



Research Paper

# Liver Function Parameters in Hip Fracture Patients: Relations to Age, Adipokines, Comorbidities and Outcomes

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## Abstract

**Aim:** To assess liver markers in older patients with hip fracture (HF) in relation to age, comorbidities, metabolic characteristics and short-term outcomes.

**Methods:** In 294 patients with HF (mean age 82.0±7.9 years, 72.1% women) serum alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), albumin, bilirubin, 25(OH)vitaminD, PTH, calcium, phosphate, magnesium, adiponectin, leptin, resistin, thyroid function and cardiac troponin I were measured.

**Results:** Elevated ALT, GGT, ALP or bilirubin levels on admission were observed in 1.7% - 9.9% of patients. With age GGT, ALT and leptin decrease, while PTH and adiponectin concentrations increase. Higher GGT (>30U/L, median level) was associated with coronary artery disease (CAD), diabetes mellitus (DM), and alcohol overuse; lower ALT (≤20U/L, median level) with dementia; total bilirubin >20μmol/L with CAD and alcohol overuse; and albumin >33g/L with CAD. Multivariate adjusted regression analyses revealed ALT, ALP, adiponectin, alcohol overuse and DM as independent and significant determinants of GGT (as continuous or categorical variable); GGT for each other liver marker; and PTH for adiponectin. The risk of prolonged hospital stay (>20 days) was about two times higher in patients with GGT>30U/L or adiponectin >17.14 ng/L (median level) and 4.7 times higher if both conditions coexisted. The risk of in-hospital death was 3 times higher if albumin was <33g/L.

**Conclusions:** In older HF patients liver markers even within the normal range are associated with age-related disorders and outcomes. Adiponectin (but not 25(OH)vitaminD, PTH, leptin or resistin) is an independent contributor to higher GGT. Serum GGT and albumin predict prolonged hospital stay and in-hospital death, respectively. A unifying hypothesis of the findings presented.

Key words: liver function, adipokines, hip fracture, comorbidities, outcomes

## Introduction

Global population ages rapidly, and by the year 2050 the percentage of people >60 years of age is expected to reach 22%. Advanced age is the single major independent risk factor for most chronic diseases and functional deficits, accounting for 60% of all deaths

worldwide. The liver because of its multitude metabolic, homeostatic and detoxification functions plays a central role in aging and susceptibility to age-related diseases. Ageing is associated with significant loss of hepatic volume and blood flow, structural changes in

all liver cells, accumulation of ageing pigments at the cytoplasm and pseudocapillarization of the sinusoid.<sup>1-4</sup> Over the past several years, substantial research has shown that alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) activities decrease with old age.<sup>5-10</sup> However, the relationship between ageing and liver function and diseases remains obscure.<sup>11</sup> For example, a number of reports concluded that in the elderly low ALT level is a strong and independent predictor of mortality,<sup>6, 8, 12-15</sup> while in other studies of community-dwelling older adults<sup>14, 16-18</sup> and older twins,<sup>19</sup> elevated serum ALT,<sup>16</sup> aspartate aminotransferase (AST), alkaline phosphatase (ALP)<sup>14, 17, 20</sup> and, especially, GGT levels<sup>12, 14, 18, 20-29</sup> predicted all-cause, cardiovascular, and liver mortality.

Numerous investigators found a positive association between ALT and/or GGT activities and metabolic syndrome, non-alcoholic fatty liver disease, type 2 diabetes mellitus (DM), cardiovascular disease (CVD), including coronary artery disease (CAD), hypertension, heart failure and stroke, chronic kidney disease, and cancers,<sup>19, 27, 30-44</sup> conditions which are highly prevalent in older individuals. The aforementioned associations were often evident across ALT and GGT activities values within the normal range,<sup>13, 21, 23, 31, 45-47</sup> independent of alcohol intake and other risk factors. Notably, although most of these studies did not focus specifically on the elderly population and the results have not been entirely consistent,<sup>48</sup> these associations were strong in young individuals, but weakened with age.<sup>21, 22, 24, 36, 42, 49, 50</sup>

Liver is critically involved in metabolism of many factors contributing to bone health and hepatic osteodystrophy is a common well-documented complication in patients with chronic liver disease.<sup>51-58</sup> Surprisingly, limited research has examined the liver function in older patients with hip fracture (HF).<sup>59, 60</sup> Liver markers, except low serum albumin concentration for mortality<sup>61-68</sup> and increased postoperative complications,<sup>61, 69</sup> are not currently included in the prognostic criteria.

