NAFLD & NASH: Epidemiology & Burden, Risk Factors and Pathophysiology

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DR. MANTZOROS: Hello, everybody. Welcome to our meeting. In the next hour or so, I'm planning to present to you the topic of NAFLD and NASH, starting with an introduction going over epidemiology, pathophysiology, and from pathophysiology, we will discuss screening and therapeutics.

Why talk about NAFLD and NASH? This is an unmet clinical need. Most of the time in clinical medicine we don't think about it. But, as we learn more and more about what causes NASH, and that there are diagnostics and treatments, we also learn that this is part of the epidemic of the 21st Century, which is cardiometabolic disease. So it is part of the metabolic syndrome and insulin resistance. And this is why this is part of this program. So, it's an epidemic, very common. It is an unmet clinical need. We need to think about it. Let's go step by step over what we need to cover.

My name is Christos Mantzoros. I am a professor of medicine at Harvard Medical School. I run the Endocrinology Section in the state for the V.A. system, which includes four hospitals and ten clinics, and I run Human Nutrition for Beth Israel Deaconess, and I am a professor at Harvard, and the Editor-in-Chief of the journal, Metabolism, with an impact factor of 8.7.

Today I will talk to you about not only our own research, but what we are learning as a community.

In my group, we focus primarily on obesity and diabetes at Beth Israel Deaconess Medical Center. Obesity, according to the obesity society, today has 98 co-morbidities. Diabetes is well known. Cardiovascular disease is another one. Now, the new kid on the block is NASH and NAFLD, and I will explain to you why.

We started with interest in leptin and adeponectin. Our claim to fame is leptin in low energy states. We proceeded with obesity and its complications. We now study gastrointestinal hormones, hormones that are hepatokines, or secreted by muscle, and I am going to present all of these to you and hope you make sense out of what we are learning, and apply in the clinic, with a specific focus today on what we call NAFLD or NASH. And we will go over noninvasive diagnostic algorithms, and then over clinical evaluations and therapies.

So, as a very brief introduction, very simply, what is NAFLD or NASH? So, the diagnosis is a negative one. It is nonalcoholic. So, it is not caused by alcohol. In the past, we used to call it cryptogenic. We did not know what it was. Now we are learning more and more. So we have to exclude a few diagnoses. But, in simple terms, what we call NAFLD out there is a nonalcoholic accumulation of fat, or steatosis. More than 5% of the liver is fat. With MRI 5.5%, but we need to remember more than 5% fat in the liver is abnormal, and we call it nonalcoholic fatty liver disease (NAFLD), or fatty liver steatosis. Our bodies recognize that this is not normal, and tries to limit it. So, in this case, it sends micro - -, and inflammatory cells to remove it. And it is successful, most of the time, 80% of the time this is controlled. But 20% of the time this progresses and from physiology becomes pathophysiology, and become inflammation steatohepatitis, ballooning inflammation. And, then, with prolonged period of time, scarring, fibrosis, and we move now to cirrhosis and, in a smaller number of people, to hepatocellular carcinoma. Now, we realize, as I am going to tell you later, that this is caused by either overall obesity, or sarcopenia, so, because of metabolic factors. And we also realize that, in addition to liver complications, our subjects will have cardiometabolic complications. So, this is metabolism-associated fatty liver disease, and many of us out there have proposed that we need to change the name to Metabolism Fatty Liver Disease (MAFLD), or, Dysmetabolism Associated Fatty Liver Disease (DAFLD). And this is work in progress. A lot of work has happened with the previous diagnoses and the previous definition. But, it is time. So, it is Nonalcoholic

Fatty Liver Disease, and in the future it may be called Metabolic, or Metabolism Associated Fatty Liver Disease.

Okay. Let's go over the current gaps in NASH awareness. In summary, I can tell you that most of us out there in the clinics don't look for NASH, and we don't look for NASH because we don't think about it. And we don't think about it, as I am going to tell you in a few minutes, because, until recently, we did not have a lot of diagnostic tools, and we did not have proper treatments. But, now, things have changed.

So, let's go over what is happening in out there. Very recently, professional societies in the USA, the American Gastroenterology Association, the Obesity Society, American Diabetes Association, the Endocrine Society, convened a panel of experts. And I was representing the Endocrine Society. And we needed to assess participants' knowledge. We said, let's go out there, and all 750 participants from all walks of medicine, most of them were primary care doctors. And, on average, they had about twenty years in practice, and we sent out a questionnaire with 24 questions, on screening, diagnosing, and monitoring NASH. I can tell you that the results were rather disappointing. Probably, not different than random. But, let me go over a few of the questions with you, so that we can see together what you know, and before we say what we need to know.

