Nonalcoholic Steatohepatitis and Endpoints in Clinical Trials

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is now the leading cause of liver disease in developed countries, and the rates of NAFLD continue to rise in conjunction with the obesity pandemic. While the majority of patients with isolated steatosis generally have a benign course, a diagnosis of nonalcoholic steatohepatitis (NASH) carries a significantly higher risk for progression of disease, cirrhosis, and death. Pharmacologic therapeutic interventions in NASH have largely proven to be ineffective or unappealing due to long-term side-effect profiles, and the majority of patients cannot achieve or sustain targeted weight loss goals, necessitating an urgent need for therapeutic trials and drug development. The complex molecular mechanisms leading to NASH and the long duration of time to develop complications of disease are challenges to developing meaningful clinical endpoints. Because of these challenges, surrogate endpoints that are linked to all-cause mortality, liver-related death, and complications of cirrhosis are much more likely to be beneficial in the majority of patients.

As obesity rates have steadily increased around the globe, the landscape of chronic liver disease in developed nations has morphed accordingly. Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in Westernized nations and is associated with the metabolic syndrome. Estimates of the prevalence of NAFLD range from 2.8% to 46%, depending upon the study population and diagnostic criteria used.[1] Even higher rates of NAFLD have been demonstrated in obese patients undergoing bariatric surgery, where as many as 91% have been shown to have NAFLD.[2] Autopsy studies have placed the prevalence of nonalcoholic steatohepatitis (NASH) at 2.7% among lean patients, with rates increasing to 18.5% among markedly obese patients.[3] A prospective cohort study utilizing ultrasound and liver biopsy in asymptomatic middle-aged Americans who were referred for routine colon cancer screening determined the prevalence of NASH to be 12.2%.[4] A much lower NASH prevalence of 1.1% was demonstrated in a living donor transplant population,[5] and expert

opinion suggests the true prevalence of NASH outside of the highly selected populations of clinical trials to be 1% to 3%.[6]

The natural history of NAFLD is highly variable, often nonlinear in progression, and most dependent on the presence or absence of NASH as determined by hepatic histology obtained from liver biopsy. While there is some evidence that NAFLD with mild necroinflammation may progress to NASH, and the paradigm of non-NASH NAFLD vs NASH is overly simplistic, this distinction is still an important outcome predictor. Liver biopsy enables pathologic evaluation for lobular and portal inflammation as well as hepatocyte ballooning in order to distinguish non-NASH NAFLD from NASH and to quantify hepatic fibrosis. The prognosis in NAFLD patients without significant inflammation or fibrosis is generally good with a lower potential for histologic or clinical progression and similar mortality rates to the general population.[7,8] In contrast, the presence of NASH is associated with a reduced life expectancy from cardiovascular, malignancy, or liver-related sequelae.[9,10] Cardiovascular disease is the primary cause of death in NAFLD patients.[11] NASH with fibrosis predicts a worse prognosis than NASH without fibrosis, particularly from liver-related mortality.[12,13] While the time course is extremely variable, one author suggested that approximately 11% of NASH patients progress to cirrhosis over a 15-year period.[14] NASH cirrhosis has comparable outcomes to other causes of cirrhosis and can lead to hepatocellular carcinoma (HCC).[15,16] NAFLD-related HCC is the fasting growing indication for liver transplantation.[17,18]

