NAFLD & NASH Diagnosis: A Deeper Dive

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Diagnosis of NAFLD: From Non-invasive to Invasive

KENNETH CUSI: Welcome to the foundations of cardiometabolic health certification course. Thank you for considering this certification opportunity. I'm Dr. Kenneth Cusi. I'm a professor of medicine at the University of Florida at Gainesville and chief at the division of endocrinology and diabetes. And following that detailed explanation by Dr. Mantzoros on the pathophysiology of NASH, we're going to go now into the diagnosis. And this is a very important topic as we learn more and more. It is brought implication for the management of obesity and diabetes.

These are my disclosures, largely for grants and agents that are in the pipeline which I'm going to talk about in the last half of this presentation. And for those who don't know where Gainesville is, I am in North Florida, and you're welcome to visit if you happen to be around.

Now after hearing what the mechanisms are that lead to fatty liver and steatohepatitis and fibrosis, what I would like to cover is a little bit the aspects that have to do with the diagnosis and the management. The diagnosis is going to be involved, number one, into thinking that there are some patients that are particularly at risk. And these are divided in three groups. Those who have insulin-resistance or features of insulin-resistance syndrome or metabolic syndrome that you've heard before in the prior lectures; people with type 2 diabetes; or those that on your electronic medical records or an imaging study has an incidental finding of steatosis or has been found to have elevated liver enzymes. Step two will be to assess if they have a history of excess alcohol intake or other medical conditions and that requires a good history. The first step will be now digging into identifying if they have fibrosis or not. And we're going to do a very simple test we're going to talk about. It's called FIB-4. And with that, we're going to be dividing patients into their risk being high, indeterminate, or low. And I'm going to go over this in detail in this brief lecture.

NAFLD and T2DM

With that, let's first discuss the link between type 2 diabetes and fatty liver disease. With this - - so I don't want to add to that, because I know Dr. Mantzoros has done an outstanding job already. But just to remember that this is part of a multi-system disease which the liver is behaving to the insult of lipotoxicity in the way that it can, which leads to a pathological response of inflammation and in the end of fibrosis. Remember that this sort of fat attack on the liver is going to be from visceral fat that drains directly into the portal - -. Also from systemic fat as obesity progresses, there's more fatty acid from non-visceral sources to the liver. And again how fat gets sick with that reduction adiponectin, which plays also a critical role, among many mechanisms.

This is overall global prevalence of type 2 diabetes, patients with type 2 diabetes and how often they would have NAFLD. Notice this is from many different countries and regions, but largely using ultrasound. And this has been published about two years ago. We have published very recently, and it's still in press, but it is in the *Lancet Gastroenterology a*n update to this that included several studies in the past two years using MR elastography and magnetic resonance imaging to establish fat and fibrosis. And we think that that number of people with steatosis is higher than 55 percent in the population with type 2

diabetes and more like 70 percent. And what you can notice is that many, many studies now have linked type 2 diabetes to fatty liver, and the link is very complex. But I would have to say that if you have diabetes your chance of having fatty liver would increase. But not only that, the chances of you having inflammation and fibrosis increased markedly, and of cirrhosis and hepatocellular carcinoma. For decades people have wondered why this is the finding, that people with diabetes have two or three times the risk of developing adenocarcinoma of the liver and now we know that that link is because they have more NASH. And on the other hand, if individuals with fatty liver have diabetes, that diabetes becomes more difficult to control. They're very insulin-resistant, have more atherogenic dyslipidemia and also more cardiovascular disease. The ADA paid attention to this in 2019. And of course every year since then, they have recommended that if you have prediabetes or type 2 diabetes, and elevated liver enzyme, that you think whether this patient has fibrosis or not and do the work-up which we're going to be talking about today.

Another important thing is that if you have a fatty liver and don't have diabetes yet, your chances of getting it are much higher. And with this study, that included 19 observational studies, almost 300,000 individuals, with 16,000 cases of insulin diabetes for followup, found again a 2.22 greater risk of insulin diabetes. And if you had more severe NAFALD, only in few studies has it been looked at, you also had a higher chance. But overall you see that most of the studies have shown this association.

Now another important thing is the most recent data that has been published on trends in the prevalence of diabetes in U.S. adults. And you see this is not going to go down. And this is published in *JAMA* in 2021. You see that we continue to have more and more diabetes and something has to be done about it. And, again, early screening and management of with loss is very, very important. But this is not only a problem in the United States. It's a worldwide problem. And you see by 2045, you're going to have roughly more than 50 percent growth in the prevalence of diabetes and it's across all regions, so major, major public health problem. Again, literally the ADA has done this, but many societies across the world, liver societies, and even the American Association of Clinical Endocrinology has new guidance that is coming out now in early 2022, in collaboration with the American Association for the Study of Liver Disease, set the basis for endocrinologists, diabetologists, to work up these patients. There's also a new guideline, an updated guideline, by the American Association for the Study of Liver Disease. And a clinical care pathway that was a multidisciplinary effort that Dr. Mantzoros also participated in, trying to set some clear strategies for the screening and diagnosis of patients. That's what I'm going to share with you.

