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“Comprehensive assessment of frail phenotype determinants in cirrhotic patients evaluated for liver transplantation”

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SUMMARY

BACKGROUND. Frailty is defined as a distinct biologic syndrome of decreasing physiologic reserve and increasing vulnerability to health stressors that predispose affected individuals to health adverse outcomes. So far, frail phenotype in cirrhotic patients has been regarded to a large extent as a manifestation of a wasting disorder, with sarcopenia as the major pathogenic mechanism. However, specific investigations on the pathogeneis of cirrhosis-related frailty are lacking. Therefore, the aim of this study was to perform a comprehensive assessment of potential determinants of frail phenotype, testing known risk factors for cirrhosis-related sarcopenia and exploring other pathogenic mechanisms derived from aging-related frailty

METHODS. Observational prospective study on a cohort of cirrhotic patients, aged 50-70 years, who underwent the clinical workup for deceased-donor LT listing at the Hepatology and Liver-Kidney Transplant Unit of the Udine Academic Hospital, from June 2019 to November 2021. Patient physical performance was assessed using the Liver Frail index (LFI) and a LFI≥4.5 was used as diagnostic criteria for frailty. Body mass composition was assessed on cross-sectional computer tomography images at the third lumbar spine level, as follows: skeletal muscle mass index (SMI), visceral adipose tissue index (VATI), subcutaneous adipose tissue index (TATI); total adipose tissue index (VATI+TATI), visceral-to-subcutaneous adipose tissue ratio (VSR) and skeletal muscle radiodensity (SMRD). Osteoporosis was diagnosed based on T score<-2.5 on dual-energy X-ray absorptiometry. For endocrine, inflammatory and metabolic assessment, the following

parameters were evaluated on blood samples: thyroid stimulating hormone, freeT4, 17-beta estradiol, testosterone, dehydroepiandrosterone solphate, cortisol and IGF-1 [endocrine profile], neutrophil-to-lymphocyte ratio, C-reactive protein (CRP) and erythrocyte sedimentation rate [inflammatory profile], 25(OH) Vitamin D, prognostic nutritional index, triglyceride, cholesterol HDL, cholesterol LDL, total proteins, albumin, cholinesterase, ammonia, uric acid, creatinine and percent glycated hemoglobin [metabolic profile]

RESULTS. One hundred ten patients were assessed. The median LFI was 3.9 [IQR 3.6-4.4], with a frail status prevalence of 23.6% (n=26). Frail patients were not significantly older than non-frail ones but showed a tendency toward an higher prevalence of female sex (46.1% vs 26.2%, p 0.054). Frailty was associated with higher MELD-Na score (median,19 vs 13, p <0.001). Moreover, it was associated with higher NASH prevalence (15.4% vs 0.24%, p 0.027), higher TATI (124.8 cm2/m2 vs 69.7 cm2/m2, p 0.001) and higher metabolic syndrome prevalence (23.1% vs 1.4%, p<0.001). Vitamin D levels were significantly lower, irrespective of pre-existing vitamin D oral supplementation, but frailty was not associated with osteoporosis. Cortisol (431 nMol/L vs 332 nMol/L, p 0.041) as well as CRP (6.5 mg/L vs 3.5 mg/L, p 0.032) levels were significantly higher, while and IGF-1 levels were significantly lower (37 pg/mL vs 45 pg/mL, p 0.032). Frail women specifically showed higher VATI and VSR (0.80 vs 0.41, p 0.27), but comparable SMI and SMRD, as well as significantly lower testosterone (0.7 nMol/L vs 1.4 nMol/L, p<0.001) and estrogen levels (40 pMol/L vs 69 pMol/L, p 0.017). Conversely, frail men showed significantly lower SMI (47.8 cm2/m2 vs 50.9 cm2/m2, p 0.012) and lower SMRD, higher SATI but comparable VSR, with significantly lower testosterone levels (4.5 nMol/L vs 8.7 nMol/L, p<0.001).

CONCLUSIONS. Frailty in cirrhotic patients should not be primarily considered as a wasting disorder and the pathogenic role of adipose tissue may be at least as important as that of skeletal muscle. Therefore, beyond sarcopenia and malnutrition, diagnostic and therapeutic interventions should also possibly target obesity, chronic inflammation, hypogonadism, IGF-1 deficiency and vitamin D deficiency.

1. INTRODUCTION

1.1 Frailty definition, prevalence and morbidity

Frailty is defined as a distinct biologic syndrome of decreasing physiologic reserve and increasing vulnerability to health stressors that results from multi-dimensional derangements across musculoskeletal, cardiovascular, neurologic, endocrine, and/or immune systems, as well as psychosocial factors [1-5]. Frail patients are characterized by sudden, disproportionate changes in health following seemingly minor stressor events, followed by an extended period of recovery and frequent failure to return to previous level of function [4]. Therefore, they are associated with an increased risk of a range of adverse outcomes, such as low quality of life, disability, hospitalization, falls and premature death, which all have a considerable impact not only on the individual health status but also on the health care system, with increased costs and resources consumption [1-5]. The frail phenotype was initially recognized in geriatric setting and its pathogenesis was mainly related to processes of senescence [1-5]. Nonetheless, its clinical recognition has been progressively expanded even to chronic diseases setting in adult patients, identifying frailty as a fundamental determinant of long-term outcomes [1-5]. In particular, it has been shown that cirrhotic patients are at major risk of frailty, with a reported prevalence that ranges from 18% to 43%, depending on the population evaluated, the assessment methods, and the operational definitions used [1,2]. As a matter of fact, chronic liver failure, portal hypertension and the frequently associated comorbidities that cirrhotic patients present with, all predispose to the development of features that are characteristic of frailty syndrome, such as sarcopenia, malnutrition, cognitive impairment and progressive immobility [1,2]. Furthermore, frailty has been consistently and independently associated

with the risk of cirrhosis-related events (ascites, encephalopathy, varices bleeding, acute decompensation, hepatocellular carcinoma (HCC), etc.) [6,7]. In 2018, Kahn et al. [8] performed a systematic review and meta-analysis to assess the prognostic value of frailty for waitlist mortality in cirrhotic liver transplant (LT) candidates. Six studies with a total of 1702 patients were included, and frailty was found to be highly related to waitlist mortality or delisting due "too sick for transplant" status. In Figure 1.1 the major clinical series investigating the prognostic impact of frailty during waitlist time are reported. Moreover, several studies have also demonstrated that preoperative frailty is an independent strong risk factor for early mortality even after LT [6,7]. Despite the criteria for LT listing and prioritization are currently based on the "sickest-first principle", the prognostic severity of frailty represents a critical issue in transplant decision-making process [1,2,9]. Is severe frailty an urgent call for LT priority or a predictor of transplant futility, particularly in a context of liver graft shortage? Furthermore, the relevance of the topic is also determined by the evidence that in recent year liver disease severity at transplantation is worsening, the proportion of older adults (≥65 years) awaiting transplantation is rising, and the prevalence of obesity-related liver disease is rapidly escalating—all of which are contributing to a cohort of LT patients who are sicker, more medically complex, and increasingly being described as “frail” [1,9]. Nonetheless, compared with other transplant risk factors, a unique feature of frailty is that its individual components are potentially modifiable with therapeutic interventions, such as physical and nutritional rehabilitation [1,2,10].

Tool	Study	N	Score	Association with outcomes (overall mortality unless otherwise specified)
ADL	Lai 2014[6]	294	≥1 disability (24%)	HR: 1.23 95% CI (0.91-1.66)
	Samoylova 2017[28]	458	≥1 disability (49%)	sHR: 1.8 95% CI (1.4-2.4)
	Tapper 2015[14]	734	ADL < 12: 9.2% without HE and 24% with HE**	ADL < 12: HR 1.8 95% CI (1.1-3.2)
CFS Range 1-9	Tandon 2016[16]	300	CFS > 4: 18% CFS > 3: 51%	OR (per 1 unit): 1.9 (1.4-2.6)
	Ney 2018[4]	355	MoCA-CFS score (cognitive + physical frailty)	OR of an HE-related hospitalization:
			0	1
			1	3.3 (1.5-7.7)
			2	5.7 (1.9-17.3)
Karnofsky Performance Scale (range A-C or 0-100)	Malinis 2014[29]	35 686	KPS (B or C): 63.4%	5-yr mortality: sHR 1.30 (1.23-1.37)
	Orman 2016[11]	70 092	KPS (B or C): 56%	1-year mortality by KPS: A (11.4%), B (15.5%), C (27.4%) KPS B: HR 1.08 95% CI (1.04-1.111) KPS C: HR 1.26 95% CI (1.20-1.33)
	Tandon 2017[15]	954	KPS (B or C): 68%	3-month postdischarge mortality: By KPS: A (5%), B (11%), C (23%) KPS (per 1-unit): OR 0.97 95% CI (0.96-0.98)
Braden Scale Range 6-23	Tapper 2015[14]	734	Moderate- to high-risk Braden Scale: ≤18 (28.1% HE, 13.7% without HE)	90-day mortality Score 16-18: 2.71 95% CI (1.88-3.90) Score < 16: 1.85 95% CI (0.83-4.12)
	Sundaram 2017[13]	341	Moderate- to high-risk Braden Scale: 16-18: (17%), ≤16 (20%)	Posttransplant mortality: insufficient outcomes
FFP Range 0-5	Lai 2014[6]	294	FFP ≥ 3: 17%	Per point: 1.45 95% CI (1.04-2.02)
	Tandon 2016[16]	300	FFP ≥ 3: 35%	OR 4.0
	Sinclair 2017[13]	587	FFP ≥ 3: 32%	Hospitalization days per 12 months IRR: 1.2 95% CI (1.02-1.44)
	Tapper 2018[5]	685	FFP ≥ 3: 41%	Transplant-free survival HR per FFI point: Without HE: 1.37 (1.20-1.58) With HE: 1.14 (0.98-1.33)
6MWD Meters walked	Carey 2010[9]	121	Mean 6MWD 69 ± 122 m	Per 100 m: 0.58 95% CI (0.37-0.93)
	Yadav 2015[30]	213	Mean 6MWD 371 ± 121 m 12% ≤250 m	250 m cutoff: HR 2.1 95% CI (0.9-4.7)
	Faustini Pereira 2016[12]	86	Mean 6MWD 410 ± 27.8 m	<410 m walked (unadjusted): RR 4.21 95% CI (1.25-6.41)
Gait speed (meters/second)	Dunn[17]	373	Mean gait speed 0.95 ± 0.25 m/s	Hospital bed-days Per 0.1 m/s: RR 0.85 (0.74-0.98)
SPPB Range 0-12	Lai 2014[6]	294	SPPB < 9: 31%	Per point: 1.19 95% CI (1.07-1.32)
	Tandon 2016[16]	300	SPPB < 10: 38%	OR 2.5
Liver Frailty Index per point	Lai 2017[7]	529	Median LFI: 3.8 (3.4-4.3)	Waitlist mortality Per point: HR 2.2 95% CI (1.7-2.9)
Cardiopulmonary Exercise Testing mL/kg/min	Ney 2016[10]	1107	Ventilatory anaerobic threshold (AT) Peak exercise oxygen uptake (peak VO2)	Posttransplant mortality (mean difference between survivor and nonsurvivors) AT: 2.0 95% CI (0.42-3.59) Peak VO2: 0.77 95% CI (−1.36-2.90)