Pharmacologic Treatment

Weight loss via dietary interventions and exercise remains the most recommended treatment strategy for NAFLD, but only a minority of patients are able to achieve and sustain therapeutic targets. Thus, there is a continued need and ongoing efforts to develop pharmacotherapy for the treatment of NAFLD and NASH. Unfortunately, there are currently no medications approved by the US Food and Drug Administration. Classes of medications that have been investigated include weight loss agents, diabetic medications, anti-inflammatory agents, and antifibrotic agents. As insulin resistance has been thought to be essential to the pathogenesis of NAFLD, diabetic medications have been the most investigated for NASH treatment. Metformin has been studied with disappointing results in terms of efficacy for the treatment of adult and pediatric NASH.[19-21] Thiazolidinediones, including pioglitazone and rosiglitazone, have shown more promise in the treatment of NASH, improving hepatic steatosis and necroinflammation, although fibrosis improvement has not been consistently seen with therapy.[22-26] However, the associated side effects of weight gain, small bone fracture risk in women, myocardial ischemia, osteopenia, and congestive heart failure exacerbation (which carries a black box warning) make the long-term use of thiazolidinediones solely for a NASH indication difficult to recommend. Pioglitazone remains a more viable choice given a neutral effect on lipid profiles and no association with myocardial infarction as seen in earlier studies with rosiglitazone. Other novel diabetic medications such as the glucagon-like peptide-1 agonists exenatide (Byetta, AstraZeneca) and liraglutide (Victoza, Novo Nordisk) have been suggested to have benefit in NASH populations, both in terms of metabolic as well as histologic parameters.[27-29] Larger randomized, controlled trials with well-defined clinical endpoints are necessary to confirm benefit.

Antioxidants represent another potential therapeutic target for NAFLD patients. Vitamin E, vitamin C, and betaine have all been studied in NAFLD populations, with evidence supporting some modest benefit from vitamin E, although this is tempered by concerns about effects on cardiovascular health, all-cause mortality, and prostate cancer.[6,30] Medications classified as cytoprotective agents, including ursodeoxycholic acid and pentoxifylline, have also been investigated with mixed results.[31,32] Similarly, medications designed to improve serum cholesterol panels have been evaluated in NAFLD populations. The evidence to date has suggested that 3-hydroxy-3-methylglutaryl-coenzyme A reductase or statins can be used safely in NAFLD populations for hyperlipidemia, but cannot be recommended for histologic benefit in NASH.[33] Weight loss medications such as orlistat have shown modest efficacy, but only when accompanied by weight loss of at least 9% body weight.[34] Newer weight loss medications have not been studied. Multiple novel classes of agents, particularly those targeting hepatic fibrosis, are under investigation. From preliminary trials, it has become apparent that well-defined clinical endpoints need to be established to assess the efficacy of medications in NASH patients.

Challenges in Developing Clinical Endpoints

Given the increasing disease burden associated with NASH and the lack of widespread weight loss success, therapeutic pharmacologic interventions are desperately needed. Developing outcome measures to assess this at-risk population and validate clinically meaningful study endpoints is imperative. Despite the high global prevalence of NAFLD, the vast majority of patients are asymptomatic or have nonspecific symptoms such as fatigue. This lack of symptom specificity to aid in the diagnosis of NASH creates a substantial obstacle in developing symptom- and

functional-based outcomes in early disease.[35] Fortunately, the majority of patients with NAFLD will have limited or minimal progression of disease.[36]

The underlying pathologic process by which NAFLD progresses to NASH involves multiple molecular pathways that are complex and incompletely understood. In addition, NAFLD itself represents a heterogeneous disease process that is influenced by the interaction between environmental factors, genetic susceptibility, and multiple modifiable risk factors.[37] While this pathogenetic background provides several potential targets for therapeutic intervention, this same complexity limits the ability to define clear, measureable, and objective clinical endpoints. Given these factors, surrogate endpoints, which can be used to predict outcomes on clinically meaningful endpoints, are likely to be the most effective in all patients but those with advanced NASH and fibrosis (Table).