The potential pathogenic role of factors affecting both liver and bone, such as low vitamin D status, elevated PTH levels, dysregulation in secretion of adipokines (especially, adiponectin, leptin and resistin), all of which are common in the elderly, virtually have not been investigated systematically in HF patients, despite growing evidence that the liver, the bone and adipose tissue are functionally interrelated organs.<sup>70</sup> The aforementioned metabolic mechanisms are important for optimal function of many organs and tissues throughout the body and involved in numerous age-related comorbidities which may substantially contribute to poor outcomes in HF patients.

To our knowledge, no published study has evaluated the relationship between liver function parameters and age, comorbidities, adipokines, vitamin D and PTH, as well as short-term outcomes in HF patients.

The aim of this prospective observational study was three-fold: 1) to determine liver function parameters in older HF patients in relation to age, and whether markers of hepatic function are associated with comorbidities, 2) to evaluate the relationship between serum liver markers, on one hand, and serum concentrations of vitamin D, PTH, adipokines adiponectin, leptin, and resistin, on the other, and 3) to assess the value of liver function markers on admission as predictors of short-term outcomes.

## Materials and Methods

### Patients

The study population consisted of 294 patients (212 females and 82 males) aged 60 years and older with low-trauma osteoporotic HF admitted to The Canberra Hospital. Patients with high trauma, pathological HF, Paget's disease, primary hyperparathyroidism or who did not have all serum variables of interest measured were not considered for the study. A detailed medical history, full physical examination and medication use were obtained along with demographic and anthropometric variables in all patients.

Informed consent was obtained from all individuals or their carers. The study was approved by the regional ACT Health Human Research Ethical Committee and conducted according to the Helsinki Declaration (as revised in 2008).

### Laboratory Analyses

After 12-hour overnight fast usually within 24 hours after arrival at the Emergency Department venous blood samples were taken and sera were isolated. One serum sample was frozen and stored at -70°C until further analysis of adiponectin, leptin and resistin. All other haematological and biochemical assessments were performed at the day of collection. All patients had the following tests performed: liver function markers (ALT, GGT, ALP, albumin and total bilirubin), complete blood count, urea, creatinine and electrolytes, fasting blood glucose (and HbA1C in diabetic patients), thyroid function tests (TSH, T4 and T3 if indicated), 25 (OH) vitamin D [25(OH)D], intact PTH, total calcium, phosphate, magnesium, C-reactive protein (CRP) and cardiac troponin I (cTnI), adiponectin, leptin and resistin.

Liver function tests were evaluated by using commercially available standard enzymatic reagents and diagnostic kits by spectrophotometry on the bio-

chemical autoanalyzer Abbott Architect CI16200 (Abbott Laboratories, IL 60064, USA). ALT, GGT and ALP were measured with enzymatic colorimetric methods, total bilirubin was analysed using diazonium salt, total protein was tested by a Biuret method and albumin was measured using bromocresol green. The mean inter-assay and intra-assay coefficients of variations (CV) for these tests were within 1.1% – 6.6%.

Serum levels of leptin were determined by enzyme-linked immunosorbent assay (ELISA) method (Diagnostic System Laboratories, Webster, TX, USA), total adiponectin and resistin by human ELISA kits (B-Bridge International, Mountain View, CA, USA). Intra- and interassay CV were less than 7% for these tests. All assays were performed according to the manufactures' instructions with kits of the same lot number.

Serum levels of 25(OH)D were determined by a radioimmunoassay kit (Dia Sorin, Stillwater, MN, USA; interassay CV 5.9–9.4%, intraassay CV<11.5%), intact PTH by solid-phase two-site chemiluminescent enzyme-linked immunometric assay on a DPC Immulite 2000 analyzer (Diagnostic Products, Los Angeles, CA, USA; interassay CV 6.2–7.0%, intraassay CV < 6%), cTnI by two-step chemiluminescent microparticle immunoassay (Chemiflex, Abbott Labs, Mississauga, Ontario, Canada; the minimum detectable limit 0.03 µg/L and the upper limit of reference range is 0.06 µg/L). Serum calcium concentration was corrected for serum albumin. Glomerular filtration rate (eGFR) was estimated using a standardized serum creatinine-based formula normalized to a body surface area of 1.73 m<sup>2</sup> (Levey A 2010; Johnson D 2012). All serum samples were processed in the same laboratory using the same methods and the same reference values.

For liver enzymes 2 times the upper normal limit (UNL) cut-off levels were used to define abnormal tests. When aminotransferase activities were analysed as categorical variables median values were used: for ALT 20U/L and for GGT 30U/L, levels comparable with data in the literature (Ruhl C 2012; Wu W 2012).

For the analyses, insufficiency of vitamin D was defined as 25(OH)D < 50 nmol/l and deficiency as 25(OH)D < 25 nmol/l based on current recommendations. Secondary hyperparathyroidism (SHPT) was defined as elevated serum PTH (>6.8pmol/l, the upper limit of the laboratory reference range).

