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Research Article

Identifying Novel Biomarkers of Frailty in Cirrhosis: Results from an Unbiased Proteomics Search from the Functional Assessment in Liver Transplantation (FrAILT) Study

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Abstract

***Background*:** Patients with cirrhosis suffer not only from commonly-diagnosed portal hypertensive complications such as ascites and hepatic encephalopathy but also from more insidious effects of chronic liver failure including muscle wasting, under-nutrition, and functional decline. These manifestations of physical frailty have been demonstrated to predict mortality in patients with cirrhosis independently of liver disease severity, but objective biomarkers associated with physical frailty in cirrhosis are needed. The aim of this work is to identify biomarkers associated with physical frailty among patients with cirrhosis.

***Methods*:** Mass spectrometry was used to identify serum proteins that were differentially expressed between frail and non-frail patients with cirrhosis (15 frail and 15 non-frail, matched for age, gender, and MELDNa). Then, eleven proteins were selected for further analysis based on those with the lowest p-value and largest differential. ELISA assays were

used to validate and quantify differences in protein concentration (37 frail and 37 non-frail patients with cirrhosis, matched for age, gender, and MELDNa).

***Results*:** Mass spectrometry identified twenty-five proteins that were differentially expressed in frail versus non-frail patients with cirrhosis ($p<0.05$). Subsequently, ELISA assays were performed for eleven of the most promising candidate proteins and three proteins were identified that were differentially expressed in frail vs. non-frail patients with cirrhosis: vitronectin, leucine-rich alpha-2 glycoprotein, and alpha-1 acid glycoprotein ($p<0.1$).

***Conclusions*:** This study identifies three serum proteins (vitronectin, leucine-rich alpha-2 glycoprotein, and alpha-1 acid glycoprotein) as possible novel biomarkers of physical frailty in patients with cirrhosis. Prior literature supports that these proteins may have biologically plausible associations with frailty in other patient populations, providing a rational basis to conduct larger cohort studies to validate and expand these findings.

Keywords

Frailty; cirrhosis; biomarker; vitronectin; leucine-rich alpha-2 glycoprotein; alpha-1 acid glycoprotein

1. Introduction

Patients with cirrhosis suffer not only from commonly-diagnosed portal hypertensive complications such as ascites and hepatic encephalopathy but also from more insidious effects of chronic liver failure including muscle wasting, under-nutrition, and functional decline. The concept of frailty – a classically geriatric construct defined as a state of decreased physiologic reserve that leads to adverse health outcomes [1, 2] has been applied to patients with cirrhosis to describe these insidious "extra-hepatic" effects of cirrhosis. Importantly, frailty predicts mortality in patients with cirrhosis independently of liver disease severity [3, 4]. Recently, we developed the Liver Frailty Index (consisting of grip strength, chair stands, and balance testing) to operationalize this construct specifically for patients with cirrhosis. Specifically, we demonstrated that the addition of the Liver Frailty Index improves risk prediction of waitlist mortality above and beyond the Model for End-Stage Liver Disease (MELDNa) score alone [5].

While the Liver Frailty Index is objective and low-cost, it must be administered in person by trained individuals. This limits its utility for use in the transplant setting where assessments of mortality risk prediction must be drawn as frequently as every seven days. Patients often live hours away from their transplant center and therefore cannot come to the clinic for assessments at frequent intervals. Therefore, it would be useful to identify one or more blood-based biomarkers that correlate with physical frailty in patients with cirrhosis in order to enhance the applications for frailty in clinical practice. Such biomarkers may even be able to serve as additional, unbiased biomarkers to incorporate into the transplantation selection process.

In this study, we aimed to identify potential serum biomarkers of physical frailty in patients with cirrhosis using an unbiased mass spectrometry-based approach. Then, we quantified and validated the most promising biomarkers using enzyme-linked immunosorbent assay (ELISA) assays.