Endpoints

All-Cause Mortality and Liver-Related Death

Patients with NASH have increased risk for disease progression, cirrhosis, and all-cause mortality. In fact, the presence of inflammation, regardless of whether it meets the specific criteria for NASH, is a predictor for progression to advanced fibrosis and subsequent complications.[38] Similar to other chronic medical conditions, all-cause mortality as a clinical endpoint remains the most important clinical outcome to assess in studies of NASH. Likewise, liver-related death, which is a component of overall mortality, is also a useful hard clinical outcome. Liver-related death is generally linked with the development of cirrhosis and its associated complications.[39] Progression to cirrhosis, which is the leading cause of liver-related death in NAFLD patients, occurs very slowly, with an average progression of 11% over a 15-year period.[36] In addition, the overall prevalence of NASH is estimated to be as low as 1% to 3%.[6] Thus, in order to effectively evaluate hard clinical outcomes, especially in patients with early-stage NASH, clinical trials would require large numbers of patients followed for a decade or more. In an effort to hopefully shorten this timeline, current phase 3 clinical trials in NASH have looked to enrich their studies with patients who have moderate to advanced fibrosis (stage 2-3) at baseline. Given these problematic issues and the high costs associated with such long clinical trials, endpoints in NASH involving all-cause mortality and liver-related death will be challenging. The development of HCC is increasingly important as NASH continues to increase. Unfortunately, HCC can arise in NAFLD in the absence of cirrhosis, with data suggesting that this may occur in as many as 50% of cases, making HCC unreliable as a clinical endpoint.[40]

Development of Cirrhosis

Cirrhosis leads to clinical decompensation and an increase in mortality risk through the development of variceal hemorrhage, ascites, hepatic encephalopathy, infection, and other factors. In a study by Bhala and colleagues, 19.4% of NAFLD patients with well-compensated advanced fibrosis or cirrhosis developed liver-related complications over a mean of 7.1 years.[39] In the NAFLD cohort of 247 patients, there were 33 deaths or liver transplants. When coupled with other studies assessing the progression of NASH cirrhosis, approximately 31% of patients may progress to decompensation over an 8-year time horizon.[16,41,42] Cirrhosis is defined by objective histopathologic assessment,[43] although not without limitations, such as sampling error and possible inadequacy of sample and lack of expertise of the pathologist.[44] However, despite these limitations, the development of cirrhosis appears to be a useful clinical endpoint in clinical trials to assess progression of NASH.

Because of the invasive nature of liver biopsy and some of the concerns with histologic assessment, newer noninvasive methods to assess cirrhosis may demonstrate potential. In particular, imaging modalities based upon transient elastography have shown an ability to confidently exclude fibrosis stage 2 or greater with a negative predicative value approaching 90%.[45] Because the positive predictive value of transient elastography for advanced fibrosis is modest at best, this imaging modality is best used as a screening test to exclude advanced disease. Additionally, magnetic resonance elastography has demonstrated similar efficacy in a small comparative trial.[46] While these noninvasive imaging techniques are an attractive option for use in clinical trials, further study is necessary to directly correlate liver stiffness measures on elastography with clinical outcomes. Other noninvasive imaging modalities to assess fibrosis and cirrhosis, such as multiparametric magnetic resonance imaging, are being developed. In a pilot study of patients with chronic liver disease, corrected T1 correlated closely with fibrosis stage and identified NASH patients with fibrosis with an area under the receiver operating characteristic curve (AUROC) of 0.90.[47] Validation of multiparametric magnetic resonance imaging in NAFLD patients is currently lacking, and larger studies are needed.

Surrogate Markers in Nonalcoholic Steatohepatitis Patients With Cirrhosis

With the development of NASH cirrhosis, a number of surrogate markers for morbidity and mortality, such as the Child-Turcotte-Pugh (CTP) score, the Model for End-Stage Liver Disease (MELD) score, and the hepatic venous pressure gradient (HVPG), have been used as