So, we put this paper together in several journals at the same time, Obesity, Metabolism, Gastroenterology, Diabetes. And, as I told you, the result was that we said we are called to action. We realize that most of us don't think about NASH, although it is very common, about 24-37% of the population have fat in their livers. And 20% of people who have fat in their livers will progress to steatohepatitis, as I told you earlier, and 20% will progress to cirrhosis and liver failure. So we don't want to see patients in end-stage liver failure, or cirrhosis. We need to get to the bottom of it very early, because we need to stop it in its tracks. So, we suggested in this paper that we need to screen at the primary care level, at the endocrinology level. We need to diagnose earlier, and we need to optimize patient management consistently, timely, and accessibly. So, NAFLD and NASH is not a disease only for hepatologists in the 55, or so, hepatology centers in the USA. It is a problem that all of us in our clinics need to think about, and do as much as we can before, of course, we refer extreme cases to hepatologists.

All right. Let's go over some of the questions from our survey, which, I think, highlight significant gaps in our knowledge.

One of the questions was, Are almost all patients with severe obesity likely to have NAFLD? Think about it before I give you the answer. Are almost all patients with severe obesity likely to have NAFLD? The answer is yes, and unfortunately, only a few primary care doctors and endocrinologists and gastroenterologists out there said yes. But, obesity is very strongly associated with NAFLD. And I am going to show you some data.

Is NAFLD common in patients with type 2 diabetes? Think about it for a minute. Yes. Unfortunately, most people got it wrong. But, yes. Diabetes is very closely associated with it. We will go over the data in a few minutes.

Should initial evaluation of patients with suspected NAFLD include cross-sectional abdominal imaging? So, someone comes to our clinic with diabetes and obesity, we think about NAFLD. Should we send them

to radiology for computed tomography to screen for HCC? Think about it for a minute. And the answer is no. We shouldn't. It's too early.

Are pioglitazone or vitamin E recommended as treatment in select patients with NASH? Think about it. The answer is yes. And only 50% of participants answered correctly. Keep in mind nondiabetics. Vitamin E 800 units per day works. We will go over it. Pioglitazone works in diabetics. It is not approved specifically for NASH, but it helps NASH in diabetics. Vitamin E – nondiabetics. Pioglitazone is good for diabetics.

Can abdominal ultrasound be used as a diagnostic in identifying NAFLD patients with NASH? Well, most people said yes, but the answer is no. Ultrasound is not specific enough. It is very specific if fat in the liver is 25-30%. But not in patients with fat in the liver on the order of 5% or 10%. So, in late stages, yes. But for most people, no. It is not indicated.

Alright. Let's go on with this survey.

To the best of your knowledge, which of the following statements accurately define nonalcoholic fatty liver disease?

Evidence of hepatic steatosis

Evidence of steatosis and lack of secondary causes.

Fat, but no specific other causes.

Steatosis with other causes?

Not sure? Would like to receive more information.

Think about it for a minute. I will give you the answer, and we will discuss it in more detail later. The answer is Steatosis and lack of secondary causes of hepatic fat accumulation. We will go over that.

To the best of your knowledge, which of the following statements is accurate?

Presence of 5% of fat.

Presence of 5% of fat with hepatocellular injury.

Presence of 5% of fat without hepatocellular injury.

So, if we talk about nonalcoholic fatty liver disease, we only need steatosis. If we talk about NASH, steatohepatitis, as I told you earlier, you need both. Hepatic steatosis and inflammation. We will go over this in a minute. We will go over who to screen, how to diagnosis, how to treat patients, and, of course, the disparities between published practice guidelines and clinical practice. So, we have just published the guidelines in Gastroenterology this month.

So, let's go step-by-step. Describe what is happening out there. Epidemiology. Risk factors. Then move from epidemiology to pathophysiology. Current understanding of why what is happening is happening. And, then, from pathophysiology, let's move to potential diagnostics and therapeutics. If we understand mechanisms, we can design better novel therapeutics.

We all know that as time progresses, obesity is becoming more and more prevalent. Thirty percent of Americans today are obese, 30% are overweight. And with the increasing prevalence of obesity, the prevalence of comorbidities goes up. We all realize, for example, that the prevalence of diabetes is going up from 5% or 6% in 1990 to more than 10% or 12% in 2030. What we don't see, most of the time, is that the prevalence of NAFLD also goes up, and it is twice as many patients who have NAFLD as the patients who have diabetes. And this is a problem that we need to address.

