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Endpoint Selection of Non-alcoholic Steatohepatitis Clinical Trials

Wang Q[1], Tong X[1], Zhang W[1], Ma H[1], Ou X[1], Kong Y[2], You H[1], Wei L[3*] and Jia J[1*]

[1]Liver Research Center, Beijing Friendship Hospital, Capital Medical University, Beijing Key Laboratory of Translational Medicine on Liver Cirrhosis, National Clinical Research Center of Digestive Diseases, Beijing, China

[2]Clinical Epidemiology and EBM Unit, Beijing Friendship Hospital, Capital Medical University; National Clinical Research Center for Digestive Disease, Beijing, China

[3]Hepatopancreatobiliary Center, Beijing Tsinghua Changgung Hospital, Tsinghua University, Beijing, China

***Corresponding author:**

Jidong Jia,
Liver Research Center, Beijing Friendship Hospital, Capital Medical University, Beijing Key Laboratory of Translational Medicine on Liver Cirrhosis, National Clinical Research Center of Digestive Diseases, No. 95 Yong-An Road, Xi-Cheng District, Beijing 100050, China. Tel: +86-10-63139246, Fax: +86-10-63139246, E-mail: jia_jd@ccmu.edu.cn

Lai Wei,
Hepatopancreatobiliary Center, Beijing Tsinghua Changgung Hospital, Tsinghua University, No. 168 Litang Road, Chang-Ping District, Beijing 102218, China. Tel.: +86-10-88325730,
E-mail: weilai@mail.tsinghua.edu.cn

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Abbreviations:

NAFLD: Nonalcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; FDA; Food and Drug Administration; EMA: European medicines agency; NASH-CRN; NASH Clinical Research Network; SAF: Steatosis activity fibrosis; NAS: NAFLD activity score; FLIP: Fatty liver inhibition of progression; DHN: Division of hepatology and nutrition; AI: Artificial intelligence; MRI-PDFF; MRI-proton density fat fraction; TE: Transient elastography; SWE; Shear wave elastography; MRE: Magnetic resonance elastography; CK-18: Cytokeratin-18; ALT; Alanine aminotransferase; ELF; Enhanced liver fibrosis; APRI; AST-to-platelet ratio index; NFS; NAFLD fibrosis score; HVPG: Hepatic venous pressure gradient; MELD: Model for end-stage liver disease; CTP: Child-turcotte-pugh

1. Abstract

With the increasingly global epidemic of obese and metabolic syndrome, non-alcoholic steatohepatitis (NASH) has become a growing common cause of cirrhosis, hepatocellular carcinoma, and end-stage liver disease. It is imperative to develop safe and effective drug especially for those with fibrosis, to improve clinical outcomes and reduce the burden of disease. Selection of meaningful endpoints is an essential precondition for the success of NASH clinical trials. Considering the heterogeneity of its pathogenesis and fluctuation of its natural history, use of hard clinical endpoints including liver related outcomes and cardiovascular events are not feasible in clinical trials. Currently accepted endpoints are mainly based on standardized evaluation of paired liver histology, including resolution of NASH without worsening of fibrosis and/or improvement in fibrosis without worsening of NASH. Validation and refinement of these histological surrogate endpoints in longer term studies is critical for the ongoing development of new therapies for NASH. In consideration of the invasiveness and other limitations, the development and application of reliable non-invasive biomarkers that parallel with drug induced changes and clinical outcomes as a surrogate endpoint is an attractive approach in the future.

2. Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) is an emerging global health issue closely associated with insulin resistance and metabolic syndrome. With significantly increased prevalence both in western countries and Asia, NAFLD has become the leading cause of liver-related morbidity and mortality worldwide [1-3]. Histologically, NAFLD represents a spectrum of diseases, ranging from simple fat-

ty liver, to non-alcoholic steatohepatitis (NASH), which is the more aggressive form, characterized by hepatocellular injury, ballooning, and often accompanied with fibrosis [2, 4, 5]. Although all subtypes of NAFLD patients have increased risk of death, especially from cardiovascular disease, NASH is the fastest growing causes of cirrhosis, hepatocellular carcinoma, and liver-related complications, and will become the main indication for liver transplantation in the next 10 years [6, 7].