Table. Endpoints in NASH Clinical Trials

Clinical Endpoints	Surrogate Markers	Future Research
All-cause mortality • The most important clinical outcome • Requires large numbers of patients followed for 10-15 years	CTP and MELD scores • Not specific to NASH • Progression from CTP class A to CTP class B is associated with increased mortality. • Subjective CTP components may create bias. • MELD is useful to assess mortality in advanced liver disease. • MELD score >14 is a valid endpoint linked to mortality and clinical outcomes.	Direct and indirect biomarkers • Currently lack sensitivity and/or specificity to make them useful in identifying NASH • Specific markers, combinations, or sequential algorithms could better differentiate disease stage, track disease progression, and measure fibrogenesis or fibrosis regression. • May better provide cardiometabolic data or liver-related outcomes
Liver-related death • Surrogate for all-cause mortality • Linked with cirrhosis and associated complications • Impractical endpoint given lengthy study duration and sample size for all but advanced NASH	HVPG • HVPG >10 mm Hg reliably predicts clinical outcomes. • >20% reduction in HVPG appears to be useful (mortality benefit). • Its invasive nature and need for serial monitoring limit its usefulness outside of clinical trials.	Quality of life • Functional assessments • Symptom-based assessments • Medical costs • Health care utilization rates
Hepatocellular carcinoma • May occur in the absence of NASH cirrhosis • Unreliable as a clinical endpoint for NASH	NASH resolution • Reversal of NASH has not been shown to reduce overall mortality or liver-related death. • Inflammation can diminish as fibrosis progresses. • Unreliable as a clinical endpoint	Noninvasive imaging • Current imaging methods can accurately detect advanced fibrosis and cirrhosis and quantify steatosis. • Lacks discriminatory power to differentiate adjacent stages • Correlation of imaging results with clinical outcomes is required. • Potential to avoid the need for liver biopsy
Development of cirrhosis • Linked to clinical decompensation and mortality • Useful clinical endpoint in a subset of patients with advanced fibrosis • Limited by the need for histopathologic assessment • Noninvasive imaging may play a role in the assessment of cirrhosis, avoiding the need for biopsy.	Histologic scores • NAS has not been shown to predict mortality or long-term prognosis. • SAF score and algorithm may be more useful than NAS, but clinical relevance has yet to be determined. • Other scoring systems lack validity to be useful.	Genomic targets • Genetic pathways in the pathogenesis of NAFLD are incompletely understood. • Specific genes or polymorphisms may be linked to advanced disease or higher risk of disease progression. • Need to develop biologically plausible pharmacogenomic targets with treatment endpoints • Limited clinical application currently
	Fibrosis regression • Fibrosis stage is the most significant predictor of mortality. • Improvement in fibrosis ≥1 stage is the optimal endpoint. • May replace NAS or NASH resolution in clinical trials • Noninvasive imaging methods may play a future role, but currently lack the ability to differentiate between closely related stages.	

CTP, Child-Turcotte-Pugh; HVPG, hepatic venous pressure gradient; MELD, Model for End-Stage Liver Disease; NAFLD, nonalcoholic fatty liver disease; NAS, Nonalcoholic Fatty Liver Disease Activity Score; NASH, nonalcoholic steatohepatitis; SAF, Steatosis, Activity, and Fibrosis.

endpoints in clinical trials.[35] The use of these instruments is not specific to NASH, and some data suggest that patients with NASH have different clinical outcomes than other forms of chronic liver disease. For example, when compared to patients with compensated cirrhosis from hepatitis C virus, NASH patients with CTP class A had a significantly lower mortality over a 10-year period.[48] Nevertheless, in this same study, CTP and MELD scores independently predicted death. CTP score has the disadvantage that 2 variables (ascites and encephalopathy) can be subjective and variable according to the use of medications, and both CTP and MELD scores use the international normalized ratio, which may not appropriately capture the degree of coagulopathy in cirrhosis.[49] Multiple studies have evaluated CTP and MELD scores to assess the prognosis of cirrhosis, although neither has consistently proven to be superior to the other. Both CTP and MELD scores are useful instruments to assess mortality in patients with advanced liver disease. Uncertainty remains on the usefulness of specific point changes in the CTP or MELD scores as a clinical endpoint, although a MELD score greater than 14 does appear to be a valid endpoint that is linked to mortality and clinical outcomes.[35]

