Clinical Validation of the sFlt-1:PlGF Ratio as a Biomarker for Preeclampsia Diagnosis in a High-Risk Obstetrics Unit

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Guests: Dr. Victoria Higgins is a Clinical Biochemist at DynaLIFE Medical Labs and a Clinical Lecturer at the University of Alberta in Edmonton, Alberta. Dr. Jessica Miller is a Clinical Biochemist at Dynacare in Toronto, Ontario, Canada.

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.

Preeclampsia is a progressive multi-system disorder that complicates 3% to 5% of all pregnancies and is a major cause of fetal and maternal morbidity and mortality. Diagnostic criteria for preeclampsia have evolved over time, but the hallmarks remain as new onset of hypertension with either proteinuria or significant end-organ dysfunction, with symptoms typically presenting after 20 weeks of gestation.

Preeclampsia is typically treated by delivery of the fetus to prevent complications. However, due to the risks associated with premature delivery, better biomarkers are needed to enable earlier diagnosis and to aid in determining the optimal time of delivery. Promising biomarkers for preeclampsia include the proangiogenic factor "placental growth factor" (PlGF) and the antiangiogenic factor "soluble fms-like tyrosine kinase-1" (sFlt-1).

Studies have demonstrated that pregnant women diagnosed with preeclampsia have elevated sFlt-1 and decreased PlGF, and therefore an elevated sFlt-1 to PlGF ratio. The May 2023 issue of *JALM* features an article that provides a clinical validation and cutoff analysis for an automated test method for sFlt-1 and PlGF in a high-risk obstetrics unit.

Today were joined by the articles' co-first authors Drs. Victoria Higgins and Jessica Miller. Dr. Higgins is a Clinical Biochemist at DynaLIFE Medical Labs and a Clinical Lecturer at the University of Alberta in Edmonton, Alberta. Dr. Miller is a Clinical Biochemist at Dynacare in Toronto. Welcome Drs. Higgins and Miller.

Let's start with this. What exactly is preeclampsia and can you elaborate on why there is a need for novel biomarkers for its diagnosis?

Victoria Higgins: So the definition of preeclampsia varies between different clinical guidelines, but in general is defined as a patient presenting after 20 weeks' gestation with new onset of hypertension. So this is usually defined as systolic blood pressure greater than or equal to 140 millimeter mercury and/or diastolic blood pressure greater than or equal to 90 millimeter mercury, as well as proteinuria or end-organ dysfunction, which the latter can include renal insufficiency, liver dysfunction, pulmonary edema, or thrombocytopenia for example.

So I'll give a brief overview of how this disease is thought to develop. So during the early stages of a normal pregnancy, maternal spiral arteries undergo remodeling allowing increased blood flow to the fetus, and this allows optimal exchange of nutrients and oxygen.

However, in women who develop preeclampsia, the remodeling of the spiral arteries isn't normal. So vessels remain narrow and this leads to placental hypoperfusion. Now, this subsequently causes placental ischemia and oxidative stress, causing that imbalance of the proangiogenic and antiangiogenic factors that we will discuss in more detail.

So this imbalance will alter the maternal systemic endothelial function and eventually causes those clinical manifestations I mentioned previously, such as hypertension. So it's very important to identify preeclampsia as early as possible because it leads to both maternal and fetal complications. So worldwide, about 10% to 15% of maternal deaths that result from obstetric complications of pregnancy are associated with preeclampsia or eclampsia. And then for the fetus, it can lead to growth restriction and preterm birth.

So the reason novel biomarkers of preeclampsia are important to investigate is that it can be difficult to distinguish preeclampsia from other types of hypertensive disorders of pregnancy. So this could be gestational hypertension or HELLP syndrome, and it can be difficult to diagnose preeclampsia in the presence of other vascular comorbidities, such as chronic hypertension.

So traditionally pregnant women are simply evaluated for risk factors such as previous pregnancy with preeclampsia, multifetal gestation, or renal disease, and then simply based on this they can be given low dose aspirin for prophylaxis. However, there is a need for predictive biomarkers of preeclampsia that are more sensitive and specific than these simple risk factors, and this would enable early diagnosis, monitoring, and optimal time of delivery.

Randye Kaye: Thank you. That was Dr. Higgins. Dr. Miller, I'll direct this next question to you. Why did you choose to perform a study specifically on the biomarkers sFlt-1 and PlGF?

Jessica Miller: So as Victoria mentioned, the diagnosis of preeclampsia is based on elevated blood pressure above 140/90 millimeters of mercury and to the presence of proteinuria in a pregnant woman presenting after 20 weeks' gestation. But these symptoms however, can represent a late-stage biomarkers where end-organ damage has actually already started to occur. And so there's definitely a need for biomarkers to both rule in and rule out preeclampsia that present prior to onset of clinical manifestations.

And so with regards to sFlt-1 and PlGF specifically, there have been studies since 2003 that have identified abnormal concentrations of these two markers in women with preeclampsia where sFlt-1 is abnormally high and then PlGF is abnormally low. And since then, much more about the pathophysiology of preeclampsia has been uncovered. And so, in a healthy pregnancy, the maternal spiral arteries that connect the placenta to the fetus need to expand and this is in part achieved by an increased concentration of PlGF. But in preeclampsia however, the damaged placenta actually secretes increased concentrations of sFlt-1 and then sFlt-1 is an antagonist of PlGF, where it binds the PlGF and prevents it from interacting with appropriate receptors in order to induce its biological function.

And so as a result, in preeclampsia, expansion of the maternal spiral arteries is impaired, which leads to decreased blood flow to the fetus and an increased blood pressure in the mother. And so this characteristically high ratio of sFlt-1 to PlGF is therefore observed in women with preeclampsia and has shown utility in previous publications both for ruling in and for ruling out preeclampsia. And then importantly, the extent of elevation of the ratio has actually been shown to correlate with disease severity and risk of adverse outcomes.

