Supplementary Online Content 1

Association of Bariatric Surgery with Major Adverse Liver and Cardiovascular Outcomes in Patients with Biopsy-Proven Non-Alcoholic Steatohepatitis

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STUDY PROTOCOL (January 11, 2020) **S**urgical **P**rocedures and **L**ong-term **E**ffectiveness in **N**ASH **D**isease and **O**besity **R**isk (**SPLENDOR**)	
Association of Metabolic Surgery with Major Adverse Liver and Cardiovascular Outcomes in Patients with Biopsy-Proven Non-Alcoholic Steatohepatitis: The SPLENDOR Study	
Summary	Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are the major cause of chronic liver disease in the US. Medical treatment for NAFLD/NASH is very limited. Surgical weight loss through metabolic surgery can improve NAFLD/NASH and reverse some histological changes associated with inflammation and fibrosis. The long-term effects of metabolic surgery on serious cardiovascular and hepatic endpoints in patients with NAFLD/NASH is largely unknown. The goal of SPLENDOR study is to examine the long-term incidence of serious clinical outcomes of patients with biopsy-proven fibrotic non-cirrhotic NASH and obesity who underwent surgical (metabolic surgery) versus medical treatment at the Cleveland Clinic Health System (CCHS).
Background	The growing pandemics of obesity and diabetes represent major global public health threats. NAFLD is commonly associated with obesity, diabetes, and dyslipidemia. NAFLD is currently the most common cause of abnormal liver function tests in western countries and affects 25–30% of western countries' population and more than 80% of people with obesity. It refers to a spectrum of liver damage ranging from simple steatosis to NASH, advanced fibrosis, cirrhosis, or even hepatocellular carcinoma. Unfortunately, there is no FDA-approved medication for NASH. In addition, no therapy has been shown to be effective in reducing the risk of major adverse cardiovascular and hepatic outcomes in patients with NASH. There is substantial evidence that metabolic surgery can lead to significant improvement of components of metabolic syndrome including type 2 diabetes, dyslipidemia, and hypertension in patients with obesity. Furthermore, the growing body of evidence, derived from the observational or case-control studies, demonstrates that metabolic surgery in patients with NAFLD/NASH is safe and improves NASH-related histologic changes, including fibrosis.
Study Questions	1. In patients with NASH and liver fibrosis, is metabolic surgery associated with a reduction in the risk of major adverse hepatic clinical outcomes (composite outcome of ascites, esophageal variceal bleed, hepatic encephalopathy, hepatocellular carcinoma, liver transplantation, repeat liver biopsy of showing fibrosis stage 4, or liver-related mortality) compared with nonsurgical treatment? 2. In patients with confirmed NASH and liver fibrosis, is metabolic surgery associated with a reduction in major adverse cardiovascular events (coronary and cerebrovascular events and/or mortality) compared with nonsurgical treatment?

Inclusion Criteria	1. Liver biopsy at the CCHS between 2004 and 2016 2. Age 18-80 3. BMI ≥30 kg/m^2 4. Presence of NASH in liver biopsy based on the NASH Clinical Research Network (NASH CRN) definitions 5. Presence of steatosis, inflammation, and ballooning (all 3, any grade) in liver biopsy 6. Liver biopsy with presence of liver fibrosis (stage 1, 2, or 3)
Exclusion Criteria	1. Presence of other chronic liver disorders (drug-induced, viral hepatitis, autoimmune, and genetic) [codes in the eTable 1] 2. Absence of NASH (ballooning) in liver biopsy 3. Absence of liver fibrosis in liver biopsy 4. Presence of liver cirrhosis (stage 4 fibrosis) in liver biopsy 5. Presence of ascites before liver biopsy 6. Presence of esophageal varices before liver biopsy 7. History of hepatocellular carcinoma before liver biopsy 8. Diagnosis of cancer (any type) within 1-year before liver biopsy 9. History of excessive alcohol use or alcoholic-related medical conditions prior to liver biopsy (codes in the eTable 1) 10. History of organ transplant (heart, lung, liver, kidney, pancreas, intestine, bone marrow) prior to liver biopsy 11. History of severe heart failure (any ejection fraction <20%) prior to liver biopsy 12. History of dialysis prior to liver biopsy 13. Known cases of human immunodeficiency virus infection or AIDS before liver biopsy 14. Total parental nutrition within 6 months prior to liver biopsy
Study Design	SPLENDOR is a retrospective observational study. Data of adult patients who underwent liver biopsy between 2004 and 2016 at CCHS will be reviewed. Only patients with confirmed NASH and fibrosis (stage 1-3) in liver biopsy will be included. Surgical group includes patients who had liver biopsy at the time of metabolic surgery (limited to 2 procedures: Roux-en-Y gastric bypass [GB] and sleeve gastrectomy [SG]). The routine practice at the CCHS is to perform simultaneous liver biopsy for all patients at the time metabolic surgery, regardless of preoperative LFT, liver ultrasound findings, or intraoperative size and shape of liver. Nonsurgical group includes patients who had percutaneous or trans-jugular liver biopsy and did not have codes for any type of metabolic surgery (GB, SG, and other metabolic surgical procedures) [codes in the eTable 2]. Eligible patients will be followed until the last follow-up available in the CCHS electronic health record (EHR) to identify the occurrence of one of study outcomes (mentioned below).
Estimated Sample Size	Based on estimates from our previous studies, we expect that we would require to review about 10,000 patients in each group and would eventually include about 500-1000 patients in each study arm.

