Plasma Protein Profiling by Proximity Extension Assay Technology Reveals Novel Biomarkers of Traumatic Brain Injury – A Pilot Study

Article:
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Guest: Michelle Chen is a medical student at McMaster University in Ontario, Canada and completed her Master's of Science at the University of Toronto. Under the supervision of Dr. Eleftherios Diamandis at Mount Sinai Hospital in Toronto, ON, her research centers on traumatic brain injury biomarkers and their application to disease diagnosis and prognosis.

Randye Kaye: Hello and welcome to this edition of JALM Talk from the *Journal of Applied Laboratory Medicine*, a publication of the American Association for Clinical Chemistry. I'm your host, Randye Kaye. The CDC defines a traumatic brain injury, or TBI, as a bump, blow, or jolt to the head that disrupts the normal function of the brain. TBIs affect nearly 69,000,000 people worldwide per year. The degree of a TBI may vary from mild to moderate or severe. Symptoms may be physical, cognitive, or behavioral, and may persist for months to years. Unfortunately, it's difficult to predict patient outcomes and long-term effects of TBIs. The identification of serum biomarkers that could accurately delineate the severity of trauma and prognostic outcomes following TBI would be beneficial in managing affected patients. An article in the May 2021 issue of JALM describes a high-throughput plasma protein profiling approach to identify novel candidate TBI biomarkers. The first author of the report, and our guest for this podcast, is Michelle Chen. Michelle completed her Master's of Science at the University of Toronto and is currently a medical student at McMaster University in Ontario Canada. Under the supervision of Dr. Eleftherios Diamandis, the senior author of the report, Michelle's research centers on TBI biomarkers and their application to disease diagnosis and prognosis. Michelle, welcome. How common is TBI and why did your group see a need to identify TBI biomarkers?

Michelle Chen: So unfortunately, TBIs are quite common and they really stem from things like falls, car accidents, or things like sports related injuries. So, we do know that in the U.S., there's an estimated 2.8 million cases of TBI per year and these injuries are really associated with quite significant mortality as well as morbidity as well as long-term physical and neurological deficits. So unfortunately, more severe TBIs are associated with worse outcome. Our research team really thought that there was an unmet need for both diagnostic and prognostic TBI biomarkers. The idea being that these serum biomarkers are a non-invasive way to stratify patients so that we can guide management and predict outcome.

Randye Kaye: Okay. So, what you use was a novel technology referred to as high throughput proximity extension assays or PEA. Can you tell me how does PEA work?

Michelle Chen: Yeah, of course. So, PEA is a targeted multiplex immunoassay and it uses a combination of one, targeted antibodies and two, coupling with quantitative PCR. And what this does is it allows us to quantify protein's abundance in patient samples with really high sensitivity as well as specificity. And our team chose this platform in particular because it really enabled us to use very low volumes of patient samples so we know that when we do kind of discovery work like this, patient samples can be quite valuable, so we've really liked this platform for that reason. So overall, as I mentioned previously, we selected this method because of the very high sensitivity and the minimal cross reactivity which we really felt were issues that we had experienced on different multiplex platforms in the past and we actually independently compared this technology with commercially available ELISA kits, and we saw that it was highly robust and very sensitive. So using PEA, we were able to conduct this high-throughput discovery phase on severe TBI patient samples.

Randye Kaye: All right, thank you. Can you say more or describe the patient samples that you used in the study?

Michelle Chen: Yeah, absolutely. So, we had two major cohorts in the study. The first was our TBI cohort where we used plasma samples from patients that were drawn within 24 hours of hospital admission and so the patients that we included in this pilot study were 10 adult patients with severe TBI which we defined as patients having a Glasgow Coma Scale score of less than or equal to 8 with CT findings. So, the Glasgow Coma Scale score is this clinical scoring system to determine the severity of TBI. We also had 10 healthy, aged, and sex MeSH control samples to go along in our TBI cohort. Our second group was a reference cohort that consisted of ovarian cancer and dementia patients. The idea being that we're trying to look for proteins that are very specific for TBI, and so when we look at our biomarkers, if they're highly abundant in these ovarian cancer and dementia patients, they may be less specific for TBI and more reflective of perhaps other neurological disorders or a highly inflammatory state.

Randye Kaye: So, your article explains that you did identify six novel TBI biomarker candidates, so what were those biomarkers? Tell us a little bit about them.

Michelle Chen: So, using this PEA platform, we simultaneously measure the abundance of over a thousand proteins across all sample groups and with this data, we applied a quantitative and qualitative filtering criteria in order to identify our candidate

TBI biomarkers. Overall, we identified six highly promising markers that we saw were significantly elevated in our severe TBI patients compared with our healthy controls. We also saw that there was an increased abundance of these markers in non-surviving patients compared with surviving patients. So, what we're seeing is that these markers are elevated in severe TBI patients and even more elevated in patients that did not survive their injuries. Additionally, all of our candidate proteins showed a low abundance in the reference cohorts that I mentioned previously.

Randye Kaye: All right, thank you. So, were any of these biomarkers related to clinical outcomes and severity of injury in the patient cohorts?

Michelle Chen: Yeah. Actually, of the six markers, two stood out as particularly interesting. So, the first one is EN-RAGE or also known as S100A12, and this protein was significantly and negatively correlated with patient GCS score. So, the Glasgow Coma Scale score that I talked a little bit about before and so, this basically showed that we had a higher abundance of this biomarker in more severe cases of severe TBI. Another interesting biomarker that we saw was chitinase-3-like-protein 1 where we observed a positive correlation with hospital length of stay.

Randye Kaye: So, what's next for the evaluation of these biomarker candidates? I mean, when could we potentially see these types of biomarkers used in a clinical setting?

Michelle Chen: Yeah, great question. So, we conducted this discovery study to identify interesting and promising candidate biomarkers. This was a pilot study and the results that we found will have to be validated in a larger independent severe TBI cohort with extended controls and we're currently in the process of planning this stage for a follow-up study. Ideally, in a clinical setting, these markers could potentially be used in combination with other modalities such as imaging, perhaps other markers as well to guide patient care or to aid in prognosing patient outcome.

Randye Kaye: Very interesting pilot study. Thank you so much for joining us, Michelle.

Michelle Chen: Thank you Randye.

Randye Kaye: That was Michelle Chen from McMaster University, describing the JALM article "Plasma Protein Profiling by Proximity Extension Assay Technology Reveals Novel Biomarkers of Traumatic Brain Injury – A Pilot Study." Thanks for tuning in to this episode of JALM Talk. See you next time and don't forget to submit something for us to talk about.