What about diabetes and NAFLD that I told you about earlier? Seven out of ten diabetics who come to my clinic will have NAFLD. So, in the USA, anywhere between 30-70% of the diabetics who come to our clinics have NAFLD. The numbers are even higher in Europe, and Southeast Asia. Africa has fewer patients with NAFLD. And Latin America also has very high prevalence. So diabetes is very strongly associated with NAFLD. Think about NAFLD, what you see in an obese, diabetic person. The global population 25% of people out there, one out of four patients who come into our clinics, will have NAFLD. And patients with diabetes, more than 60%. Seven percent in my clinic, and, then, out of those, about 20% will have NASH. So, about up to 30% of patients will have NASH. It is very expensive for our society and our health care systems, and the costs are going up.

What is the spectrum of the disease? Healthy liver – less than 5% of fat in the liver by histology, 5.5 with MRS imaging. So, if fat exceeds 5%, we talk about steatosis, or nonalcoholic fatty liver. And, if this persists over time, and we have evidence of inflammation of hepatocyte damage, then we talk about NASH. 20% of people with NAFLD on average will proceed to develop NASH. And, then, if this, over the years, becomes prolonged, it takes about seven years to go from each stage to the next. We may have scarring and fibrosis, which is a condition leading to cirrhosis, and hepatocellular carcinoma later in life.

Interventions would make a difference. The past approach was that we would intervene with severe fibrosis and try to block the progression to cirrhosis and hepatocellular carcinoma. This was an approach that was influenced by successes with hepatitis C or hepatitis B. Now we realize that this is a metabolic condition. We think that we need to intervene earlier. Why not intervene when fat accumulates, or when we have the first signs of inflammation? Why do we have to wait until cirrhosis? We need to prevent it rather than wait until major and advanced manifestations are present.

This is what is happening in a more pictorial way. So ballooning is fat in the liver. It progresses in 30% of the patients. It may take five years, may take seven years to inflammation. And, then, they progress about one stage of fibrosis every 7 years for NASH, and every 14 years for NAFLD. So, it takes seven years to get to NASH, and about seven years to get to fibrosis. It is a slow progressing but relentless disease process. We need to intervene earlier.

In terms of fibrosis, we have several stages. F1 is perisinusoidal fibrosis. Most of the FDA clinical trials focus on F2, the second stage of fibrosis, and above. F2 is perisinusoidal and portal fibrosis. F3 is more advanced fibrosis due to NASH, bridging fibrosis. And F4 is cirrhosis, a different package by itself. So, this is how we classify NASH and NAFLD.

From NAFLD to NASH to fibrosis takes several years of progression. hat is our current understanding of the pathophysiology, disease progression, and complications? I told you that, until recently, we did not know much. That is why the negative name, nonalcoholic was differentiated from alcohol, right? But, as we learn more about it, and again, it is a significant percentage of the population. So we need to focus on it. We are learning that there is genetic and environmental factors that contribute

to the development of steatosis. So, we will talk about genetic predisposition. We will talk about the main cause of NAFLD, which is obesity, insulin resistance. And then, for each and every metabolic condition, we will talk about diet and exercise, and how all these obesogenic environments lead to fat in the liver. In about 20%, as I told you, inflammation develops, and then how this leads to hepatocellular damage, and death, and fibrosis, and through fibrosis to cirrhosis, liver failure, and through the metabolic component to cardiometabolic complications.

Now we will look at risk factors for NASH. So if 100 patients come to our clinic, 80% of them will have obesity, and the components of the metabolics -- hypertension, hyperlipidemia, diabetes, and obesity. Let's talk about this 80%, and let's talk about the remaining 20%. So, the question here is, who are those in the 20% who don't have obesity?

We realize today that we have an obese, and nonobese nonalcoholic fatty liver disease. So, if one looks into lean patients with NAFLD, as I told you, the minority at 20%, but this is a distinct percentage, right? There is the prevalence of NAFLD even among the lean. And you can go over the numbers among the NAFLD population 20% are nonobese. I showed you in the previous slide. In the general population, 5%. And in the lean population, 10%. But, remember, people who have NAFLD 80% obese, 20% are lean. And the incidence is going up progressively in both groups, as I told you. Why is this happening?

Let's focus first on obesity. The basics. All of us have genetically, epigenetically determined adipose tissue storage space in our subcutaneous adipocytes. In our subcutaneous fat, we have storage space which is expandable. So, if we are not planning to use the calories we ate recently, we store it as energy in adipocytes, and to the extent that we are normal, or that we are obese, but we have a lot of storage space. And no energy is stored outside the subcutaneous adipose tissue. There is normal muscle, normal liver, everything is fine. Metabolically healthy obese subjects, 25% of the obese are metabolically healthy. The lucky ones, with big storage space, nor abnormalities. The rest, 75%-80% of the obese metabolically are unhealthy. What happens in them? Or, in people who are metabolically healthy obese, if they become even more obese in the future.

When the storage space is exceeded, fat will have to go somewhere. Will it go into the muscle, and cause insulin resistance, diabetics. Or will it go into the liver and cause fatty liver disease? This is how obesity is associated with NAFLD and NASH.

