Protocol Title: Usefulness of Controlled Attenuation Parameter (CAP) for the assessment of liver steatosis in liver donors

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Abstract

The shortage of liver allografts for transplantation causes thousands of patients dying in the liver transplant (LT) waitlist every year. The use of marginal livers is a strategy to close the gap between demand and supply of organs. Steatosis is one of the main parameters determining a marginal liver, and to date there is no method to quantify it in a noninvasive fashion. Controlled-attenuation parameter (CAP) from transient elastography (TE) has successfully been used to evaluate steatosis in patients with liver disease, but its usefulness to address steatosis before LT in organ donors has never been tested. Our primary aim is to determine the accuracy of CAP in the quantification of liver steatosis using liver biopsies as reference. Secondarily we will correlate TE and CAP results, analyze possible associations between CAP/TE and post-LT clinical outcomes, and evaluate the change in CAP after LT. A portable TE device (Fibroscan 530) will be used to quantify the degree of liver steatosis (CAP) and stiffness (TE) in donors before organ procurement. Liver biopsies will be obtained after CAP or at the time of organ procurement. The following post-transplant outcomes will be investigated: primary non-function, early allograft dysfunction, ICU length of stay, retransplantation, and death. A follow up CAP/TE with Fibroscan 502 or 530 will be performed in transplant recipients having an outpatient visit 3-6 months after LT. Based on power calculations the study aims to include as many donors as needed to achieve 100 transplanted liver allografts.

Background

Worldwide, there is a shortage of liver allografts to meet the number of patients with end-stage liver disease in need for a liver transplant (LT). As a result of this mismatch between organ demand and supply, about 5000 listed patients do not undergo LT each year and are added to the transplant waiting list pool in the United States.(1) It is estimated that 10-20% of patients in the waiting list will either die or become "too sick to transplant", depending mostly on the degree of liver failure. With increasing waiting list times and LT occurring at higher MELD scores, it is expected that the number of patients dying or becoming too sick to transplant will progressively increase in the forthcoming years.

In order to meet the LT demands and to best serve patients, most LT centers are increasingly using marginal donor allografts at risk for poorer outcomes after LT. These so called "extended donor criteria" (EDC) allografts include the use of livers with a high percentage of steatosis, livers derived from older or sicker donors, livers from donors infected with viral hepatitis B or C, and donation after circulatory death (in counterpart to donation after brain death). Although various donor-risk scales have been designed to weigh the consequences of using EDC allografts, none of them provide an accurate risk assessment. Moreover, the trade-off defining the use of EDC allografts is commonly made under far from ideal circumstances (overnight, at facilities with limited resources, with non-specialized on-call personnel), and flawed by subjective evaluations such as determination of the degree of hepatic steatosis.

The percentage of liver steatosis in the donor allograft liver biopsy is of greatest importance to define prognosis of LT, and plays a paramount role in the decision to use or not an allograft. To address this, the amount of large fat droplets (macrosteatosis) within the hepatocyte is semi-quantitatively estimated against the degree of non-fatty liver parenchyma on a given liver biopsy specimen. Although there is controversy as to whether very small lipid droplets (macrosteatosis) should be accounted for in this evaluation, most centers would only consider macrosteatosis in their decision. In general, allografts with >30% of steatosis are more prone to experience primary non-function, early allograft dysfunction, and constitute a risk factor for acute kidney injury, biliary complications, poor allograft survival, and retransplantation.

Controlled-attenuation parameter (CAP) constitutes a non-invasive method of evaluating liver steatosis. It has several advantages over liver biopsy and other methods to assess steatosis in that it is non-invasive, easy to perform, reliable, not expensive, and provides immediate and objective evaluation on a continuous scale. CAP is based on a novel technology measuring the degree of ultrasound attenuation of liver fat from ultrasonic signals acquired during transient elastography (TE) with a

Fibroscan (Echosens, Paris, France) device. A cross-sectional study aiming to define the accuracy of the CAP when compared to liver biopsy found an area under the receiver-operating characteristic (AUROC) curve of 0.81 (0.74-0.88) to identify a degree of ≥10% of steatosis in the 153 patients studied. Although CAP was not able to accurately tell apart moderate (>33%) from severe (>66%) steatosis, the technique seemed to be more reliable at higher degrees of steatosis, and in patients with no to minimal fibrosis (50% of patients had stage ≥2 of fibrosis).(2) In a more recent study including 440 paired CAP-liver biopsies the AUROC to identify >33% steatosis with CAP was 0.84 (0.80-0.88),(3) and in a meta-analysis including 1771 subjects it was 0.88 (0.85-0.91).(4) The last study reported positive and negative predictive values of 58% and 94% for the detection of >33% of steatosis (median cut-off point 255 dB/m), and 57% and 93% for the detection of >66% of steatosis (median cut-off point 290 dB/m), respectively.