2. Materials and Methods

2.1 Patient Selection

Participants in the ongoing Functional Assessment in Liver Transplantation (FrAILT) Study, a prospective study assessing physical frailty in liver transplant candidates at the University of California, San Francisco (UCSF), were further consented to participate in this arm of the study. The FrAILT Study enrolls patients who are ≥18 years of age, actively listed for liver transplantation, and seen in the outpatient UCSF Transplant hepatology clinic [5]. Patients listed for transplantation for causes other than cirrhosis were excluded. Patients provided informed written consent for both functional frailty testing and biospecimen collection, and both modes of testing were completed on the same day. At the time of this study, there were 224 patients enrolled in the FrAILT cohort who had completed both the clinical frailty assessment and biomarker specimen collection. These 224 patients were sorted by their Liver Frailty Index, and frail patients were selected from the top thirty percent, and non-frail patients were selected from the bottom thirty percent. Patients in the frail and non-frail cohorts were then matched in pairs by age, sex, and MELDNa, to minimise confounding of biomarker results based on these factors. For the mass spectrometry analysis, 15 frail and 15 non-frail patients with cirrhosis were selected. For the ELISA analysis, 37 frail and 37 non-frail patients with cirrhosis were selected. Most patients were not previously studied in the mass spectrometry analysis in order to validate findings in additional patient samples (14 tested in both assays, 60 additional patients only tested in the confirmatory ELISA testing).

2.2 Frailty Assessment

At enrollment into the FrAILT Study, all patients underwent a single objective measurement of frailty using:

1) Grip strength: the average of three trials, measured in the subject's dominant hand using a hand dynamometer;
2) Timed chair stands: measured as the number of seconds it takes to do five chair stands with the subject's arms folded across the chest;
3) Balance testing: measured as the number of seconds that the subject can balance in three positions (feet placed side-to-side, semi-tandem, and tandem) for a maximum of 10 seconds each.

These three tests were administered by trained study personnel. With these three individual tests of frailty, the Liver Frailty Index was calculated using the following equation (calculator available at: http://liverfrailtyindex.ucsf.edu) [5]: (-0.330 * gender-adjusted grip strength) + (-2.529 * number of chair stands per second) + (-0.040 * balance time) + 6.

The FrAILT study was approved by the Institutional Review Board at UCSF. All patients enrolled in the FrAILT study provided informed consent to be involved. Additional consent was obtained for the drawing of serum for the purpose of this study.

2.3 Serum 2-D Dimensional Difference in Gel Electrophoresis (2D-DIGE)

Serum was collected from the subject participants, stored at -80C, and sent to Applied Biomics in Hayward, California for Serum 2-D Dimensional Difference in Gel Electrophoresis (2D-DIGE) and mass spectrometry analysis. Samples from 30 subjects were paired to allow comparison of 15 pairs of frail/non-frail patients with cirrhosis. Each pair was matched with an internal standard to ensure complete protein identification and reduce gel-to-gel variation. Internal standard samples were labelled with CyDye 2, frail samples with CyDye 3, and non-frail with CyDye 5, to enable simultaneous co-separation and analysis of samples on a single multiplexed gel. 2D gel electrophoresis was used to separate the 3 labelled samples on a single 2D gel using isoelectric focusing and SDS polyacrylamide gel electrophoresis (SDS-PAGE) to create an overlay image with 3 different colors representing the 3 different samples. The gel was then scanned using a Typhoon image scanner to create an image specifically targeting each dye, thus creating a separate image for Cy2, Cy3 and Cy5 respectively. ImageQuant and DeCyder computer software was used to quantify the difference in protein ratios between samples in order to identify protein differences between the frail and non-frail groups. The Ettan^{TM} spot picker (GE Healthcare Life Systems, >99.9% accuracy) was then used to identify spots that represent proteins for which there existed significant differences. This procedure was performed on all 15 pairs, and then a cluster analysis was performed to identify the spots that were statistically significantly different between the frail and the non-frail patient samples. A p-value of <0.05 was used to identify proteins of interest.