In a very, very small percentage of people, extremely muscular, lean-appearing people, we have what we call generalized lipodystrophy—extremely rare genetic causes that destroy the subcutaneous adipose tissue storage space. Or medications, say highly active antiretrovirals that people take for HIV, autoimmune conditions that destroy the adipose tissue. If we don't have adipose tissue storage space, even if we are lean, the energy we take, the fat that we consume, will have to go somewhere, and even in those people will go into muscle which causes insulin-resistance similar to the extremely obese, or the liver and cause fatty liver disease. You see the bright fatty liver here. So, in a very, very small percentage of the people, they don't have adequate storage space, lipodystrophy. A huge percentage, 80% of - - being extreme obesity.

This slide shows lipodystrophies, and these people have low leptin levels, and low adiponectin levels. So, in extreme obesity we have high leptin levels, and when fat starts getting deposited in the liver we have low adiponectin. In people with lipodystrophy we have low leptin, no fat, a lot of fat, but it is deposited where it is not supposed to be deposited. So this is associated with low levels of adiponectin, low levels

of insulin, and those who are insulin sensitized develop insulin resistance. It goes without saying that medications that would increase adiponectin, or medications that would increase leptin, would be of therapeutic importance, and we have worked on leptin for many years. Now, leptin has been approved for complete lipodystrophies, complete congenital lipodystrophy with metabolic abnormalities, triglycerides, and high glucose. So this is the tip of the iceberg. And, as I will show you later, leptin receptor analogs are in clinical trials for more common manifestations of the disease.

Alright. So I told you about the 80%. So, this is lack of adipose tissue storage space, namely obesity, in a small percent it's. What is the rest, the 20%?

Well, as we grow older, a lot of us will develop sarcopenia, low muscle mass. We lose muscle mass. This chart is from one of our epidemiology studies about to be published, 2,500 subjects were followed for about ten years. So, low muscle mass equals high risk for NAFLD. High muscle mass, this is the highest muscle mass, about 50% have the risk to develop NAFLD than people with low muscle mass. So, if you have high muscle mass, you have half of the risk. If you have high muscle mass and central obesity, then your risk goes up. High muscle mass, no central obesity, your risk goes down. So these two factors, sarcopenia and obesity, lead for NAFLD, and, of course, they work together in creating the metabolics.

In a very small percentage of people, there is another group of subjects who have genetic causes of NAFLD and NASH. This is a different bucket we are understanding. This is the most common mutation; this is a molecule called adiponectin, and certain polymorphisms of this molecule increase hepatic fat content and cause liver damage, not because they create an abnormal metabolic organism level, but because, luckily, inside the liver, they don't let the fat in the liver metabolize. And I will show you in a few minutes how this happens. Well, this is not very common in African Americans. It is not very common in Caucasians. But it is very, very common among Latinos. So, you need to be screened for this, and this, of course, will be the target for interventions with medications.

Other mutations like HSD increase fat in the liver, but because they act locally in the liver, they don't progress to fibrosis or inflammation. In my opinion, lower cancer rates. Remember, also, three other mutations that are less common than adiponectin that interfere with hepatic storage, fat storage, and beta-oxidation also. I will show you in a graphic in a few minutes.

Let's go over adiponectin. I told you adiponectin failure is more common in Hispanics, and causes hepatic steatosis in this specific group of people. What happens? These - - inside the liver, decreases the activity of adipose triglyceride lipase that normally mobilized fatty acids, increases triglycerides in hepatocytes, and this steatosis leads to inflammation cirrhosis and hepatocellular cancer.

So, if you look at the alleles of this adiponectin molecule, the more Gs one has, very simplified, every G increases increase for NASH twofold. And, then, if you have 2 Gs in comparison to 2 Cs that are normal, the risk goes up to four. So each G allele increases twofold the risk for HCC and NASH and fibrosis. So this is a big problem for this population. And I would predict that in the context of personalized medicine, we will need to screen for genetics in the future; it is not available yet. And medications will be available in the future.

We know from mice, for example, that, if one knocks out this molecule in adipose tissue, one will see the abnormalities that we see in humans. So, we have the right animal models, and this is an intensive area of studies.

Very briefly, if we go over the other polymorphisms that I told you about earlier, this is a very interesting one, TM6SF2. What is does is decrease the hepatic VLDL excretion. So, serum triglycerides, LDL, in the serum everything looks normal, right? But, at the level of the liver, things are not so good. So there are increases in cardiovascular disease and increased steatosis.

So, I told you about HSD increases fat in the liver, but decreases inflammation, so not bad from my point of view. So, steatosis may be there, but they will not progress as frequently to inflammation and steatosis.

