Future Considerations of Biological Disparities in Drug Development for NAFLD/NASH: Trial Design and Analysis

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Abbreviations:

ANCOVA, analysis of covariance; BMI, body mass index; ESLD, end-stage liver disease; HCC, hepatocellular carcinoma; MAPK, mitogen-activated protein kinase; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NHANES, the National Health and Nutrition Examination Survey; ROS, reactive oxygen species; TGF, Transforming growth factor.

1. Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) has become a significant health concern not only in the US but also worldwide due to the global obesity epidemic. Although the natural course in the majority of NAFLD patients is relatively benign, those with non-alcoholic steatohepatitis (NASH) are at an increased risk of disease progression, leading to hepatic fibrosis, cirrhosis, end-stage liver disease (ESLD), and hepatocellular carcinoma (HCC). Owing to its rapidly increasing prevalence in the US, NASH has become a leading liver transplant indication across racial/ethnic groups. NAFLD is also associated with an increased risk of cardiovascular disease, chronic kidney disease, and liver-related and overall mortality, posing a heavy burden both clinically and economically. No FDA-approved pharmacological agents are currently available to treat NAFLD/NASH. This is likely due to the multifactorial nature of NAFLD and biological disparities. Safe and effective treatments are thereby overly needed to mitigate the NASH progression, prevent complications, and reduce future medical and economic burdens. There are several challenges in the process of testing new agents, which include but are not limited to 1) multifactorial pathogenesis and 2) lack of sufficient considerations of biological disparities by age and sex/gender in clinical trial designs. Since current study designs rarely take these disparities into

consideration, this paper will focus on the second issue and provide an overview of NASH pathogenesis, epidemiology, and disparities by age and sex/gender and propose possible methodological solutions such as adaptive design and post hoc analysis, while discussing advantages and disadvantages of the proposed solutions.

2. Introduction

Owing to the obesity epidemic, nonalcoholic fatty liver disease (NAFLD) has grown as a significant health concern in the US. The NAFLD prevalence among the adult population has risen from 18% in 1988-1991 to 31% in 2011-2012, according to the National Health and Nutrition Examination Survey (NHANES) studies. [1] NAFLD was once considered as a disease condition prevalent in western countries, but due to the global obesity epidemic, NAFLD became a global health issue; the estimated global prevalence of NAFLD among adults is about 25%. [2] This reflects global westernization of diets and lifestyle, resulting in less-physically activity, sedentary lifestyle, and diets that are high in saturated fats and sucrose and low in dietary fiber, which in turn contributed to a growing health risk of obesity, metabolic diseases, and NAFLD.

The majority of NAFLD patients have simple steatosis, a relatively benign condition, but among those with nonalcoholic steatohepatitis

(NASH), there is an increased risk of hepatic fibrosis progressing to cirrhosis, end-stage liver disease (ESLD), and, in some patients, hepatocellular carcinoma (HCC). [3] About 25-30% of patients with NAFLD are estimated to have the progressive form of NAFLD. [4-6] Over time, approximately 32% to 53% of patients with NASH experience fibrosis progression. [7, 8] Until recently, viral hepatitis (especially hepatitis C) and alcoholic liver disease were the two leading indications for liver transplants in the US. [9] However, NASH is a rapidly growing liver transplant indication across racial/ethnic groups. [9] NAFLD-related ESLD and HCC became a leading cause of liver transplant consideration in the US: the second leading cause among men and the leading cause among women. [9, 10] NAFLD is also associated with increased risk of cardiovascular disease, cancer, and chronic kidney disease and significantly increases overall mortality. [11-14] Thus, NAFLD and its associated comorbidities pose heavy clinical and economic burdens, with a staggering estimated annual direct medical cost of patients with NAFLD of $103 billion in the US and €35 billion in Europe. [15, 16] Therefore, safe and effective treatments to mitigate the NASH progression and prevent complications (i.e., cardiovascular diseases, chronic kidney disease, HCC) are desperately needed.