Short-term outcomes included in-hospital all-cause mortality, prolonged length of stay (>20 days), post-operative myocardial injury defined by cardiac troponin I rise (>0.06 µg/L), high inflammatory response (CRP>100 mg/L) and being discharged to a permanent residential care facility.

## Statistical Analyses

Statistical calculations were carried out using the Stata software version 10 (StataCorp, College Station, TX, USA). The summary statistics are presented as the mean ± standard deviation (SD) for continuous variables and as the number (percentages) for categorical variables. Comparisons between groups of patients were made by use Student's t test for normally distributed continuous variables and a  $\chi^2$  test for categorical variables. The relationships between variables were examined by Pearson's linear correlation test and multivariate logistic regression analyses after logarithmic transformation of continuous variables with a skewed distribution. When the dependent parameter was stratified by level to further assess the independent participation of each of the factors studied, odds ratios with 95% confidence intervals (CI) were measured in multivariate logistic regression models, incorporating into the models biomarkers as continuous variables and clinical characteristics (sex, presence of CVD, DM, excessive alcohol consumption, history of smoking, etc) as categorical/ binary variables (yes, no). Bonferroni and Sidak adjustments for multiplicity were performed. To assess the significance of multicollinearity phenomena in multivariate regression analyses, the variance inflation factor was calculated. Results were considered statistically significant if P values <0.05 (two-sided).

## Results

### Characteristics of patients

Demographic, biochemical and haematological characteristics of the study population on admission are summarized in Table 1. The mean age of the patients was 82.0 years, 72.1% were women who were slightly older than men (+2.0 years, p=0.053), and 89(30.2%) subjects were admitted from long care residential facilities. There were 152 patients with a cervical HF and 142 with an intertrochanteric HF. The prevalent comorbidities at the time of hospitalisation for HF included CVD (66.3%), chronic kidney disease (CKD)≥stage 3 (eGFR<60 ml/min/1.73m<sup>2</sup>, 43.2%), dementia (27.8%), history of stroke or transient ischaemic attack (19.7%), type 2 DM (16.4%), chronic obstructive pulmonary disease (COPD, 11.0%), and Parkinson's disease (4.6%); 28(9.5%) patients consumed alcohol 3 or more times a week. In the study population 51(17.3%) of patients had neither CVD, CKD, or DM, nor were alcohol overusers. The prevalence of abnormal liver tests was as follows: ALT (>2ULN, 80 U/L) in 5(1.7%) patients, GGT (>2ULN, 128 U/L) in 23 (7.8%), ALP (>2ULN, 120 U/L) in 26 (8.8%), bilirubin (>1ULN, 20µmol/L) in 29 (9.9%) and albumin (<33 g/L) in 64 (21.8%) subjects. The liver

function abnormalities were small in magnitude and not associated with clinically apparent hepatic disease. There were no gender differences in indicators of liver functions, haemoglobin, and glycosylated haemoglobin (HbA1c) values. However, women, compared to men, had higher PTH, leptin, adiponectin and T4 concentrations and significantly lower mean 25(OH)D and eGFR levels.

**Table 1.** General characteristics of patients with hip fractures by gender

	Total Sample (n=294)	Men (n=82)	Women (n=212)	P Value
Age, years	82.0 ± 7.9	80.6 ± 8.3	82.6 ± 7.7	0.053
Cervical/Trochanteric fracture, n	152/142	38/44	114/98	0.298
GGT, U/L	54.1 ± 95.6	57.9 ± 106.0	52.6 ± 91.5	0.670
ALT, U/L	23.0 ± 42.6	18.9 ± 11.1	24.6 ± 49.6	0.302
ALP, U/L	105.4 ± 80.1	98.6 ± 64.6	108.2 ± 85.3	0.343
Total bilirubin, µmol/L	12.4 ± 7.4	13.6 ± 7.1	11.9 ± 7.5	0.073
Albumin, g/L	37.1 ± 6.3	37.4 ± 5.8	37.0 ± 6.5	0.573
Haemoglobin, g/L	124.9 ± 17.1	127.8 ± 16.8	123.7 ± 17.1	0.064
Leptin, ng/ml	18.5 ± 23.2	11.7 ± 18.6	21.1 ± 24.3	0.002
Adiponectin, ng/ml	17.5 ± 7.4	15.6 ± 7.6	18.3 ± 7.1	0.007
Resistin, ng/ml	18.7 ± 10.5	18.7 ± 10.7	18.6 ± 10.4	0.978
Leptin/adiponectin ratio	1.5 ± 2.4	1.1 ± 1.6	1.6 ± 2.7	0.121
Leptin/resistin ratio	1.4 ± 2.1	0.8 ± 1.0	1.6 ± 2.3	0.006
Adiponectin/resistin ratio	1.3 ± 1.5	1.1 ± 0.9	1.4 ± 1.7	0.148
25 (OH) D, nmol/L	37.3 ± 18.0	42.4 ± 18.2	35.3 ± 17.6	0.003
PTH, pmol/L	6.9 ± 5.6	5.5 ± 3.5	7.4 ± 6.1	0.009
TSH (mU/L)	1.55 ± 2.17	1.36 ± 1.35	1.62 ± 2.41	0.382
Free T4 (pmol/L)	15.9 ± 3.54	15.2 ± 3.53	16.2 ± 3.50	0.021
eGFR, ml/min/1.73m <sup>2</sup>	65.1 ± 23.7	71.2 ± 26.4	62.7 ± 22.2	0.006
*HbA1c, %	6.6 ± 1.04	6.7 ± 1.29	6.6 ± 0.94	0.825