Figure 1.1. Prognostic value of frailty in cirrhotic patients, according to different metrics of physical frailty, fitness, and/or disability (from [1])

2.2 Frailty assessment

In clinical practice, the assessment of frailty presents certain complexities: it requires a composite examination to comprehensively evaluate the multidimensional construct of frailty; the main assessment tools are functional and performance tests, which can be only performed prospectively, require a specific expertise and are time and resource consuming; cirrhotic patients can present with extremely heterogeneous features of frailty and a wide range of frailty severity [2]. A useful model of frailty in patients with liver failure should set and meet several targets [1,7]. First, it should instruct an accurate (i.e. associated with important outcomes), reliable (limited inter-observer variation) and validated classification of 'frail vs not-frail' [1,7]. Second, it should clarify the sources and potential reversibility of frailty. Third, it should be straightforward to operationalize the concept of frailty as a measure that can be obtained in the course of clinical practice for an effective and pragmatic clinical management [1,7].

Several tests have been proposed for cirrhotic patients, mainly derived from geriatric or oncologic setting [1,3,7]. These can be broadly categorized into subjective, mixed and objective tests. A summary of the most relevant methods and a critical analysis of their performance are reported in Figure 1.2 and 1.3. Interestingly, all these tools have shown a relevant predictive value for mortality on LT waitlist, hospitalizations and overall mortality, independently of MELD score, which is currently the main prognostic parameter used in LT prioritization [1,3,7,10,11]. In 2021, an expert opinion statement from the American Society of Transplantation Liver and Intestinal Community of Practice recommended that every patient with cirrhosis awaiting liver transplantation should be assessed at baseline and longitudinally using a standardized frailty tool, and provided a tool kit comprising the Karnofsky Performance Scale (KPS), Activity of Daily Life (ADL), Liver Frailty Index (LFI), and the 6-minute walk distance (6MWD). [1]

Tool	Refs	Where has it been studied?	Special devices/ training	Outcome prediction before or after transplant	How to perform
Subjective					
Eye-ball test	(2)	(Inpatient) or outpatient	No	Before	Experienced clinicians' general assessment during examination
Activities of Daily Living (ADL)	(6–8)	Inpatient, outpatient	No	Before / After	Grade ability to independently (1 point) or require assistance with (0 points) bathing, dressing, toileting, transferring, maintain continence, and feeding self
Clinical Frailty Scale (CFS)	(9)	(Inpatient) or Outpatient	No	Before	Grade patients on scale of 1 (Very Fit) to Terminally ill (9) based on impact of illness and ability to manage symptoms and daily activities.
Karnofsky Performance Scale (range A-C or 0-100)	(10, 11, 13, 25)	Inpatient, outpatient	No	Before / After	Grade function as a percentage from 0% (dead) to 100% (working, thriving); Grade A (80-100) normal activity with variable symptoms; B (50-70) requiring assistance and variable medical care; C (10-40), disabled, seriously ill.
Mixed subjective and objective					
Braden Scale Range 6-23	(8, 15)	Inpatient, (outpatient)	No	Before / After	Grade pressure ulcer risk from 6-23. Higher scores, lower risk. Sensory perception (1, unresponsive, - 4,no impairment); skin moisture (1,constantly moist – 4, rarely); activity (1, bedfast – 4, walks frequently); mobility (1, immobilized – 4, no limits); nutrition (1, poor intake – 4 excellent); transferring (1, difficult – 4, no problem)
Fried Frailty Index (FFI) Range 0-5	(6, 9, 16)	Inpatient, outpatient	Yes	Before	Frailty defined by poor performance on ≥3 of: weight loss (>10lbs in year), exhaustion (>3 days/week of feeling 'everything was an effort' and 'could not get going', hand-grip strength in gender-BMI stratified 20th percentile, 15 ft walk-speed in gender-height stratified 20th percentile, activity on Minnesota Leisure Time Activity scale in gender-stratfied 20th percentile.
6 Minute Walk-Distance (6MWD) Meters walked	(22, 23, 26)	Outpatient	No	Before	Meters walked in 6 minute timed trial
Gait speed (meters / second)	(24)	Outpatient	No	Before	Meters per second to walk set distance (e.g. 10 meters).
Short Physical Performance Battery (SPPB) Range 0-12	(6, 9)	Outpatient	Yes	Before	Three tests of balance (yes/no 10 seconds) with variable foot placement (side-by-side, semi-tandem, tandem), 4-meter walk-speed, chair-stands (time to performed 5 rises from a chair)
Liver Frailty Index Range 1-7	(5)	Outpatient	Yes	Before	Three tests of balance (yes/no 10 seconds) with variable foot placement (side-by-side, semi-tandem, tandem), hand-grip, chair-stands (time to performed 5 rises from a chair).
Cardio pulmonary Exercise Testing mL/kg/min	(21, 27)	Outpatient	Yes	Before / After	CPET is performed on a treadmill or in a stationary cycle ergometer by increasing the patient's workload with continuous heart-rate, blood pressure, and electrocardiographic monitoring. Through a respirator, gas exchange is measured to yield indices of oxygen uptake, ventilation, and carbon dioxide output ($VC0_2$)

Figure 1.2 Tools for the assessment of disability and frailty in patients evaluated for LT (from [7])

Figure 1.3. Properties of different frailty assessment tools (from [1])

		Subjective ←——————————→										Objective
		CFS	KPS	ADL/IADL	Braden scale	FFP	SPPB	LFI	Grip strength	Gait speed	6MWT	CPET
Subjectivity	Requires clinician judgment	✓	✓	✗	✓	✗	✗	✗	✗	✗	✗	✗
	Can be biased by patient reporting	✓	✓	✓	✓	✓	✗	✗	✗	✗	✗	✗
Predictive validity	For pretransplant outcomes	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	For posttransplant outcomes	–	✓	–	–	–	–	–	–	–	–	✓
Test characteristics	Reliability (internal consistency and repeatability)	✓	–	–	–	–	–	–	✓	✓	–	–
	Responsiveness to change over time	✗	✗	✗	✗	✗	✓	✓	✓	✓	–	–
Clinical feasibility	Estimated time taken (minutes)	<1	<1	<2	<5	<10	<5	<5	<1	<2	<10	<60
	Need for specialized equipment	✗	✗	✗	✗	✗	✗	✓	✓	✗	✗	✓✓
	Need for highly trained personnel	✗	✗	✗	✗	✗	✗	✗	✗	✗	✗	✓✓

Abbreviations: 6MWT, 6-minute walk test; ADL, Activities of Daily Living; CFS, Clinical Frailty Scale; CPET, cardiopulmonary exercise testing; IADL, Instrumental Activities of Daily Living; FFP, Fried Frailty Phenotype; KPS, Karnofsky Performance Status; LFI, Liver Frailty Index; SPPB, Short Physical Performance Battery.
Note: Double check mark indicates that these tests really need specialized technicians and equipment more so than the other tests that have only one check mark.
[a]No data available; ✓ yes; ✗ no.

2.2.1Subjective and mixed tests.

Subjective tools are based on patient self-reporting and/or clinician judgment, thus they are limited by heterogeneous reliability and reproducibility. Nonetheless, they are simple, quick, intuitive and inexpensive [7]. Those validated in cirrhotic patients are mainly the Activity of daily living (ADL), the Karnofsky Performance Scale (KPS) and the Clinical Frailty Scale (CFS).

ADL is patient-reported, it assesses six aspects of daily life (hygene, dressing, toiletting, locomotion, continence and meals) and, in cirrhotic patients, it has been associated with the prediction of pre-transplant death, delisting, discharge to a nursing facility and 30-day hospital readmission after discharge A difficulty in at least 2 ADLs marks severe frailty [12,13].

Karnofsky Performance Scale (KPS) has been extensively validated in cirrhotic patients since it is universally recorded by the United Network for Organ Sharing (UNOS) [7]. It combines patient-reported outcomes and clinical assessments, with a scale ranging from 0 (death) to 100 (perfect health), which can also be graded as A (80-100, able to work), B (50-70, unable to work but completes ADLs), C (0-40, disabled). Poor KPS is associated with pre-LT mortality as well as early post-LT mortality and graft loss [14]

Similarly, the Clinical Frailty Scale grades patients from very fit (1) to terminally ill (9) based on the severity of disease and performance of ADLs. A CFS score >4 is associated with unplanned hospitalization or death in pre-transplant patients with decompensated cirrhosis [15]

Among the mixed subjective and objective tests, there are the Braden Scale and the Fried frailty index. The Braden Scale is a standard index of pressure ulcer risk that is widely used by inpatient nurses. Its dimensions include an assessment of sensory perception,

skin moisture, activity, mobility, and nutritional intake [16]. The Braden has been found to be associated with mortality in pre-transplant patients (TAPPER15). Following transplant, Braden scores have been linked with prolonged hospital length of stay, bedridden status at discharge, and discharge to a rehabilitation facility [17]

The Fried frailty index combines 5 domains of physical frailty: exhaustion, weight loss and low activity (patient-reported); weakness and slowness (measured). It scores 0-5 and a FFI ≥3 marks a significant frailty resulting in increased mortality. It is among the most frequently used score in cirrhotic patients [12,18]

2.2.2 Objective Tests

Objective studies are based on measurements of patient physical performance [1,7,10,11]. One of the first tests of frailty studied in transplant waitlisted patients was the 6-minute walking distance (6MWD, meters) [19]. It has been shown that each 100 meters walked was associated with incrementally improved MELD-adjusted survival [19]. A walk distance <250m is currently accepted as a mark of severe frailty [7]

The Short Physical Performance Battery (SPPB) is an objective test to evaluate lower-extremity physical performance status. It evaluates performance on 3 timed tasks: walking speed, standing balance, and chair stand. Scored on a scale of 12 to 0, each point decrement has been associated with an increased risk of waitlist mortality [12,15].