Weight reduction and regular exercise have been proven to reverse steatosis, NASH, and NASH fibrosis. [17] However, achieving and sustaining therapeutic weight reduction and exercise habits is a significant challenge in patients with NAFLD. Identifying an effective, safe pharmacological treatment to mitigate NASH activity and fibrosis is critical to the prevention of NASH progression and liver cancer development. During the past decades, tremendous advancement was made in understanding the pathogenesis of NAFLD, which led to numerous clinical trials. Despite the identified therapeutic targets and promising candidates tested in clinical trials, no established pharmacological agents exist today to treat NAFLD. Many trials have been terminated prematurely due to the lack of sufficient efficacy at the interim analysis. The challenges in NASH/NAFLD drug development are multifactorial. NAFLD pathogenesis is complex, involving a cascade of pathophysiologic changes. Thus, the therapeutic approach should be formulated based on understanding the multiphasic NAFLD pathogenesis in patients with NAFLD (e.g., a combination of drugs targeting multiple pathways, drug therapy in combination with diet and/or exercise). Another limitation is the lack of sufficient consideration of biological disparities by age and sex/gender in clinical trial design and analytic planning. Robust data exists, demonstrating sex/gender differences in human health and diseases. [18] Many aspects of the NAFLD pathogenesis and the disease progression are regulated in sex-specific manners. [19, 20] Aging also leads to functional senescence in adipocytes, cellular stress response, inflammation, immune response, and regenerative capacity. [21-23] Thus, treatment response and safety profiles may significantly vary by sex, reproductive status, and age. Some medications may exert sex-/age-specific therapeutic effects or safety signals. Since men and women age differently, the effects could be age- and sex-specific. [24]

Such disparities are rarely considered in current clinical trials, probably because it would complicate the study design and increase the required sample size. [18, 25] In this article, we will briefly summarize the NAFLD pathogenesis, review biological disparities in NAFLD/NASH mechanisms, discuss necessary considerations in the analysis, and propose possible methodological solutions, including the application of adaptive design and posthoc analysis of the trial data to inform later phase clinical trials.

## 3. Overview of NASH Pathogenesis, Epidemiology, and Disparities by Age and Sex

NAFLD is a disease caused by excess lipid accumulation in the liver. Beside a few exceptions such as drug-induced NAFLD and lysosomal acid lipase deficiency, most NAFLD cases are associated with metabolic derangement induced by obesity (i.e., metabolic-associated fatty liver disease or MAFLD). The key disease drivers of NAFLD are abdominal obesity and insulin resistance, both of which fuel the liver with increased free fatty acids delivery, causing metabolic stress in the hepatocytes. [3] When the increased lipid burden encounters failed hepatocellular adaptation, it then leads to hepatocellular damage, chronic liver injury (i.e., nonalcoholic steatohepatitis or NASH), fibrosis, and tumorigenesis. [3] The disease severity and progression, i.e., NASH and fibrosis, are considered a consequence of failed adaptation to the increased metabolic stress, impaired homeostasis, and dysregulated wound-healing process. [3] Figure 1 depicts the multiphasic aspects of NALFD pathogenesis.

NAFLD occurs in both sexes and spans a wide range of age groups, from children to the elderly. However, the prevalence, clinical and histologic features, and risk factors of NAFLD are not necessarily homogeneous across different age groups or between sexes. [25-28] As reviewed in a recent article, population-based studies consistently demonstrated the prevalence of NAFLD to be higher in men than in women during the reproductive age, while the prevalence in women increases after the age at menopause and exceeds the prevalence in men in older age. [25] This age-sex interaction on the NAFLD prevalence is partly explained by estrogen's protective effects on visceral obesity and insulin resistance. [25] Hormone replacement therapy among postmenopausal women appears to be protective against liver enzyme elevation presumably associated with NAFLD. [29, 30] Similar age-sex interaction is also observed in the severity of NASH fibrosis; premenopausal women are protected from hepatic fibrosis compared to men and postmenopausal women, [31-33] which is likely attributed to estrogen's inhibitory effects on satellite cell proliferation and fibrogenesis. [34, 35] Of note, the prevalence of NAFLD is positively correlated with body size (i.e., BMI), but this association is weak among the older population [36, 37] reflecting the heterogeneity in the NAFLD pathogenesis; [38] This observation may suggest that in older subjects, cellular homeostasis/senescence may more significantly contribute to the disease progression than the upstream disease–driving factor, i.e., increased lipid influx to the liver.