2.4 Mass Spectrometry

Selected spots were then identified using mass spectrometry matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) [6]. Washed spots were dried and then rehydrated in modified trypsin at 37C. Extracted peptides were spotted into wells of a MALDI plate, and mass spectrometry was performed using Applied Biomics Proteomics Analyzer. The 10-20 most abundant peptides were then further fragmented and tandem mass spectrometry was performed to identify the proteins. Specific protein identification was performed by matching peptide mass mapping and peptide fragmentation mapping in primary sequence databases.

2.5 ELISA

After reviewing the twenty-five candidate proteins identified by mass spectrometry analysis, we selected a subset of these proteins to quantify and validate with ELISA assays. We considered several factors in this selection process: 1) the p-value in the mass spectrometry analysis, 2) the magnitude of difference in protein concentration between frail and non-frail groups, 3) the presence of a commercially available ELISA assay, and 4) the plausibility that the protein is correlated with the frail phenotype based on a literature search. Based on these factors, eleven proteins were selected. Serum was collected from the subject participants, stored at -80C, and sent to BioBank and Research Laboratory Services, Maine Medical Center Research Institute in Scarborough, Maine. The following commercially-available ELISA assays were utilized for this study: alpha-1 acid glycoprotein (R&D, DAGP00), alpha-1 antitrypsin (IDK, K6752), alpha-2 macroglobulin (R&D, DY1938, DY008), alpha-2 hs glycoprotein (ALPCO, 43-FETHU-E01), apolipoprotein A1 (ALPCO, 41-APOHU-E01), haptoglobin (R&D, DHAPG0), Inhibitor of nuclear factor k-b kinase subunit

(MyBioSource, MBS2883662), leucine-rich alpha-2 glycoprotein (My BioSource, MBS701497), transthyretin (MyBioSource, MBS762549), vitronectin (Thermo Sci, EHVTN), and zinc alpha-2 glycoprotein (BioVendor, RD191093100R). ELISAS were run in duplicate. Differences in protein quantity between frail and non-frail cohorts were compared using unpaired t-tests.

3. Results

3.1 Patient Characteristics

At the time of this study, there were 224 patients enrolled in the FrAILT cohort who had completed both the clinical frailty assessment using the Liver Frailty Index and biomarker specimen collection. These 224 patients were sorted by their Liver Frailty Index, and then patients were selected from the top and bottom thirty percent of frail and non-frail patients, matched for age, sex, and MELDNa. Of the 30 patients selected for mass spectrometry analysis (15 frail, 15 non-frail), the mean Liver Frailty Index was 4.86 (standard deviation 0.81) in frail patients vs. 2.91 (standard deviation 0.42) in non-frail patients. Patient characteristics are displayed in Table 1. For the 74 patients included in the confirmatory ELISA analysis (37 frail, 37 non-frail), Liver Frailty Index results were similarly separated 4.86 (standard deviation 0.59) in frail patients vs. 2.94 (standard deviation 0.56) in non-frail patients (Table 2).

Table 1 Patient demographics for patients tested in the mass spectrometry analysis

	FRAIL (n=15)	**NON-FRAIL (n=15)**
Age (median, yrs)	62	55
Gender		
Female (%)	33%	33%
Male (%)	66%	66%
Etiology of liver disease (*)		
Alcohol (%)	47%	40%
HCV (%)	47%	53%
NAFLD (%)	7%	13%
Other (%)	13%	13%
MELDNa (median)	16	16
HCC present (%)	20%	20%
BMI (median)	29	28
Liver Frailty Index		
Median	4.72	2.75
Mean, SD	4.86 (0.81)	2.91 (0.42)

(*) If multiple etiologies of liver disease, listed in all applicable categories

Table 2 Patient demographics for patients tested in the ELISA protein quantification analysis

	FRAIL (n=37)	NON-FRAIL (n=37)
Age (median, yrs)	61	59
Gender		
Female (%)	43%	38%
Male (%)	57%	62%
Etiology of liver disease (*)		
Alcohol (%)	22%	32%
HCV (%)	35%	51%
NAFLD (%)	35%	14%
Other (%)	19%	19%
MELDNa (median)	14	14
HCC present (%)	32%	46%
BMI (median)	30	31
Liver Frailty Index		
Median	4.86	2.91
Mean, SD	4.86 (0.59)	2.94 (0.56)

