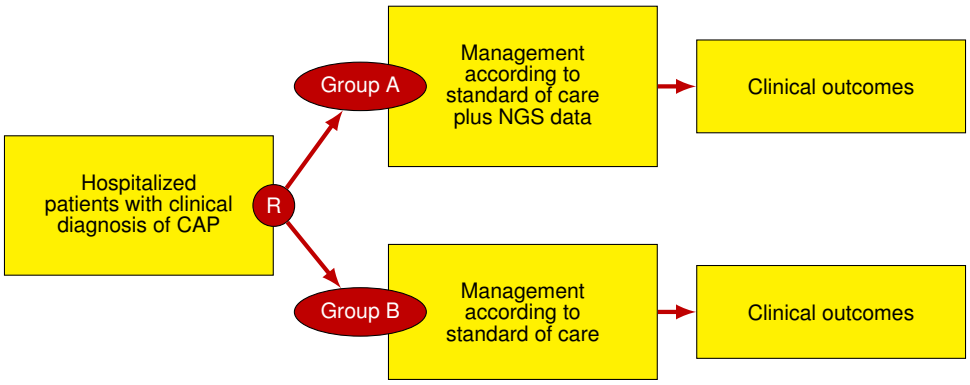

Figure 1. Study design for the evaluation of NGS in patients with CAP.
Abbreviations: CAP, community-acquired pneumonia; NGS, next generation sequencing; R, randomization;

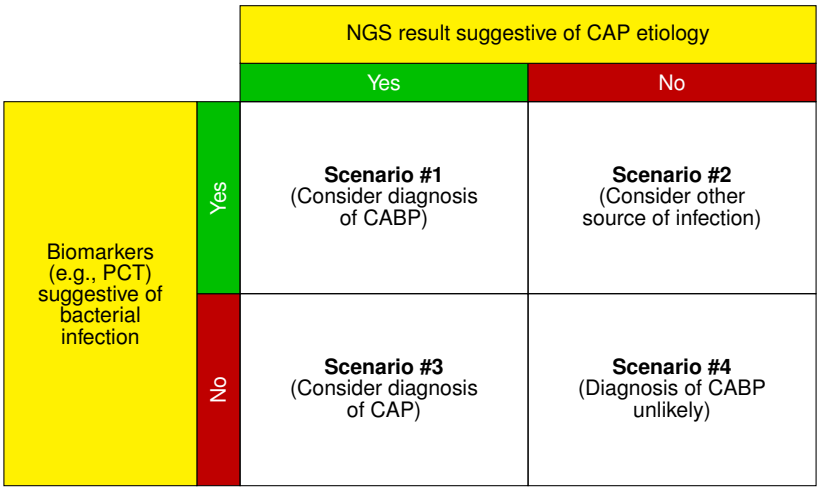

Figure 2. Proposed framework for research using next generation sequencing in patients with suspected community-acquired bacterial pneumonia (CABP).
Abbreviations: CABP, community-acquired bacterial pneumonia; CAP, community-acquired pneumonia; PCT, procalcitonin.

tures. However, antimicrobials may be unnecessarily escalated without a clinical benefit, leading to increased risk of antimicrobial adverse events, selection for multidrug resistant organisms, or risk of *Clostridioides difficile* infection. Additionally, NGS provides little information on antimicrobial susceptibility, thereby increasing the likelihood of antimicrobial escalation in the NGS group. Selection of definitive antimicrobial would be challenging if the predominant organism identified is not traditionally associated with CAP.

Other studies in Scenario #1 include describing the yield of concordant NGS results from different anatomical samples. For example, does NGS of oropharyngeal swabs match NGS results from lower respiratory samples (i.e., sputum or bronchoalveolar lavage [BAL])? Does NGS of a patient's blood or urine identify the causative pathogen?

Scenario #2

In Scenario #2, a patient will have biomarkers suggestive of a bacterial infection, but the NGS result does not identify a bacterial pathogen. Research studies in Scenario #2 could focus on the ability of NGS to guide clinicians towards an alternative diagnosis. In comparing the NGS group to the SOC group, an outcome of interest may be time to alternative diagnosis or rate of alternative diagnosis at the point of discharge. However, the threshold for designating an NGS of a respiratory sample as "positive" for bacterial organisms remains to be defined. Should a different threshold for positivity of NGS be used for immunocompromised patients if CABP is still the most likely etiology?