Lastly, The Liver Frailty Index (LFI), specifically designed for ESLD patients (Figure 1.4 [20]. It consists of 3 performance-based tests of physical frailty, including grip strength, chair stands, and balance testing. Grip strength is a marker of nutritional status; chair stands are a marker of lower extremity strength, and impaired balance is a marker of neuromotor cordination. it was developed by Lai et al. [20] as an extension of the SPPB,

deriving and validating the components in patients awaiting LT. In the landmark study of LFI [20], the final battery was derived from a broader set that also included the FFI's subjective domains, ADL performance, and walk-speed. Hand-grip and chair-stands without balance showed equal performance for the prediction of mortality compared to the LFI, however the three assessments together were felt to provide a greater range for the discrimination of risk within a cohort. the LFI can be graded in 3 classes of frailty: mild/absent (<3.2), moderate (3.2-4.4), severe(≥4.5). To date, the LFI has the broadest applicability among all the frailty instruments for practical frailty assessment in the LT setting and has the advantages of being entirely objective, performance-based, multidimensional and suitable for longitudinal measurement [1,7,10]. Given its simple nature, it can be feasibly carried out in the outpatient setting at baseline and be followed over time [21]

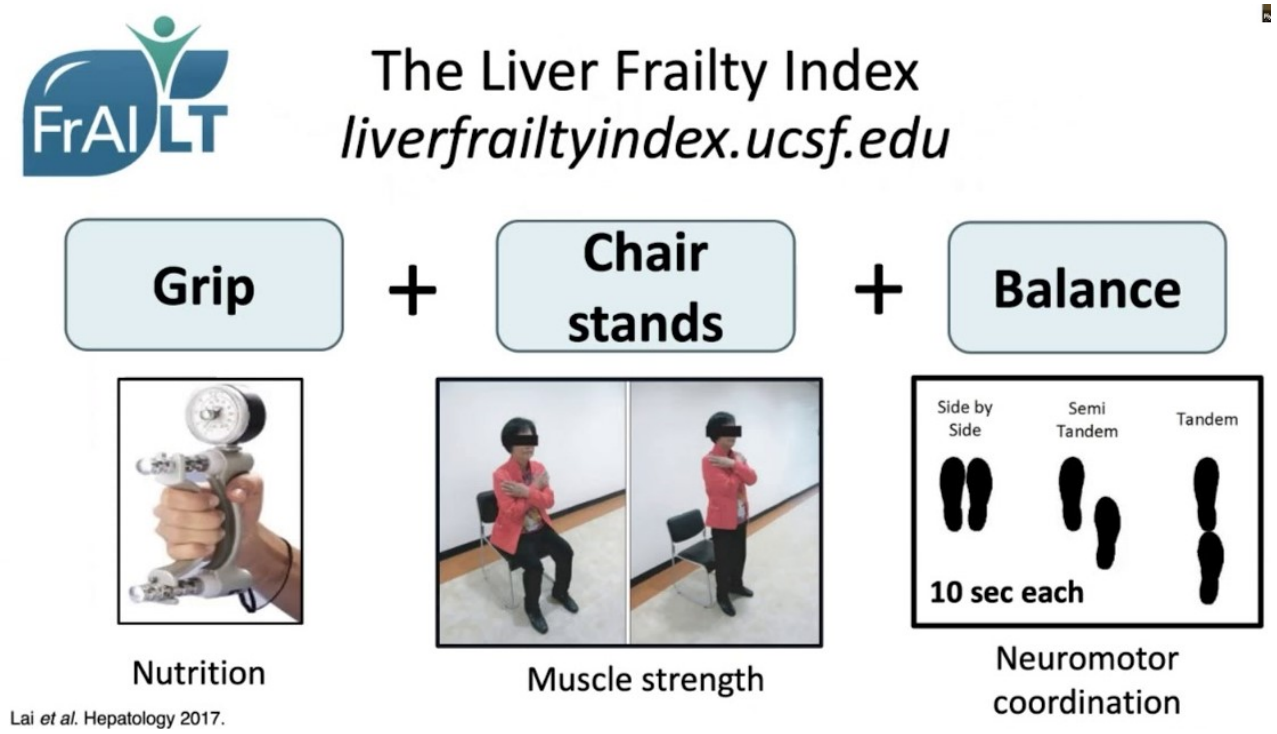

Figure 1.4. The Liver Frailty Index [20]

1.3 Frailty pathogenesis

From the pathophysiological point of view, frail phenotype in cirrhotic patients is currently regarded to a large extent a manifestation of a wasting disorder, with sarcopenia as the major mechanism [1,7,11, Figure]. Sarcopenia is defined as a pathologic, generalized loss of skeletal muscle mass. It can be primarily associated with aging or secondary to an underlying condition [1]. The reduced muscle mass and strength that accompanies aging is termed primary sarcopenia. Conversely, the causes for secondary sarcopenia can be activity-related (being bedridden, ataxia), nutrition related (malabsorption) or disease related (malignant tumors, severe organ failure, chronic inflammatory diseases, endocrine diseases). In cirrhotic patients, sarcopenia has a multifactorial pathogenesis, including nutritional, inflammatory, metabolic and neuroendocrine factors [6,22]. In Figure 1.5, a scheme of the sarcopenia pathogenic pathway is shown while in Figure 1.6 some hypothesis of frailty pathogenesis are graphically described.

1.3.1 Malnutrition and hyperammonia

Cirrhosis-related malnutrition comprises both inadequate calories intake and deficit of essential nutrients [23]. Patients tend to suffer from anorexia, dyspepsia and early satiety. These are mainly caused by altered taste perception due to zinc and magnesium deficiency and neuropathy, chronic hyperglycemia, chronic inflammatory state, compressive effect of tense ascites, gastroparesis and delayed bowel transit due to autonomic neuropathy and portal hypertensive gastroenteropathy [23,24]. Malabsorbtion is secondary to portal hypertensive gastroenteropathy and altered enterohepatic circle with intestinal mucosal atrophy and oedema, bacterial overgrowth and changes in the gut microbiota [25]. Consequently deficits of essential amino acids, essential lipids and lipid-

soluble vitamins occurs [23,25]. In particular, deficit of BCAAs (leucine, isoleucine, and valine) impairs muscle proteins synthesis and lower ammonia blood clearance capacity with a subsequent neurotoxic and myotoxic effect [26]. In skeletal muscle, BCAA are the primary source for proteins synthesis and essential substrates for ammonia detoxification via glutamine synthase [27-30]. Thus, BCAA deficit as well as the increase of absolute ammonia serum levels due liver metabolic dysfunction result in proteolysis and intramuscolar ammonia accumulation with mithocondrial dysfunction and hyperactivation of myostatin pathways [27-30]. Likewise, it has been recently shown that Vitamin D deficiency induces atrophy of type II fibers (fast muscle fibers) in the skeletal muscle of cirrhotic patients [31].

1.3.2 Chronic inflammation and hypercatabolic status

Cirrhotic patients tend to develop a chronic inflammatory state, which is primarily induced by local inflammation of cirrhotic liver parenchyma, increased bacterial translocation due to portal hypertensive as well as by precipitating events such as spontaneous peritonitis, variceal bleeding and frequent large volume paracentesis [1,2,23,26]. Increased levels of pro-inflammatory cytokine such as tumor necrosis factor-alpha (TNF-a), interleukin-6, leptin and myostatin are normally detected [1,2,22,27,30]. These factors induce and chronically maintain a hypercatabolic status with increased basal energy expenditure, which makes cirrhosis metabolically mimicking a state of starvation, with inappropriate use of body fat and protein stores for gluconeogenesis [1,2,22,27,30]. Sarcopenic patients tend to use fat and protein as an energy source instead of storage carbohydrates, mainly due to impaired liver glycogenosyntesis, inflammation-induced insulin resistance, chronic hyperinsulinemia due impaired serum clearance and metabolic acidosis due to renal impairment [24]. The resulting lipid peroxidation and mobilization of amino acids from the

skeletal muscles and visceral proteins causes muscle depletion and decrease in subcutaneous fat [1,2,22,27,30]. Moreover, myostatin has a direct negative effect on muscles, inducing muscle authophagy, proteolysis and suppressed protein synthesis [27-30].

1.3.3 Neuroendocrine dysfunction

The skeletal muscle mass is also regulated by hormonal pathways, in particular of insulin growth factor 1 (IGF-1) and testosteron. IGF-1 is produced by the liver and mediates most of the growth-promoting effects of growth hormone [22,26,27,30]. Cirrhotic patients tend to have low plasma levels of IGF-1, and the resulting severe growth hormone resistance has been associated with muscle wasting [22,26,27,30]. Likewise, ESLD is associated with sex hormone dysfunction, due to increased peripheral aromatase activity as well as hypothalamic–pituitary–gonads axis dysfunction [6,22,26,27,30]. Physiologically, testosterone increases muscle protein synthesis through direct stimulation of muscle androgen receptors, activation of the intramuscular IGF-1 system and inhibition of myostatin pathways [4]. Thus, the frequently detected hypogonadism in cirrhotic men, represents another important pathogenic mechanism for the development of sarcopenia [6,22,26,27,30].