Diagnosis of Steatosis and Fibrosis in NAFLD/NASH

Now let's first look at the tools that we have to establish steatosis and establish the presence of fibrosis in our patients. This is how a liver of somebody with steatosis looks like if you did a plain ultrasound. And when you look at these patients, you see that you compare the echogenicity of the liver to that of the renal cortex. And you see this is rental cortex, this is brighter. This liver has too much fat. And the reason for the report of increased echogenicity is just indicating fat. The limitations of ultrasound are they're very operator-dependent. Second is that if you're more obese, the diagnosis is not so good.

Third, in studies that we did we compared with MRI methods and ultrasonography doesn't detect fat below about 12 percent, so it's not very sensitive. And lately, and lastly, what important is also to know that it cannot tell really if you have fibrosis or not. It can be very bright and just negative of having cirrhosis, so not a great test, but it's available and can be helpful.

More valuable is the use of elastography techniques. The most widely used being the FibroScan with more validation studies. But there are other methods like shear-wave elastography and Sonic Incyte is another company that has a new device that's FDA-approved. And you see that's the typical device. And what it gives, there's smaller devices with elastography. The typical report that you will see is going to look like this, with the left showing fat. Above 284 is considered steatosis, definite steatosis. And the other parameter that you see is the transient elastography reading or fibrosis. I would say a number greater than 8 is clearly suggestive of needing additional workup. And, again, it's a very simple test that takes about 10, 15 minutes to perform and can give you a lot of valuable information. Again, the slope of this reading by the vibration-controlled transient elastography is going to tell you what the risk of steatosis that a given patient has.

We used this and we found that this is a greater problem than we have considered before. This has been published now a year ago in early 2021. Dr. Lomonaca is a faculty member of the team. And this was a multidisciplinary effort done in family medicine clinics, internal medicine clinics, and endocrinology clinics. And here's the key message. These were 561 patients that did not have any knowledge of ever having been told that they had a fatty liver. If they were, they were excluded and didn't have any other measurements. And, again, seven out of ten had steatosis. And one in five patients with type 2 diabetes had fibrosis. Again, even using live enzymes alone was not very informative. And that's why relying on elevated liver enzymes of 40 or greater is probably not the way to go. You should probably use a cutoff of at least 30, but probably use the FIB-4 which I'll tell you in a moment. But when you look at those, mild is any reading above 7, moderate fibrosis is 8 or greater, and then all the way to cirrhosis is when that cutoff is 13.6 on the transient elastography. We see that this is something broadly, a big problem that's underdiagnosed. And, again, only a minority of people had liver enzymes above 40 - - would suggest you to do additional screening. That's why we think liver enzymes are useful, but not enough. And that's why we advocate additional tests to screen for this.

Diagnosis and Management of NAFLD/NASH: A 2022 Update

What are these additional tests? Let's dive now into the diagnosis and management of NAFLD. And let's say what a multidisciplinary group of experts decided. Dr. Kanwal is a hepatologist based in Houston, and Baylor College of Medicine, Dr. Shubrook is a primary care physician. We have a number of prominent of hepatologists. You see Dr. Mantzoros there, the other lecturer in this series, and myself. Again, going back to what we talked about in the beginning, risk factors are patients with obesity, people with type 2 diabetes, people with a metabolic syndrome. Again, rule out alcohol intake, other preexisting conditions that are listed down there, and do the biochemistry tests that allow you to rule out these other conditions, which you can see there on the slide.

Now there's been a lot of debate of what to look at in terms of when to refer to the hepatologist. And in this paper, there was a survey in which there were different opinions of when to send. And, again, the guidelines, these are different guidelines from the World Gastroenterology Association, European Association for the Study of Liver Disease, American Gastroenterology Association, all of these have different views. We felt that this was very confusing for the primary care. And with people who are involved in many of these, this is a multinational group of experts, we came up with something simpler. The first test to do will be the FIB-4, and what this allows you is to separate people at low risk, which will be about at least half of the people with diabetes and two-thirds of those with obesity. And, again, these will not need any additional workup. And then those who have high-risk would go straight to the hepatologist. Those with indeterminate risk can go to the hepatologist or we can do an imaging study as I showed you before, which would be an elastography test. And, again, if those risks were considered low, you repeat the test in two or three years. We don't think there's a rapidly progressive disease in most patients. And then that way use a cost-effective approach.