Outcome Variables	**Primary Composite Endpoints:** • Major Adverse Liver Outcomes (MALO): first occurrence of progression to clinical (e.g. development of esophageal varices, ascites, or hepatic encephalopathy) or histologic (F4 on repeat liver biopsy) cirrhosis, development of HCC, liver transplantation, or liver-related mortality based on the first occurrence after the index date (eTable 4 for definitions and codes). • Major Adverse Cardiovascular Events (MACE): first occurrence of coronary artery events (unstable angina, myocardial infarction, or coronary intervention/surgery), cerebrovascular events (ischemic or hemorrhagic stroke, transient ischemic attack, or carotid intervention/surgery), heart failure, or cardiovascular mortality, recording the first occurrence after the index date as the event date (eTable 5 for definitions and codes). ○ For the composite adverse cardiovascular events, the conditions/events in which a patient had in their history at baseline (coronary or cerebrovascular events before liver biopsy) will be omitted from the composite definition in follow-up. **Other Endpoints:** • Depending the sample size and number of events, individual components of primary composite endpoints may be studied and reported (if statistically sound and feasible). • Changes from baseline in body weight and glycated hemoglobin (HbA1c, only for patients with diabetes at baseline) will be compared among the study groups over time.
Data Source	The data source for all analysis will be Cleveland Clinic's EHR.
Data Elements	The following data elements will be extracted from the EHR and used for analysis (codes and definition in eTables 1-5). Note: Specific diagnosis, visit, encounter, procedure, prescription, and lab dates will be collected with each data element, where applicable, to track changes over time. Demographics • Date of birth • Sex • Race • Zip code income • Weight • BMI • Insurance • Smoking status • Alcohol use • CCHS location Medical history • Alcohol consumption and alcoholic-related disorders • Other chronic liver disorders (drug-induced, viral hepatitis, autoimmune, and genetic) [codes in the eTable 1]

	• Ascites • Type 2 Diabetes (using the modified Kho algorithm in eTable 3) • Hypertension • Dyslipidemia • Coronary artery disease • Cerebrovascular events • Nonalcoholic steatohepatitis • Esophageal varices • Esophageal variceal bleed • Encephalopathy • Portal hypertension • Hepatocellular carcinoma • Liver transplant • Other organ transplants • Being on dialysis • AIDS • Charlson comorbidity index Laboratory and Clinical data • Systolic/diastolic BP • HbA1c • Creatinine • Platelet count • ALT • AST • Bilirubin level • Serum albumin • INR • LDL • HDL • Triglycerides Medication • Insulin • Non-insulin diabetes medication • Antihypertensive medications • Vitamin E • Cholesterol lowering medications (including statins) Cancer diagnosis • Based on the codes in the eTable 1. Survival and Mortality • If deaths are unknown (i.e. not entered into Cleveland Clinic's EHR), the Social Security and State Death Indices will be searched. Liver biopsy data Grading and staging of biopsies will be based on the NASH CRN definitions. • Steatosis grade in liver biopsy