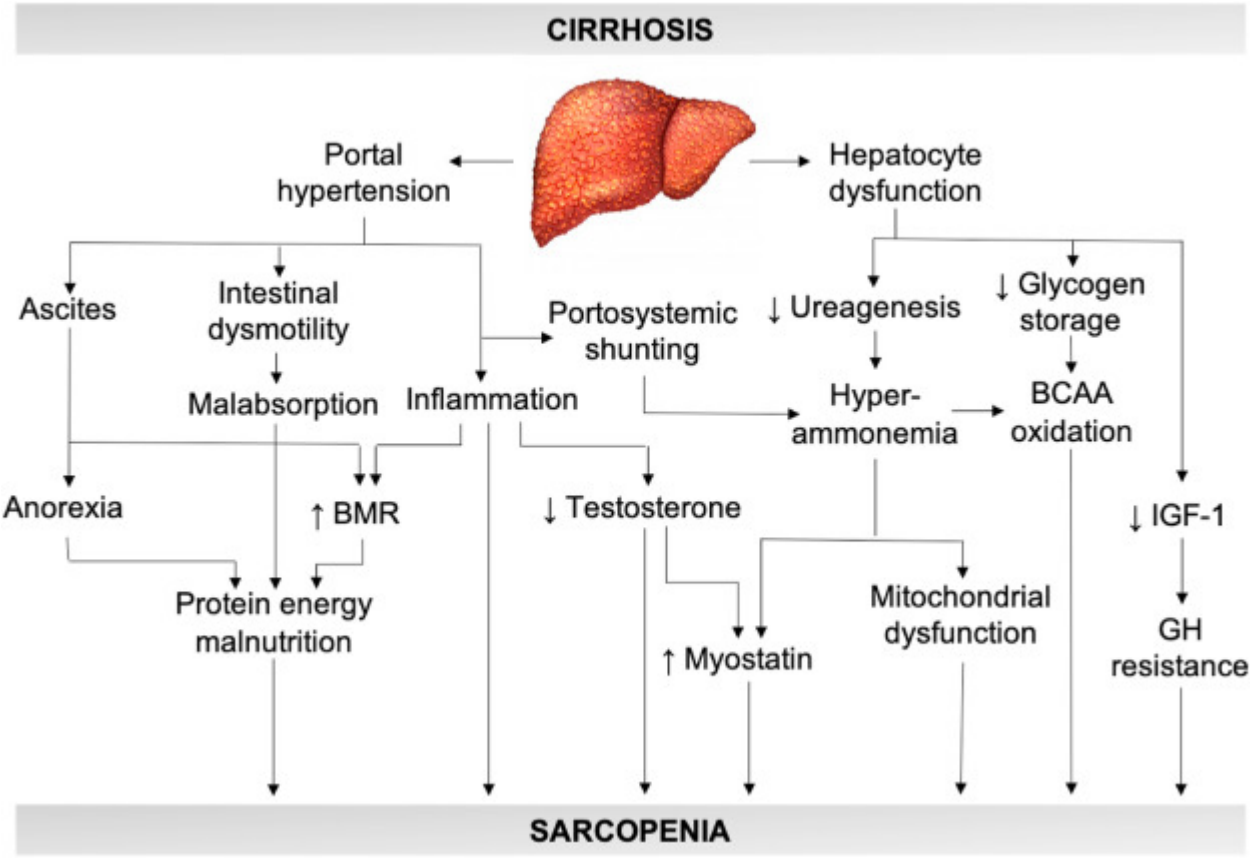

Figure 1.55 Pathogenesis of sarcopenia in cirrhotic patients (from [35])

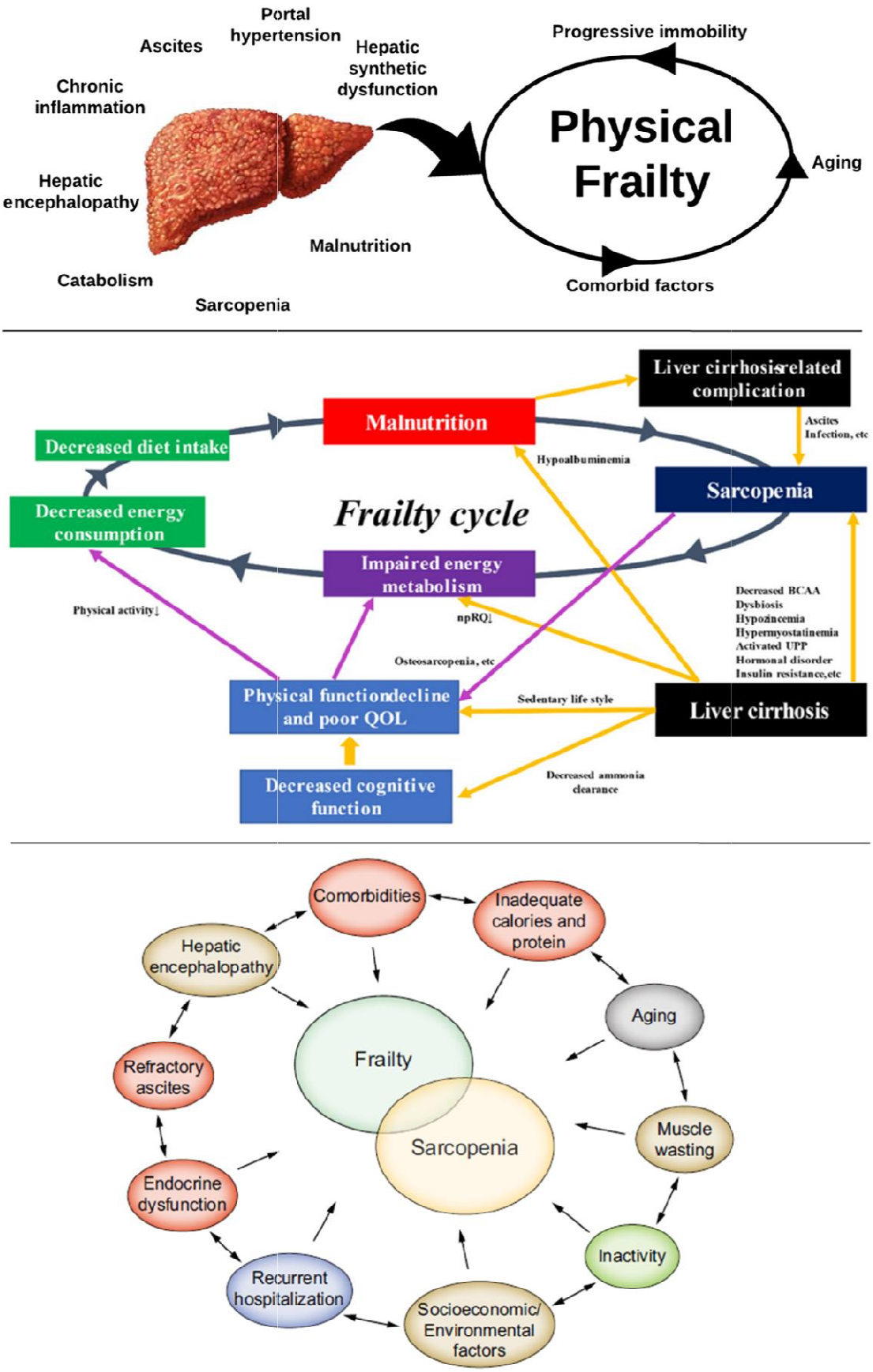

Figure 1.7 Different pathogenic models of frailty in cirrhotic patients (from [1,2,6])

1.3.4 Frailty in older patients

Senescence processes cause frailty trough mechanisms that are frequently analogues to those of cirrhosis-related sarcopenia, such as malnutrition, vitamin D deficiency, chronic inflammation, neurocognitive degeneration, insuline resistance, and hypogonadism [4,32-34]. Moreover, even chronic hyperactivation of glucocorticoid pathways, thyroid dysfunction, postmenopausal status, decreased energy expenditure and metabolic syndrome have been also implicated in the pathogenesis of aging-related frailty, but their pathogenic role in cirrhosis-related frailty has not been adequately explored so far [4,32-34].

1.4 Aim of the study

Frailty has been progressively recognized as one of the most impactful determinant of morbidity and mortality in cirrhotic patients. Advances in clinical research have recently significantly improved the performance of the diagnostic tools for frailty assessment and diagnosis. Nonetheless, the real pathogenesis of frailty in cirrhotic patients is still not completely elucidated. Many of the clinical features of frail phenotype in the elderly patients are also identified in those with ESLD, but no specific investigations have been undertaken so far to determine why and how frailty occurs at an earlier age in cirrhosis. Moreover, despite sarcopenia seems to be a primary driver of frailty in cirrhotic patients, it still does not meet and comprehend the multidimensional pathogenesis of frailty. Sarcopenic patients tend to have different clinical and demographic characteristics compared with frail patients and the negative pathogenic effect of obesity and osteoporosis are not adequately evaluated in sarcopenia assessment. Moreover, beside skeletal muscle quantity, even muscle quality is an important determinant of muscle function and muscle steatosis has been recently identified as an independent predictor of LT outcomes. Lastly, the pathogenic role of glucocorticoids, thyroid hormones and female sex hormones has never been clinically investigated in frail/sarcopenic patients with cirrhosis. Therefore, the aim of this study was to perform a comprehensive assessment of the potential determinants of frail phenotype, testing known risk factors for cirrhosis-related sarcopenia and exploring other pathogenic mechanisms derived from age-related frailty.

2. METHODS

2.1 Study population

This is an observational prospective study on a cohort of cirrhotic patients who underwent the clinical workup for deceased-donor LT listing at the Hepatology and Liver-Kidney Transplant Unit of the Udine Academic Hospital, from June 2019 to November 2021. The following inclusion criteria were used:

- patient age between 50 and 70 years, to control the effect of age on frailty;

- a post-menopausal status for female patients, to obtain an homogenous sex-hormones profiling;

- liver cirrhosis due to either alcohol abuse, non-alcoholic steatohepatitis (NASH), viral hepatitis or autoimmune hepatitis;

- elective outpatient clinical evaluation for non-urgent LT listing (no UNOS status 1-2A).

Exclusion criteria comprised neurological or endocrine comorbidities, re-LT cases, clinical evaluation for combined LT, LT indications other than end-stage liver disease (ESLD) and/or hepatocellular carcinoma (HCC), hospitalized patient with acutely decompensated liver cirrhosis.

Diagnosis of liver cirrhosis was based on clinical examination, liver function laboratory tests, and imaging [ultrasound scan, contrast-enhanced computer tomography (CT) scan, magnetic resonance imaging (MRI)] features of cirrhotic liver parenchyma degeneration and of portal hypertension (ascites, splenomegaly, portosystemic shunts). Cirrhosis etiology was determined on the base of past medical history, HBV and HCV serology,

autoimmunity serologic panel, and liver biopsy (when required). ESLD-severity was evaluated using the MELD-Na score. HCC diagnosis was based on mRECIST criteria and on percutaneous biopsy (when required). Patient comorbidities were evaluated and graded according to the Charlson comorbidity index [36]. Moreover, a specific clinical assessment of metabolic syndrome features (body mass index >30, arterial hypertension, diabetes and dyslipidemia) was performed. Arterial hypertension, diabetes and dyslipidemia were considered when a specific pharmacological therapy was active at the time of examination.

2.2 Frailty assessment

Frailty was assessed at an outpatient clinic visit using the LFI score [20]. LFI was selected, among other frailty tools, based on the evidence that LFI has currently the broadest applicability and an extensive validation in cirrhotic patients. It is entirely objective, performance-based, multidimensional and suitable for longitudinal measurement. It consists of 3 performance-based tests [20]:

- Grip strength: the average of 3 trials, measured in the patient's dominant hand using a hand dynamometer;

- Timed chair stands: measured as the number of seconds it takes to do 5 chair stands with the patient's arms folded across the chest;

- Balance testing: measured as the number of seconds that the patient can balance in 3 positions (feet placed side to side, semitandem, and tandem) for a maximum of 10 seconds each.