Now what is the FIB-4? The FIB-4 is a very simple test in which what we look at is age, liver enzymes, and platelets. For example, in our electronic medical record, we have developed the FIB-4. We just have to click a calculator and it does it for us. But if not, you just go to any web browser-type FIB-4 and fatty liver, and there are many websites that can do it for you. And it's a good test to at least rule out cirrhosis. It has very high negative predictive value. It means you're probably not going to miss a lot of people with cirrhosis. Not so great in its positive predictive value, what we call sensitivity. But it's free because most of our patients will have platelets and liver enzymes. If it's greater than 2.67, high-risk of cirrhosis. If it's below 1.3, low risk but it's never perfect. And then there's a lot of people in the middle. That's what the calculator typically looks like. You put in age, liver enzymes, and platelets, and you get the number that was discussed.

Now how useful are these tests? Well, first we took, this is a little example that we published mid-last year. We tried here to address what is the appearance of steatosis. This is done by a test called US FLI, United States Liver Index. And you see that even within the overweight range, the rate of steatosis is pretty high, 50 percent. And when you move into the obesity range, by the time you hit number of 35 in your BMI, most people have steatosis. But the real important thing for you to remember is that, A, diabetes itself increases significantly the risk of steatosis. Diabetes is in orange. But it seems that an even an individual without diabetes, with obesity, also have a very high risk.

But getting back to the FIB-4, if you look here, those with a FIB-4 greater than this magic number of 2.67, where did the number come from? That cutoff came from a lot of epidemiological studies done in the past 20 years. It's not new. This is something being broadly used in the liver field. It's just new to us as non-hepatologists. But, again, about 10 percent of people with diabetes, whether overweight or obese, about half of them with obesity will have this number. But if you take the gray zone number, and, again, we published a paper showing going all the way done to this gray number between 1.3 and 2.67, if you cut that in half and just use 1.67, it's still about one out of four patients are at risk of fibrosis. Remember sending those to the hepatologists.

Now getting back to the natural history of the disease, and I know we talked about this, there are many forces at play. One is first development of fat in the liver. And, again, that depends on insulin resistance. You can say that whether you're lean, overweight, or obese, if you have steatosis, we've seen this many times, you're most likely insulin-resistant, if we did a high-end metabolic test to find out. Steatosis: think about insulin-resistance. However, there's a biology of steatohepatitis, which we think is linked defects in the accumulation of toxic lipid metabolites, like diacylglycerols, ceramides, and some sort of malfunction or collapse in the mitochondria. The thinking is by this chronic sustained flux of fatty acids in some way. The key thing that happens once inflammation is triggered, for example, some animal studies have shown that Kupffer cells, that are like the macrophages in the liver, you could remove them and inflammation would not happen. Clearly there's a talk between hepatocytes, Kupffer cells, and ER stress that drives this. And then stellate cells receive this message and take it over to the next level by promoting collagen synthesis and fibrosis. Very complex biology. And, again, this is not something I'm going to discuss with you.

But we just talked about FIB-4. You should know there are other panels. I'm just going to mention a couple of things here. The ELF panel has been also extensively studied in Europe and many other countries. And, again, it uses hyaluronic acid, TIMP1, and aminoterminal peptide or procollagen 3. It's a good index, again, and it's been recently approved in the United States. Again, it is now commercially available in the United States. None is perfect. The areas under the curve vary in the population where they're applied. Better in liver clinics, not so good or less predictive in primary care, but with an area under the curve of about .75 to .85. Pro-C3 is used in research studies, is developed in a commercial platform for clinical care, used in research studies. There's NiS-4, another panel recently approved in the United States, and so forth. U.S.-based imaging tests, we talked about transient elastography. We didn't have time, shear wave is another method. And then we talk about MRIs, there's MR-elastography is the gold standard for fibrosis, short of doing a biopsy. And there's also the LiverMultiScan, which looks at fibro-inflammation, necroinflammation as a surrogate marker of NASH, and I'll show you that in a moment.

Emerging Biomarkers and Diagnostic Tools

Let's look at these emerging biomarkers and later I'll show you a little bit about the imaging. Let's pay a visit to what are these emerging biomarkers and diagnostic tools. Number one, I always get asked, well, why is it so hard? Why don't we have an A1C as we have diabetes to diagnosis NASH? Well, what happens, we need to find out what's happening in an organ that's relatively small, considering an individual, that has a poor substance in the blood that we later sample to do like a liquid biopsy. But to confuse the thing more, fibrosis is not just a static thing. It has two ends, right? One is generation of new collagen deposition and extracellular matrix. And then it's removal. This is constantly in formation and removal, same as other tissues, bone, skin, whatever you want to call it. And you have to say that the many collagens tested and looked at, and, again, the one that has been proven most valuable so far has been PRO-C3, which has been evaluated in clinical studies.