Because Scenario #2 describes discordant procalcitonin and NGS results, researchers need to be cautious in situations with potentially falsely elevated procalcitonin concentrations or false negative NGS results. A possible research study could examine the influence of antimicrobials prior to collection of samples for NGS. Additionally, a study could be done to examine the influence of viral respiratory infections leading to false negative NGS results.

Scenario #3

In Scenario #3, a patient will have an NGS result of a respiratory specimen that is suggestive of CABP but will not have significantly elevated biomarkers. As in Scenario #2, the procalcitonin and NGS results are discordant and therefore would need to be explained. Atypical organisms (*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and *Legionella pneumoniae*) in CABP may not cause a significant elevation in procalcitonin. Additionally, fungal pathogens may cause respiratory infections in immunocompromised patients. However, the predominance of a low-virulence bacteria may simply be a sign of bacterial dysbiosis and may not require antimicrobial therapy for CABP.

Scenario #4

In Scenario #4, a patient will demonstrate concordant procalcitonin and NGS results suggesting an unlikely diagnosis of CABP. This should result in prompt discontinuation of antimicrobials for CABP in the NGS group. Not only should patients benefit from decreased risk of unnecessary antimicrobials, but they may also benefit from additional diagnoses or treatments once CABP has been removed from the differential. Such benefits have been demonstrated by de Jong *et al.*, where procalcitonin-guided discontinuation of antimicrobials resulted in improved 28-day mortality in critically ill patients.[6] However, patients in Scenario #4 may still have diagnostic uncertainties as some organisms, such as *Mycobacterium tuberculosis* or *Bacillus anthracis,* may prompt treatment regardless of NGS predominance.

Conclusion

Significant research questions remain regarding the clinical use of NGS in CAP. NGS in CAP may result in a diagnostic pathway that can quickly and reliably differentiate between bacterial respiratory tract infections versus other causes. However, the clinical utility of widespread or routine NGS remains largely unknown. Prospective randomized controlled trials are urgently needed to define the role of NGS in patients with CAP. Our proposed research framework outlines a programmatic approach to designing research of NGS in CAP.

Published: October 29, 2021

Copyright: © 2021 The author(s). This original article is brought to you for free and open access by ThinkIR: The University of Louisville's Institutional Repository. For more information, please contact thinkir@louisville.edu. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding Source: The author(s) received no specific funding for this work

Conflict of Interest: All authors declared no conflict of interest in relation to the main objective of this work.

References

1. Gu W, Miller S, Chiu CY. Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection. Annu Rev Pathol **2019**; 14:319-38. doi: 10.1146/annurev-pathmechdis-012418-012751. PMID: 30355154.

2. Tarabichi M, Shohat N, Goswami K, et al. Diagnosis of Periprosthetic Joint Infection: The Potential of Next-Generation Sequencing. J Bone Joint Surg Am **2018**; 100(2):147-54. doi: 10.2106/jbjs.17.00434. PMID: 29342065.

3. Zhang H, Zhan D, Chen D, et al. Next-generation sequencing diagnosis of severe pneumonia from fulminant psittacosis with multiple organ failure: a case report and literature review. Ann Transl Med **2020**; 8(6):401. doi: 10.21037/atm.2020.03.17. PMID: 32355845.

4. Wu X, Li Y, Zhang M, et al. Etiology of Severe Community-Acquired Pneumonia in Adults Based on Metagenomic Next-Generation Sequencing: A Prospective Multicenter Study. Infect Dis Ther **2020**; 9(4):1003-15. doi: 10.1007/s40121-020-00353-y. PMID: 33170499.

5. Self WH, Balk RA, Grijalva CG, et al. Procalcitonin as a Marker of Etiology in Adults Hospitalized With Community-Acquired Pneumonia. Clin Infect Dis **2017**; 65(2):183-90. doi: 10.1093/cid/cix317. PMID: 28407054.

6. de Jong E, van Oers JA, Beishuizen A, et al. Efficacy and safety of procalcitonin guidance in reducing the duration of antibiotic treatment in critically ill patients: a randomised, controlled, open-label trial. Lancet Infect Dis **2016**; 16(7):819-27. doi: 10.1016/s1473-3099(16)00053-0. PMID: 26947523.