Data are mean ± SD. GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; 25(OH) D, 25-hydroxyvitamin D; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; T4, thyroxine; eGFR, estimated glomerular filtration rate; \* HbA1c, glycosylated haemoglobin, measured only in patients with type 2 diabetes mellitus.

### Ageing mediated changes in serum metabolic parameters

The results of Pearson's correlations between liver markers, other parameters and age are shown in Table 2. GGT, ALT, leptin and eGFR were inversely associated with age, whereas adiponectin, resistin, PTH, creatinine, and urea were positively correlated with age. As might be expected, age showed a significant positive association with American Society of Anaesthesiologists (ASA) score ( $r=0.224$ ;  $p=0.001$ ) and presence of dementia ( $r=0.214$ ;  $p=0.001$ ). There was no significant association between any other studied parameters and age.

Study subjects were further classified into three age groups: <75, 75 – 85 and >85 years (Table 3). In the oldest patients (aged > 85 years), compared to aged < 75 years, the mean GGT activity was 50.6% and the ALT activity 48.4% lower; the mean leptin, 25(OH)D

and eGFR levels were also 36.5%, 14.6% and 29.4% lower, respectively, while adiponectin (+42.2%), resistin (+25.1%) and PTH (+72.3%) demonstrated an opposite trend (all  $p$  for trend <0.05). No associations between age and ALP, albumin, bilirubin, TSH, T4, HbA1c were found.

**Table 2.** Association between age and parameters of liver, renal, thyroid functions, adipokines and haemoglobin in older patients with hip fractures (Pearson correlation coefficients)

Variable	r	P Value
GGT	- 0.198	0.001
ALT	-0.134	0.022
ALP	-0.076	0.192
Bilirubin	0.006	0.920
Albumin	-0.060	0.308
Leptin	-0.159	0.007
Adiponectin	0.248	<0.001
Resistin	0.148	0.018
Egfr	-0.313	<0.001
Creatinine	0.202	0.005
Urea	0.296	0.001
25 (OH)D	-0.003	0.958
PTH	0.250	<0.001
TSH	0.032	0.594
T4	-0.027	0.662
Haemoglobin	-0.110	0.058
ASA score	0.224	0.001
Dementia	0.212	0.001

Data were log transferred before analysis (to achieve normal distribution).

GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; T4, thyroxine; eGFR, estimated glomerular filtration rate; ASA, American Society of Anaesthesiologists.

**Table 3.** Liver and renal parameters, adipokines, vitamin D, PTH and haemoglobin levels in older patients with hip fracture by age

Variable	Age (years)			P Value
	<75 (n=52)	75 – 85 (n=145)	>85 (n=97)	
Age, mean ± SD	69.3±5.0	81.2±2.7	90.1±3.5	<0.001
GGT, U/L	85.6±65.1	50.7±77.2	42.3±61.5	0.010
ALT, U/L	37.8±29.0	21.1±24.1	19.5±18.5	0.010
ALP, U/L	114.6±89.5	104.1±76.1	102.5±66.4	0.564
Bilirubin, µmol/L	12.3±8.3	12.5±7.7	12.2±6.4	0.948
Albumin, g/L	38.1±5.4	36.8±6.1	36.9±6.1	0.438
*HbA1c, %	6.6±1.0	6.6±1.0	6.5±1.2	0.971
Leptin ng/ml	20.8±24.7	21.2±26.5	13.2±14.9	0.026
Adiponectin, ng/ml	13.5±6.5	17.8±7.9	19.2±6.2	0.001
Resistin, ng/ml	16.7±10.3	17.9±9.6	20.9±11.4	0.044
25 (OH) D, nmol/L	39.7±15.9	37.1±19.0	33.9±17.5	0.035
PTH, pmol/L	4.7±3.1	6.8±5.0	8.1±7.0	0.002
eGFR, ml/min/1.73m <sup>2</sup>	82.9±25.2	63.1±21.5	58.5±21.5	<0.001
Hb, g/L	129.8±17.4	124.4±18.0	122.9±15.1	0.051

Data are the mean ± SD. P value for trend. GGT, gamma-glutamyltransferase; ALT alanine aminotransferase; ALP, alkaline phosphatase; \*HbA1c, glycosylated haemoglobin, measured only in patients with type 2 diabetes mellitus; 25(OH) D, 25hydroxyvitamin D; PTH, parathyroid hormone; eGFR, estimated glomerular filtration rate; Hb, haemoglobin.