The growing burden of NASH on public health underscores the need for the development of effective therapies. To date, lifestyle modification with weight loss remains the standard of care for NAFLD [8, 9]. However, the effectiveness of life style modification is difficult to achieve and sustain due to the poor compliance [10, 11]. During the last 20 years, a variety of agents, which primarily target metabolic or inflammatory pathways to improve steatohepatitis and/or hepatic fibrosis have been investigated in randomized controlled clinical trials. Unfortunately, none of them has been approved by the US Food and Drug Administration (FDA) or the European Medicines Agency (EMA) for the treatment of NASH till now [12]. The prospect of new drug development for NASH is challenging for a variety of reasons, one of which is the optimal definition and selection of the endpoints in NASH clinical trials.

The improvement of clinical outcome is the hard endpoint for evaluating drug efficacy. However, for NASH patients, especially those without cirrhosis, the observation of clinical outcome usually takes several years. Therefore, the FDA and EMA allow the rational use of surrogate endpoints in NASH drug research. How to define the surrogate endpoints and whether it can reflect clinical outcomes and benefits is a new challenge. This review article aims to discuss the selection and the challenges of treatment endpoints at different stages in the current clinical trials for new drug development of NASH.

3. Hard Clinical Endpoints in NAFLD: The Improvement of Clinical Outcomes

Clinical outcomes are traditionally used to assess drug efficacy in many chronic diseases. Improving clinical outcomes including the reduction of liver-related mortality and all-cause mortality, the decrease of incidence in cirrhosis, hepatocellular carcinoma, or liver transplantation, are the key goals of drug therapy in NASH. Liver-related mortality is closely related to the decompensation of cirrhosis such as ascites, hepatic encephalopathy, gastroesophageal variceal bleeding, hepatocellular carcinoma, acute or acute on chronic liver failure [13] and would be the best clinical outcome to evaluate therapeutic efficacy [14]. Besides, cardiovascular events and extrahepatic malignancy are the leading causes of death in non-cirrhotic NASH patients, therefore, they also need to be considered [15, 16].

Although the improvement of clinical outcomes is the ideal endpoint for the evaluation of the efficacy of NASH drugs, some factors limit their wide application as clinical endpoints in clinical trials. Firstly, the progression of NASH to cirrhosis is slow and there is usually a long asymptomatic period before the development of clinical outcomes. Studies [17, 18] show that liver fibrosis progresses by 1 stage in an average of 7 years, with 15% ~ 20% of NASH patients can progress to cirrhosis during this period (an annual incidence of NASH cirrhosis of 4%). To prove that therapeutic drugs can reduce the mortality of the disease, an observation period of 10 to 15 years is required for early-stage NASH patients; this makes it less feasible to use the improvement of clinical outcomes as the end point of new drug development, especially for patients with non-cirrhotic NASH [19]. Secondly, some outcomes may not occur in all NASH phenotypes during the study period, highlighting the importance of purposeful patient enrollment and deliberate segregation of these study groups.

In view of difficulties to assess clinical outcomes for NAFLD and NASH, surrogate endpoints may be allowed for FDA approval through the accelerated/conditional pathways. It is a major challenge to identify and validate surrogate markers that predict a reduction in progression to hard outcomes.