And so we've performed this study at Sunnybrook Health Sciences Centre in Toronto, Canada and we wanted to clinically validate these biomarkers for preeclampsia in our high-risk obstetrics clinic.

Randye Kaye: Thank you. Dr. Higgins, so what were the specific aims of your study and what would you say made your study unique?

Victoria Higgins: So our study really had three specific aims. So the first was to evaluate the clinical performance of the Roche Elecsys sFlt-1:PlGF ratio assay as well as the PlGF assay alone for predicting preeclampsia in a high-risk obstetrics unit by using various cutoffs that have been previously published.

The second aim was to use the optimal ratio cutoff that we established in aim one to investigate whether the ratio has improved diagnostic performance over traditionally used parameters such as proteinuria and/or hypertension.

And then our third and final aim was to examine the predictive ability of the sFlt-1:PlGF ratio to rule in and rule out preeclampsia within seven days and 28 days respectively.

So, our study was unique because we were able to assess how the sFlt-1:PlGF ratio performs clinically in a high-risk obstetrics unit, which is a different cohort from most previous studies and it has a much higher prevalence of preeclampsia.

Also, Sunnybrook Health Sciences Centre in Toronto where we performed our study was also the first North American site to clinically implement these tests. So we have the opportunity to report on our experience clinically implementing this assay.

Randye Kaye: So to address those aims, what types of methods did you use and what types of analysis did you perform? Dr. Miller?

Jessica Miller: Yeah. So, in order to validate these biomarkers, we worked really closely with Sunnybrook physicians Dr. Nir Melamed and Dr. Michelle Hladunewich, and so when patients presented to their clinics with symptoms and a clinical suspicion of preeclampsia, the physicians would order serum sFlt-1 and PlGF, and then we are provided with clinic notes for these patients with information such as blood pressure and whether proteinuria was present. And then importantly, we are provided with whether they were diagnosed with preeclampsia or an alternative diagnosis. And so with this information, we then analyzed the sFlt-1 to PlGF ratio in the patients that were diagnosed with preeclampsia and compared it to those diagnosed with an alternative diagnosis, and using various cut-offs that were published in the literature including 33, 38, and 85, and it was a cutoff of 38 in terms of the sFlt-1 to PlGF ratio that showed the greatest diagnostic accuracy over other cutoffs such as 33 and 85. And so, we use these cutoffs in our subsequent analysis.

And what we did next using the sFlt-1 to PlGF ratio cutoff of 38, we determined the clinical sensitivity, specificity, and the diagnostic accuracy of the ratio to diagnose preeclampsia, and then we compared its performance to the traditional preeclampsia markers such as blood pressure and proteinuria.

And then finally, as Victoria outlined, a major objective of the study was to determine the ability of the sFlt-1 to PlGF ratio to rule out preeclampsia within one week after the first visit

and to prognosticate preeclampsia within the next four weeks.

And so to do this, we stratified the patients that were diagnosed with preeclampsia within seven days and 28 days from the date of testing, and then we looked at the negative predictive value of the ratio in order to rule out preeclampsia within seven days, and then looked at the positive predictive value of the ratio to rule in preeclampsia within the next 28 days.

Randye Kaye: Thank you. That's a very interesting study. Can you summarize your key findings and the importance for preeclampsia?

Victoria Higgins: So the key findings from our first aim were that the sFlt-1:PlGF ratio cutoff of more than 38 yielded the highest diagnostic accuracy of 90.8%. A similar diagnostic accuracy was observed where we stratified by both early and late gestational age as well. Then compared to measuring PlGF alone, the sFlt-1:PlGF ratio had a higher sensitivity and diagnostic accuracy.

So, to give this some context, we've found that six pregnant patients in our cohort that developed preeclampsia would have been missed if PlGF were used alone but they all had the sFlt-1:PlGF ratio greater than the cutoff of 38.

So, in terms of our second aim of comparing the ratio to traditional measures such as hypertension and proteinuria, we found that compared to hypertension alone, the ratio had higher specificity and diagnostic accuracy. And then when compared to proteinuria alone, the ratio had higher sensitivity and diagnostic accuracy.

In terms of our third aim, we found that an sFlt-1:PlGF ratio cutoff of 38 had a negative predictive value of 96.4% to rule out preeclampsia within seven days and it had a positive predictive value of 84.8% to rule in preeclampsia within 28 days.

We had a couple additional findings outside of our three main aims. So we also found that those who had an sFlt-1:PlGF ratio more than 38 were admitted for delivery earlier on average, so around 16 days compared to patients with the ratio that was less than or equal to 38, which was around 35 days.

Similarly, we found pregnant patients with an elevated sFlt-1 to PlGF ratio had a younger gestational age when they gave birth. So these findings both support the impact of using the ratio on timing of delivery.

So in conclusion, our study was able to show the utility of sFlt-1:PlGF ratio to both rule-in and rule out preeclampsia and its improved clinical utility over the use of traditional markers like hypertension or proteinuria. And then we were able to demonstrate this in a high-risk obstetrical unit at the first North American institution to adopt this test clinically.

Randye Kaye: All right. Well, thank you so much for joining us today.

Jessica Miller: Thank you very much.

Victoria Higgins: Thank you.

Randye Kaye: That was Dr. Victoria Higgins from DynaLIFE Medical Labs and Dr. Jessica Miller from Dynacare discussing their *JALM* Article, "Clinical Validation of the sFlt-1:PlGF Ratio as a Biomarker for Preeclampsia Diagnosis in a High-Risk Obstetrics Unit." 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.