With these 3 individual tests of frailty, the LFI was calculated using the following equation (calculator available at: http://liverfrailtyindex.ucsf.edu): (-0.330 * gender-adjusted grip strength) + (-2.529 * number of chair stands per second) + (-0.04 * balance time) + 6.

Patients were categorized as frail based on a previously established cutoff of LFI score>=4.5.

Furthermore, the patient physical performance was additionally assessed with the gate speed test: the patient is asked to take 8 steps at the greatest speed; the meters walked and the time (sec) required are measured and the gait speed is calculated (m/sec) [37].

The patient's ADL was evaluated using the Duke activity status index [38], which is a validated functional capacity assessment tool for preoperative risk evaluation. It is a 12-item questionnaire that assesses daily activities such as personal care, ambulation, household tasks, sexual function and recreation with respective metabolic costs. Each item has a specific weight based on the metabolic cost. The participants are asked to identify each activity they are able to do. The final score ranges between zero and 58.2 points. The higher the score, the better the functional capacity.

2.3 Body mass composition assessment

Using cross-sectional CT at the L3 level, skeletal muscle and adipose tissue areas were examined by using AQUARIUS INTUITION software, version 4.4.13.P4 (TeraRecon, San Mateo, CA), as previously described [39-41] Skeletal muscle mass was evaluated as the skeletal muscle mass index (SMI) at the level of the third lumbar vertebra (L3). This is the most validated method of muscle mass assessment, with good linear association with whole body muscle mass (r= 0.86-0.94) and minimal bias due to ascites or fluid retention [39,40]. At L3, skeletal muscle area includes psoas, erector spinae, quadratus lumborum, transversus abdominis, external and internal obliques, and rectus abdominis muscles, which are identified and quantified by using attenuation values of - 29 to 150 Hounsfield units (HU) (Figure 2.1 A). SMI-L3 is calculated by normalizing skeletal muscle areas to the

square of the patient's height (cm2/m2). Sarcopenia was diagnosed on the base of validated gender specific cutoffs (SMI < 50 cm2/m2 in male and < 39 cm2/m2 in female patients), recommended by the "North American Working Group on Sarcopenia in Liver Transplantation" [22]. Muscle quality was examined as muscle attenuation of the entire muscle mass area at L3 level (skeletal muscle radiodensity, SMRD), since its indirectly measures fat infiltration in muscles, as previously reported [42,43]. Subcutaneous and visceral adipose tissue areas were quantified by using attenuation values of 190 to 30 HU (Figure 2.1 B) and 150 to 50 HU (Figure 2.1 C), respectively. Thus, visceral adipose tissue index (VATI), subcutaneous adipose tissue index (SATI) and total adipose tissue index (TATI, VATI+SATI), were calculated normalizing the respective fat areas to the square of the patient's height (cm2/m2) [41]. Visceral-to-subcutaneous ratio (VSR) was calculated by dividing VATI by SATI.

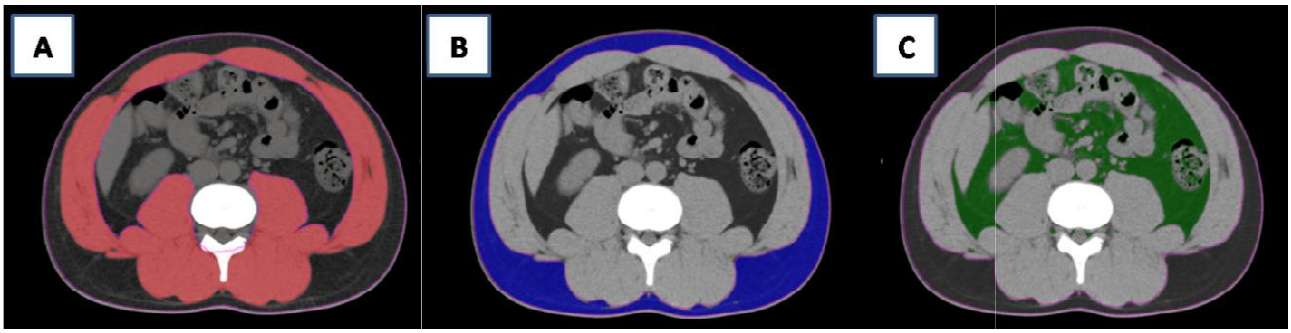

Figure 2.1 Cross-sectional computed tomography at the level of the third lumbar vertebra. (A) Skeletal muscle areas were identified and quantified by using a computed tomography attenuation value of - 29 to 150 HU. (B) SAT areas were quantified by using attenuation values of -190 to -30 HU. (C) VAT areas were quantified by using attenuation values of -150 to -50 HU. HU, Hounsfield units;. (from [38,39])

2.4 Bone mineralization and vitamin D assessment

Bone mineralization was evaluated with dual-energy X-ray absorptiometry (DXA) at lumbar and femoral level. A T score<-2.5 was diagnostic for osteoporosis in both sexes, according to the recommendations of the "International Society for Clinical Densitometry" for postmenopausal women and men over 50 years old [44]. Moreover, pre-existing Vitamin D oral supplementation was assessed and serum levels of 25(OH) Vitamin D were measured.

2.5 Endocrine, inflammatory and metabolic assessment

In fasting patients, the morning plasma levels of the following hormones were tested: thyroid stimulating hormone (TSH), freeT4, 17-beta estradiol, testosterone, dehydroepiandrosterone solphate (DHEAS), cortisol and IGF-1.

Neutrophil-to-lymphocyte ratio (NLR) as well as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were used to evaluate the patient proinflammatory state. NLR is a validated biomarker of systemic immuno-inflammatory function, with prognostic value in cirrhotic patients [45-48]. The prognostic nutritional index (PNI) was used to assess the patient nutritional status. PNI has been demonstrated as the most reliable and consistent method to identify malnutrition in cirrhotic patients [49]. It is calculated using the following formula: 10 * serum albumin (g/dL) + 0.005 * Total lymphocyte (cells/mm^2)]. Furthermore, plasma levels of lipids (triglyceride, cholesterol HDL, cholesterol LDL), total proteins, albumin, cholinesterase, ammonia, uric acid, creatinine and percent glycated hemoglobin (HbA1c) were measured as well.

2.7 Study end-points

Primary end-point of the study was to comprehensively explore and potentially identify significant pathogenic determinants of frailty in cirrhotic patients assessed for LT.

2.8 Statistical analysis

Categorical variables were expressed by frequencies and percentage, while continuous variables were expressed by median [interquartile range IQR]. In the comparison of frial group vs non-frial group, chi-square or Fisher exact test were used for categorical variables, and Mann-Whitney test for continuous variables. Spearman correlation coefficient was used to explore correlation between LFI and Duke .The predictive value of the variable significantly associated with frailty was tested in univariate logistic regression with ROC analysis. All analyses were performed using Stata/SE 15.1 (Stata Corp LP, United States).

3. RESULTS

3.1 Patient characteristics

One hundred-ten patients were enrolled in the study (Table 3.1-3.4). The median age was 61 years [56-65], with a male:female ratio of 76:34 and a median BMI of 24.9 [23.1-28.1]. The main underlying cause of liver cirrhosis was alcohol abuse (74.5%) while NASH was diagnosed in 5.4% of patients. Thirty-eight patients (34.5%) presented with a diagnosis of HCC. The median MELD-Na score was 14 [11-18], while ascites and signs of hepatic encephalopathy were detected in 41.8% and 30% of patients, respectively. The median albumin, creatinine and ammonia serum levels were 37 g/dL [33-40], 0.83 [0.69-1.08] and 53.7 uMol/L [39.2-74.9], respectively. Previous episodes of acute-on-chronic liver failure (ACLF) were reported by 12 (10.9%) patients. A metabolic syndrome was diagnosed in 6.3% of patients. The median HbA1c %, triglyceride and cholesterol LDL serum levels were 5% [4.3-5.5], 74 mg/dL [60-94] and 65 mg/dL [40-81], respectively. Overall, the median CCI score was 5 [5-7].

The median SMI in male and female patients was 48.5 [42.3-52.3] and 39.3 [31.8-44.8] respectively, resulting in an overall 52.7% prevalence of sarcopenia according to the "North American Working Group on Sarcopenia in Liver Transplantation" gender specific cut-offs; the VSR in male and female patients was 0.94 [0.64-1.22] and 0.60 [0.35-0.86], respectively. Thirty-six (32.7%) were on Vitamin D oral supplementation at the time of examination and the median prevalence of osteoporosis was 20.4%.

The physical and functional performances of the study population are reported in Table 5. The median LFI was 3.9 [3.6-4.4], with a frail status prevalence of 23.6% (n=26). The median Duke activity status was 39.4 [23.9-50.7] and frail patients showed a statistically

lower functional performance than non-frail ones (frail group vs non-frail group, 18.9 [15.2-24.2] vs 44.8 [31.4-52.9], p<0.001). The LFI and the Duke activity status showed an high correlation (rho -0.753, p<0.001), as shown in Figure 3.1. The correlation with the gait speed test was also statistically significant (rho -0.558, p<0.001; Figure 3.2. According to LFI-frail status, two study groups were identified: frail group (n=26) and non-frail group (n=84).