You will have all of these in your packets of slides, but I will move very fast, now, to what is happening in research. So, we go from genetics to starting transcriptomics, and then from transcriptomics over to metabolomics, and to profiling of these patients, trying to identify the molecules that lead from NAFLD to NASH, and from NASH to fibrosis. And we are finding that, for several people, there are specific gene signatures, like, several genes, groups of genes, up to twenty-five, that are associated with the progression of the disease. And these genes are candidates, if we study them deeply, to develop new medications. For example, a very interesting molecule is GDF15. We don't know as much about it as we know about adiponectin. But, this is a very active area of research, and there will be more coming down the road. Let's leave it there, at this point.

What I have told you in this part of my presentation is that in 80% of people obesity has a very important role. Twenty percent is sarcopenia. Very small percentages include lipodystrophy, and genetics, that we are learning more and more, are probably going to be a separate bucket. We will be screening for genes in those rare cases that have genetic causes, and we will be addressing the specific genetic causes. But, for the vast majority of our subjects, we will need to focus on obesity, sarcopenia, and how this leads to metabolic syndrome, insulin resistance, and then NASH.

So, what is our current understanding of pathophysiology? I told you that genetics are associated, but liver disease is a separate bucket. I will show you a slide. The MAFLD is most associated with central obesity, lipodystrophy, and sarcopenia, and cause insulin resistance. What is the result of insulin resistance? It leads to lipolysis, free fatty acids which circulate and are taken up by karyocytes—liver cells, but liver cells, also in a state of insulin resistance, there is the novel adipogenesis, more fatty acids. And, then, importantly, most people who have metabolic syndrome, obesity, insulin resistance don't watch their diet. They don't exercise. So, there are fatty acids from the diet flooding the liver.

What would be the fate of the fatty acids in the liver? They can either be oxidized in the mitochondria, so we burn them with heat. But, in insulin resistance, this mechanism does not work very well. Adiponectin is very low. There is oxidation. So most of the time they are stored, and this is what happens when we have mutations of adiponectin, right, like the genetic causes that we talked about. So these will increase VLDL, and through VLDL an abnormal metabolism will lead to cardiovascular disease, or lipotoxicity in the liver will cause inflammation. I told you the microphages will accumulate, - - , the fatty acids, and this leads to insulin resistance, and microinflammation and insulin resistance feeds back, and creates a vicious circle that, if not interrupted, will lead to fibrosis and carcinoma in the future.

Alright. Let's summarize now. I told you we have a metabolic component. Metabolic-associated fatty liver disease, and we have genetic-associated fatty liver disease. What are the differences in metabolism--obesity, sarcopenia, we have lipolysis in the blood stream. We have fatty acids, glucose, elevated insulin, VLDL is elevated, fat accumulates in the liver and is not burned, causes inflammation, and this leads to cardiovascular disease or cirrhosis.

In genetics, we don't have the abnormal metabolic profile in the picture. We have fat in the liver because it is not metabolized. And because it is not metabolized, it accumulates there. And this leads to inflammation and complications. So, in the bloodstream, you won't see much. You need to do genetic tests to find out what is happening. The LDL will be low. So, as I told you, people who have NASH, they have type 2 diabetes, they have insulin resistance, but NASH, most of the time, is recognized. So, they have all these abnormalities in the blood stream, and we tend to think about type 2 diabetes when we see high glucose, high insulin, high triglycerides, but we don't think about NASH. Why don't we think about NASH? We will come back to this in a few minutes. Because the symptoms are not there. Most of the time they are asymptomatic in the early stages. All the symptoms are nonspecific, malaise, fatigue, right upper quadrant discomfort, but nothing, nothing really pronounced. So we have to think about it. We have to test for it, in order to diagnose and treat. But, the point I want to make here, is that, when adipose tissue storage is exceeded, we have microinflammation, metabolic syndrome, insulin resistance, hyperglycemia. We recognize diabetes. We don't - - diabetes. We treat, in a holistic approach, cardiovascular disease and chronic kidney disease. And now we have a new kid on the block and it's nonalcoholic fatty liver disease, or metabolism-associated fatty liver disease. So all these go together. And probably the medications we will be using for NAFLD in the future will be significant.

Thank you for your attention. And I think this is a good point where we can take a break.

DR. MANTZOROS: In the next part of my presentation, I will move to epidemiology and risk factors. What do we need to keep in mind in terms of associations, risk factors?

As we discussed, obesity is not only linked to diabetes and metabolic syndrome and cardiovascular disease but also to nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, and the related comorbidities.

I told you about obesity and sarcopenia. Both of them lead to NAFLD and NASH through insulin resistance and abnormal cardiometabolic profile. One would think that if from obesity and sarcopenia will go to lipotoxicity, inflammation, and fibrosis, the earlier we diagnose in the spectrum of the disease and the earlier we intervene, the better it's going to be.