Robust evidence exists to support biological disparities in the NA-

FLD pathogenesis. Sex differences in mechanisms involved in the NAFLD pathogenesis are reviewed in recently published articles [19, 20], thus are not discussed here. However, there are a few crucial points relevant to the purpose of this article. Hepatic gene expression is sexually dimorphic due to the gene regulation by estrogen, androgen, and sex-specific growth hormone secretion patterns [39]. A recent computational modeling study demonstrated that female and male livers are metabolically distinct organs and identified gene regulators exerting sex-specific effects on hepatic triglycerides accumulation. [40] Such regulators include peroxisome proliferator-activated receptor (Ppar)-γ, coactivator 1-a (Pgc1a), farnesoid X receptor (Fxr), liver X receptor (Lxr), and Ppar-α [40], most of which are functionally related to current therapeutic targets in NASH, [41] reinforcing the significance of considering sex differences in evaluating efficacy and safety for drugs targeting these regulators. Besides metabolism, sexual dimorphisms are broadly observed in hepatic gene expressions across the functions qualitatively and quantitatively. An RNA sequence study assessed intra- and interspecies variation

in gene regulatory processes among primates and demonstrated sex differences across the species in gene expression involved in lipid metabolism and catabolism, steroid metabolism and biosynthesis, ATP synthesis, RNA splicing and binding, RNA processing, immune response, and wound healing (e.g., wnt signaling) in addition to genes on X-chromosome. [42] Further, hepatic fibrogenesis is regulated by sex hormones. Estradiol inhibits liver fibrosis by inhibiting stellate cell activation via estrogen receptor-β [35, 43], while progesterone activates stellate cells by inducing ROS generation, MAPK pathway activation, and TGFβ1 expression. [44] Thus, physiological estrogen levels (e.g., women vs. men, pre-menopausal vs. postmenopausal women) and the altered ratio of estrogens to progesterone (e.g., contraceptives) may modulate baseline fibrogenic activities and thus influence therapeutic response to anti-fibrogenic agents. Since hepatic lipid metabolism, inflammation, and fibrosis are frequently targeted in the NASH treatment, proper consideration of age and sex in the study design and analysis is critical in addressing variations in the treatment efficacy and safety profiles.
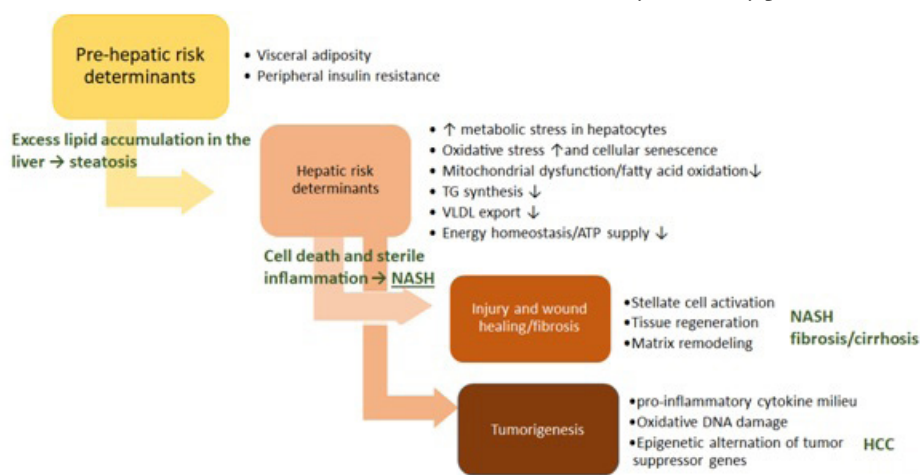
Figure 1: NAFLD pathogenesis
TG: triglyceride
VLDL: very low density lipoprotein
NASH: nonalcoholic steatohepatitis
HCC: hepatocellular carcinoma