## Links between liver markers and comorbidities

Parameters of liver function were compared between patients with and without specific co-morbid conditions. Lower ALT levels ( $\leq 20$  IU/L) were associated with dementia (OR=2.11, 95%CI 1.10 - 4.05,  $p=0.015$ ). Lower GGT levels ( $\leq 30$  U/L) were prevalent in females (OR=1.88, 95%CI 1.09 - 3.25,  $p=0.016$ ). Higher GGT levels ( $>30$  U/L) were associated with coronary artery disease (CAD, OR=2.55, 95%CI 1.37 - 4.74,  $p=0.003$ ), type 2 DM (OR=2.19, 95%CI 1.12 - 4.29,  $p=0.013$ ), and excess alcohol consumption ( $>3$  times a week, OR=6.39, 95%CI 1.6 - 29.57,  $p=0.002$ ), while total bilirubin levels above  $20 \mu\text{mol/L}$  were associated with CAD (OR=2.56, 95% 1.01 - 6.42,  $p=0.025$ ), cervical (vs. trochanteric) HF type (OR=3.26, 95%CI 1.27 - 8.72,  $P=0.006$ ) and alcohol overuse (OR=4.67, 95%CI 1.12 - 18.19,  $p=0.008$ ). Albumin concentrations above  $33\text{g/L}$  were associated with CAD (OR= 3.13, 05%CI 1.21 - 8.5,  $p=0.009$ ). On the other hand, the majority of 51 patients without prevalent CVD, CKD $\geq 3$  stage, DM, and non-alcohol overusers, had ALT $\leq 20$  U/L (70.6%), GGT $\leq 30$  U/L (66.7%), albumin $>33$  g/L (74.5%) and bilirubin $<20 \mu\text{mol/L}$  (94.1%).

## Correlations between liver markers and other parameters

Pearson's correlation coefficient between log ALT and log GGT was 0.372 ( $p<0.001$ ). Both GGT and ALT were also significantly and positively associated with ALP ( $r=0.426$ ;  $p<0.001$  and  $r=0.141$ ;  $p=0.015$ , respectively), and bilirubin ( $r=0.218$ ;  $p=0.001$ , and  $r=0.171$ ;  $p=0.003$ , respectively). ALT inversely correlated with albumin ( $r=-0.124$ ;  $p=0.034$ ). ALP was negatively associated with 25(OH)D ( $r=-0.187$ ,  $p=0.002$ ). Bilirubin correlated with resistin ( $r=0.151$ ,  $p=0.016$ ), and PTH with adiponectin ( $r=0.193$ ,  $p=0.002$ ). No other associations were found between GGT, ALT, bilirubin, albumin, on one hand, and 25(OH)D, PTH and adipokines, on the other hand. However, GGT correlated positively with TSH ( $r=0.116$ ,  $p=0.05$ ) and negatively with T4 ( $r=-0.185$ ,  $p=0.002$ ). There was also a significant inverse association between leptin and adiponectin ( $r=-0.178$ ,  $p=0.005$ ).

Multiple linear regression analysis with GGT as the dependent variable and age, sex, ALT, ALP, bilirubin, albumin, eGFR, three adipokines (adiponectin, leptin and resistin), 25(OH)D, PTH, T4 levels, alcohol consumption ( $>3$  times/week, yes/no), presence of DM (yes/no), and CVD (yes/no) as independent variables revealed eight independent and significant determinants of GGT (as a continuous variable): ALT, ALP, bilirubin, adiponectin, alcohol overuse, DM (all six positively related), albumin and age (both inversely related). This model explained 54.3% of the

variance in serum GGT activity (Table 4). Similar analyses for four other liver markers, namely ALT, ALP, bilirubin and albumin, as dependent parameters in separate regression models showed that GGT was an independent and significant predictor for each of these liver function parameters ( $\beta=0.112$ ,  $p=0.10$ ;  $\beta=0.541$ ,  $p<0.001$ ;  $\beta=0.019$ ,  $p=0.004$ ;  $\beta=-0.012$ ,  $p=0.039$ , respectively). When multiple linear regression analysis was performed with adiponectin as the dependent variable three independent significant associations were found: age ( $\beta=0.232$ ,  $p=0.001$ ), male sex ( $\beta=-2.500$ ,  $p=0.031$ ) and PTH ( $\beta=0.185$ ,  $p=0.040$ ).