What do we do in the clinic? Think about NAFLD. Most of us don't think about it. If you don't think about it, you won't find it, you won't treat it. If someone with obesity, Type 2 diabetes comes to your clinic, 80% of our obese patients have NAFLD, 7 out of 10 diabetics have NAFLD.

Metabolic syndromes, the more components of the metabolic syndrome the higher the prevalence of NAFLD, advanced stage. In some lab reports high ALT, low platelets, low albumin, especially in Hispanics, this is a red flag. This in fact, can be put together as a test called FIB-4 and in our hospital, we have added now FIB-4 as part of a Chem-20 so this comes up on our computer screen and reminds us to screen people for NAFLD and NASH.

Risk factors, obesity, diabetes, the components of the metabolic syndrome are very, very, very prevalent. Clinical presentation, as I told you earlier, we don't think about it. It's asymptomatic or symptomatic with nonspecific symptoms like fatigue, malaise, and right upper quadrant discomfort, so we need to think even in asymptomatic patients.

We went over the pathophysiology in a few minutes, this is a reminder now about what causes. Obesity, sarcopenia, we talked about it, and sarcopenia and obesity will lead not only to NAFLD and

metabolic complications, but a very significant increase in the ten-year risk of cardiovascular disease. This is from one of our papers, we followed more than 2,000 people for 10 years and we have shown that NAFLD is a predictor of cardiovascular disease, even after we adjust for known risk factors like waist circumference and diabetes, and hypertension, and other known risk factors.

Obesity, sarcopenia, are risk factors metabolic syndrome for NAFLD. What about progression from NAFLD to NASH or fibrosis? The same risk factors. Age; every year of age increases the risk of progression to fibrosis by 8%, hypertension by 20%, BMI 900%, ninefold. Diabetes 18-fold. These are the major risk factors not only for having fat in the liver but also for progression to fibrosis.

This is then where we need to focus on. Type 2 diabetes, Type 2 with NAFLD, and then a smaller percentage of 7 to 13% of patients with Type 2 diabetes, we have advanced fibrosis. Out of 100 diabetics, about 70 in my clinic have Type 2 diabetes with NAFLD. Twenty percent of those like 15%, will have NASH and fibrosis. Very significant.

What about NAFLD and Type 2 diabetes and fibrosis? As I told you earlier, most people who have fibrosis progression had Type 2 diabetes. This is where we should focus. Eighty-four percent of people who have fibrosis progression have Type 2 diabetes.

As we go from healthy liver to NASH, we develop cirrhosis in about 20% of the people and this leads to hepatocellular cancer, but sometimes patients with NAFLD may go to hepatocellular carcinoma without overt cirrhosis. People with NAFLD related hepatocellular cancer are diagnosed at a later tumor stage. We need to - - and diagnose them early have worse outcomes and potentially they have low response to treatment with PD-1s, like the immune checkpoint inhibitors.

What about diabetes in relation to chronic liver disease and hepatocellular carcinoma? I'm showing this to you in a graphical form over time, 14 years. The risk, even if you don't have diabetes increases but with diabetes the risk goes up several-fold and the same is true for hepatocellular carcinoma.

These days NASH has surpassed hepatitis C as an indication for liver transplant and the numbers are going up. This is NASH. This is going up. Unfortunately, we are going to see this more and more in the future.

Eighty million Americans have NAFLD. In about 80% of them, this will remain controlled, steatosis, 20% will develop NASH, and this is where we need to focus our attention. Twenty percent of them will have advanced fibrosis, 3.5 million Americans, and these subjects are at risk for hepatocellular carcinoma, liver failure, transplantation, or death. Big numbers; 80 million, 60 million, and about 3.5 million they will have advanced fibrosis.

Similar to what I told you earlier, going from no NAFLD to steatosis to steatohepatitis, this is a metabolic condition. Red is mortality from cardiovascular disease, it is up and it remains up as the disease progresses. What is added to cardiovascular mortality is liver-related mortality.

We have now more recent data that were published in November of 2021, in JAMA, from a very large medical system, Cleveland Clinic, in the Midwest, and let's focus on this part of the graph for now. Nonsurgical controls, 500 people, who have been followed for up to 10 years by Cleveland health team. These people had NASH and obesity, biopsy-proven. What do they die from? What do they develop over

time? Liver outcomes 40 people, so it's about 12% cumulative incidence over ten years. What about major cardiovascular outcomes? Fourteen percent. More people, 60 patients here, 40 patients there. People with NASH will develop cardiovascular outcomes. It's a metabolic condition in addition to liver outcomes, which is less.

Mortality is going up as fibrosis stage is going up. This is a meta-analysis from several studies showing that mortality goes up as the fibrosis goes up. More and more - - .