To date, no study has attempted to study CAP to quantify the degree of liver steatosis in deceased donors. Such study has been largely limited by the fact that only a few institutions have the expertise and resources to operate a Fibroscan. With the development of the Fibroscan 530, a portable TE device, this is no longer a limitation. Given the grave need to increase the number of LT by using EDC allografts, CAP has the potential of becoming a very useful tool during donor assessment (before allocation) by providing a non-invasive and objective evaluation of liver allograft steatosis. If useful, CAP could become quite advantageous to the organ procurement agencies by providing a timely evaluation on steatosis and fibrosis (this from TE), saving resources by avoiding unnecessary liver biopsies and procurement of EDC allografts posing excessive risks to LT recipients (i.e. with excessive steatosis for each given donor-recipient match).

Study Aims

The primary aim of this study is to determine the accuracy of CAP from Fibroscan in the quantification of liver steatosis in donors using liver biopsies as the gold standard. Secondary aims include:

(1) correlation between TE results and fibrosis

(2) association of CAP and TE results with post-LT clinical outcomes,

(3) measurement of change in CAP after LT.

Study Design

This is a cohort study with a cross-sectional component for the primary aim executed independently at UAMS and Stanford University. The Liver Transplant Program at

each participating center will identify study subjects during the process of liver allocation to their listed recipients. Criteria for recruitment are as follows:

Inclusion criteria – Liver Recipient

- Men and women, Age 18-years old to 80-years old inclusive

Inclusion criteria – Liver Donor

- Valid TE with Fibroscan 530, defined as:
  - At least 10 valid measurements
  - IQR/Median stiffness value <30% (only in cases with >7.1 kPa)

Exclusion criteria – Liver Recipient

- Patient did not undergo liver transplantation

Exclusion criteria – Liver Donor

- Donation after circulatory death (DCD)
- No liver biopsy obtained during organ procurement process

Investigational Approach

The chronological process of organ procurement and study is explained in Table below:

Clinical or Research Event	Standard of Care	Research Only
1. When a liver donor becomes available, and only after brain death is confirmed, the Arkansas Regional Organ Recovery Agency (ARORA) contacts the UAMS Transplant coordinator and notifies the transplant surgeon.	✔	
2. The recipient next in the liver transplant waitlist is notified and is admitted to UAMS.	✔	
3. A liver biopsy (snap frozen) is performed by the Organ Recovery Agency (when applicable) before procurement.	✔	
4. The surgeon travels to perform the liver procurement.	✔	

5. Fibroscan 530 is obtained before the procurement in the donor.		✔
6. A second liver biopsy is obtained by the time of procurement (on formaldehyde). This is reviewed by UAMS pathologists.	✔	
7. Transplantation occurs.	✔	
8. Patient is discharged from hospital and continues with outpatient visits (from biweekly to every three months as per UAMS' liver transplant guidelines).	✔	
9. At one of the subjects' regularly scheduled follow-up appointments (3 to 6 months post-transplant), a second TE is performed, this time on the recipient (using either Fibroscan 502 or 530).		✔
10. Subject continues with per protocol follow up until retransplantation, death, or 5 years of follow-up.	✔	

Before organ procurement, a Fibroscan-trained investigator will bring Fibroscan 530 to the bedside of donor. TE will be performed with either the M- or XL-probe depending on the skin to liver capsule distance (M-probe if <2.5 cm, and XL-probe when between 2.5 and 3.5 cm), as per manufacturer's recommendations (Echosens, Paris, France). In brief, after applying a water-based gel, the corresponding probe will be placed against the right hypochondrium at the level of the mid axillary line (exerting a slight pressure), and then 10 valid TE measurements will be obtained. The procedure should take no more than 5-7 minutes. Data from TE will be stored for future extraction of CAP. In order to assure an adequate fasting period before TE (≥3 hours) the surgeon leading organ procurement would request stopping of enteral nutrition after being initially contacted by the organ procurement organization (in the case of UAMS, the Arkansas Regional Organ Recover Agency [ARORA]), when applicable, and only after confirmation of brain death.