(*) If multiple etiologies of liver disease, listed in all applicable categories

3.2 Mass Spectrometry Identifies Candidate Serum Proteins Associated with Frailty in Patients with Cirrhosis

2D-DIGE gels were performed for each of the 15 pairs of serum samples. This proteomic approach identified twenty-five serum proteins that were differentially expressed between frail and non-frail patients ($p<0.05$). Positive average ratios indicate proteins that had a higher concentration in frail than non-frail patients with cirrhosis (fourteen proteins, Table 3); negative average ratios indicate proteins that had a lower concentration in frail than non-frail patients with cirrhosis (eleven proteins, Table 4). Interestingly, many of these proteins had previously been correlated with aging or physical frailty in other populations (see references in Tables 3 and 4).

Table 3 Mass spectrometry identified serum proteins that are expressed at a higher concentration in frail patients with cirrhosis

Protein	Avg ratio frail: non-frail	P-value	Biologic function; Association with frailty in other patient populations
Haptoglobin (*)	+5.04	0.043	• Inflammatory marker; Hepatic recycling of heme • Has anti-microbial and anti-oxidant activity • Associated with frailty in persons ≥65yrs [7, 8]
Leucine-rich alpha-2 glycoprotein (*)	+4.59	0.006	• Involved in signal-transduction, cell adhesion, neovascularization, and development • Associated with inflammation in patients with musculoskeletal diseases [9]
Alpha-1 acid glycoprotein (*)	+3.75	0.004	• Acute phase reactant that is upregulated in response to inflammation or infection [10] • Associated with increased in-hospital mortality for elderly patients [11] and overall mortality in persons ≥65yr [12]
Zinc alpha-2 glycoprotein (*)	+2.56	0.003	• Stimulates lipolysis • Associated with cancer cachexia [13]
Zinc finger MYM-type protein 4	+2.47	0.042	• Regulates cell morphology and cytoskeletal organization
Nicolin-1	+2.45	0.048	• Part of the tubulin polyglutamate complex
Transthyretin (*)	+2.17	0.01	• Carries thyroxine (T4) and retinol-binding protein • Associated with sarcopenia in the elderly [14] • Associated with protein-calorie malnutrition [15, 16]
Keratin, Type 2 skeletal	+1.99	<0.001	• Structural protein important for cytoskeletal organization
Serum albumin fragment	+1.99	0.001	• Regulates colloidal osmotic pressure of blood, binds and carries zinc • Associated with oxidative stress and frailty in the elderly [17]
Protein AMBP	+1.60	0.017	• Important for cell adhesion, heme catabolic processes
Apolipoprotein A1 (*)	+1.64	0.005	• Involved in immunity, inflammation, apoptosis • Associated with CV risk in older men [18]
Trypsin 2	+1.63	<0.001	• Involved with extracellular matrix disassembly and cell growth regulation • Involved in defensin processes in the ileum, thus affecting innate immunity [19]
Ig alpha-1 chain	+1.57	0.003	• Caspase-like protease in chromosome segregation
Vitronectin (*)	+1.42	0.029	• Upregulated during wound healing [20] and plays an important role in apoptosis [21] • Associated with frailty in older women [22]

(*) = Selected for further analysis with ELISA assays

Table 4 Mass spectrometry identified serum proteins that are expressed at a higher concentration in non-frail patients with cirrhosis