If you review in a systemic way, all the studies in relation to fibrosis stages, all-cause mortality goes up. The difference between no fibrosis and fibrosis stage 1 is not that big. Either for all-cause mortality or liver-related mortality. All-cause mortality goes up linearly after fibrosis stage 1, goes up linearly after fibrosis stage 1 and goes significantly up in fibrosis stage 4 which is cirrhosis, in essence. That's why most insurance companies, most pharmaceutical companies when they develop medications, they don't develop medications for this group of people, but they want to focus on fibrosis 2 and above to prevent mortality. In my opinion, we need to prevent this and focus on earlier status. Of course, we cannot ignore that most people will die from liver or all-cause mortality when they have advanced fibrosis higher than F2.

Fibrosis stage is indeed the strongest predictor for disease specific mortality in nonalcoholic fatty liver disease. If we follow patients up to 33 years after diagnosis. This is the entire cohort. This is F0 to 2, this is F3 to 4. You can see if you go over numbers that for overall mortality, risk goes up with increased fibrosis stages, but irrespective of what we look at, cardiovascular or liver-related or GI malignancies or infectious diseases, the risk is significantly higher with advanced stages of fibrosis.

Let's now move to earlier stages. Not NASH and fibrosis, but mortality in people of have NAFLD. I told you that I would focus on prevention. If you have NAFLD and no component of the metabolic syndrome, your risk to die over the next 20, 25 years is relatively low. As one accumulates more and more components of the metabolic syndrome the risk to develop NASH and progress is not classified.

Let's talk about a few cases to put this in context. We have an overweight 58-year-old man coming to our clinic. Past medical history of controlled hypertension, Type 2 diabetes, came to the office for a checkup. What else do we need to be aware of? I told you obesity, diabetes, hypertension, most of us will think about cardiovascular disease, and chronic kidney disease, we need to think about NAFLD.

Which of the following is the stronger predictor of advanced fibrosis in patients with NAFLD? If we think that this person has NAFLD what else do we need to think as a risk assessment? BMI more than 30, hypertension, age of 58, or diabetes.

It's diabetes and BMI. I'm not trying to say that hypertension does not play a role. I'm not trying to say that age does not play a role. I showed you in the graph earlier diabetes and BMI.

Which is the crucial histological element for considering NASH? Think about now if we could scan the liver, if we're thinking about NAFLD steatosis versus NASH. Is it when fat exceeds the diagnostic threshold? No. This is steatosis, this is NAFLD. Is it inflammation and liver cell damage? Yes, this is NASH. And severe fibrosis.

Which of the following is correct? Fibrogenesis is not a linear process but progresses or regresses in up to 20 to 30% of patients during a mean period of five years. NASH and NAFLD progress one stage of

fibrosis every 7 and 14 years, respectively. The presence and stage of fibrosis is the strongest histological determinant of hepatic and overall mortality in patients with NAFLD. Or all of the above. Would you go with A, B, C, or D.?

It's all of the above. I told you earlier NASH and NAFLD they progress 1 stage of fibrosis every 7 years of NASH and every 14 years for NAFLD. It's earlier in the disease process and the stage of fibrosis is the strongest predictor for not only liver but also cardiovascular and overall mortality.

The most important point here is that fibrogenesis not a linear process, it could progress, but we can make it regress if we remove the risk factors and the causes; obesity, diabetes, the metabolic syndrome.

This is a recent paper that was published in the New England Journal of Medicine. It's a prospective study of outcomes in adults with nonalcoholic fatty liver disease and makes the point that I wanted to make with the previous slide, mortality and new onset nonfatal outcomes according to fibrosis stage. The numbers are going up as we go from F0, F1 to F3 and 4 irrespective of whether we look at all-cause mortality or cardiovascular mortality or liver-related mortality.

Again, from Lancet, 2020, irrespective of whether we look at obese NAFLD or non-obese lean NAFLD, we have the same process and the same outcomes.

What do we see in terms of cardiometabolic? We see everything that is associated with this metabolic condition. Mainly atherosclerosis, increased risk for MI, but also risk for strokes, valvular disease, aortic valve stenosis, mitral calcification. We've seen problems with left ventricular diastolic dysfunction and we see cardiac arrythmias.

Why is that? As I told you earlier, and I don't mean to belabor the point, this is a metabolic condition. The same risk factors that lead to NAFLD, they also lead to cardiometabolic conditions, but the presence of NAFLD and NASH is not an innocent bystander. As I told you earlier, inflammation develops, insulin resistance develops, and this leads to proinflammatory factors circulating in the bloodstream, and this leads to ischemic heart disease and arrythmias and cardiovascular outcomes.

