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Detection of Intravascular Hemolysis in Newborn Infants Using Urinary Carbonic Anhydrase I Immunoreactivity.

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Guest: Dr. Pawel Swietach is an associate professor at the department of physiology, anatomy, and genetics at the University of Oxford.

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.

Intravascular hemolysis is a potentially severe condition that can cause life-threatening anemia. Intravascular hemolysis may be inherited or acquired and is especially important to recognize early in newborns. However, common blood tests often use to assess intravascular hemolysis are nonspecific. Further, there is a need for more rapid and non-invasive assessments to diagnose newborns in resource-limited settings. An article in the September 2020 issue of JALM describes the measurement of urinary carbonic anhydrase I as a marker of intravascular hemolysis in a cohort of neonates. The senior author of the article is Dr. Pawel Swietach. Dr. Swietach is an associate professor at the department of physiology, anatomy, and genetics at the University of Oxford. His research applies acid-based physiology to studies of cardiac disease, blood disorders, and cancer. Dr. Swietach is our guest for this podcast. Welcome Dr. Swietach.

Pawel Swietach: Well hello Randye. Thank you for having me today.

Randye Kaye: Let's start with the basics. Your article describes the detection of intravascular hemolysis in newborns. What is intravascular hemolysis, and under what clinical circumstances might it occur?

Pawel Swietach: Hemolysis is the rupturing of red blood cells, the carriers of oxygen in the body. And when this occurs inside blood vessels, we call it intravascular hemolysis. Hemolysis can be a major event because every second cell in our body is a red cell, that's 20 trillion cells in circulation. But the lifespan of a red cell is only three months. So the process of forming and destroying red cells is very expensive in terms of body resources. Not surprisingly, our body controls red cell turnover very, very carefully. However, abnormal breakdown of red cells can occur in various disorders. For example, certain genetic traits, like glucose 6-phosphate dehydrogenase deficiency, with infections such as bacterial sepsis or malaria, or even after taking certain drugs. The

loss of oxygen-carrying capacity during a hemolytic crisis can have catastrophic consequences on health. Clinicians must be able to detect a hemolytic event rapidly, particularly in at-risk groups.

Randye Kaye: All right, thank you. So, there are probably many at-risk groups. Why did you choose to focus on the detection of hemolysis in this particular patient population of neonates?

Pawel Swietach: So, hemolysis is actually quite a common occurrence in the first few days of life. However, the degree of hemolysis can vary. A mild form of hemolysis is entirely physiological, and it facilitates the normal replacement of fetal red cells with their adult counterparts, and this is because newborns have to extract oxygen from the air as opposed to the mother's blood. However, some babies will develop life-threatening hemolysis. They will appear jaundiced and will require immediate medical attention. Now life-threatening hemolysis can be due to a blood group incompatibility like rhesus, may be due to infections during birth, or certain genetic traits carried on. Now modern healthcare facilities offer tests, which usually assess the downstream consequences of hemolysis and require high tech equipment and appropriate facilities. However, these tests usually provide a delayed readout and often they don't have the resolving power to distinguish whether there is a mild or more severe crisis. But critically, the tests currently used in hospitals are not suitable for point-of-care testing, and that's because they require apparatus and also blood taking, and therefore these are not really suitable for work in the most deprived regions of the world. Now incidentally, the triggers for hemolysis such as malaria are more prevalent in places where modern testing is simply not available. Therefore, there is a medical and economic justification to find new markers of neonatal hemolysis.

Randye Kaye: Can you tell us more about the hemolysis biomarker that you assessed in your study, carbonic anhydrase I. Why did you choose to study this marker and what makes this a better marker than other ways of assessing hemolysis?

Pawel Swietach: So, a good biomarker of hemolysis in our opinion should meet at least three criteria. These are -- it should be associated or even unique to the red blood cell. It should be released in large amounts during red cell rupture, so it's detectable. And finally, it should be present and detectable in accessible body fluids like urine, that would make diagnosis much, much simpler particularly in neonatal patients. Now we've hypothesized that carbonic anhydrase I, or CAI, could meet these criteria, but this was never tested before. CAI is an enzyme which is present in the red cells and together with another isoform called CAII, it catalyzes the hydration carbon dioxide gas. Now this

protein is very, very abundant inside red cells, but it's not normally found elsewhere in the body, at least in such concentrations as it is in red cells. So, we proposed that the appearance of CAI in blood plasma could be indicative of hemolysis and critically, we believe that the level of CAI, in plasma, would gauge the extent of hemolysis, so provide a quantitative marker. Additionally, the carbonic anhydrase I is a relatively small protein. It's less than 30 kilodaltons which means that once it is released from a red cell, it should be able to pass through the glomerular filtration barrier of the kidney and then become excreted into the urine and that will make detection pretty simple. The aim of our study was therefore to test whether the presence of carbonic anhydrase I in urine could be an indicator of neonatal hemolysis.

Randye Kaye: All right. Thank you. So, can you summarize some of the major findings from your study? Did CAI perform well in detecting hemolysis?

Pawel Swietach: With parents' consent, we recruited 26 full-term babies born in a London hospital. These babies included patients with blood group incompatibility, babies suffering from infections, babies receiving phototherapy, which is a treatment for jaundice, as well as a group of control babies. These samples of urine were then sent to our lab in Oxford and using antibodies raised against human carbonic anhydrase I we tested for the presence of the protein in the urine samples. The study was blinded, which means that the investigator who was undertaking the measurements didn't actually know which babies the samples are coming from. And our assays were strikingly able to detect carbonic anhydrase one immunoreactivity in the urine of babies who were undergoing hemolysis. Moreover, the highest signal was detected in the most severely affected patients, with infections. Importantly, carbonic anhydrase I signal was undetectable in control babies. So it's a good marker in our hands and our findings show that urinary carbonic anhydrase I excretion could be used as a biomarker of hemolysis in the newborn

Randye Kaye: So now, what are the next steps to further validate this biomarker for clinical use?

Pawel Swietach: Every year around 5 million babies die prematurely and half of these deaths are considered avoidable by modern medical practice. An early screening of babies for these hemolytic crises using our method could reduce infant deaths. What we would like to do is we'd like to undertake a larger scale clinical trial involving the most deprived areas of the world such as sub-Saharan Africa, where the triggers for hemolysis are more common, such as malaria. If successful, the next steps would be to implement the

technology into a cheap testing kit. Lateral flow ELISA kits such as those for pregnancy testing are cost effective, non-invasive, and appropriate for point-of-care use under minimal laboratory conditions. At the moment, the COVID pandemic is putting our plans on hold, but we are keen to hear from potential collaborators and investors across the world who are keen to help us in reaching this ambition.

Randye Kaye: Very exciting research. Thank you so much for joining us today.

Pawel Swietach: Thank you.

Randye Kaye: That was Dr. Pawel Swietach from the University of Oxford describing the JALM article, "Detection of Intravascular Hemolysis in Newborn Infants Using Urinary Carbonic Anhydrase I Immunoreactivity." Thanks for tuning in to this episode of JALM Talk. See you next time and don't forget to submit something for us.