Protein	Avg ratio frail: non-frail	P-value	Biologic function; Association with frailty in other patient populations
Inhibitor of nuclear factor kappa-B kinase subunit epsilon (*)	-2.34	<0.001	• Regulates inflammatory response to viral infections • Associated with frailty in animal models [23]
Ig mu chain C region	-1.82	0.061	• Plays a role in primary defense mechanisms
Ras-related protein Rab37	-1.73	0.001	• GTPase involved in vesicle trafficking and neutrophil degranulation
Immunoglobulin lambda-like polypeptide 5	-1.72	0.032	• Involved with B cell receptor signaling, complement activation
Ig kappa chain C region	-1.6	0.015	• Involved with B cell receptor signaling
Alpha-2-HS glycoprotein (*)	-1.46	0.066	• Regulates inflammatory response and phagocytosis • Associated with sarcopenia in older adults [24]
Serotransferrin	-1.44	0.041	• Binds and transports iron
Glutathione peroxidase 3	-1.43	0.014	• Prevents oxidative damage
Keratin, type 2 cytoskeletal	-1.41	0.015	• Structural protein involved in cytoskeletal organization
Alpha-1 antitrypsin (*)	-1.39	0.027	• Protects tissues from enzymes of inflammatory cells, especially neutrophil elastase
Alpha-2 macroglobulin (*)	-1.36	0.021	• Antiprotease, reduces inflammation • Associated with frailty/mortality in aging in rats [25] • Associated with Alzheimer's disease [26] • Associated with inflammation and aging [27]

(*) = Selected for further analysis with ELISA assays

3.3 ELISA Assays Quantify Protein Expression in Frail Versus Non-Frail Patients with Cirrhosis

After reviewing the twenty-five candidate proteins identified by mass spectrometry analysis, eleven proteins were selected using the four selection criteria that we established (p-value <0.05, magnitude of difference, availability of a commercial ELISA assay, and biological plausibility). Commercially-available ELISA assays were utilized to measure protein concentration for each of these eleven proteins in frail (n=37) vs. non-frail (n=37) patients with cirrhosis matched for age, gender, and MELDNa. Three proteins had differential expression between the frail and non-frail cohorts: vitronectin (6.1 x 10-6g/mL in frail vs. 5.2 x 10-6g/mL in non-frail, p=0.049), leucine-rich

alpha-2 glycoprotein (1.6 x 10-5g/mL in frail vs. 1.2 x10-5g/mL in non-frail, p=0.070), and alpha-1 acid glycoprotein (4.9 x10-4g/mL in frail vs. 3.7x10-4g/mL in non-frail, p=0.085) (Table 5).

Table 5 Quantification of serum protein concentration in frail vs. non-frail patients with cirrhosis by ELISA assay

Protein	Protein concentration units	Mean protein quantity (SD) in frail patients with cirrhosis (n=37)	Mean protein quantity (SD) in non-frail patients with cirrhosis (n=37)	P-value
Vitronectin	X 10-6 g/mL	6.1 (2.6)	5.2 (1.3)	**0.049**
Leucine-rich alpha-2 glycoprotein	X10-5 g/mL	1.6 (1.0)	1.2 (0.7)	**0.070**
Alpha-1 acid glycoprotein	X10-4 g/mL	4.9 (3.5)	3.7 (2.2)	**0.085**
Alpha-2 macroglobulin	X10-3 g/mL	2.5 (1.1)	2.9 (1.4)	0.10
Inhibitor of nuclear factor k-b kinase subunit	X10-9 g/mL	7.6 (4.2)	9.1 (4.9)	0.337
Zinc alpha-2 glycoprotein	X10-5 g/mL	2.9 (0.7)	3.9 (7.2)	0.394
Haptoglobin	X10-5 g/mL	4.3 (7.2)	3.3 (5.9)	0.520
Apolipoprotein A1	X10-4 g/mL	1.2 (0.5)	1.2 (0.5)	0.600
Alpha-2 hs glycoprotein	X10-4 g/mL	3.3 (1.1)	3.4 (1.0)	0.637
Transthyretin	X10-5 g/mL	4.4 (4.2)	4.0 (3.5)	0.667
Alpha-1 antitrypsin	X10-1 g/mL	5.8 (3.2)	5.7 (2.2)	0.961

