University of Virginia

Is adrenal insufficiency under-diagnosed in hospitalized cirrhosis patients?

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PROTOCOL

Background

1. Provide the scientific background, rationale and relevance of this project.

Answer/Response:

Cirrhosis is known to cause various end-organ dysfunction, most notably the hepatorenal and hepatopulmonary syndromes. However, the increased recognition of adrenal insufficiency in cirrhotic patients has led to postulation about the presence of a so-called "hepatoadrenal syndrome[1]." While originally this syndrome was recognized in cirrhotic patients with sepsis and shock states[1,2], further investigation has also identified adrenal insufficiency in cirrhotic patients with variceal bleeding[3] as well as stable patients[4]. Although there is controversy about the best diagnostic test to assess adrenal insufficiency in cirrhotic patients[5,6], it is well established that cirrhotic patients with adrenal insufficiency have increased mortality, particularly as they become more decompensated[7].

In addition to this endocrinological complication, cirrhosis also has a profound effect on a patient's hematologic parameters. In a study of rheologic properties of 38 patients (18 cirrhotic – mostly of cryptogenic origin, 20 control), cirrhotic patients were noted to have increased erythrocyte rigidity and decreased plasma and blood viscosity[8]. The authors argued that their findings were related to a deficiency of high-density lipoprotein (HDL), although the correlation was admittedly weak in their study. A follow-up study by the same authors also confirmed an increase in erythrocyte rigidity in patients with alcoholic and post-hepatic cirrhosis; importantly, they noted the prior association between alcoholic liver disease and development of macrocytosis as a function of lecithin-cholesterol acetyltransferase (LCAT) enzyme deficiency[9]. This enzyme is responsible for esterification of cholesterol and subsequent incorporation into normal red cell membranes. Interestingly, its deficiency is directly manifested as an altered free cholesterol to phospholipid ratio[10], with the increased cholesterol fraction causing the membrane rigidity previously described.

Another abnormality well documented in cirrhotic patients, more frequently in patients with advanced disease, is spur cell anemia. Although first described in alcoholic cirrhosis, it is seen in all etiologies and is a poor prognostic marker. A spur cell burden of ≥5% was found to be associated with a 67% 90-day mortality rate in a recent study[11]. Furthermore, patients with spur cell anemia have decreased red blood cell (RBC) lifespan and are transfusion resistant as transfused RBCs quickly acquire the pathologic morphology. Spur cells are thought to occur secondary to red cell membrane abnormalities secondary to altered cholesterol composition, similar to the abnormal erythrocyte rigidity rheologic property described above. Interestingly, the lecithin-cholesterol acetyltransferase enzyme has been investigated as a potential key factor in spur cell anemia. A 1972 study by Cooper et al[12] measured LCAT enzyme activity by way of a surrogate method involving change in serum free cholesterol after 6-hour incubation. Lower levels of LCAT were found in patients with spur cells compared to normal controls; however, there was only a weak association with red cell lipid content.

Furthermore, platelet dysfunction in cirrhosis has also been tied to lipid abnormalities. A review of dyslipidemia in liver disease by Miller noted that platelets, similar to erythrocytes, have an imbalanced cholesterol to phospholipid ratio and associated impaired aggregation[13]. Thus, the deficient primary hemostasis noted in cirrhotic patients may not be just a function of

thrombocytopenia itself but also an intrinsic abnormality of platelet function related to a disruption in normal cholesterol pathways.

It has been demonstrated that LCAT is a key enzyme for many homeostatic functions within the human body and its deficient production in cirrhosis appears to correlate with various hematologic abnormalities. However, it may very well also play an important role in adrenal steroidogenesis and help explain the so called "hepatoadrenal" syndrome that has begun to permeate through the literature. More importantly, however, is that cholesterol esterification levels, a surrogate marker of LCAT enzymatic activity, are directly associated with mortality and thus can serve as a prognostic indicator. A recent study by Kaiser et al.[14] found that the cholesterol esterification fraction was more accurate than INR for estimation of liver synthetic capability and both 3-month and 1-year mortality. Thus, if one can quantify this deficiency in cirrhotic patients and demonstrate association with several related pathologic conditions, it may soon become a useful biomarker to direct treatment and more reliably predict mortality.