The HVPG measures the pressure differential between the wedged hepatic pressure and the free hepatic venous pressure and has become the gold standard for detection of clinically significant portal hypertension.[50] Ripoll and colleagues have demonstrated that the HVPG can predict the development of clinical decompensation in patients with compensated liver disease who have portal hypertension but no varices with a hazard ratio of 1.11.[51] In fact, the authors suggest that each 1–mm Hg increase in the HVPG is associated with an 11% increase in decompensation.[51] Another study specific to patients with biopsy-proven NASH demonstrated that a HVPG greater than 10 mm Hg predicts clinical outcomes with 86% accuracy.[50] For NASH patients with compensated cirrhosis, a HVPG greater than 10 mm Hg does appear to be a reliable surrogate marker to predict clinical decompensation. A meta-analysis by D'Amico and colleagues also demonstrated that a reduction of the HVPG to no more than 12 mm Hg or by at least 20% significantly decreased the risk of variceal bleeding.[52] Furthermore, a mortality benefit was seen with at least a 20% reduction in the HVPG.[52] Thus, reduction in the HVPG may also be considered a useful surrogate endpoint in clinical trials. However, this usefulness is somewhat limited by the invasive nature of the HVPG and the need for serial monitoring.

Resolution of Nonalcoholic Steatohepatitis

Given the prolonged amount of time to progress to cirrhosis in NASH, other histologic endpoints for tracking the progression of NASH may serve as useful clinical endpoints in the short term. In particular, NASH has been linked to an increased risk of liver-related mortality compared with NAFLD patients without NASH.[53] This association with disease progression is likely linked to fibrosis, which is a major histologic feature of NASH.[12,13,54] In fact, the presence of fibrosis has the strongest predictor of overall mortality followed by portal inflammation, diagnosis of NASH, and the presence of ballooning.[55] However, reversal of NASH has not been shown to reduce overall or liver-related mortality, and the presence of inflammation can diminish as fibrosis progresses.[56] Thus, further clinical trials are needed to demonstrate that changes in NASH over time are associated with improved clinical outcomes for it to become a meaningful clinical endpoint.

Histologic Scores

Because NAFLD represents a clinicopathologic spectrum, differentiating definite NASH from NAFLD can be difficult.[12] Semiquantitative scoring systems have been developed to assess disease severity and progress in an effort to increase reproducibility among pathologists, reduce homogeneity based upon histologic features, and compare biopsies over time.[44] The Nonalcoholic Fatty Liver Disease Activity Score (NAS) was developed to provide a numerical pathologic score that differentiates between necroinflammatory activity and fibrosis,[44] and, until recently, it had been the standard primary endpoint for histologic effectiveness in NASH clinical trials. While a NAS of at least 5 has been shown to correlate with the histologic diagnosis of NASH, definite NASH is not always present and a NAS of 4 or less does not indicate benign histology.[57] The diagnosis of NASH still requires a gestalt diagnosis by a pathologist. Furthermore, neither fibrosis nor portal inflammation—both of which have been linked to increased mortality risk—is a component of NAS.[54] Regardless, NAS has not been shown to predict mortality or long-term prognostic information.[13,54] Other histologic scoring systems such as the Steatosis, Activity, and Fibrosis (SAF) score have been able to discriminate the presence of NASH with excellent interobserver agreement.[58] The SAF score was developed to overcome some of the recognized limitations of the NAS and specifically to differentiate between NASH and NAFLD without NASH. In a validation study of the SAF score, an activity score (ballooning + lobular inflammation) of at least 2 correctly identified all cases with NASH.[58] However, this boundary between NASH and not NASH may be somewhat artificial given the histopathologic spectrum of this disease and evolution over time. The SAF score and algorithm may prove to be more useful than the NAS over time, although the clinical and prognostic relevance has yet to be determined.[44]

Fibrosis Regression

As stated previously, fibrosis stage is the most significant predictor of mortality in patients with NAFLD, with bridging fibrosis (stage 3) and cirrhosis (stage 4) being associated with the highest risk.[55] In a study by Angulo and colleagues, fibrosis stage 4 was 10.9 times more likely to be associated with the outcome of death or liver transplantation and 51.5 times more likely to be associated with a liver-related event compared with fibrosis stage 0.[54] Based upon these data as well as others,[12,13,54] improvement of at least 1 stage in patients with advanced fibrosis appears to be the optimal endpoint in clinical trials and should replace endpoints based upon NAS or NASH resolution. Unfortunately, regression of fibrosis occurs over a long period of time, with a median of 7.1 years in patients with NASH.[59] Given this lengthy time interval, noninvasive imaging modalities may play a role in assessing fibrosis regression, although current elastography-based methods lack the ability to adequately differentiate between closely related fibrosis stages.