4. Translating the Biological Disparities in The NAFLD Pathogenesis into Statistical Consideration

Keeping the biological disparities in mind, we discuss a few statistical considerations in analyzing NAFLD/NASH data. First, age and sex are often considered covariates or variables for matching to remove confounding effects. Given the biological effects of age and sex on the NAFLD pathogenesis, these variables should also be considered potential effect modifiers. Second, when analyzing sex-/age-differences in the efficacy, it is common to stratify the data by age or sex separately. As many of the key mechanisms in NAFLD are regulated by sex hormones, age does not equally affect the disease mechanisms in men and women. Age-sex interaction (two-way interaction) needs to be considered in the analysis (i.e., menopausal status). Sex-specific analysis including all age groups is not sufficient to address sex differences and may mask important associations.

5. Key Limitations in Current Trial Design and Analysis

In liver disease clinical development, it is recognized that there are some limitations in the design and analysis of NAFLD/NASH clinical trials. These key limitations include, but are not limited to, (i) the lack of information on sex differences from preclinical experiments to inform study design, (ii) the lack of blocked or stratified randomization, which often leads to uneven distribution of sex and age (or treatment imbalance in sex and age), (iii) the lack of information on women's menopausal status and reproductive health which may have an impact on enrollment and/or final data analysis, and (iv) there are no considerations of potential sex/age differences and possible sex-by-age interaction. To overcome some of the limitations, we suggest the following approaches be considered: the use of adaptive trial design and post-study subgroup analysis. In what follows, these two approaches will be briefly described.

6. Future Considerations for NAFLD/NASH Clinical Trials

In practice, two approaches can be employed in NAFLD/NASH clinical trials to address the key limitations described above. The first approach is to utilize a pre-study stratified randomization with stratification factors of interest such as age and sex under a valid trial design such as adaptive trial design. Stratified randomization allows the assessment of possible confounding and/or interaction effect between treatment and the stratification factors. The second approach is post-study subgroup analysis provided there are sufficient number of subjects in the study. These two approaches are briefly summarized below.

The Use of Adaptive Trial Design – First, we suggest employing an adaptive trial design to address some of the limitations discussed above. As indicated in Chow and Chang (2011), there are ten different types of adaptive trial design (see also FDA, 2019). Selecting an appropriate adaptive design for an intended NAFLD/NASH clinical trial depends upon the study objectives of the intended trial. For example, an adaptive-randomization design may be considered if the objective is to detect potential sex and/or age differences and possible sex-by-age interaction.

We can consider a stratified randomized parallel-group design with stratification factors such as sex, age, obesity, menopausal status (female only), and/or other key factors for the specific pathway of interest and a planned interim analysis.

For instance, we can divide the target population into 4 strata using age and gender:

a. Female patient aged over 50 (i.e., age surrogate of menopause)

b. Female patient aged 50 or below

c. Male patient aged above 50 (this age can be determined using previous knowledge about the treatment effect or just the median age of the target male population)