**Table 4.** Multivariate linear regression analyses for serum gamma-glutamyltransferase (GGT) level as a dependant continuous (model 1) and categorical ( $>30$  U/L, model 2) variable

	Model 1		Model 2		
	$\beta$	P Value	OR	95% CI	P Value
Age	-2.464	<0.001	0.98	0.94-1.03	0.563
Sex (m)	2.008	0.852	2.53	1.28-5.03	0.008
ALT	0.263	0.010	1.04	1.01-1.06	0.013
ALP	0.637	<0.001	1.01	1.01-1.02	0.015
Bilirubin	1.869	0.004	1.01	0.94-1.06	0.602
Albumin	-1.554	0.039	1.01	0.95-1.06	0.738
Adiponectin	1.403	0.034	1.06	1.02-1.11	0.009
*Alcohol	92.368	<0.001	6.13	1.44-26.02	0.015
DM	30.560	0.048	2.29	1.03-5.07	0.041
	$R^2=0.5426$		$R^2=0.1924$		

$\beta$ , parameter estimate/regression coefficient; OR, odds ratio; CI, confidence interval; ALT alanine aminotransferase; ALP, alkaline phosphatase; DM, diabetes mellitus. \*Alcohol consumption  $\geq 3$  times a week.

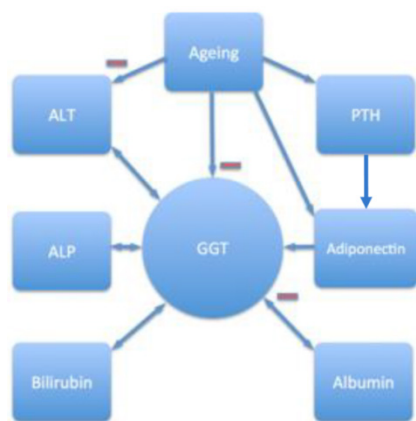
Adjustments: age, sex, ALT, ALP, bilirubin, albumin, adiponectin, leptin, resistin, 25(OH)D, PTH, estimated glomerular filtration rate (eGFR), alcohol overuse, presence of type 2 diabetes mellitus and cardiovascular disease. Only statistically significant associations (at least in one model) shown ( $p<0.05$ ).

In multivariate adjusted regression model that included GGT $>30$  U/L as the dependent variable and the same independent variables, the significant associations showed adiponectin, male sex, ALT, ALP, alcohol overuse and DM (Table 4, model 2). In multivariate regression analyses, ALT $< 20$  U/L was independently predicted only by GGT (OR=0.992,  $p=0.021$ ), and albumin $<33$  g/L was predicted only by GGT (OR=0.995,  $p=0.033$ ) and ALP (OR=1.006,  $p=0.040$ ). Compared to the rest of the cohort, the patients with GGT $>30$  U/L ( $105.7 \pm 65.5$  vs.  $19.6 \pm 5.1$  U/L) had also significantly higher levels of ALT ( $31.9 \pm 35.8$  vs.  $17.2 \pm 8.2$  U/L,  $p=0.004$ ), ALP ( $129.4 \pm 109.4$  vs.  $89.3 \pm 45.8$  U/L,  $p<0.001$ ), bilirubin ( $13.7 \pm 9.0$  vs.  $11.5 \pm 6.0 \mu\text{mol/L}$ ,  $p=0.011$ ), adiponectin ( $18.8 \pm 7.4$  vs.  $16.6 \pm 7.2$  ng/ml,  $p=0.015$ ) and resistin ( $20.3 \pm 10.9$  vs.  $17.5 \pm 10.0$  ng/ml,  $p=0.038$ ).

On subgroup analysis of patients with vitamin D insufficiency (25(OH)D $<50$  nmol/L,  $n=222$ ), the subjects with elevated PTH levels ( $>6.8$  pmol/L,  $n=98$ ) compared to those with PTH within the reference

range (n=124) demonstrated a significantly higher mean adiponectin concentration ( $19.8 \pm 7.3$  vs.  $16.0 \pm 7.1$  ng/ml,  $p=0.001$ ), while 25(OH)D levels were similar ( $29.0 \pm 11.96$  vs.  $31.3 \pm 11.83$  nmol/L,  $p=0.194$ ). It appears, therefore, that elevated PTH (not vitamin D insufficiency *per se*) modulates adiponectin production by adipose tissue.