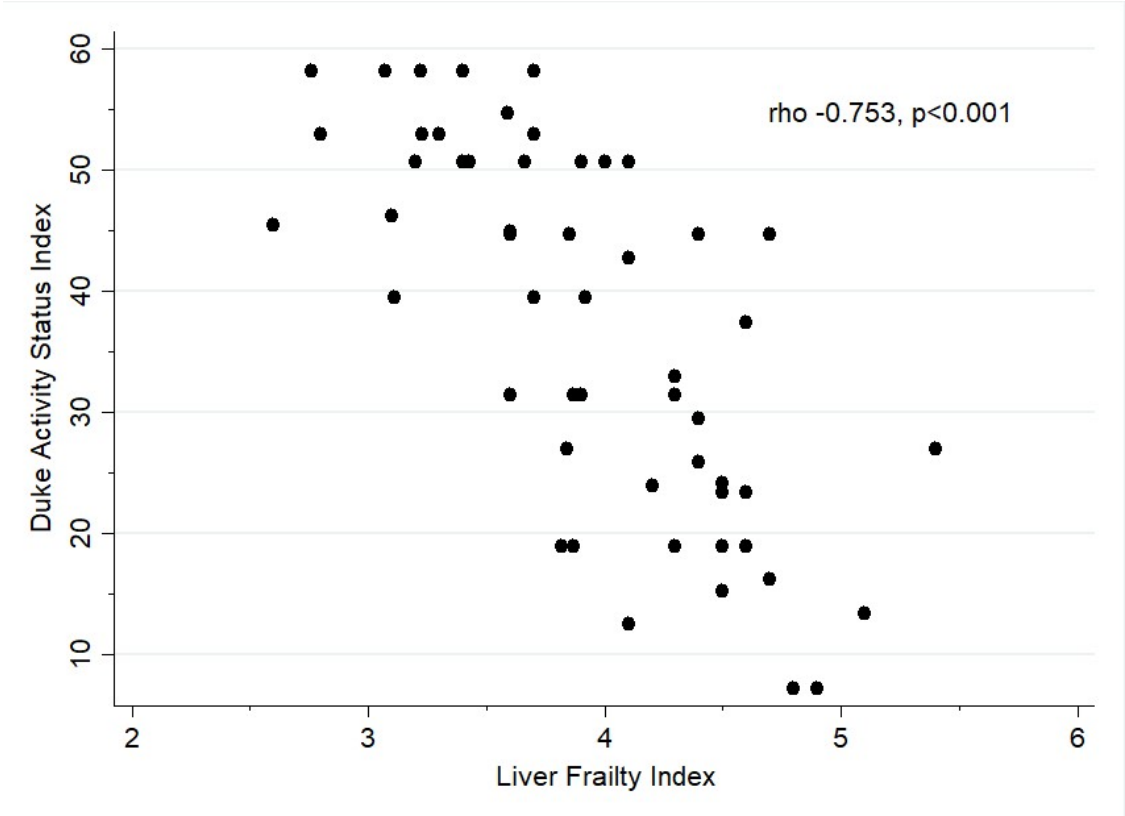

Figure 3.1 Correlation between LFI and Duke activity status index

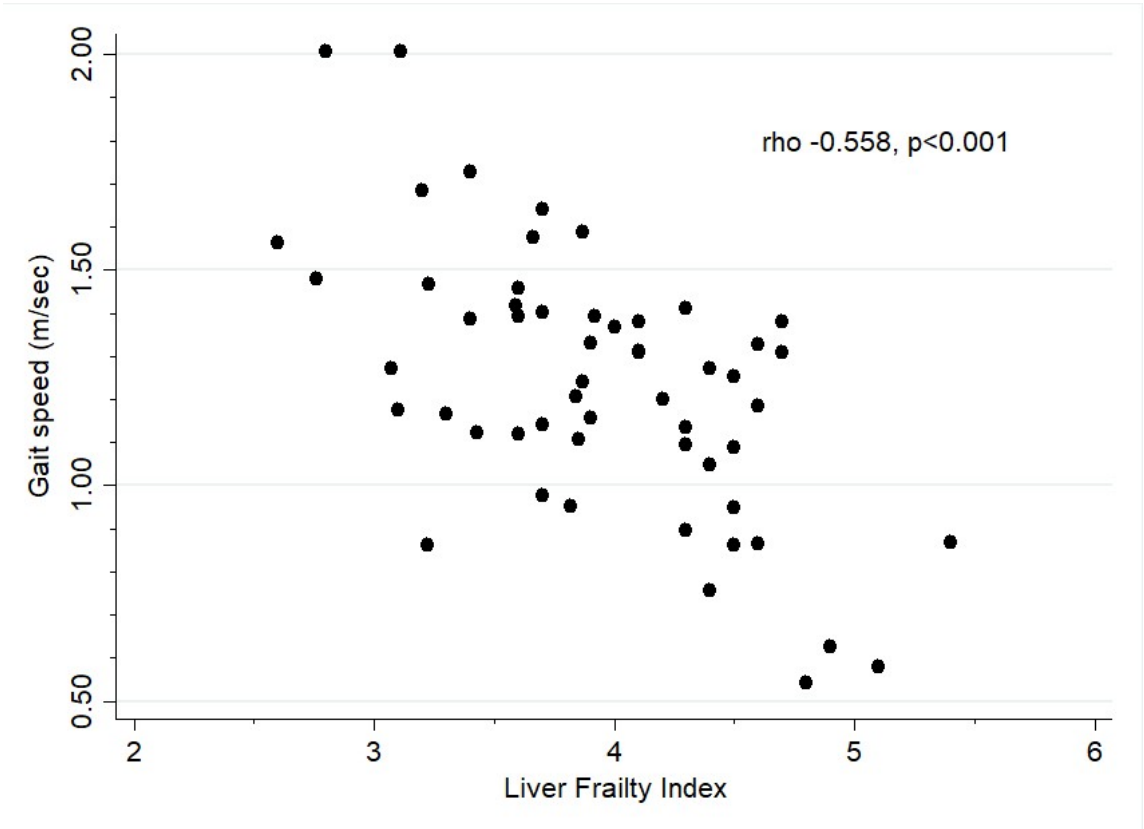

Figure 3.2 Correlation between LFI and gait speed.

3.2 Frailty determinants and outcomes

Frail patients were not significantly older than non-frail ones (frail vs non-frail group, 63 yrs [57-66] vs 60 yrs [56-64], p 0.517) but showed a significantly higher BMI (28.1 [23.9-29.3] vs 24.6 [23.1-26.8], p 0.037) and a tendency toward an higher prevalence of female sex (46.1% vs 26.2%, p 0.054). Frailty was associated with a significantly higher MELD-Na score (19 [15-21] vs 13 [10-16], p <0.001) and with a nearly significant higher prevalence of ascites (50% vs 39.3%, p 0.060). Albumin plasma levels were similar between study groups while creatinine serum levels were significantly higher in the frail group (0.96 mg/dL [0.84-1.27] vs 0.81 mg/dL [0.69-0.96], p 0.009). The prevalence of hepatic encephalopathy as well as ammonia serum levels were comparable between the groups. Coherently with the concept of frailty, previous ACLF episodes were more frequently reported by frail patients (23.1% vs 7.1%, p 0.033). The prevalence of NASH as underlying cause cirrhosis was significantly higher (15.4% vs 0.24%, p 0.027) and frail patients showed a higher prevalence of obesity (26.9% vs 10.7%, p 0.040) and dyslipidemia (15.4% vs 2.4%, p 0.027), with higher triglyceride (120 mg/dl [73-126] vs 73 mg/dl [60-86], p<0.001) and lower cholesterol HDL (50 mg/dL [31-53] vs 55 mg/dL [44-63], p 0.059) serum levels. Overall, frailty and a metabolic syndrome were significantly associated (p<0.001).

Frailty was associated with a significant derangement of body mass composition, but with different patterns between sexes. Frail men showed a significant decrease in muscle mass (SMI, 47.8 cm2/m2 [38.1-48.3] vs 50.9 cm2/m2 [44.8-52.9], p 0.012) associated with a significant increase in subcutaneous adipose tissue (SFI, 67.7 cm2/m2 [44.9-97.5] vs 43.7 cm2/m2 [27.4-61.1], p 0.009) resulting in a non-significant decrease of VSR. SMRD was significantly lower (34.3 UI [25.5-42.5] vs 43.4UI [36.6-46.6], p 0.004).

Conversely, frail women did not show a significant change in skeletal muscle mass or radiodensity but sustained a significant increase in visceral fat (VATI, 54.3 cm2/m2 [29.8-

64.5] vs 16.6 cm2/m2 [8.9-24.8], p<0.001) which resulted in a significant VSR increase (0.95 [0.61-1.18] vs 0.41 [0.32-0.84], p 0.027).

Overall, both frail men and women showed significantly higher TATI than non frail patients (men: 114.1 cm2/m2 [93.6-159.7] vs 87.1 cm2/m2 [52.1-120.9], p 0.02; women: 155.9 cm2/m2 [50.9-172.5] vs 53.9 cm2/m2 [33.2-77.1], p 0.011).

Frail patients were more frequently treated with vitamin D oral supplementation but no significant association between frailty and osteoporosis was noted. Nonetheless, in both treated and untreated frail patients, the 25(OH) vitamin D serum levels were significantly lower than in non-frail patients (treated, 20.3 ng/mL [12.9-24.4] vs 28.7 ng/mL [27.4-39.3], p<0.001; untreated, 7.7 ng/mL [7.6-8.8] vs 13.4 ng/mL [6.8-24], p 0.016). A potential confounding effect related to worse renal function in frail patients could be excluded.

The endocrine assessment identified several dysfunctions associated with frailty. Frail patients showed significantly higher level of morning plasma cortisol (431 nMol/L [286-668] vs 332 nMol/L [240-502], p 0.041) and lower level of IGF (37 pg/mL [23-46] vs 45 pg/mL [32-68], p 0.026). Moreover both men and women in frail group showed significantly lower testosterone levels (men, 4.5 nMol/L [3.2-4.7] vs 8.7 nMol/L [5.4-16.8], p<0.001; women, 0.7 nMol/L [0.5-0.7] vs 1.4 nMol/L [0.7-3.2], p<0.001). Frail women showed also significantly lower estrogen levels. Conversely, the thyroid function was comparable between groups.

A part from dyslipidemia, in the frail group, the metabolic assessment detected lower levels of cholinesterases and higher level of uric acid, while protein profile, HbA1c% and PNI were comparable. Among the tested inflammatory biomarkers, only CRP was significantly associated with frailty.

Assessing patients just on the base of skeletal muscle mass showed that sarcopenic patients (defined by the cutoff of the "North American Working Group on Sarcopenia in

Liver Transplantation [22], sarcopenic group, n=58; non-sarcopenic group, n=52) were characterized by a non significant higher prevalence in men (sarcopenic vs non-sarcopenic group, male:female, 42:16 vs 34:18, p 0.426), significantly lower BMI (24.5 [21.6-26.8] vs 26.9 [24.2-28.7], p 0.008) and obesity prevalence (6.9% vs 23.1%, p 0.028). Sarcopenia was not significantly associated with either SMRD (male: 39.1 UI [34.6-46.6 vs 44.6 UI [34.5-46.4], p 0.391; female: 38.7 UI [53.5-63.6] vs 57.5 UI [55.8-63.5], p 0.762) or VSR (male: 0.95 [0.42-1.27] vs 0.96 [0.72-1.17], p 0.404; female: 0.56 [0.35-1.05] vs 0.65 [0.38-0.83], p 0.762). Conversely, it was significantly associated with osteoporosis (31.0% vs 7.7%, p 0.003).