d. Male patient aged 50 or below

Then we may recruit patients in each category with known ratio in the whole target population. By randomly assign them with 1:1 ratio to two treatment groups, we will finally result in a 2-arm parallel design in stage 1, each contains patients from all 4 strata with specific ratio. Stage 2 should also be a 2-arm parallel design, but after interim analysis, we may decide to drop patients from some strata if there is no significant treatment effect or there are some considerations about safety. (Figure 2) illustrates the proposed adaptive trial design with stratified randomization.
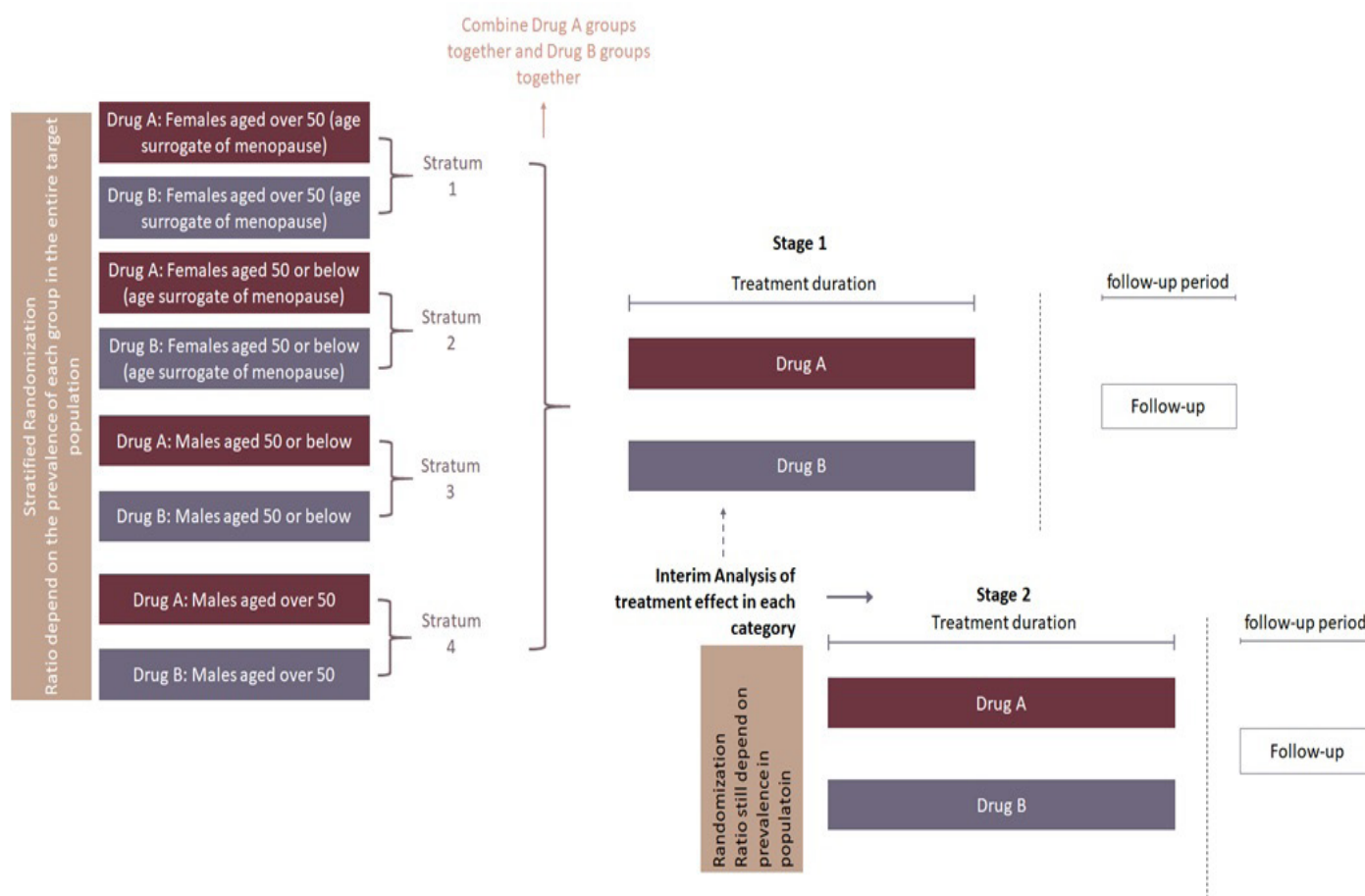
Figure 2: Proposed adaptive trial design with stratified randomization