As part of the standard evaluation of the donor allograft a liver biopsy is obtained to address the degree of steatosis (one standard frozen core). Investigators will make sure this biopsy is performed by the time of TE. Only bedside biopsies on the mid-axillary line or biopsies from segments VI/VII after allograft procurement will be allowed. Although liver biopsies should ideally be performed immediately after

performing TE and before organ procurement, liver biopsies obtained from the time of donor identification and up until back table preparation (inclusive) will be acceptable. In all cases where the organ is allocated to a recipient, the transplant surgeon will be asked to grade the appearance of the liver before implantation.

During liver transplant procedure and immediately after finalization of venous, arterial and biliary anastomoses, a second biopsy will be obtained (formaldehyde). This biopsy is taken routinely as part or the liver transplant protocol at UAMS to address quality of the implanted allograft including damage from reperfusion. The allograft site from which biopsy will be obtained will be left to the discretion of the transplant surgeon, although it should be documented in the operative note.

Before transplantation, the following data will be collected about the donor (data will be collected from the United Network for Organ Sharing [UNOS] portal, which compiles deidentified clinical data relevant to liver transplantation):

- height,
- weight,
- gender,
- age,
- ethnicity,
- cause of death,
- comorbidities,
- platelets,
- bilirubin,
- ALP,
- ALT and AST on the day of TE,
- peak ALT and AST,
- presence of any infectious disease
- degree of steatosis from snap frozen biopsy
- NPO status will be documented as well (in hours), from last oral intake or stopping of enteral nutrition to TE procedure,
- Cold and warm ischemia will be documented as part of the standard of care

On the day of transplantation, the following will be recorded from the transplant recipients:

- Routine labs on the day of transplantation (CBC, chemistry, liver panel, INR).

After transplantation, the following liver transplant outcomes will be prospectively documented:

- development of primary non-function (PNF),

- early allograft dysfunction (EAD),
- peak ALT,
- ICU length of stay,
- retransplantation, and
- death.

PNF will be defined as AST>3000, plus INR ≥2.5 or arterial pH ≤7.30 within 7 days from implantation; whereas EAD as total bilirubin >10 mg/dL on day 7, INR ≥1.6 on day 7, and ALT or AST >2000 U/L during first week post-LT. Need for retransplantation or death at 3- to 6- months post-transplant (depending on follow up protocol at each participating center) will be recorded as well. Where available, a follow up TE with Fibroscan 502 or 530 will be performed at a standard-of-care follow outpatient visit at 3-6 months post-transplant. The patient will lie over his/her back and both the M and XL probes will be used following the same steps described above.

All liver biopsies will be evaluated by two independent liver pathologists blinded to the results of CAP. In all cases, both the snap frozen and formaldehyde liver biopsy specimens will be reviewed and the degree of macrovesicular steatosis graded as per standard recommendations (<5, 5-33%, 34-66%, >66%). Liver pathologists will independently stage fibrosis of liver specimens using the Batts-Ludwig scoring system and the Brunt classification.

Data Analysis

The primary aim is to correlate the CAP results with the degree of hepatic steatosis on the liver biopsy fixed in formaldehyde. Spearman's rho will be used for this analysis. Parallel analyses will be performed with the snap frozen sample, and computerized histomorphometry to determine accuracy of CAP in different possible clinical scenarios. In order to assess whether a threshold value of CAP with a sufficiently high positive predictive value can be defined to obviate the need for a liver biopsy during organ procurement, Receiver Operating Characteristics (ROC) curves will be plotted, and areas under the curve (AUROC) reported as a measure of accuracy of CAP. For this analysis, the magnitude of steatosis in the formaldehyde samples will be dichotomized as ≥34 or <34% in order to address steatosis as for EDC allografts. A sensitivity analysis using computed histomorphometry results and dichotomizing grading of steatosis as ≥20 or <20% will be performed in case the prior analysis lacks power, as this degree of steatosis has been found to be associated with poor post-transplant outcomes as well.

CAP will be performed with either the M- or XL-probe depending on the skin to liver capsule distance (M-probe if <2.5 cm, and XL-probe when between 2.5 and 3.5 cm) as per manufacturer's recommendations (Echosens, Paris, France).

The first secondary aim is to examine correlation between TE results and fibrosis stage (Spearman's rho). In addition to correlating the TE results with fibrosis stage, we will explore whether the combination of CAP and TE results can define unacceptability and subsequently help in the decision to discard and organ. To achieve this goal a sensitivity analysis with various combinations of CAP and TE will be tested in logistic regression models, and then cut-off valued determined by constructing AUROC. Agreement between pathologist for both steatosis and fibrosis will be examined with the kappa statistic. A sensitivity analysis for donors on vasoactive amines or a peak ALT or AST >300 before procurement is planned to better determine accuracy of CAP/TE in liver donors.