4. Histological Surrogate Endpoints

4.1. Histology for Diagnosis and Evaluation of NASH

Liver biopsy remains the gold standard for confirmation of the diagnosis and assessment of the severity of histological findings in NAFLD. The histological features of NASH include steatosis, ballooning of hepatocytes, scattered lobular inflammation and Mallory-Denk bodies. In the early stage of the disease, the most serious changes in steatosis are in the centrilobular zone (zone 3). As the disease progresses, steatosis can be evenly distributed throughout the liver acinar irregularly. Inflammation is mainly a mixed inflammatory infiltration dominated by lobular distribution. Ballooning is the most important feature for the diagnosis of NASH. Fibrosis usually starts in zone 3 and perisinus with typical "chicken-wire" changes, then it may progress to bridging fibrosis or even cirrhosis in certain patients.

There are two semiquantitative scoring systems commonly used for the histological evaluation in NAFLD, namely the NASH Clinical Research Network (NASH-CRN) system and the steatosis activity fibrosis (SAF) scoring system. The former was designed and validated in 2005, including the NAFLD activity score (NAS) score which was proposed for use in clinical trials rather than for diagnosis of NASH in clinical practice [20, 21]. The latter, developed by the Fatty Liver Inhibition of Progression (FLIP) European consortium in 2012, was designed for histopathologic classification of liver lesions [22]. The new parameter “activity” was defined as the sum of lobular inflammation and ballooning. A diagnostic algorithm for NASH was also proposed and based on the activity. Using the SAF system, any case could be descripted as NAFLD ($S_{\geq 1}A_{any}F_{any}$) or NASH ($S_{\geq 1}A_{\geq 2}F_{any}$), makes it easily to compare the changes observed in paired biopsies during clinical trials [22]. So far, the majority of clinical trials have used the NASH-CRN system for the screening of enrolled patients and the evaluation of surrogate endpoints. Recent studies also validated the SAF system as a tool for patient enrollment in therapeutic

trials [23, 24].

4.2. Histology as a Clinical Trial Endpoint

To meet the needs of NASH new drug development, surrogate markers need to be explored and validated to predict clinically meaningful end-points, which can reflect changes in the disease process and are also closely related to the pathogenesis of the disease. Surrogate endpoints usually need to meet the following criteria [14]: (1) predict clinical benefit; (2) predict irreversible morbidity or death. The histopathology assessment can provide information on the progression or reversal of the disease, and have the characteristics of flexibility and short-term evaluation. The progression of liver fibrosis is closely related to liver-related outcomes. A number of recent studies [16, 25-29] have shown that the degree of liver fibrosis is the most closely related factor for the long-term prognosis of NAFLD. Advanced fibrosis is related to overall survival. The regression of fibrosis is associated with a decrease in liver-related mortality. The activity of NASH is also a contributing factor to the progression of liver fibrosis [30]. Data from PIVENS and FLINT clinical trials have demonstrated the strong link between histological resolution of NASH (improved NAS ≥2) with at least 1-stage decrease or more in fibrosis [31].

In 2018, the FDA issued a draft guideline for the development of therapeutic drugs for NASH with liver fibrosis [32]. For NASH with moderate or bridging fibrosis (fibrosis stage 2 and stage 3), liver histology improvement can be used as a treatment endpoint and accelerated approval process. In a joint report of the NAFLD clinical trial endpoints published by the American Society of Liver Diseases and the European Society of Liver Diseases in 2019 [14], histopathology can be accepted as a surrogate endpoint for new drug trials of non-cirrhotic NASH especially in phase 2b and phase 3 (please see another review in this issue for details). For clinical trials of compensated NASH-related cirrhosis, it is still recommended to use the improvement of clinical outcome as the endpoint evaluation, but the improvement of histology is also one of the important treatment endpoints of phase 2/3 clinical trials.

