Discussion: 'Predicting spontaneous preterm birth' by Esplin et al

In the roundtable that follows, clinicians discuss a study published in this issue of the Journal in light of its methodology, relevance to practice, and implications for future research. Article discussed:

Esplin MS, Merrell K, Goldenberg R, et al; the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal-Fetal Medicine Units. Proteomic identification of serum peptides predicting subsequent spontaneous preterm birth. Am J Obstet Gynecol 2011;204:391.e1-8.

DISCUSSION QUESTIONS

- Was the overall study design a good choice?
- What was the Preterm Prediction Study?
- Was it reasonable to use data from the early 1990s?
- What were the main findings?
- What does ITIH4 do?
- What were the study's strengths and weaknesses?

INTRODUCTION

One of every 8 pregnancies in the United States ends in a preterm birth.[1] This worrisome statistic makes preterm birth an important issue; probably the number 1 concern in perinatal medicine today. Promising data on interventions, such as vaginal progesterone, progesterone injections, and perhaps, cerclage, have emerged in recent years. Still, despite extensive research, we have had little influence on the incidence of preterm birth; it remains higher than it was in the 1980s or 1990s.[1] Now another intriguing clue has emerged: using proteomics, researchers have learned that low levels of 3 maternal serum peptides might herald early spontaneous labor.

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STUDY DESIGN

Macones: ***Can you tell us a bit about the overall study design?***

Stamilio: Yes. The authors described this as a case-control study nested within the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units (MFMU) Network Preterm Prediction Study.

Macones: ***Yes, the Preterm Prediction Study—an oldie but goodie. Can you tell us about that?***

Stamilio: Sure. It is an older study, but we are still reaping the benefits of the data from it. The Preterm Prediction Study was performed by the MFMU Network in the early 1990s. Essentially, a large cohort of women were identified and enrolled at network sites and followed over the course of their pregnancy. They had many tests and specimens collected while participating in the study. Ultimately, this study compared a variety of predictors between women who delivered preterm and those who did not. The Preterm Prediction Study has generated some important data, especially on fetal fibronectin and cervical length and their relations to preterm birth.

Macones: ***OK, so let's go back to the study design.***

Stamilio: As I said, this is essentially a nested case-control study—actually 2 nested case-control studies—using data from the Preterm Prediction Study. One of these nested studies looked for markers in serum banked at 24 weeks; 40 samples were from women who delivered preterm, and 40 were from women who delivered at term. The other investigation was similar, except that it used serum banked at 28 weeks. Again, 40 samples came from women who had a spontaneous preterm birth and 40 came from women who delivered at term after spontaneous onset of labor. The authors then looked at the concentration of proteomic markers among those who delivered preterm compared to those who did not.

Macones: So, the people in the 24-week case-control study were not necessarily included in the 28-week case-control study.

Stamilio: Correct. And the outcome for the study was spontaneous preterm birth at <35 weeks.

Macones: ***Was that outcome clinically important?***

Stamilio: Good question—I would say yes. Your question, in epidemiology-speak, is whether preterm birth is a reasonable surrogate for health outcomes that really matter, such as intraventricular hemorrhage and necrotizing enterocolitis. I think the answer to this is yes, especially since the authors' definition of preterm birth is less than 35 weeks, a point when the link to adverse outcomes is stronger.

Macones: ***Is it reasonable to use data from the early 1990s? We are in a new century.***

Cahill: Indeed we are, but I have no problem with it at all. I think this population is still apt to be representative of today's population, and it is quite efficient to use existing data like this.

Macones: ***Does it matter that different women are in the 24- and 28-week case-control studies?***

Cahill: I think it's fine but certainly not ideal. The main disadvantage is that you can't look at the change in proteomic measurements between these 2 gestational ages. If you had longitudinal data on each woman, you would be able to do that. I understand the practicalities of what they did, and it's a good start. But I do wish the same women were in both case-control studies.

Macones: ***Do you think a case-control study is a good choice? I am sure the network has more serum banked than this. Why not use everyone?***

Cahill: Great question. I think this is an issue of efficiency and cost. This is not my area of expertise, but I imagine the methods for isolating peptides are time-consuming and expensive. Thus, narrowing your study to a logistically and economically reasonable size is a good idea.

Macones: ***What do you think of the group's lack of a priori markers to assess?***

Odibo: You can take 2 approaches. In a candidate approach, you identify markers of interest based on biological plausibility. The other is a bit more of a "shotgun" approach, where you see what markers turn up and then try to understand the biology. I see merits in both, but with these modern techniques, taking a broader approach makes sense. Of course, the shotgun approach means an increase in type I error or finding associations by chance. That is the price you pay.