The second secondary aim is to correlate CAP and TE results with various post-LT outcomes. Various (logistic, linear or proportional hazards) regression analyses will be used depending on the outcome variable of interest (PNF, EAD, retransplantation or death). Predictor variables will include CAP and TE as well as well-known donor and recipient predictors of LT outcomes (i.e. age, MELD, ICU-stay, etc).

The third secondary aim is to determine the change in CAP after LT. Baseline and follow up CAP determinations will be compared with paired t-test or Wilcoxon, depending on distribution of data. Normal distribution will be tested with Shapiro-Wilk. Categorical changes (i.e. ≥34% to <34%) will be addressed with McNemar's test.

Accurate sample size determination is difficult in this pilot study because the test characteristics are not defined. We propose to examine as many donors as needed to achieve 100 transplanted liver allografts, which will allow detection of meaningful differences between livers with high versus low CAP values. If the prevalence of unacceptably high CAP value is as low as 5% of the livers, the sample size will permit detection of a threshold that has 80% positive predictive value or higher. Between the two participating institutions, it is expected to complete inclusion of 100 transplanted liver allografts in one year. It is expected to include no more than 60 patients at UAMS.

Data Collection and Handling

A database will be created to accumulate all of the clinical information generated during this study. No identifiers, apart from UNOS identification number for donors, and name and DOB for recipients, will be collected. All data will be stored in databases kept in a study folder created at PI's computer (password-protected). Main

database will include aforementioned identifiers, however, each pair of subjects (recipient and respective donor) will have a unique study ID assigned, and once an analytic table is created, all PHI will be removed from the table (after approval by the PI). Only this de-identified dataset with unique study ID's will be used for statistical analyses. Therefore, the risk to the privacy of the individuals will be minimized.

The access to the research folder and its contents will be restricted to the research staffs listed in this submission form. The PHI will be kept in the main database for no more than 10 years. The main database containing PHI will be kept in the study folder and will be never transferred out without de-identification of the data. No hard copy research data will be generated in this project.

Each of the participating centers (UAMS and Stanford University) will work independently in gathering their own data. The PI's from each center will share on a monthly basis their subject accrual figures and after study is completed each center will share its de-identified database and a unique database will be built.

Risks and Benefits

TE is a noninvasive procedure and no adverse effects were reported in the registration trials. FDA approval for Fibroscan 502 was obtained in April/2013, and for Fibroscan 530 (the lighter and portable version of Fibroscan 502) in March/2016. CAP received FDA approval in June/2015.

Liver biopsies in an allograft can theoretically result in bleeding, hemobilia, arteriovenous fistulas, or infection in the recipient. However, apart from bleeding, which can be controlled by the transplant surgeon during the surgical procedure, no other complications have been reported. Moreover, liver biopsies are performed routinely during the organ procurement process or by the time of organ implantation, and thus this study would not expose recipients to unusual or unnecessary risks.

Each institution will keep its own database until finalization of the study. Afterwards, a combined database will be built by each center sharing a de-identified version of its database (datasets will be combined at UAMS). Thus, PHI from UAMS' patients will remain within UAMS firewall and only used for specific research purposes in this project. Aggregated data will be analyzed and published, but specific data elements will not be made available. Further, subjects will not forfeit any rights by participating - their healthcare and eligibility for health benefits will not be affected in any way. Although a breach in confidentiality is always a risk when collecting personal information from patients, we believe this is small given the system that has been developed to obtain and handle data.

Participants (liver transplant recipients) will not benefit from this study as clinically meaningful results will only be available until after completion of study and analysis of data.

Ethics

Applicable government regulations, University of Arkansas for Medical Sciences research policies and procedures will be followed. This protocol and any amendments will be submitted and approved by the University of Arkansas for Medical Sciences Institutional Review Board (IRB) to conduct the study.

All subjects (recipients) for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The formal consent of a subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent. The subject will be approached for signing of informed consent at any point from being placed on the waitlist to the admission ending up in liver transplantation (either during a routine outpatient visit or as an inpatient). It will be emphasized to recipient that receipt of a transplant in no way depends upon research participation.

Liver Donors and/or their family will execute a consent process with the Arkansas Regional Organ Recovery Agency (ARORA) (Consent form provided with documentation).

References

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