Biomarkers for Nonalcoholic Steatohepatitis and Its Progression

The ability to discriminate patients with NASH from those with non-NASH NAFLD through noninvasive means such as biomarkers is an area of significant interest. Serum biomarkers or algorithms combining direct and indirect biomarkers with clinical features may be beneficial in estimating the severity of NAFLD and risk-stratifying those patients who need a biopsy.[60] For example, markers such as keratin 18 fragments (a marker of hepatocyte apoptosis), acute-phase reactants, cytokines, and markers of inflammatory stress and lipotoxicity have been evaluated to differentiate NASH from isolated steatosis. Unfortunately, none of these biomarkers alone or in combination have proven to be reliable in identifying NASH with enough sensitivity or specificity to make them effective surrogate endpoints for clinical trials.[44]

Similarly, serum biomarkers could prove to be useful either in combination or sequentially over time for progression of disease in NAFLD. The most validated method to assess disease severity is the NAFLD fibrosis score, which has reasonable accuracy in identifying patients with and without advanced fibrosis.[60] From a meta-analysis of 13 studies, the NAFLD fibrosis score had an AUROC of 0.85 in identifying patients with NASH and advanced fibrosis (stage ≥3).[61] Additional methods such as the Fibrosis-4 calculator and the aspartate transaminase/alanine transaminase ratio have also proven to be reliable in excluding advanced fibrosis in most NAFLD patients.[62] Other composite and proprietary algorithms exist to assess fibrosis severity, although none have been designed to track disease progression in NASH. Furthermore, current biomarkers do not have the ability to differentiate between closely related stages of disease. As a result, serum biomarkers are unlikely to be effective surrogate endpoints in NASH until markers that can adequately diagnose NASH and/or assess disease progression or regression are developed and validated.

Quality of Life and Other Measures

NAFLD has been associated with a reduction in quality of life (QOL), particularly in physical health when compared to the general population. Furthermore, adults with NASH report significantly poorer physical health when compared to patients with non-NASH NAFLD, and QOL is particularly low for patients with cirrhosis.[63] Other studies have demonstrated that NAFLD patients have lower health-related QOL compared with other chronic liver diseases,[64] and NASH has an increased lifetime incidence of depression and generalized anxiety disorder.[65] Still others have demonstrated that fatty liver disease, although not limited to NAFLD, is associated with higher medical costs and health care utilization.[66] With progressive disease and severity of NASH, functional assessments may become more practical in addition to other measures such as hospitalizations, number of physician visits, or lost work days. While changes to QOL, symptoms, and functional measures are acceptable primary endpoints,[35] meaningful changes in health status are likely to be captured only over long periods of time or in subsets of NASH patients with advanced disease. Further study is necessary to define which QOL measures will have the most impact and which instruments are best suited to measure them.

Summary

The development and acceptance of meaningful, readily obtainable, and well-defined clinical trial endpoints in NAFLD are imperative to develop new and effective therapies to treat this growing epidemic. Collaborative assessment of current and future technologies for measuring these endpoints is critical. One of the greatest challenges currently faced, prior to enrolling patients into NASH clinical trials, is deciphering which patients with NAFLD have NASH, particularly those with advanced fibrosis. Once these at-risk patients have been identified, the endpoints that appear to be the most readily attainable and reliable include monitoring for fibrosis regression, development of cirrhosis, and surrogate measures of liver-related outcomes. Longer-term follow-up to assess for all-cause mortality (mainly cardiovascular death) and liver-related mortality is also important but will take longer to evaluate. Developing novel, noninvasive technology to assess these endpoints

is imperative to achieve global success in finding effective therapies for NASH.

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