4.3. Challenges in using histology as a surrogate endpoint

The rationale for using histology as a surrogate endpoint for NASH clinical trials is mainly based on the relationship between histology and prognosis in previous studies. However, there is still insufficient evidence to prove that those who reach the surrogate endpoint of histology have significantly reduced NASH-related liver events liver, including cirrhosis decompensation, hepatocellular carcinoma, liver-related mortality, and all-cause mortality. In the development of new NASH drugs, further research is needed to prove the clinical benefits and the improvement of clinical outcomes. The Division of Hepatology and Nutrition (DHN) at the FDA emphasizes in the latest guidance that if the new NASH drug development is accelerated approved based on histological endpoints, it still needs to conduct a phase 4 clinical outcomes trial to verify the efficacy [32]. In addition to the evaluation of the above-mentioned outcome events, evaluation of progression to cirrhosis is also needed to be consider for patients with non-cirrhotic NASH.

Although histology is the gold standard for diagnosis and grading of NASH, there are still limitations, such as appreciable (about 40%) sampling error [33], insufficient sample length without enough number of portal areas [34], which may lead to inaccurate evaluation. Inconsistency of diagnosis between different pathologists (inter-reader variability) is another important challenge, sometimes even inconsistent with their own previous diagnosis (intra- reader variability). In response to the above problems, the standard operating procedures for pathological samples should be strictly followed in NASH clinical trials. Double-blind reading should be conducted by liver pathologists. Training of pathologists is recommended by the FDA to improve the consistency of pathology evaluation. An adjudication committee of central pathologists should be established. At least two trained pathologists will read the baseline and treatment slices together to determine the scores of the various components of the NAS. It is also recommended that sponsors should review their plan for liver biopsy procurement, processing, and interpretation in detail before starting the phase 3 trial [32].

Recently, artificial intelligence (AI) has been applied in the evaluation of NASH pathology. A study [35] used two-photon microscopy-based fibrosis parameters (q-FP) to quantitatively evaluate the level of NASH fibrosis. Another study [36] used a two-photon/second harmonic method to quantify components such as lipidosis, lobular inflammation, and ballooning in NASH histology. Using the digital images of the trichrome-stained slides of liver biopsies, an integrated AI-based automated tool has been developed recently to detect both the continuous measurement of amount of fibrosis (collagen proportionate area) and the architectural pattern NASH related fibrosis [37]. The application of AI in NASH pathological evaluation may bring new possibilities for improving the consistency of pathological diagnosis.

5. Exploratory Endpoints: Based on Non-Invasive Markers

NASH drugs currently under development are mainly divided into three categories according to the mechanism: metabolic improvement, anti-inflammatory and anti-fibrosis [38, 39]. In the early exploratory phase of clinical trials, both the metabolic, anti-inflammatory, anti-fibrotic effects, and the ability of histological improvement still need to be further verified. The endpoint selection for this phase depends on the intrinsic mechanism of the new drug. Therefore, serological makers, imaging, and non-invasive models based on the above can be considered as exploratory endpoints for the short course of pharmacokinetic/pharmacodynamic evaluation in the early stage of clinical trials with small sample size.

At present, the regulatory agencies usually encourage the use of non-invasive biomarkers from proof-of-concept early phase 2 stud-

ies to late stages of drug development (phase 2b and phase 3 trials) as a secondary and exploratory endpoint, hopefully to provide more evidence for the discovery of reliable non-invasive markers as a surrogate efficacy endpoint to predict clinical benefits [32].

5.1. Imaging

Hepatocyte steatosis is an important pathological feature of NAFLD. In non-cirrhotic patients, the complete resolve of hepatic steatosis may be accompanied by the improvement of NASH [40]. MRI-proton density fat fraction (MRI-PDFF) is a new imaging method of measuring the liver fat, with an excellent correlation with steatosis grade and high diagnostic accuracy in NAFLD/NASH [41-44]. The advantages of non-invasive, quantitative, and reproducible make it a promising tool for non-invasive diagnosis of NASH. In phase 1/2a trials of NASH drugs that target liver fat reduction, more and more studies tend to use MRI-PDFF to quantitatively assess liver steatosis as the primary efficacy endpoint [45-47]. In the 36-week open-label extension study of resmetirom (MGL-3196) for the treatment of NASH, liver fat reduction assessed by MRI-PDFF was used as primary endpoint [48]. With standardized training and good quality control, the absolute value or relative percentage of MRI-PDFF change can be used to evaluate drugs for reducing liver fat content.