Due to the limited number of frailty cases, the significant sex-differences and the high number of variables to be potentially included in the model, it was not possible to perform a multivariate analysis. Nonetheless, a ROC analysis on clinically relevant variables which showed significant association with frail phenotype was performed to test their predictive value, as reported in Table 3.5.

Frailty was associated with a significant risk of not LT listing or LT list drop-out due to clinical contraindications to LT (30.7% vs 9.5%, p 0.021). Moreover, after the study assessment, 11 patients died: none of them were actively LT listed and 5 (45.5%) were frail. During the study period, 57 LT procedure were performed in 54 patients. However, among the patients assessed for the present study, only 21 were transplanted a no death cases were recorded.

Table 3.1. Demographic and clinical characteristics

	Total (n=110)	**Frail group (n=26)**	**Non-frail group (n=84)**	**p**
Age (years)	61 [56-65]	63 [57-66]	60 [56-64]	0.517
Sex (M:F)	76:34	14:12	62:22	*0.054*
BMI	24.9 [23.1-28.1]	28.1 [23.9-29.3]	24.6 [23.1-26.8]	**0.037**
MELDNa	14 [11-18]	19 [15-21]	13 [10-16]	**<0.001**
HCC diagnosis (%)	38 (34.5%)	2 (7.7%)	36 (42.8%)	**0.001**
Ascites (%)	46 (41.8%)	15 (50%)	31 (39.3%)	0.060
Hepatic encephalopathy (%)	33 (30%)	10 (38.4%)	23 (27.4%)	0.281
Previous episodes of ACLF (%)	12 (10.9%)	6 (23.1%)	6 (7.1%)	**0.033**
Aetiology (%)				
Alcohol abuse	82 (74.5%)	18 (69.2%)	64 (76.2%)	0.477
Viral hepatitis	23 (20.9%)	4 (15.4%)	19 (22.6%)	0.584
Autoimmune	8 (7.2%)	0	8 (9.5%)	0.102
NASH	6 (5.4%)	4 (15.4%)	2 (0.24%)	**0.027**
Metabolic syndrome (%)	7 (6.3%)	6 (23.1%)	1 (1.4%)	**<0.001**
Metabolic syndrome features (%)				
BMI>30	16 (14.5%)	7 (26.9%)	9 (10.7%)	**0.040**
Diabetes	20 (18.2%)	6 (23.1%)	14 (16.7%)	0.561
Dyslipidemia	6 (5.4%)	4 (15.4%)	2 (2.4%)	**0.027**
Hypertension	26 (23.6%)	8(30.7%)	18 (21.4%)	0.327
Charlson comorbidity index	5 [5-7]	5 [5-7]	5 [4-7]	0.597

ACLF: acute-on-chronic liver failure, BMI: body mass index, HCV: hepatitis C virus, HCC: hepatocellular carcinoma, MELD: Model for end-stage liver disease, NASH: non-alcoholic steatohepatitis

Table 3.2.Physical and functional performance

	Total (n=110)	**Frail group (n=26)**	**Non-frail group (n=84)**	**p**
Mean grip strength (Kg)				
- male	34.5 [25.1-38.9]	21.8 [17.4-29.9]	35.1 [31.6-41.3]	**<0.001**
- female	17.7 [16.7-20.7]	17 [16-17.1]	20.0 [16.7-22.6]	**0.003**
5 chair stands (sec)	13 [10-17]	17 [15-21]	12 [9-14]	**<0.001**
Balance (sec)	30 [25-30]	25 [21-26]	30 [30-30]	**<0.001**
Liver frailty index	3.9 [3.6-4.4]	4.6 [4.5-4.8]	3.7 [3.4-4.1]	**<0.001**
Gait speed (m/sec)	1.25 [1.08-1.40]	0.94 [0.86-1.25]	1.31 [1.13-1.45]	**<0.001**
Duke activity status index	39.4 [23.9-50.7]	18.9 [15.2-24.2]	44.8 [31.4-52.9]	**<0.001**

Table 3.3.Body mass composition and vitamin D levels

	Total (n=110)	**Frail group (n=26)**	**Non-frail group (n=84)**	**p**
SMI (cm2/m2)				
- male	48.5 [44.8-52.4]	47.8 [38.1-48.3]	50.1 [44.8-53.1]	**0.012**
- female	39.3 [31.8-44.8]	41.3 [30.2-45.0]	39.3 [31.8-44.8]	0.942
SMRD (UI)				
- male	41.8 [34.5-46.4]	34.3 [25.5-42.5]	43.4 [36.6-46.6]	**0.004**
- female	37.6 [35.2-43.5]	36.5 [36-43.6]	38.7 [34.8-43.7]	0.935
TATI (cm2/m2)				
- male	93.9 [61.2-129.9]	114.1 [93.6-159.7]	87.1 [52.1-120.9]	**0.021**
- female	54.2 [41.1-102.1]	155.9 [50.9-172.5]	53.9 [33.2-77.1]	**0.011**
VATI(cm2/m2)				
- male	45.4 [25.7-65.7]	50.6 [32.8-78.5]	42.3 [20.4-65.3]	0.147
- female	23.7 [14.9-42.1]	54.3 [29.8-64.5]	16.6 [8.9-24.8]	**<0.001**
SATI (cm2/m2)				
- male	45.4 [31.6-67.3]	67.7 [44.9-97.5]	43.7 [27.4-61.1]	**0.009**
- female	34.7 [22.5-55.4]	91.4 [21.7-95.7]	29.7 [23.3-53.1]	0.121
VSR				
- male	0.94 [0.64-1.22]	0.82 [0.73-0.96]	0.97 [0.61-1.27]	0.204
- female	0.60 [0.35-0.86]	0.80 [0.71-1.20]	0.41 [0.32-0.84]	**0.027**
Vitamin D oral supplementation (%)	36 (32.7%)	14 (53.8%)	22 (26.2%)	**0.009**
25(OH) Vitamin D (ng/mL)				
- Vitamin D oral supplementation	27 [20.3-35]	20.3 [12.9-24.4]	28.7 [27.4-39.3]	**<0.001**
- no Vitamin D oral supplementation	12.8 [7.4-20.1]	7.7 [7.6-8.8]	13.4 [6.8-24]	**0.016**
Lumbar-DXA (T-score)				
- Vitamin D oral supplementation	-2.05 [-2.9 to -1.5]	-2 [-2.4 to -1]	-2.1 [-3.3 to -1.5]	0.328
- no Vitamin D oral supplementation	-1.2 [-2 to 0.5]	-1.5 [-2.3 to 0.9]	-1 [-2 to 0.5]	0.740
Femoral-DXA (T-score)				
- Vitamin D supplementation	-1.5 [-2.3 to -0.9]	-0.9 [-2.5 to -0.5]	-1.5 [-2.3 to -1.1]	0.241
- no Vitamin D supplementation	-1.2 [-2 to -0.3]	-1.3 [-2 to -0.5]	-1.2 [-2 to -0.5]	0.605
Ostheoporosis (%)	22 (20.4%)	8 (23.1%)	14 (16.7%)	0.116

DXA: dual-energy X-ray absorptiometry; SMI: skeletal muscle index; SMRD: skeletal muscle radiodensity; VATI: visceral adipose tissue index; SATI: subcutaneous adipose tissue index; TATII: total adipose tissue index

Table 3.4.Endocrine, inflammatory and metabolic profile

	Total (n=110)	**Frail group (n=26)**	**Non-frail group (n=84)**	**p**
TSH (uUI/mL)	1.83 [1.13-2.53]	2.03 [1.40-3.01]	1.82 [1.13-2.51]	0.151
fT4 (pg/mL)	10.62 [9.4-12]	11.30 [9.54-11.60]	10.50 [9.40-11.99]	0.652
17- beta estradiol (pMol/L)				
- male	150 [124-166]	148 [108-159]	151 [124-167]	0.658
- female	54 [40-87]	40 [32-60]	69 [48-144]	**0.017**
Testosterone (nMol/L)				
- male	7.9 [4.7-15.2]	4.5 [3.2-4.7]	8.7 [5.4-16.8]	**<0.001**
-female	0.8- [0.7-2.2]	0.7 [0.5-0.7]	1.4 [0.7-3.2]	**<0.001**
DHEAS (ug/dL)				
- male	40 [21-76]	61 [18-97]	35 [21-69]	0.126
-female	15 [24-51]	15 [15-60]	28 [15-31]	0.625
Cortisol (nMol/L)	378 [240-528]	431 [286-668]	332 [240-502]	**0.041**
IGF-1 (pg/mL)	44 [32-68]	37 [23-46]	45 [32-68]	**0.026**
NLR	3 [1.8-4.3]	2.6 [1.8-3.2]	3.1 [1.9-4.3]	0.081
CRP (mg/L)	4.1 [1.8-8.5]	6.5 [3.1-10.6]	3.5 [1.6-8.4]	**0.032**
ESR (mm/h)	35 [14-67]	46 [14-80]	34 [13-65]	0.614
PNI	42.2 [37.7-46.7]	42.1 [40.5-47.1]	41.8 [37.7-45.2]	0.278
Triglyceride (mg/dL)	74 [60-94]	120 [73-126]	73 [60-86]	**<0.001**
Cholesterol HDL (mg/dL)	53 [44-61]	53 [31-56]	53 [44-63]	0.146
Cholesterol LDL (mg/dL)	65 [40-81]	58 [36-72]	65 [42-81]	0.248
Total proteins (g/L)	72 [63-76]	72 [68-76]	72 [63-76]	0.697
Albumin (g/L)	37 [33-40]	37 [34-39]	36 [33-41]	0.534
Cholinesterase (UI/L)	3386 [2307-4331]	2466 [2273-3386]	3558 [2317-4377]	**0.037**
Ammonia (uMol/L)	53.7 [39.2-74.9]	70 [44-77]	51.3 [37.4-74.7]	0.248
Uric acid (mg/dL)	5.3 [4.1-6.7]	6 [5.4-6.7]	5.1 [4.1-6.3]	**0.019**
HbA1c %	5 [4.3-5.5]	5.3 [4.5-5.9]	5 [4.3-5.4]	0.112
Creatinine(mg/dL)	0.83 [0.69-1.08]	0.96 [0.84-1.27]	0.81 [0.69-0.96]	**0.009**

CRP: C-reactive protein, DHEAS: dehydroepiandrosterone solphate ESR: erythrocyte sedimentation rate; IGF-1: insulin growth factor 1; NLR: neutrophil-to-lymphocyte ratio; PNI: prognostic nutritional index, TSH: thyroid stimulating hormone

Table 3.5. Predictive value of clinical variables significantly associated with frailty