Macones: Thanks. I really have come around on this issue. When I was young and idealistic, I was in favor of a candidate marker approach. But now I see the merit of using this amazing technology to generate hypotheses for future research. So I agree with you, Dr Odibo.

Macones: ***How did they identify markers?***

Odibo: The laboratory methods are dense, but it seems like the authors have taken a thorough and thoughtful approach. I can't comment on the specifics, since I do not do that type of work. Essentially, the researchers first removed high molecular weight proteins from the samples, since the target markers were of low molecular weight. The remaining material was then analyzed with capillary liquid chromatography and mass spectrometry.

Macones: ***What do you think of the statistical analysis?***

Cahill: I know this seems complex, but from an epidemiologist's viewpoint, the study and analysis are straightforward. Essentially, they are identifying new predictors for preterm birth—in this case, markers related to the proteome or the range of proteins expressed in the serum of pregnant women—and combining these with other predictors in a multivariable model. It's like other prediction studies, but they included some novel predictors.

RESULTS

Macones: ***That's great and very helpful. What were the main findings?***

Stamilio: Table 1 shows demographics, which don't reveal anything too new. The heart of the findings is that the authors identified 3 markers that were significantly reduced in cases of spontaneous preterm birth at <35 weeks. These markers were noted in both the 24-week and 28-week case-control-studies, which I think is reassuring with regards to type I error, since it was replicated in different patients. Upon peptide identification, these markers were all found to be derived from 1 region of a protein called inter-alpha-trypsin inhibitor heavy chain 4 (ITIH4).

Macones: ***What does ITIH4 do? What is the biology of this protein?***

Stamilio: I think the authors did an excellent job explaining what is—and more importantly, isn't—known about this protein. Apparently, ITIH4 is a kallikrein-sensitive acute-phase reactant, which is increased in inflammatory states. However, the biological activity of the parent protein or the fragments identified in this study are unknown.

Macones: ***Very interesting; clearly, we have a lot to learn. Can you describe the prediction piece of this study?***

Odibo: Well, each individual peptide was a marginal predictor. For example, the best of the peptides had a sensitivity of 65% and a specificity of 82.5%. A multivariable model including all 3 peptides was not better than the individual peptides alone. The authors then added a number of other possible serum-based predictors into a 9-variable model and found a high sensitivity (86%) and specificity (80%).

CONCLUSIONS

Macones: ***With those results in mind, can you comment on the strengths of the study?***

Cahill: First, the authors used a very well-characterized and valid dataset. Second, they used novel techniques to assess new predictors, and that is a good way to push the field forward. Third, I think overall, they had a sound analytic approach. Lastly, I think the authors were very forthright about their findings in that they aren't sure exactly what ITIH4 does biologically.

Macones: ***What were the study's weaknesses?***

Odibo: No study is perfect, and I have some comments. While the fact that these 3 peptides turned up in both the 24- and 28-week studies is somewhat reassuring, I still worry about type I error. And I have 1 small analytic issue. In multivariable prediction rules, you have to always look at the number of predictors relative to the number of cases. If you include too many predictors, you can end up with a problem called "overfitting," and it makes the model unreliable and unstable. A general rule of thumb is 1 predictor per 10 cases. Using that rule, the multivariable models should have no more than 4–5 predictors, and the full model included 9 variables. So I would suggest that future researchers think more carefully about the number of predictors in any type of multivariable models. Finally, when it comes to prediction rules, I think it is always good to have

some validation, even if it is internal. The techniques most commonly used for internal validation of prediction rules are "bootstrapping" or "jackknifing." I know that the prediction rule was not necessarily the focus of this paper, but that is a purist's view.

Macones: Terrific, Dr Odibo. I think you do hit on an important point. In many ways, this is a first step toward thinking about how we can incorporate proteomics into the way we think about the etiology of preterm birth and its prediction. Hopefully, studies like this will give us some insights about biology and ultimately lead to studies of prevention. So to sum up, I think we are all very enthusiastic about this study, and I congratulate the authors on a job well-done! ■

REFERENCE

1. Reproductive health: preterm birth. Centers for Disease Control and Prevention Web site. Available at: http://www.cdc.gov/reproductivehealth/MaternalInfantHealth/PretermBirth.htm. Accessed Feb. 28, 2011.