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Fifty Shades of Yellow

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Guest: Dr. Dennis Dietzen is Professor of Pediatrics and Pathology, and Immunology at Washington University School of Medicine in Saint Louis.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

The May 2017 issue of *Clinical Chemistry* includes an editorial by Dr. Dennis Dietzen entitled "Fifty Shades of Yellow." The title is a play on the bestselling book trilogy by British author E. L. James, but it is also a nod to the multiple forms of bilirubin found in the blood and the various ways it is measured.

These differences are discussed in the context of their role in earlier diagnosis of biliary atresia, a rare disease of the liver and bile ducts that presents in infancy. If detected early enough, a surgical procedure is available to establish an alternate path for bile flow and delay the eventual need for liver transplant.

Dr. Dietzen joins us for this podcast. He is Professor of Pediatrics and Pathology, and Immunology at Washington University School of Medicine in Saint Louis where he has directed the Core Laboratory and Metabolic Genetics Laboratory at Saint Louis Children's Hospital since 2002. So, Dr. Dietzen, tell us about the significance of this title, "Fifty Shades of Yellow."

Dr. Dennis Dietzen: So, when I was asked to write the editorial for this paper, I think the most striking thing that comes out of this is the way that different eyes approach different patients. The laboratory test that's in question here is a relatively simple test and typically its interpretation is relatively simple, but in small babies it's not quite so simple depending upon who is looking at the patient. The neonatologist looks at a bilirubin result and tends to worry about the invasion of unconjugated bilirubin into the brain where as a neonatal gastroenterologist would look at the same patient and the same test result and see a broad range of disorders that might potentially be there, so I think that the way I look at it is those physicians -- those patients that look at the same colorimetric chemistry assay and see very different shades of that color. So, that's where the title came from.

Bob Barrett: What is biliary atresia?

Dr. Dennis Dietzen: So, biliary atresia is -- it's a very insidious disorder that starts most commonly in the perinatal period. It's not detectable typically in utero, but it is a very slowly progressive disorder in which the bile flow from the liver to the intestine slowly gets interrupted to the point where there is obstruction and there's no longer bile flow into the intestine.

The result of that is that bile backs up into the liver and causes hepatocytes to die and then there's a fibrotic and inflammatory response in the liver that eventually leads to total liver failure.

It is not uncommon. It is most common in Taiwan and Japan where about one in five thousand infants are affected by this. It's a little less common in the United States and Europe where it occurs in about 1 to 15,000, or 1 to 20,000 babies. So, it's rare but it's not -- it's something that's encountered at least at my institution several times a year, but the question of its existence comes up much more frequently than that.

The cause of the disorder is still the subject of much investigation and I'm afraid we don't have a good handle on why it occurs. There are number of hypotheses as to why it occurs. Some hypotheses involve a neonatal viral infection, some sort of toxic exposure, maybe in utero, or shortly after birth, there are autoimmune hypotheses that are involved in mechanisms that destroy these bile ducts.

So, we really, really do not have a handle on what causes it, therefore our ways to detect it and treat it are rather coarse at this point. So, it's something that is always in the back of the neonatologist's brain when they look at a child that has conjugated hyperbilirubinemia.

Bob Barrett: Well, with all that then how is biliary atresia diagnosed?

Dr. Dennis Dietzen: So, it is a long arduous process. The initial key is to identify-- so when you measure bilirubin in the laboratory, you can measure -- most commonly you measure total bilirubin. And total bilirubin is the key to making sure that the new born brain is okay. There are nomograms and algorithms that are available for the -- to dictate what should be done to an infant who has a high total bilirubin, but a lot of times people are so focused on that total bilirubin that they forget that there's another fraction of bilirubin that sometimes is measured and sometimes is not measured depending on the physician, that is -- and in that particular shade of bilirubin is--conjugated bilirubin. And

that doesn't have consequences for the new born brain, but it does have other consequences.

So, the initial diagnostic process involves a recognition of conjugated hyperbilirubinemia, but then the differential diagnosis in a child of conjugated hyperbilirubinemia is dozens of disorders long.

So, once you identify that there's cholestasis as we call it, there are number of other disorders that must ruled out before you can diagnose biliary atresia and ultimately the diagnosis of biliary atresia requires typically some imaging studies where a tracer is used to follow the bile flow through the liver and into the intestine, and most often there is a liver biopsy that's also involved. So it takes a long time from recognition of conjugated hyperbilirubinemia to the diagnosis of biliary atresia.

Bob Barrett: Must have some limitations of those methods used for diagnosis?

Dr. Dennis Dietzen: Absolutely, I mean the problem is that the tool -- the initial tool we have, this measurement of conjugated bilirubin, is not our best -- it's not our best test. So, the issue, there are a number of different ways to measure this. There is a -- on one particular platform -- one particular chemistry platform, you can measure a little more specifically, you can measure that conjugated bilirubin fraction. But in other on -- most other chemistry analyzers, the technique involves measuring bilirubin without the presence of what we call an accelerant, to make sure that you can measure all of the bilirubin, so we're only measuring a little fraction of the bilirubin.