Liver stiffness determined by elastography, including transient elastography (TE), shear wave elastography (SWE) and magnetic resonance elastography (MRE), provide an accurate evaluation of hepatic fibrosis in patients with various chronic liver diseases including NAFLD. TE can be affected by multiple factors such as liver inflammation, cholestasis and subcutaneous fat content. High failure rates in obese patients limits reliable measurement of liver stiffness by TE [49, 50]. The optimal cut-off value to be utilized for diagnosis and screening advanced fibrosis in NAFLD patients still remains to be determined [51].

MRE has been proved to be more accurate in identifying different stages of fibrosis [52, 53]. Recent research has shown that changes in liver stiffness detected by MRE are correlated with changes in body weight. A decrease in MRE-estimated liver stiffness of approximately 15-19% corresponds to a weight loss of 5% [54]. It has been also used in the longitudinal studies to assess changes in liver fibrosis. In a phase 2 trial of selonsertib [45], MRE-estimated liver stiffness was assessed pre-and post-treatment, the area under the receiver operating characteristic curve (AUROC) of MRE-stiffness to predict fibrosis improvement was 0.62 (95% CI 0.46-0.78). These studies indicate that MRE may become a surrogate end point for the assessment of liver fibrosis. However, further validation with larger sample size is needed. Due to the higher cost and the limited availability, the wide application of MRE may be hindered, which is another factor to consider.

5.2. Biochemical Markers

In view of the difficulty and potential risks of repeated liver biopsy, the development and use of reliable non-invasive biomarkers as a surrogate endpoint is an attractive approach. Serological makers reflecting inflammation or damage of hepatocytes in NASH include serum aminotransferase, cytokeratin-18 (CK-18) fragments and different kinds of cytokines. Although the serum aminotransferase is an indicator of hepatocyte damage, its diagnostic accuracy for NASH is modest [55]. Data from the PIVENS and TONIC trials showed the relationship between the decrease in aminotransferase levels and the histological improvement of NASH. Some phase 2 trials have used the percentage change of alanine aminotransferase (ALT) from baseline as the primary efficacy endpoint [56].However, both the degree of reduction and the cut-off levels of aminotransferase have not been established [57].

CK-18, a biomarker of hepatocyte apoptosis, was used to be considered one of the promising non-invasive tools for the diagnosis and grading of NASH [58, 59]. However, data from a large study showed more limited value than ever thought, the AUROC to predict NAFLD, NASH or fibrosis using CK-18 were 0.77 (95% CI = 0.71–0.84), 0.65 (95% CI = 0.59–0.71) and 0.68 (95% CI = 0.61–0.75), respectively. And there was less correlation with lobular inflammation, ballooning, and fibrosis, which were the most meaningful histological features of NASH. Whether the combination of it with other valuable biomarkers could prove more accurate needs to be investigated in the future [60].

A large number of biomarkers are studied and validated to evaluate the degree of liver fibrosis including the FibroTest, the Enhanced Liver Fibrosis (ELF), the AST-to-platelet ratio index (APRI), the NAFLD fibrosis score (NFS), the FIB-4 index, and the Fibrometer. As diagnostic makers, these models have substantial ability to rule out liver fibrosis; however, the ability to distinguish different stage of fibrosis is limited. The ability to estimate the dynamic change of fibrosis is also need to be further verified. Data from recent clinical trials showed that non-invasive biomarkers may predict improvement in NASH related fibrosis [61-64]. APRI, NFS and FIB-4 index are the most widely used models with the advantages of lower costs, convenient calculation, and wide availability. In the FLINT trial, the relationship between the three models and the liver fibrosis improvement has been studied. It is demonstrated that these non-invasive markers may serve as surrogate end points in NASH clinical trials [62].

