Editorial Commentary

Rapid and accurate diagnosis of hemoglobinopathy and β-thalassemia by ultrahigh-resolution mass spectrometry and tandem mass spectrometry from blood: review of a benchmark study

Laszlo Prokai

Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX, USA

Correspondence to: Laszlo Prokai, PhD, DSc. Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107, USA. Email: Laszlo.Prokai@unthsc.edu.

Provenance: This is an invited article commissioned by Guest Section Editor Ying Zhao, MM (Department of Laboratory Medicine, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China).

Comment on: He L, Rockwood AL, Agarwal AM, *et al*. Diagnosis of Hemoglobinopathy and β-Thalassemia by 21 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometry and Tandem Mass Spectrometry of Hemoglobin from Blood. Clin Chem 2019;65:986-94.

Submitted Aug 06, 2019. Accepted for publication Aug 11, 2019.
doi: 10.21037/atm.2019.08.69
View this article at: http://dx.doi.org/10.21037/atm.2019.08.69

Many point mutations of the human hemoglobin (Hb) genes have been documented (1-4) and, without a doubt, more remain to be identified. Most of the mutations are single base replacements in the genes and, thus, result in single amino acid substitutions in the corresponding globin chains. Fortunately, most of the approximately 1,700 reported variants are harmless; however, several mutations are known to cause diseases such as sickling disorders and thalassemias that are considered the most common monogenic diseases worldwide (5,6). Therefore, screening for and identification of globin chain variants have received huge attention in hematological diagnostics (7).

While electrophoresis and high-performance liquid chromatography of globin chains have become widely used diagnostic screening tools, inherent ambiguities of these techniques owing to their limited power to resolve the many documented and yet to be documented variants will require genetic testing for accurate molecular diagnoses in today's clinical practice (7). Mass spectrometry has been considered an emerging secondary screening tool to analyze globin chains since the introduction of the method to measure molecular masses of intact proteins at ±1 Da accuracy even relying on low-resolution instruments routinely used by bioanalytical laboratories (8). Combined with proteolytic digestion followed by tandem mass spectrometry (MS/MS) of the resultant peptides, an apt demonstration of mass spectrometry's power included the accurate identification of the "electrophoretically silent" Quebec-Chori β F3 variant with threonine (Thr) to isoleucine (Ile) replacement at position 87 of the β-globin chain because of single base substitution in the gene (9). The general consensus has been that traditional phenotypic methods combined with electrospray ionization (ESI) mass spectrometry can identify up to 95% of known Hb variants rapidly (in a few hours of turnaround time including sample preparation). Nevertheless, traditional phenotypic methods were recommended as the first-line screening approach for detecting some common clinically important variants (e.g., Hb C, E, D-Punjab and O-Arab) that differ from normal by 1 Da in mass (10). Additional developments have included proteolytic digestion with trypsin followed by liquid chromatography–electrospray ionization tandem mass spectrometry (LC-MS/MS) (11), which is commonly referred to as a "bottom-up" strategy. However, this comes at the cost of extended sample preparation and data acquisition reducing turnaround times.

The above consensus is challenged by He *et al*.'s recent evaluation that has relied on ultrahigh-resolution ESI mass spectrometry and MS/MS performed on intact globin chains; i.e., without proteolytic digestion. This is known as a "top-

down" strategy that obviously would not require additional sample preparation steps and lengthy acquisition times of the bottom-up approach (12). In a limited study involving blinded analyses, the authors utilize a Fourier transform ion cyclotron resonance (FT-ICR) instrument custom-built at the National High Magnetic Field Laboratory of the Florida State University, Tallahassee, FL, in the United States (13). Therefore, it is unmatched in magnetic field strength (21 Tesla) and resultant mission-critical performance measures among commercial mass spectrometers. The paper reported correct identification of all 18 hemoglobin variants tested—including resolving a heterozygous variant Hb AD differing in mass from the normal Hb A by less than 1 Da, as well as the characterization of homozygous Hb Himeji variant for the first time. The authors deduced all results through manual interpretations of ultrahigh-resolution ESI mass spectra, as well as top-down MS/MS analyses utilizing two complementary ion-dissociation techniques (known to mass spectrometrist as collision-induced dissociation and electron transfer dissociation). In this publication intended principally for mass spectrometry experts, they explained in meticulous details the generic "dilute and infuse" de novo sequencing approach, deciphering the sequences of selected Hb variants in their test samples, as well as relative quantification of Hb subunits for β-thalassemias diagnosis.