Taken together, these data show strong bidirectional associations between GGT and ALT, ALP, bilirubin (all positive) and albumin (negative), and confirm that both DM and alcohol overuse are independent predictors of higher GGT. More importantly, the results indicate that in older patients with HF circulating adiponectin, which rises with ageing in parallel with PTH, is an independent and significant determinant of serum GGT activity. Multiple regression analysis adjusted for possible covariates, including alcohol consumption and DM, identified that for every 1ng/ml increment of serum adiponectin concentration the probability of higher GGT(>30 U/L) increases by 6.2%. With age both GGT and ALT decrease, while PTH and adiponectin levels increase, and the latter seems to have a greater effect on serum GGT activity than age. These complex relationships are depicted in Figure 1.



**Figure 1. Relationships between age, liver function parameters, circulating levels of adiponectin and PTH (independent and significant associations shown).** GGT, gamma-glutamyltransferase; ALT alanine aminotransferase; ALP, alkaline phosphatase; PTH, parathyroid hormone; ■ negative correlation. Ageing is associated with decreased GGT and ALT activities and higher serum PTH and adiponectin levels. PTH correlates with adiponectin and adiponectin is associated with higher GGT levels. A bidirectional relationship links GGT with ALT, ALP and bilirubin (positively) and albumin (negatively).

### Liver markers and short-term outcomes

In our cohort, the prevalence of prolonged LOS (>20days) was 31.6% (n=94), and the in-hospital death rate was 4.8% (n=14). Myocardial injury (cTnI rise) was observed in 27.2% (n=80) of patients, a high post-operative inflammatory response (CRP>100 mg/L) in 60.2% (n=177) and 49% of patients admitted from home have been discharged to a permanent

residential care facility.

When serum parameters (log transformed) of liver function were analysed as continuous variables by Pearson correlation, GGT activity correlated positively with prolonged hospital stay ( $r=0.140$ ,  $p=0.020$ ), and ALP correlated with a higher post-operative inflammatory response ( $r=0.123$ ,  $p=0.036$ ); albumin correlated inversely with in-hospital death ( $r= -0.137$ ,  $p=0.005$ ), postoperative cTnI rise ( $r=-0.292$ ,  $p<0.001$ ) and need to be discharged to a permanent residential care facility ( $r= -0.151$ .  $p=0.002$ ).

When each liver marker was examined as a categorical variable in multivariate adjusted analysis (with age, sex, other liver parameters and eGFR as independent variables), GGT >30U/L was associated with 1.9-fold increases in prolonged hospital stay and albumin<33g/L was associated with a 3-fold higher in-hospital mortality (Table 5). These findings remained consistent or even stronger after further adjustment for serum leptin, adiponectin, resistin levels, ratios leptin/adiponectin, leptin/resistin and adiponectin/resistin, 25(OH)D and PTH (model 2), as well as such potential confounders as alcohol consumption, smoking (current or previous), presence of DM and CVD (Table 5, model 3). Of note, in multivariate analyses, adiponectin ( $p=0.031$ ), PTH ( $p=0.001$ ), age ( $p=0.051$ ) and smoking history ( $p=0.007$ ) were also independent predictors of prolonged hospital stay, while PTH ( $p=0.001$ ) and male sex ( $p=0.03$ ) were independently associated with in-hospital death. In patients with LOS>20 days, compared to the rest of the cohort, the mean adiponectin levels were significantly higher ( $18.9 \pm 7.9$  vs.  $16.4 \pm 7.0$  ng/ml,  $p=0.022$ ). Serum adiponectin concentration above the median level (17.14 ng/ml) 2-fold increases the risk of prolonged hospital stay (OR 2.01, 95%CI 1.16-3.77,  $p=0.014$ ) after adjustments for age, sex, liver markers, 25(OH)D, PTH, eGFR, presence of CVD and DM.

**Table 5. Liver function parameters at admission as independent predictors of poorer outcomes in older patients with hip fracture**

Parameter	Outcome	Model	OR (95%CI)	P Value
GGT>30U/L	LOS>20 days	1	1.86 (1.09 - 3.19)	0.023
		2	1.98 (1.12 - 3.49)	0.019
		3	1.74 (1.08 - 3.10)	0.038
Albumin<33g/l	In-hospital death	1	3.13 (1.01 - 9.09)	0.049
		2	3.45 (1.08 - 11.11)	0.038
		3	11.11 (2.33 - 50.00)	0.003

GGT, gamma-glutamyltransferase; LOS, length of hospital stay; OR, odds ratio; CI, confidence interval.