Table 1: Pros and Cons of the Two Approaches

	Advantages	Disadvantages
Pre-Study	1) Pre-planned study so that we may make adaptions to sample size and endpoints so that we can maximize the chance to achieve a desired power of the trial. 2) May use stratified sampling to avoid the problem of treatment imbalance within each characteristic group.	1) With dropping out unwanted strata, generalizability of the study will decrease. 2) After dropping out the unresponsive strata, sample size may become too small to make the conclusion statistically significant even if we recruit some more patients in the treatment sensitive groups.
Post-Study	1) Can assess whether patients in different subgroups benefits or experience harm due to a treatment differently.	1) Multiple analyses may cause Type I error to inflate and may also increase the probability of observing different or even opposite treatment effect across subgroups. 2) May result in treatment imbalance since the stratification of target population is not done in advance. 3) May results in small sample size which cause the power to decrease. 4) Problem of generalizing the conclusion of a single subgroup to the whole population.

The purposes of the planned interim analysis are multi-fold. First, it is to verify the assumptions made upfront for power calculation of sample size requirement based on data observed at interim. Second, it is to perform sample size re-estimation to determine whether we will achieve the study objective with the desired power in the end if the observed clinically meaningful difference (treatment effect) preserves till the end. Third, we may stop the trial early due to safety, futility and/or efficacy after the review of interim data. (May drop both the arms which are unresponsive to the treatment or adjust safety measures if any arms exert safety concerns, and continue with the treatment sensitive arms.) Fourth, it provides the opportunity for adaptations to the study protocol after the review of interim data. Adaptations could include (i) change in study endpoint, (ii) change in randomization, (iii) change in hypothesis (e.g., from superiority hypothesis to non-inferiority hypothesis), and etc. These adaptations may shorten the development process and increase the probability of success.

Under the adaptive trial design, the collected clinical data can be analyzed using the method of analysis of covariance (ANCOVA) with sex, age, and obesity as fixed effects and other demographics and patient characteristics such as menopausal status as covariates. The mixed effects model will allow us not only to test potential sex/age differences, but also to assess possible sex-by-age interaction. In addition, odds ratios and their corresponding 95% confidence intervals between the levels of class variables (fixed effects) can be obtained for necessary adjustment of study design.

7. Post-Study Subgroup Analysis

If the study already done, one may consider post-study subgroup analysis. A subgroup (or subpopulation) may be defined by sex, age, obesity, and/or menopausal status (for female) if the information is available. Subgroup analysis allow us not only to test potential sex/age differences, but also to assess possible sex-by-age interaction. However, subgroup analysis has been criticized that (i) sample size is often small and hence may not have sufficient power for detection of clinically meaningful difference, (ii) subgroup may not be representative of the entire patient population under study and hence we cannot draw any conclusions regarding the entire patient population, and (iii) it is most likely there are treatment imbalance with respect to sex/age, obesity, and other factors as deemed important by PI. To overcome these problems, it is suggested that a clinical trial simulation in conjunction with a sensitivity analysis be conducted in support of subgroup analysis.

Under the framework of post-study subgroup analysis, data can be similarly analyzed using the method of analysis of covariance (ANCOVA) with sex, age, and obesity as fixed effects and other demographics and patient characteristics such as menopausal status as covariates. The mixed effects model will allow us not only to test potential sex/age differences, but also to assess possible sex-by-age interaction. In addition, odds ratios and their corresponding 95% confidence intervals between the levels of class variables (fixed effects) can be obtained.

8. Summary

This article summarizes current knowledge of age-/sex- disparities in the NAFLD pathogenesis, opportunities/challenges in investigating the disparities in clinical trials of NAFLD/NASH. As detailed above, there are differences in biology between males and females. Proper consideration of these variations may aid in delineating the heterogeneity in therapeutic response in patients with NAFLD/NASH and inform personalized therapeutic approaches in patients with NAFLD.

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