	• Lobular inflammation grade in liver biopsy • Hepatocyte ballooning grade in liver biopsy • NAFLD Activity Score • Fibrosis stage in liver biopsy
Statistical Plan	Time-to-event analysis will be conducted and patients will be followed from time zero until the first occurrence of either study outcomes (mentioned above) or until the last known follow-up date as a censoring date (i.e. last hospital discharge date or last office visit, whichever was later). Time zero will be the date of liver biopsy (i.e. the same as date of surgery for the surgical patients). To minimize the effects of confounding factors, doubly robust estimation combining the overlap weighting and outcome regression will be used to compare outcomes in surgical and nonsurgical groups. Six a priori–identified potential confounders including age at index date, sex, smoking status, presence of type 2 diabetes, histologic NAFLD Activity Score, and histologic liver fibrosis stage will be used for overlap weighting. Subsequently, Firth's penalized method in fully-adjusted Cox proportional hazard framework will be utilized by adjusting models for the index date, baseline BMI, race, income, location (Ohio versus Florida), Charlson Comorbidity Index score, presence of hypertension, dyslipidemia, heart failure, coronary artery disease, cerebrovascular disease, baseline serum bilirubin, albumin, international normalized ratio (INR), creatinine levels, and use of insulin and non-insulin diabetes medication. Cumulative incidence estimates (Kaplan-Meier method) for 10-years after the index date and unadjusted absolute risk difference for MALO and MACE will be calculated. The adjusted absolute risk difference for the composite endpoints between the study groups and number needed to treat (NNT) at 10-years from adjusted Cox models will be estimated. The 95% confidence intervals (CIs) for the difference in 10-year risk will be obtained by the percentile method from 2000 bootstrap iterations. Within each outcome dataset, missing values will be multiply-imputed with multiple imputation by chained equations to create 5 imputed datasets. Predictive mean matching, logistic regression, and polytomous logistic regression will be used for numeric, binary, and categorical variables, respectively. Imputation-corrected standard errors of model estimates and contrasts will be obtained by Rubin's formula. The percent (%) of weight loss and HbA1c values will also be evaluated over time and compared among the surgical and non-surgical groups using a linear mixed effect model. Depending on number of patients and events rates, subgroup analysis between GB and SG may be performed (if statistically sound and feasible). A significance level of 0.05 for 2-sided comparisons will be considered statistically significant, and hazard ratios (HRs) and 95% CIs will be reported.

Sensitivity Analysis	• To adjust for baseline differences, instead of overlap weighting, matching with a conventional propensity score method will be used as sensitivity analysis. • Depending on availability of control subjects, various matching ratios may be used as sensitivity analysis (if statistically sound and feasible).
References	• Diehl AM, Day C. Cause, Pathogenesis, and Treatment of Nonalcoholic Steatohepatitis. N Engl J Med. 2017;377:2063-2072. doi: 10.1056/NEJMra1503519 • Schauer PR, Bhatt DL, Kirwan JP, et al. Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes. N Engl J Med. 2017;376:641-651. doi: 10.1056/NEJMoa1600869. • Aminian A, Zajichek A, Arterburn DE, et al. Association of Metabolic Surgery With Major Adverse Cardiovascular Outcomes in Patients With Type 2 Diabetes and Obesity. JAMA. 2019;322:1271–82. doi: 10.1001/jama.2019.14231. • Manco M, Mosca A, De Peppo F, et al. The Benefit of Sleeve Gastrectomy in Obese Adolescents on Nonalcoholic Steatohepatitis and Hepatic Fibrosis. J Pediatr. 2017;180:31-37.e2. doi: 10.1016/j.jpeds.2016.08.101. • Mattar SG, Velcu LM, Rabinovitz M, et al. Surgically-induced weight loss significantly improves nonalcoholic fatty liver disease and the metabolic syndrome. Ann Surg. 2005;242:610-7. doi: 10.1097/01.sla.0000179652.07502.3f. • Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313-21. doi: 10.1002/hep.20701. • Milinovich A, Kattan MW. Extracting and utilizing electronic health data from Epic for research. Ann Transl Med. 2018;6:42. • Kho AN, Hayes MG, Rasmussen-Torvik L, et al. Use of diverse electronic medical record systems to identify genetic risk for type 2 diabetes within a genome-wide association study. J Am Med Inform Assoc. 2012;19:212-8. • Li F, Morgan KL, Zaslavsky AM. Balancing Covariates via Propensity Score Weighting. J Am Stat Assoc. 2018; 113:390-400. doi:10.1080/01621459.2016.1260466. • Heinze G, Schemper M. A solution to the problem of monotone likelihood in Cox regression. Biometrics. 2001;57:114-9. doi: 10.1111/j.0006-341x.2001.00114.x. • van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. Stat Methods Med Res. 2007;16:219-42. doi: 10.1177/0962280206074463. • Rubin DB. Inference and missing data. Biometrika. 1976; 63:581–592. https://doi.org/10.1093/biomet/63.3.581

AMENDMENT TO STUDY PROTOCOL (September 23, 2021) **S**urgical **P**rocedures and **L**ong-term **E**ffectiveness in **N**ASH **D**isease and **O**besity **R**isk (**SPLENDOR**)	
In order to address the comments raised during the journal pee-review process, the following parts are added to the study protocol.	
Complications of Metabolic Surgery	Early and late major adverse events after metabolic surgery will be collected and reported.
E-value for Sensitivity Analysis	Additional sensitivity analysis to assess the potential effects of unmeasured confounders will be added by the E-value methodology. The E-value is the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the treatment and outcome, conditional on the measured covariates, to fully explain away a specific treatment–outcome association. For MALO and MACE, the E-value will be calculated and interpreted for the HR estimates and for their limit of 95% CI closest to the null.
References	• VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. Ann Intern Med. 2017;167:268-274. • Haneuse S, VanderWeele TJ, Arterburn D. Using the E-Value to Assess the Potential Effect of Unmeasured Confounding in Observational Studies. JAMA. 2019;321:602-603.