4. Discussion

This study employs proteomics as an unbiased approach to identify differences in protein profiles between frail and non-frail patients with cirrhosis on the liver transplant waitlist. This is critical preliminary work to guide the search for objective biomarkers of physical frailty in this population, and serves as a proof of concept that it is possible to use this approach to identify serum biomarkers of physical frailty in patients with cirrhosis using these methods. Specifically, using an unbiased mass spectrometry-based approach, we identified twenty-five proteins that differ between the frail and non-frail cohorts. The proteins identified are involved in many systemic processes such as the inflammatory response, immune function, cytoskeletal formation, and lipolysis, which offer important clues as to the mechanisms underlying frailty in cirrhosis. Subsequently, ELISA assays confirmed that three proteins – vitronectin, leucine-rich alpha-2 glycoprotein, and alpha-1 acid glycoprotein – have higher protein concentrations in frail vs. non-frail patients with cirrhosis ($p<0.1$), identifying these proteins as potential biomarkers of physical frailty in patients with cirrhosis.

Many of the proteins identified by our proteomics approach are involved in the systemic inflammatory response, such as haptoglobin, apoplipoprotein A1, and albumin, which were all

found at higher concentrations in frail vs. non-frail patients with cirrhosis. This is biologically plausible, as cirrhosis is known to be a pro-inflammatory state and sarcopenia may be the end-manifestation of chronic inflammation [28]. Moreover, several of these proteins have previously been associated with frailty in other patient populations. For example, the inflammatory marker haptoglobin has been noted to be elevated in frail geriatric populations [7, 8], albumin fragments have been associated with oxidative stress and frailty in the elderly [17], and apolipoprotein A1 has been associated with cardiovascular disease risk in older men [18]. Collectively, these findings may reflect that inflammation is a key driver of frailty in patients with cirrhosis, which is similar to hypothesized mechanisms of frailty in older adults without chronic liver disease [1].

The second broad category of candidate proteins identified by our mass spectrometry analysis is proteins that are involved in immune function and regulation. Specifically, immunoglobulin mu heavy chain disease protein, immunoglobulin lambda-like polypeptide 5 and immunoglobulin kappa chain C protein are all proteins involved in immune function, and each of these proteins was present at lower levels in frail than non-frail patients with cirrhosis. It is known that immune dysfunction occurs during cirrhosis, so these findings may indicate the immune dysfunction is even more severe in frail patients with cirrhosis. Further supporting this concept, infection risk has been shown to be higher in studies of sarcopenic patients with cirrhosis [29].

Many of the proteins identified in this study are synthesized by the liver, including haptoglobin, leucine-rich alpha-2-glycoprotein, zinc alpha-2-glycoprotein, apolipoprotein A1, vitronectin, and alpha-2-HS glycoprotein. Therefore, the observed alterations in protein levels may simply reflect changes in hepatic synthetic function not otherwise captured by the MELDNa score in some cases. For example, both alpha-1-antitrypsin and alpha-2-glycoprotein are thought to be almost exclusively produced in the liver, and thus the lower levels observed in frail patients with cirrhosis may reflect reduced hepatic synthetic function. This requires further study into the potential clinical utility of these biomarkers as measures of hepatic synthetic function that more precisely indicate the frail phenotype compared to other synthetic biomarkers like albumin and INR. In contrast, other proteins synthesized by the liver such as alpha-2-HS glycoprotein were found to be higher in frail patients with cirrhosis, suggesting that frail patients do not purely represent more severe liver synthetic dysfunction, but may have more complex metabolic derangements.