	AUC	95% CI	p.
Metabolic syndrome	0.649	0.540-0.758	**0.003**
NASH	0.565	0.492-0.637	**0.025**
Creatinine level	0.668	0.529-0.808	**0.009**
SMI - male	0.285	0.163-0.408	**0.039**
SMRD - male	0.239	0.104-0.374	**0.011**
VATI - female	0.800	0.623-0.976	**0.007**
SATI - male	0.733	0.586-0.879	**0.009**
VSR - female	0.745	0.570-0.920	**0.032**
Testosterone level - male - female	 0.159 0.106	 0.061-0.255 0.006-0.205	 **0.005** **0.050**
Estrogen levels - female	0.461	0.281-0.493	**0.014**
Cortisol levels	0.635	0.511-0.768	**0.050**
IGF-1 levels	0.355	0.228-0.482	**0.038**
Vitamin D	0.336	0.224-0.436	**0.006**

AUC: area under the cureve; NASH: non-alchoolic steatohepatitis; SATI: subcutaneous adipose tissue index; SMI: skeletal muscle index; SMRD: skeletal muscle radiodensity; TATI: total adipose tissue index; VATI: visceral adipose tissue index;

4. DISCUSSION

The present study identified several potential determinants of frail phenotype among endocrine, metabolic and inflammatory mechanism; these confirmed its multifactorial pathogenesis, which only partially matched the pathogenesis of sarcopenia in cirrhotic patients. As a matter of fact frail phenotype was associated with NASH-related cirrhosis, metabolic syndrome as well as higher BMI, tryglicerid serum levels and TATI. Such result is in line with previous studies both in cirrhotic [1,3,50,51] as well as older patients [33,34] and outlines some crucial new evidences: (i) frailty should not be regarded as a primarily wasting disorder; (ii) the pathogenic role of adipose tissue may be at least as important as that of skeletal muscle. Indeed excessive adipose tissue leads to reduced physical capabilities, increased metabolic instability, increased inflammation and low antioxidant capacity, which all have been identified as direct determinant of frailty [33,34,51]. Moreover, the majority of the differences noted in the endocrine profile of frial and non-frail patients tended to have a direct or indirect pathogenic interaction with the adipose tissue, even though the precise hierarchy could not be further explored. IGF-1, that was actually tested in relation to its myotrophic function, has also a specific action even on fat metabolism [52,53]. As a matter of fact, IGF-1 it induces lipolysis in VAT and decreases lipogenesis and triglyceride accumulation in liver. Moreover, it increases systemic insulin sensitivity and contrasts oxidative stress [52,53]. Thus IGF-1 deficiency resulting from liver cirrhosis may results in visceral obesity, dyslipidemia, hypertension, and frailty [52,53]

Frail patients showed significantly higher cortisol serum levels than non frail, which was consistent with evidences in geriatric setting [4] but was quite surprising considering that cirrhotic patients tend to present with a relative adrenal insufficiency [54]. However, the

chronic proinflammatory state associated with cirrhosis and related complications, as well as the excessive adipose tissue might be a probable trigger for chronic hyperactivation of glucocorticoid pathways, which in turns mediates pro-frailty metabolic effects [4]. Indeed frailty was associated even with significantly higher levels CRP, in line with previous results in geriatric setting and with the pro-inflammatory status pathogenesis [55].

The association between adipose tissue disorder and frailty was even more evident in women. In line with previous studies, it was noted a gender difference of frailty, both in terms of prevalence and association with body mass composition. In the overall geriatric population, frailty is consistently more frequent in woman than men [56]. A systematic review based on data from 11 studies (17,746 women and 22,596 men) has concluded that older women (9.6%) were almost twice as likely as older men (5.2%) to be frail [57]. Likewise, the multicenter FrAILT study [58], assessing 1405 cirrhotic patients awaiting LT, has shown that after adjusting for age, MELDNa score, ascites, and hepatic encephalopathy, LFI persisted at 0.16 (95% CI, 0.08-0.23) units higher in women than in men. The cumulative incidence of wait list mortality at 24 months was significantly higher in women than in men and it was estimated that 13.0% of this sex gap was attributable to frailty. Fozouni et al [5] has reported that, after adjusting for MELDNa, sarcopenia among males was associated with a 2.81 times increased odds of frailty (95% CI 1.19–6.67, p 0.02), whereas sarcopenia among females was not significantly associated with frailty. As a result, two-thirds of frail men had sarcopenia, but only one quarter of frail women had sarcopenia. Malnutrition has been targeted as the prevalent pathogenic mechanism for sarcopenia in women, and a prevalent fat loss rather than muscle mass loss as the probable protective mechanism yielding a significantly lower sarcopenic risk compared with men [59]. However, in the present investigation, frail women showed no muscle disorder in terms of mass or quality but an absolute (VATI) and relative (VSR) increase of

visceral fat mass, assuming an android fat distribution. Furthermore, frailty was associated with significantly lower estrogen and testosterone serum levels. Physiologically, estrogens regulate muscle trophism, in particular type II fibers, which consist of fast moving units. Moreover, they have important regulatory role in energy balance, adipose tissue metabolism and inflammation. In particular, by upregulating leptin pathway and downregulating glucocorticoid one, estrogens increase energy expenditure, oppose excessive total body fat accumulation and visceral distribution [60]. In post-menopausal healthy women, estrogen deficiency correlates with visceral obesity and frailty [61]. No specific data are available in cirrhotic women. The observed specific increase of VATI has even greater pathogenic implications for frailty. As a matter of fact, adipocytes within visceral adipose tissue have increased response to catecholamines and actively produce proinflammtory cytokines such as interleukin-6, tumour necrosis factor-α and monocyte chemotactic protein, which promotes chronic inflammation and insulin resistance [41,62]. Such effect has been particularly evident in postmenopausal women [62]. Free fatty acids released from lipolysis of visceral adipose tissue are delivered directly to the liver through portal vein and subsequently lead to increased triglyceride deposition in liver. Another interesting finding was that in women, like in men, frailty was associated with low serum testosterone levels. While no specific data are available in cirrhotic patients, several reports in geriatric population have demonstrated a significant association between performance status and testosterone even in women [63-65]. In 2021, a cross-sectional and longitudinal analysis from the prospective population-based Korean Frailty and Aging Cohort Study [65], comprising 890 community-dwelling older women aged 70–84 years, showed that low free testosterone serum levels were associated with a significant decrease in handgrip strength (b=-0.61; p=0.010), irrespective of skeletal muscle mass.

Skeletal muscle does also play a role in the pathogenesis of frailty, particularly in men. As a matter of fact, frail men showed significantly lower skeletal muscle mass and quality as well as a significant testosterone deficiency which is considered the major pathogenic determinant of male sarcopenia [6,59]. Furthermore, in the overall study population frailty was associated with low 25(OH) Vitamin D serum levels, irrespective of pre-existing oral supplementation or osteoporosis [31]. Vitamin D deficiency causes frailty through sarcopenia, arteriosclerosis and proinflammatory state induction [3-33,66]

The present results, although preliminary and weakened by a small size, may have important clinical implications, if further confirmed. The overall aging of the general population as well as the exponential incidence of metabolic syndrome are causing a significant increase in the number of frail cirrhotic patients to be managed in clinical practice [50,51]. Moreover, LT seems not to guarantee a curative effect on frailty. Lai et al. [21], assessed the longitudinal changes of LFI before and after LT in 214 patients. Compared to pre-LT scores (median LFI 3.7), it was shown that median LFI worsened 3 months post-transplant (3.9), was similar at 6 months (3.7) and improved only by 12 months (3.4). A similar trend is also detected for sarcopenia, although the post-transplant recovery does not usually reach a statistically significant improvement [67]. Moreover, the weight gain frequently observed in LT recipients is surely a feature of recovery from cirrhosis-related malnutrition, but it is usually sustained by a more robust increase of fat mass rather skeletal muscle mass [68,69]. It has been reported that more than 20% of non-obese transplant recipients become obese over a two-years follow-up [68,70]. Frailty and its determinants (sarcopenia, myosteatosis, metabolic syndrome, visceral obesity, vitamin D deficiency, proinflammatory states, hypoandrogenism) [41,31,71] have been proven to be strong prognostic factors for morbidity and mortality before and after LT. Therefore a comprehensive understanding of its pathogenesis and a systematic evaluation

in clinical practice may yield a more precise and effective patient risk assessment as well as the identification of potential new therapeutic opportunities. Under this perspective, the use of the Fried frailty index [12,18] as diagnostic tool should be avoided since the use of weight loss as parameter, although consistent with sarcopenia and physical wasting, does not adequately explore the adipose tissue disorder which has been found so marked in frail patients. Moreover, the major therapeutic interventions for frailty developed in cirrhotic patients have so far targeted almost exclusively sarcopenia [2,10], thus somehow precluding cirrhotic women from an effective management. Physical rehabilitation and nutritional interventions should target not only the recovery of muscle mass and quality but also the reduction of the excessive total and visceral adipose tissue of frail patients. Sex-hormone replacement therapies should be evaluated even in frail women and not only in sarcopenic men [72]. Moreover, vitamin D deficiency, IGF-1 deficiency, adipokines-mediated inflammation and oxidative stress may be potential therapeutic target to evaluate (31,52,73-76). Indeed, frailty in liver cirrhosis shares the majority of pathogenic mechanisms of primary frailty, thus the solid clinical evidences in geriatric setting may be a reliable reference for further investigations in hepatology setting.

The present study has several limitations: a small sample size and lack of longitudinal data, particularly after LT, which both were determined by the extensive restrictions due to COVID pandemic; lack of a comprehensive dietary assessment; lack of a secondary level of examination for the endocrine disorders detected, which was linked to the screening approach used in the patient evaluation; a limited assessment of the patient proinflammatory status with no evaluation of relevant citokines serum levels (IL-6, TNF)

5. CONCLUSIONS

Frailty have been recognized as one of the most impactful determinant of cirrhotic patient prognosis. Its multidimensional construct allows a comprehensive but pragmatic patient evaluation, as it synthetically and simultaneously allow to evaluate several, diverse and reciprocally independent prognostic factors. Therefore, further efforts should be made to implement its assessment in the routine clinical practice. In hepatology setting, frailty has been so far regarded mainly as the functional phenotype of sarcopenia and physical wasting. However, the present study questioned or at least widened this pathogenic construct, showing how frailty is tightly linked with an adipose tissue disorder. Of course, the results of the present study should be considered as just preliminary due to the important limitations of the investigation. Nonetheless, if confirmed, it may have significant and "revolutionary" implications for the clinical recognition and management of frailty in cirrhotic patients, particularly of female sex.

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