Objectives/Hypothesis

Answer/Response:

The hepatoadrenal syndrome has been well described in the literature and is known to be associated with poorer outcomes in both stable and critically ill cirrhotic patients. In chronic liver disease, adrenal (and more specifically cortisol) insufficiency is thought to be a byproduct of altered lipid metabolism that results in decreased HDL production and thus decreased delivery of cholesterol to the adrenal for subsequent corticosteroid production. Studies to date have implicated lecithin-cholesterol acetyltransferase (LCAT) as the key enzyme which is deficient in some cirrhotic patients, leading to an impaired ability to esterify cholesterol and thus a loss of normal cellular functioning and membrane stability. We seek to quantify this LCAT deficiency in a cohort of cirrhotic patients and demonstrate its association with various abnormal physiologies associated with chronic liver disease, including spur cell anemia, low HDL levels, and adrenal insufficiency.

Study Design: Biomedical

1. Will controls be used?

Answer/Response: No a priori controls (i.e. we will not be including patients without cirrhosis in our study). We will compare cirrhotic patients with and without adrenal insufficiency.

►IF YES, explain the kind of controls to be used.

Answer/Response: N/A

2. What is the study design?

Answer/Response: prospective cohort study

3. Does the study involve a placebo?

Answer/Response: No

►IF YES, provide a justification for the use of a placebo

Answer/Response: N/A

Human Participants

Ages: _18-100___
Sex: **_Men and women___**
Race: **_All___**

Subjects- see below

1. Provide target # of subjects (at all sites) needed to complete protocol.
Answer/Response: 100

2. Describe expected rate of screen failure/ dropouts/withdrawals from all sites.
Answer/Response: 20

3. How many subjects will be enrolled at all sites?
Answer/Response: 120

4. How many subjects will sign a consent form under this UVa protocol?
Answer/Response: 120

Inclusion/Exclusion Criteria

1. List the criteria for inclusion
Answer/Response:

- age >= 18 years,
- diagnosis of cirrhosis,
- admission to hospital

2. List the criteria for exclusion
Answer/Response:

- age <18 years,
- prior enrollment in study (i.e. readmission),
- prisoner,
- prednisone or hydrocortisone use in the past 24 hours,
- pregnancy

3. List any restrictions on use of other drugs or treatments.
Answer/Response: None

Statistical Considerations

1. Is stratification/randomization involved?
Answer/Response: No

►**IF YES, describe the stratification/ randomization scheme.**
Answer/Response: N/A

►**IF YES, who will generate the randomization scheme?**
_____ Sponsor

_____ UVa Statistician. Insert name Answer/Response:
_____ UVa Investigational Drug Service (IDS)
_____ Other: Specify Answer/Response:

2. What are the statistical considerations for the protocol?

Answer/Response:

Subjects will be classified as adrenally sufficient or insufficient on the basis of standard-dose cortisol stimulation test. Variables of interest for comparison between the groups include MELD score, Child-Turcotte-Pugh (CTP) classification, high-density lipoprotein (HDL) levels, presence of spur cell anemia, serum cholesterol ester percentage (surrogate for LCAT enzymatic activity), cortisol binding globulin levels, and free cortisol levels. Student's t-test and Chi Square tests will be utilized to determine significance; a p-value <0.05 will be used as our threshold for significance. If multiple factors are found to be significantly different in a univariate fashion between classification groups, a multivariate logistic regression analysis will be performed for adjusted analysis. We will also seek to define any correlations between variables. Furthermore, we will assess correlation between MELD score and serum cholesterol ester percentage, spur cell anemia, HDL levels, cortisol binding globulin levels, and free cortisol levels; similar correlate analysis will be done using CTP classification instead of MELD score.

Prevalence of adrenal insufficiency (AI) in cirrhosis varies widely based on several factors, including severity of liver disease (i.e. MELD score or CTP classification) and the testing modality used to assess for AI. Given that admitted patients to UVA are typically CTP B or C, we expect the incidence to be about 33%. Thus, 120 patients recruited should be sufficient to provide a statistical difference in our outcome measures of interest, assuming a 20% dropout rate to lead to 100 patients completing study protocol.

3. Provide a justification for the sample size used in this protocol.

Answer/Response:

Given that admitted patients to UVA are typically CTP B or C, we expect the incidence of adrenal insufficiency to be about 33%. We feel 100 patients would be adequate to complete a pilot study to test for correlation between adrenal insufficiency, low serum cholesterol ester percentage, and spur cell anemia. There is no current literature upon which to do a formal power calculation (although low levels of cholesterol esterification is a well-established near-universal metabolic finding in cirrhotic patients), but this sample size would allow for adequate size comparator groups and decrease the likelihood of a Type II statistical error.

