Clinical Chemistry

Post-Translationally Modified Proteoforms as Biomarkers: From Discovery to Clinical Use

Article:
Ruben Y Luo, Priscilla S W Yeung, Morgan W Mann, Lichao Zhang, Yifei K Yang, Andrew N Hoofnagle.
Post-Translationally Modified Proteoforms as Biomarkers: From Discovery to Clinical Use.
Clin Chem 2025; 71(11): 1111–24. https://doi.org/10.1093/clinchem/hvaf094

Guest: Dr. Ruben Luo is an Assistant Professor of Pathology at Stanford University and Associate Director of the Clinical Chemistry and Immunology Laboratory at Stanford Health Care.

Bob Barrett: This is a podcast from *Clinical Chemistry*, a production of the Association for Diagnostics and Laboratory Medicine. I'm Bob Barrett.

Typically, clinical laboratories measure the total amount of a given protein, which can then be interpreted in the context of patient symptoms to draw conclusions about the presence or absence of disease. What these total mass assays fail to consider is the tremendous diversity within a given population of the same protein. Proteins created from alternatively spliced RNA transcripts may be larger, smaller, or simply exhibit a different amino acid sequence. Further adding to the complexity is the vast array of post-translational modifications, chemical groups added to specific locations of the protein that alter its activity in a meaningful way.

These modifications are not random but instead are controlled by specific physiological processes. By extension, measurement of these specific modifications can provide important insights into states of health and disease that would otherwise go undetected. A review article, appearing in the November 2025 issue of *Clinical Chemistry*, describes the spectrum of post-translationally modified proteoforms summarizes test methods used in their measurement and highlights scenarios where measurement is already making an impact on patient care.

In this podcast, we are joined by the article's lead author. Dr. Ruben Luo is an Assistant Professor of Pathology at Stanford University and Associate Director of the Clinical Chemistry and Immunology Laboratory at Stanford Health Care. So, Dr. Luo, let's start with this. What are post-translationally modified proteoforms and why are they important in clinical diagnostics?

Ruben Luo: Protein biomarkers are routinely measured for disease diagnosis and prognosis in clinical laboratories. Proteoforms of a protein biomarker are results of genetic polymorphism, alternative splicing of RNA transcripts, and post-translational modifications on the amino acid backbone. A detailed

analysis of the post-translationally modified proteoforms, or PTMPs, may lead to more precise diagnosis and prognosis because they are influenced by pathophysiological conditions. The details of PTMPs as protein biomarkers are overviewed and discussed in the recently published review article in the journal Clinical Chemistry.

Bob Barrett: And how do we measure post-translationally modified proteoforms?

Ruben Luo: The methodologies to accurately detect and categorize PTMPs include immunoassays, electrophoresis, chromatography, and intact and proteolysis-aided mass spectrometry technologies. Immunoassays are a mainstay of clinical laboratories today due to their relatively quick turnaround time, scalability, and ease of operation. Electrophoresis and chromatography differentiate molecules based on the molecular features of analytes, and they can be coupled with mass spectrometry.

Mass spectrometry is a powerful technology to characterize PTMP biomarkers. In the intact protein approach or top-down approach, an analyte is first purified and then followed by fragmentation of selected precursor ions for amino acid sequence and a PTM determination. In contrast, the proteolysis-aided approach or bottom-up approach involves enzymatic digestion of an analyte into peptides, which are then measured by mass spectrometry.

Bob Barrett: Are there any PTMPs currently being used in clinical diagnostics?

Ruben Luo: Yes, exactly. Certain PTMPs with significant clinical meaning are being tested in clinical laboratories. The examples described in the review article include glycated hemoglobin A_{1c}, β_2-transferrin, phosphorylated tau protein or p-tau, and fucosylated alpha-fetoprotein, or AFP-L3. It should be noted that the concentration of the undifferentiated family of each protein biomarker mentioned above, meaning hemoglobin, transferrin, total tau, and AFP, was already used as a traditional clinical diagnostic biomarker. The identification of specific PTMPs either facilitated a new diagnostic use of the protein biomarker or improved its clinical performance.