In *post hoc* analysis of the PIVENS trial (Pioglitazone vs. Vitamin E vs. Placebo for the Treatment of Nondiabetic Patients with NASH), value of the ELF has been assessed both at cross-sectional and longitudinal level [65]. Significantly association of the ELF with fibrosis stage at baseline and end of treatment (EOT) was observed. However, longitudinal change in ELF score did not relate to improvement in fibrosis or NASH resolution. Interestingly, the change in amino-terminal propeptide of type III procollagen (PIIINP), one of the components of the ELF, significantly correlated with improvement in NAS and fibrosis. It is worthy of further investigation to verify its ability as a surrogate marker for clinical endpoint.

There is limited evidence for the serological markers to predict clinical outcomes. A model based on the serum level of aspartate aminotransferase together with TE (combined with Liver stiffness measurement and controlled attenuation parameter) called Fibro Scan-AST (FAST) score has been developed in a recent prospective derivation and global validation study [66]. Diagnostic performance for patients with NASH related fibrosis (NAS≥4, stage of fibrosis≥2) was satisfactory in both the study cohort (C-statistic 0.80, 95% CI 0.76–0.85) and the external validation cohorts (C-statistic range 0.74–0.95, 0.85; 95% CI 0.83–087). Non-invasive models with a combination of serological and imaging makers may become a more promising surrogate endpoint for NASH clinical trials in the future.

5.3. Other Promising Endpoints

Among patients with NASH related cirrhosis, especially those at the decompensation stage, it is impracticable to use the histological surrogate endpoint in a short period of time. Portal hypertension is the strongest indicator of decompensate cirrhosis [67, 68], while the Hepatic Venous Pressure Gradient (HVPG) is the gold standard for the detection of portal hypertension and can be a predictor of the development of clinical decompensation. Each 1mm Hg increase in the HVPG is associated with an 11% increase in decompensation [67]. Reduction in HVPG is associated with the decreased rate of decompensation and the improvement of clinical outcomes [69]. It is recommended that the reduction in HVPG of ≥2 mmHg can be used as an endpoint for phase 2b trials in cirrhotic NASH [14]. However, the application is relatively limited by the invasiveness and technical requirements for operators. Other surrogate endpoints including the Model for End-Stage Liver Disease (MELD) score and Child-Turcotte-Pugh (CTP) score, are also prognostic of clinical outcome and can be considered in phase 2b/3 clinical trials [14].

Besides clinical endpoints that best predict prognosis, patient-reported outcomes that can be the best reflection of patient experience should also be considered as a surrogate endpoint in NASH clinical trials. The Chronic Liver Disease Questionnaire (CLDQ)-NAFLD/NASH, a disease-specific health-related quality of life instrument, has been validated to have excellent psychometric ability in recent studies in NASH clinical trials [70, 71].

6. Summary

With the increasing global disease burden related to NAFLD and NASH, it is urgent to develop effective therapeutic drugs, especially for those with fibrosis. Definition and improvement of valid, reliable and practical endpoints in clinical trials is of the essence. In the early proof-of-concept stage, non-invasive assessment tools, including change of liver-specific serological markers and imaging such as MRI-PDFF and MRE, are allowed to put in use. While in the later stage of exploratory and confirmatory trials, the endpoints for evaluating drug efficacy are focused on improving the histological lesions such as inflammation and fibrosis. For NASH related cirrhosis, the combination of clinical outcome, histology and non-invasive markers as the meaningful endpoint should be taken into account. One of the challenges is the further assessment of these histological and non-invasive surrogate endpoints in long-term follow-up to verify their ability to reflect hard clinical endpoints. Recommendations on how to quantify lifestyle changes and to evaluate their influence on clinical trial endpoints and outcomes would also be expected so as to minimize placebo response. Future studies should also be intended to monitor the concomitant metabolic disorders especially cardiovascular disease and diabetes.

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