The problem is in these babies that the conjugated fraction is almost always a very small fraction of the total, and the total bilirubin often cross reacts in those what we call direct bilirubin assays. So, there's an interference there and there's a contribution from the total bilirubin that sometimes it's hard to decipher, is the conjugated fraction elevated, or is it just the consequence that the total bilirubin is high. So that's part of the problem.

So, there's a very fuzzy area between normal and abnormal when you're measuring direct bilirubin. The other issue is that the units that we use to measure bilirubin are truncated kind of artificially. So, if the upper end of the reference interval for conjugated bilirubin is 0.2 or 0.3 milligrams per deciliter, we only report out to one decimal point. So, a change of one tenth of a milligram per deciliters is a 30 to 50% change in the amount of conjugated bilirubin that present.

So, our ability to decipher very small changes and slightly abnormal concentration is compromised with that particular assay. So, it's not a great assay to use, furthermore those assays are not -- they don't agree well with each other across various chemistry platforms.

So, it's a tough thing to diagnose very slight increases and the end result is that we end up picking up elevations in conjugated bilirubin when they're very, very advanced.

Bob Barrett: Well, from your descriptions it sounds like there's urgency in this diagnosis, why is that?

Dr. Dennis Dietzen: Left alone, biliary atresia has a poor prognosis, left alone biliary atresia, to save the life of the infant, requires a liver transplant. There is however an intervention that can be made to delay -- it doesn't -- most of the time it does not totally prevent the need for liver transplant, but you can to delay the need for liver transplant for many, many years, and that intervention is called the Kasai procedure. And the Kasai procedure -- what surgeons do, they introduce an artificial conduit for bile flow from the liver into the intestine. And it works very, very well but the issue is that it has to be done, the outcomes are best, if the procedures performed at less than 30 to 40 days of life.

So, if you wait until about 60 to 90 days of life to do that procedure the odds of success drop rather dramatically. And I think data indicates that the average age at which this surgery gets performed is about 60 to 70 days. So, we have a problem -- the issue is trying to get those kids into -- to identify the disorder and get them to a Kasai procedure as quickly as we possibly can do it, and right now we don't do that very well.

Bob Barrett: How did the paper written by Lam et al address this situation?

Dr. Dennis Dietzen: So, what they did is they took an interesting approach. They hypothesized that part of the problems in getting to Kasai quickly is that the disorder is not recognized early enough. So there's a lot in the United States in particular, there's a lot of attention given to total bilirubin values, sometimes to the point where the possibility of conjugated -- the presence of conjugated bilirubin is ignored.

So, they looked at a serious of cases of biliary atresia and they looked at some of the diagnostic schedules in these kids, they looked at the time that the first bilirubin was done, the time when the first conjugated bilirubin was done, and the time at which the Kasai was done.

And what their hypothesis was, that they thought that the Kasai procedure would be done earlier if conjugated bilirubin was performed earlier, and therefore that conjugated hyperbilirubinemia was recognized earlier.

And I think there's a hint in their data that this early recognition is possibly part of the problem. So, they did not find in those two groups where conjugated bilirubin was done earlier or later, they did not see a significant difference at the age of Kasai, but they did see a little bit of a decrease from the time at which that first conjugated bilirubin assay was done, to the Kasai procedure, there was a very small decrease in time. So, I think the answer -- the short answer in their relatively small cohort was maybe recognition is really part of the problem, but I think what is reinforced by this particular study, is that there's a lot of stuff diagnostically that has to get done between the recognition of conjugated hyperbilirubinemia and the Kasai procedure that is very slow, very onerous, and very technical, and very time consuming, and that we need to fix not only the recognition part, that is getting a conjugated bilirubin done early, that we must streamline the rest of that process as well, in order to succeed in getting these kids to Kasai quickly.

Bob Barrett: Well, finally Dr. Dietzen, what other gaps need to be filled to improve the diagnosis of biliary atresia?

Dr. Dennis Dietzen: So, again I think I mentioned earlier we use rather coarse tools to make this diagnosis initially. So, the recognition is a problem because we're using a very nonspecific marker for the diagnosis of biliary atresia. What's needed, and there are many active efforts in this is -- we need to understand the mechanism of the disease much, much better.

And from an understanding of that mechanism, we will likely be able to generate more specific biomarkers where we can rule in biliary atresia much earlier, get kids to Kasai earlier, and therefore delay as long as we can the need for those children to have a liver transplant. There have been a number of proteomic and metabolomic studies to try to identify such biomarkers, but they all have weaknesses and there's really no stellar biomarker that has come out of these particular studies.

There are number of circulating proteins that have been hypothesized to be helpful, a number of circulating bile acids, number of circulating amino acids, that seem to have some information, but the information is rather crude and it doesn't have the sensitivity and specificity that's required to streamline the diagnosis of biliary atresia and get kids to a Kasai procedure as quickly as we can.

So, there's a lot of work left to be done in order for us to improve the situation anytime soon.

Bob Barrett: Dr. Dennis Dietzen is Professor of Pediatrics and Pathology, and Immunology at Washington University School of Medicine in Saint Louis. He has been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.