Of the eleven proteins analysed by ELISA in the validation cohort, three proteins were confirmed to have different concentrations in frail and non-frail patients with cirrhosis with $p<0.10$: vitronectin ($p=0.049$), leucine-rich alpha-2 glycoprotein ($p=0.070$) and alpha-1 acid glycoprotein ($p=0.085$). All three proteins were found in higher concentrations in frail patients with cirrhosis than their non-frail matched counterparts. Previous work has demonstrated that vitronectin is upregulated during wound healing [20] and also plays an important role during apoptosis [21], so it is biologically plausible that this protein is upregulated in frail patients with cirrhosis. A recent study exploring biomarkers of frailty in elderly patients demonstrated that vitronectin is associated with frailty in older women [22]. Leucine-rich alpha-2 glycoprotein is an inflammatory marker that has previously been associated with inflammatory musculoskeletal disease [9], so it is plausible that this protein is also upregulated in frail patients with cirrhosis. Similarly, alpha-1 acid glycoprotein is an acute phase reactant that is upregulated in response to systemic tissue injury, inflammation, or infection [10]. Previous work demonstrated that elevated serum alpha-1 acid glycoprotein is an independent risk factor of in-hospital death in the elderly [11] and also

associated with all-cause mortality in elderly patients [12], so it is logical that this protein also be associated with frailty in patients with cirrhosis.

We acknowledge the following limitations to this study. First, although we validated several proteins with ELISA, failure to confirm the remaining proteins identified in the test cohort may reflect differences between the test and validation cohort (eg, the test cohort may have been a slightly sicker population given higher median MELDNa than the validation cohort). Second, the investigated cohorts included both men and women as well as patients with different etiologies of liver disease, and the underlying underpinnings of the frail phenotype may differ in these subpopulations. Clearly, a major factor in this analysis was the small sample size in both groups, and thus it may be difficult to show significant differences, particularly given the subject heterogeneity. A much larger validation cohort is required to accurately detect all significant protein differences between groups. In addition, this study demonstrates correlation but not causation, so further studies are needed to understand the mechanisms by which these proteins promote or impair physical strength in patients with cirrhosis.

The strengths of this study include the use of novel proteomics techniques to identify previously unrecognized biomarkers of frailty in cirrhosis. This work lays the foundation for future studies that could aid in the diagnosis and prognostication of patients on the liver transplant waitlist, as no objective biomarkers of physical frailty are currently available. Ultimately, we hope to utilize a similar experimental strategy in a larger cohort study to develop a composite laboratory frailty index of serum protein biomarkers to be used in combination with MELDNa to predict mortality in liver transplant candidates. A laboratory frailty index that improves mortality risk prediction in this population would enable this important concept of frailty to be incorporated into a national liver allocation system to more accurately prioritize liver transplant candidates by urgency.

5. Conclusions

In conclusion, we have identified novel biomarkers that are differentially expressed in frail and non-frail patients with cirrhosis. Confirmation of elevated vitronectin, leucine-rich alpha-2 glycoprotein, and alpha-1 acid glycoprotein in the frail cohort identifies these proteins as promising objective biomarkers of frailty in cirrhosis. These data provide a rational basis to conduct larger cohort studies to validate and expand these findings. By more effectively allocating scarce donor livers to those in greatest need, we can reduce mortality on the liver transplant waitlist and help more patients achieve a cure for their end-stage liver disease.

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Author Contributions

J.L. and her research team enrolled patients in this study. M.S. and J.L. conceived and planned the mass spectrometry experiments. L.H. and J.L. conceived and planned the ELISA experiments.

L.H. and M.S. analyzed the data. L.H. performed the literature search and took the lead in writing the manuscript. All authors discussed the results and contributed to the final manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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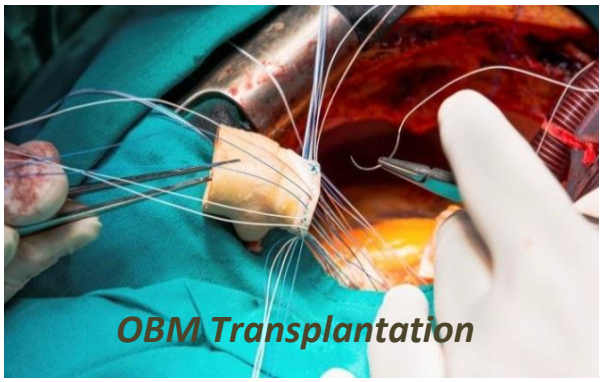

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