4. What is your plan for primary variable analysis?

Answer/Response:

Our primary outcome of interest is comparing prevalence of cholesterol esterification deficiency and spur cell anemia in cirrhotic patients with and without adrenal insufficiency.

5. What is your plan for secondary variable analysis?

Answer/Response:

- Demonstrate association between deficiency in cholesterol esterification and presence of spur cell anemia

- Demonstrate association between deficiency in cholesterol esterification and low HDL levels
- Correlate higher MELD score with presence of adrenal insufficiency
- Correlate higher MELD score with presence of spur cell anemia
- Correlate higher MELD score with lower HDL levels
- Correlate higher MELD score with deficiency in cholesterol esterification
- Correlate higher CTP classification with presence of adrenal insufficiency
- Correlate higher CTP classification with presence of spur cell anemia
- Correlate higher CTP classification with lower HDL levels
- Correlate higher CTP classification with deficiency in cholesterol esterification
- Correlate lower cortisol binding globulin levels with higher MELD score and CTP classification
- Correlate lower cortisol binding globulin levels with adrenal insufficiency
- Correlate lower free cortisol levels with higher MELD score and CTP classification
- Correlate lower free cortisol levels with adrenal insufficiency
- Multivariate logistic regression if several variables are found to be significant

6. Have you been working with a statistician in designing this protocol?
Answer/Response: No

IF YES, what is their name?
Answer/Response: N/A

7. Will data from multiple sites be combined during analysis?
Answer/Response: No

7(a). Does the study involve randomization?
Answer/Response: No

IF YES, will randomization be done at each site or among sites?
Answer/Response: N/A

7(b). Has the sample size calculation considered the variation among sites?
Answer/Response: N/A

7(c). When combining the data from multiple sites to assess the study results, is the effect of the treatment to be tested (or the association to be tested) assumed to be the same across sites or vary among sites? What is the modelling strategy?
Answer/Response: N/A

7(d). Is there a common protocol used in all sites?
Answer/Response: N/A

IF NO, how will differences among sites, such as those related to the implementation, inclusion criteria, patient characteristics, or other sites characteristics, be considered to assess the study results?
Answer/Response: N/A

Study Procedures-Biomedical Research

1. What will be done in this protocol?

Answer/Response:

Hospitalized cirrhotic patients at UVA that meet study eligibility criteria will be approached by a member of the study team to obtain consent for participation. If a patient agrees to become a study subject, women of childbearing potential will be administered a urine pregnancy test to exclude pregnancy. Eligible subjects will have an approximate total of 35ml of blood drawn by the inpatient phlebotomy team. Lab tests to be performed include: peripheral blood smear, lipid panel, free cortisol, cortisol binding globulin, serum cholesterol esters (surrogate for LCAT enzyme activity), and a standard-dose cortisol stimulation test. The latter involves blood drawn with the initial collection, administration of an intravenous 250mcg dose of synthetic ACTH by the patient's nurse, and then repeat small-volume (2 teaspoons) blood draws at 30 minutes and 60 minutes later.

The interventions listed above have the potential to identify subjects with adrenal insufficiency, which may be beneficial to their long-term care. Early identification of adrenal insufficiency may lead to earlier corticosteroid administration in critical illness or GI bleeding, thus improving morbidity and mortality. Otherwise, the blood draws themselves pose minimal risk given that it is a routine procedure done during hospitalization, often on a daily or even more frequent basis. Very small risks of bleeding, bruising, or infection do exist.

Most patients tolerate intravenous ACTH as part of the cortisol stimulation test, however known reactions include nausea, diaphoresis, dizziness, pruritus, palpitations, and facial flushing. All patients will be monitored and these side effects, if present, typically resolve within a few hours. Any patients identified as having adrenal insufficiency will be reported to the primary hepatology team caring for the patient to determine whether or not endocrinology consult and/or treatment should be considered.

2. If this protocol involves study treatment, explain how a subject will be transitioned from study treatment when they have completed their participation in the study.

Answer/Response: N/A

Subject Compliance with Study Procedures

1. **Explain how the study team will monitor the subject for compliance with the study procedures.**

Answer/Response: study team will communicate with subjects and subjects' nurses about study protocol and will follow-up to be sure labs were drawn and cortisol stimulation test was administered. This can be tracked through the electronic medical record as well.

2. **Describe criteria for when a subject is considered to be non-compliant with study procedures.**

Answer/Response: subject refuses study-related blood draw(s) or ACTH administration

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