Bob Barrett: Well, doctor, as you describe in the review, phosphorylated tau-217 is changing the landscape of early diagnosis, treatment, and monitoring of Alzheimer's disease. Could you please briefly tell the story of phosphorylated tau-217 from its discovery and to its clinical applications?

Ruben Luo: Sure. Around the year 2000, phosphorylated tau proteoforms in cerebrospinal fluid, or CSF, were first analyzed to study tau pathology in Alzheimer's disease, or AD. A pioneering amino

acid targeting p-tau181 revealed that its levels in CSF were significantly elevated in patients with AD compared to controls.

In 2017, it was first demonstrated that plasma concentrations of p-tau181 could differentiate Alzheimer's disease patients from healthy controls and were significantly correlated with CSF concentrations. In parallel, plasma concentrations of p-tau217 emerged as a particularly promising AD biomarker. Studies reported that the concentration of p-tau217 in plasma rises early in the disease process. It exhibited superior diagnostic performance in distinguishing AD from other neurodegenerative disorders with a significant higher area under the ROC curve and stronger correlation with a tau PET signal when compared with p-tau181.

In 2024, the Alzheimer's Association published the revised criteria for diagnosis and staging of AD in which p-tau217 and p-tau217 to total tau ratio were listed as plasma biomarkers for AD diagnosis. A number of diagnostic assays, including high-sensitivity mass spectrometry and immunoassays, have been developed to accurately quantify p-tau217. Recently, plasma p-tau217 to a beta-42 ratio became the first plasma biomarker for AD diagnosis approved by the US FDA.

Bob Barrett: Well, since post-translationally modified proteoforms are becoming increasingly important in clinical diagnostics, are there novel methods in development to better characterize them?

Ruben Luo: Yes. A few recent breakthroughs on analytical technologies could potentially expand our ability to identify clinically relevant PTMPs. For example, nanopore protein sequencing has recently shown significant advances including improvements in bioinformatic processing power. Several proof-of-concept studies have demonstrated the ability to resolve phosphorylated, glutathione-related, and glycosylated residues in polypeptide chains.

Furthermore, individual ion mass spectrometry is a new high-resolution mass spectrometry technology that can measure the accurate mass of single ions, thereby opening the door to the measurement of low-abundance PTMPs with increased accuracy. Overall, the ability to decipher the PTMPs from many proteins, especially at the single molecule level, may bolster our understanding about the pathophysiology of diseases, which could have potential to be translated into clinical diagnostics.

Bob Barrett: Well, finally, doctor, where there's good news, there's usually bad news. What are the main challenges in the clinical implementation of post-translationally modified proteoform measurement?

Ruben Luo: Well, you're right. While certain PTMP biomarkers hold great promise as a new generation of biomarkers, there are a few evidence gaps that remain to be overcome prior to their successful translation from discovery to clinical use.

First, demonstrating clinical validity of PTMP biomarkers. Prospective clinical studies in different disease and control cohorts are required to fully establish and verify the diagnostic performance. The diversity of PTMPs among different individuals may further complicate the clinical validation.

Second, standardizing PTMP biomarker assays. The comparability of different assays is limited by the lack of reference materials and methods. Amino acid polymorphisms, diversified post-translational modifications on the given protein, post-translational cleavage, and isoforms will affect the measurement of PTMPs and result in difficulty in producing reference materials and methods.

Third, the cost of specialized hardware and skilled labor. The analytical platforms needed to measure novel PTMP biomarkers such as high-resolution mass spectrometers are not commonly available in clinical laboratories. Also, the assay implementation and data interpretation may need personnel trained in proteomics. These financial and human resource demands can be an entry barrier for the clinical implementation of PTMP biomarkers, but these challenges can likely be addressed as the value of PTMP biomarkers becomes more widely recognized and the cost of analytical technologies decreases over time.

Bob Barrett: That was Dr. Ruben Luo from Stanford University. He wrote a review article in the November 2025 issue of *Clinical Chemistry* summarizing post-translationally modified proteoforms as an emerging class of biomarkers, and he's been our guest in this podcast on that topic. I'm Bob Barrett. Thanks for listening.