In summary, NAFLD either because of genetics that leads to inflammation inside the liver and this metabolizes inside the liver or because of obesity and sarcopenia with high leptin, low adiponectin, which is the endogenous insulin sensitizer, leads to cardiovascular morbidity and mortality. Endothelium is disrupted, aortic valves are disrupted and we have heart failure. Because of all these factors myocardial inflammation and circulating adipokines, abnormal redox status, hemostasis is abnormal, the lipid profile is abnormal, and then angiogenesis and hepatokines are abnormal.

This creates a constellation of abnormalities that work together to lead to cardiovascular events, atherosclerosis, cardiomyopathy, arrhythmias. Because of endothelial dysfunction, systemic insulin resistance, oxidative stress, systemic inflammation, and abnormal lipid metabolism.

I have this meta-analysis here to show that the incidence of cardiovascular disease is related to NAFLD, goes up with NASH, and goes up with the stage of fibrosis, as I told you earlier.

We can look at fibrosis with biopsy, we can look at simple scores that are not perfect in terms of diagnosis, we'll talk about it like FIB-4. Irrespective of how we look at it and what outcomes we look at, coronary artery calcification here on the left, or maximum CIMT values we see the same associations.

Another manifestation of cardiometabolic profile is chronic kidney disease. Again, chronic kidney disease travels together with NAFLD and NASH. People with chronic kidney disease when they also have NAFLD and NASH, they have higher mortality, and this is driven by the cardiometabolic complications. We think about NAFLD as we've seen with eGFR and albuminuria how patients with obesity and diabetes. These conditions represent common shared biology and there is linkage between kidney disease and liver histology.

If you look at for example, stages of fibrosis as I told you earlier, with advancing fibrosis, not only cardiovascular disease goes up but eGFR goes up. Kidney function is affected because we have the same common predisposing factors. If we think about common predisposing factors, if we think about these diseases traveling together, we can also think about medications that can hopefully help both.

Another component of the metabolic syndrome takes many years to develop, not very well studied, but it has started being studied more and more is cognitive function. It's Alzheimer's and this is associated with insulin resistance and obesity, but now we realize that it is also associated with nonalcoholic fatty liver disease. People who have nonalcoholic fatty liver disease and NASH, even if adjusted in models for obesity and waist circumference and the metabolic syndrome, they tend to have a higher risk to develop this problem. Cognitive function in adults is something that we need to think about and screen in our clinics.

Is it also associated with depression? A meta-analysis of recent papers has shown that the prevalence, risk factors, and outcomes of depression are also associated with nonalcoholic fatty liver disease.

As we think about NAFLD and the metabolic syndrome, think about NAFLD and depression, which may be associated with inflammatory cytokines, think about cognitive decline and Alzheimer's over the years, because this is associated with microinflammation and insulin resistance, which is part of the metabolic syndrome, especially in diabetes and obesity.

In our clinical practice, at this point, until now, we did not have any guidelines to recommend screening in the general population. Whom do we screen for NASH and fibrosis? When we see someone in our clinics with obesity, Type 2 diabetes, metabolic syndrome, or components of the metabolic syndrome like prediabetes, hypertension, hyperlipidemia. We just published the updated guidelines November 2021, and we will go over this in a few minutes. You will have access to the older guidelines, but I will focus mostly in the new guidelines.

What are the facts that are important for recognizing NAFLD? In addition to obesity and Type 2 diabetes, ALT can be high or late stages ALT and AST can be low, or they may fluctuate. Liver fibrosis as I told you earlier has been linked to morbidity and reduced patient survival. NAFLD and fibrosis are reversible with weight loss. I will show you more data in a few minutes. It progresses and regresses. We can help our patients regress and to differentiate alcoholic versus nonalcoholic fatty liver disease, in addition to getting a very good history, we can use the AST to ALT ratio.

Remember, a substantial number of patients with advanced fibrosis may have normal ALT levels. How do we screen? Let’s think about the 50-year-old man, obesity, diabetes, comes to your clinic for checkup. You think about it. You remember the pathophysiology. Which screening tests would you look for? I told you ALT/AST is not very reliable.

Who do we screen for NASH and fibrosis? Do we screen someone who has increased obesity, patients with metabolic syndrome, Type 2 diabetes more than ten years of duration, steatosis, elevated ALT, all of the above?

Which of the following genes are associated with NAFLD and NASH? Adiponitrile, PNPLA3 among Latinos, HSD, GCK, MBOAT, all of the above.

All of the above are associated with NAFLD. PNPLA3 is the main cause of progression to NASH. Development of medications for A as we speak.

Which population is more frequently present with single nucleotide PNPLA3? Very important. African Americans, Hispanics, Caucasians. Hispanics. With Hispanics think about PNPLA3. You may have to screen in the future, specific therapies are coming down the road.