The reported work begs the question whether the authors' results would offer a realistic perspective to translate the promised quantum leap in performance to actual clinical diagnostics in the near future based on data obtained with the most sophisticated mass spectrometer built thus far for molecular analyses. However, the authors put their work in the context as benchmark study "to design the most efficient experimental protocols, while at the same time improving quality and turnaround time" (12). In addition, they have pointed out that the capabilities of other, now widely available Fourier transform (lower field FT-ICR and the popular Orbitrap) instruments could be suitable for accurate characterization of Hb variants at the expense of turnaround time by relying on front-end liquid chromatographic separation performed with online coupling to mass spectrometry and MS/MS. Nevertheless, routine interpretation of ESI mass spectra and MS/MS spectra of proteins remains a challenge and, thus, requires considerable expertise (10) especially when data acquisitions are done at ultrahigh mass resolution. However, the results of He *et al.* (12) have clearly outlined the future for rapid and accurate hematological diagnoses of hemoglobinopathies and thalassemias.

Acknowledgments

None.

Footnote

Conflicts of Interest: The author has no conflict of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. The Globin Gene Server. A service provided by the laboratories of Computer Science & Engineering and Biochemistry & Molecular Biology at the Pennsylvania State University. Available online: http://globin.cse.psu.edu/ (Accessed on August 4, 2019).
2. The Globin Gene Server: Alpha-1 Globin (HBA1). Leiden Open Variation Database (Curators: Giardine B, Borg J). Available online: https://lovd.bx.psu.edu/home.php?select_db=HBA1 (Accessed on August 4, 2019).
3. The Globin Gene Server: Alpha-2 Globin (HBA2). Leiden Open Variation Database (Curators: Giardine B, Borg J). Available online: https://lovd.bx.psu.edu/home.php?select_db=HBA2 (Accessed on August 4, 2019).
4. The Globin Gene Server: Beta Globin (HBB). Leiden Open Variation Database (Curators: Giardine B, Borg J). Available online: https://lovd.bx.psu.edu/home.php?select_db=HBB (Accessed on August 4, 2019).
5. Naik RP, Smith-Whitley K, Hassell KL, et al. Clinical outcomes associated with sickle cell trait: a systematic review. Ann Intern Med 2018;169:619-27.
6. Taher AT, Weatherall DJ, Cappellini MD. Thalassemias. Lancet 2018;391:155-67.
7. Harteveld CL. State of the art and new developments in molecular diagnostics for hemoglobinopathies in multiethnic societies. Int J Lab Hematol 2014;36:1-12.
8. Covey TR, Bonner RF, Shushan BI, et al. The determination of protein, oligonucleotide and peptide molecular weights by ion-spray mass spectrometry. Rapid Commun Mass Spectrom 1988;2:249-56.
9. Witkowska HE, Lubin BH, Beuzard Y, et al. Sickle cell disease in a patient with sickle cell train and compound heterozygosity for Hemoglobin S and Hemoglobin

Quebec-Chori. N Engl J Med 1991;325:1150-4.
10. Wild BJ, Green BN, Cooper EK, et al. Rapid identification of hemoglobin variants by electrospray ionization mass spectrometry. Blood Cells Mol Dis 2001;27:691-704.
11. Haynes CA, Guerra SL, Fontana JC, et al. HPLC-ESI-MS/MS analysis of hemoglobin peptides in tryptic digests of dried-blood spot extracts detects HbS, HbC, HbD, HbE, HbO-Arab, and HbG-Philadelphia mutations. Clin Chim Acta 2013;424:191-200.
12. He L, Rockwood AL, Agarwal AM, et al. Diagnosis of hemoglobinopathy and β-thalassemia by 21-Tesla Fourier transform ion cyclotron resonance mass spectrometry and tandem mass spectrometry of hemoglobin from blood. Clin Chem 2019;65:986-94.
13. Hendrickson CL, Quinn JP, Kaiser NK, et al. 21 Tesla Fourier transform ion cyclotron resonance mass spectrometer: A national resource for ultrahigh resolution mass analysis. J Am Soc Mass Spectrom 2015;26:1626-32.

Cite this article as: Prokai L. Rapid and accurate diagnosis of hemoglobinopathy and β-thalassemia by ultrahigh-resolution mass spectrometry and tandem mass spectrometry from blood: review of a benchmark study. Ann Transl Med 2019;7(Suppl 6):S227. doi: 10.21037/atm.2019.08.69