Talking about imaging, this is the MR elastography testing. It's a device that is a little disk is the reader that goes on top of the liver. And it allows to stratify based on cutoffs. The

chance of somebody having from F1, which is mild fibrosis, to moderate, F2. This would be equivalent to the FibroScan, to the imaging by transient SLR would be done at the bedside with FibroScan or shear wave. But typically there's a relatively good correlation. Then you have advanced fibrosis or cirrhosis.

Finally something that is making significant strides has been the use of LiverMultiScan, which can detect liver fat by simply MRPFF. But more valuable is that it's measure of cT1 that gives you an idea of the degree of disease activity. And, again, this has been used in any clinical trials and it's now available in the clinic recently. And I think it's going to have potential application. Like in this person, this is a scan of one our patients at the University of Florida with elevated liver enzymes, this value being above the mid 800s. Clearly the 900s is a clear indication that this patient in addition to having a value of transient elastography that suggest fibrosis stage two, there's a lot of disease activity and chance for disease progression.

In the end, just to finish, there are many biomarkers. I just that. It's a recent review from 2021. Many have been tried, many have failed. But just to tell you that we're talking about FIB-4, but this a vast and very complex field. In a word, I have to say has been in the field of being able to diagnosis cirrhosis or advanced fibrosis. Much less in the ability to distinguish inflammation or NASH. And not great at all in determining fat by a blood sample.

And, again, where the field is heading to, just to give you a final overall view in a nutshell, there's a of genes that have been tested, SNPs. The best characterized would be the PNLPA3 that regulates fat content. But most importantly has been correlated with future fibrosis and poor liver outcomes and others. There's a lot of interest in studies looking at, again, transcriptomics, proteomics, and overall lipidomics, but that's the subject of another meeting.

Finally at University of Florida, I want to thank our patients. In the last ten years, we've learned a lot more about NASH. And I want to focus on what we can do for their management. Again, at least with the FIB-4, with elastography, and eventually in those who get a liver biopsy, we can characterize people into low risk, indeterminate risk, or high risk. And there are a number of things we can do for them. Your mission, if you're an endocrinologist, primary care doctor, weight management, a cardiologist, if you're not a hepatologist, your mission is diagnosis your patients early on and send them for a referral, because that's what's going to prevent cirrhosis. The hepatologist gets somebody ascites, it's game over. We need to learn what to do. And most importantly, know that there is pharmacotherapy under development, and I'll show where we are, but there's things you can do now, lifestyle intervention, weight loss. Again with more emphasis, always with big emphasis, but particularly when you have advanced disease, because you can regress, even cirrhosis can be halted or even improved some with significant with loss. Pharmacotherapy, currently it's limited to weight loss agents and some diabetes medications. And, again, statins should always be used in people with NASH or cirrhosis. Statins have been even used in advanced stages. But, again, you got to be careful with, not in patients with very advanced cirrhosis or decompensated cirrhosis. And finally diabetes medications today offer the best option to treat NASH, and we're going to talk about at extensively.

As a take-home for this first section, remember that as the diabetes epidemic worsens, this is going to also worsen the epidemic of NASH and of cirrhosis. As you may have heard many times now, we think that soon NASH is going to be the number one cause of liver transplantation from cirrhosis. The good news is if you do a good job in diagnosis early, thinking about the FIB-4 and some imaging, you can really prevent cirrhosis from happening. Still, there's a lot of inertia. But this is changing. Societies are getting involved. Primary care doctors and endocrinologists are now screening, so remember to do that. Again, there are some diagnostic algorithms like the one that we did with Dr. Mantzoros and Dr. Kanwal and Dr. Shurbrook, I recommend that you check them out. Check out the last ACE guidelines and the update of the American Association for the Study of Liver Disease that's out in 2022 for the latest on what to do best. And I showed you a lot of the algorithm that we do Dr. Kanwal and colleagues. And there are things you can do now. The key thing for you to reverse obesity or you change the nature and the biology of adipose tissue in this patient. We're talking about an insulin sensitizer like pioglitazone or combined.

With that, I'm just going stop here and move into pharmacological treatment. This is our group so come and visit to the University of Florida. I promise I will not do a liver biopsy at least on your first visit to us. Thank you very much.