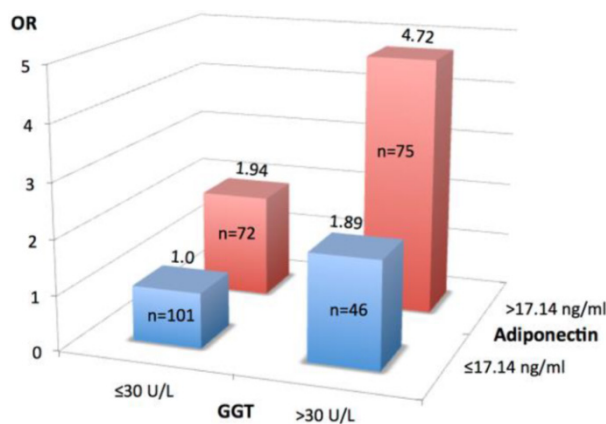
Model 1: adjusted for age, sex, liver function parameters (GGT, ALT, ALP, bilirubin, albumin, except the evaluated categorical variable), and estimated glomerular filtration rate (n=293).

Model 2: Model 1 plus serum leptin, adiponectin, resistin, ratios leptin/adiponectin, leptin/resistin, adiponectin/resistin, 25(OH)D and PTH (n=285).

Model 3: Model 2 plus alcohol consumption ( $\geq 3$  times a week), smoking (current or previous), presence of type 2 diabetes mellitus, cardiovascular disease (hypertension, coronary artery disease, history of stroke, atrial fibrillation) (n=285).

The prognostic value of GGT>30U/L for prolonged LOS [accuracy 60.4%, 95%CI 54.7 - 66.1%, sensitivity 48.9%, 95%CI 39.9 - 57.8%; specificity 65.8%, 95%CI 61.6 - 69.9%; PPV 39.8%, 95%CI 32.5 - 47.1%; NPV 73.5%, 95%CI 68.9 - 78.1%] was comparable with that of higher adiponectin level (cut-off 17.14 ng/ml) [accuracy 57.9%, 95%CI 51.6 - 63.7%, sensitivity 61.5%, 95%CI 51.8 - 70.7%; specificity 56.1%, 95%CI 51.5 - 60.4%; PPV 40.0%, 95%CI 33.6 - 45.9%; NPV 75.4%, 95%CI 69.2 - 81.3%]. However, higher GGT and adiponectin levels show synergistic prognostic value for prolonged hospital stay: the risk increases 4.7-times in patients with both these characteristics compared to subjects with none of such features (Figure 2). These two biomarkers, although interrelated, are associated with different specific pathogenetic processes; thus, for predicting LOS a two-marker approach (two biomarkers measured in parallel) is described. Compared with presence only of one of these biomarkers, combined GGT>30U/L plus adiponectin >17.14ng/ml, as would be expected, demonstrated an improvement of prognostic accuracy (72.3%, 95%CI 66.9 - 77.3), specificity (83.2%, 95% CI 79.2 - 86.8%) and PPV (57.3%, 95%CI 47.4 - 66.6%), while the sensitivity (48.9%, 95%CI 40.4 - 56.8%), and NPV (77.8%, 95% CI 74.2 - 81.3%) unchanged.

Serum albumin <33g/L has the following prognostic value for in-hospital deaths: accuracy 77.2%, 95%CI 75.0 - 79.8, sensitivity 42.9%, 95%CI 19.1-69.7%; specificity 78.9%, 95%CI 77.7 - 80.3%; PPV 9.2%, 95%CI 4.1 - 15.0%; NPV 96.5%, 95%CI 95.1 - 98.1%.



**Figure 2. Association between prolonged hospital stay (>20 days) and gamma-glutamyltransferase (GGT) and adiponectin profiles.** Study patients were classified into four groups according to serum levels of GGT (≤30U/L or >30U/L) and adiponectin (≤17.14 ng/ml and >17.14 ng/ml). Values are odds ratio (OR) adjusted for age, sex, alcohol consumption, presence of type 2 diabetes and cardiovascular disease. Number of subjects in each group is shown in the column. Group 1(n=101)-reference (OR=1.0), group 2(n=46)-high GGT and low adiponectin (OR=1.89, 95%CI 1.29 - 4.49, p=0.026), group 3 (n=72)- low GGT and high adiponectin (OR= 1.94, 95%CI 1.64 -3.22, p=0.004), group 4 (n= 75)- high GGT and high adiponectin (OR=4.72, 95%CI 2.58 - 8.65, p=0.001). Of note, both higher serum GGT (>30U/L) and adiponectin (>17.14 ng/L) levels are synergistically associated with prolonged LOS.

## Discussion

### Main findings

The results of this observational study of older HF patients showed that: (1) the prevalence of abnormal liver function tests is relatively low, but hepatic functions (even within normal range) are associated with common age-related disorders, (2) with age GGT and ALT activities decrease, while serum PTH and adiponectin concentrations increase, (3) adiponectin is an independent contributor to higher GGT, which, in turn, demonstrates bidirectional links with ALT, ALP, bilirubin and albumin levels, and (4) GGT>30U/L and albumin<33g/L on admission, regardless of other traditional risk factors, are useful independent predictors of prolonged LOS and in-hospital mortality, respectively.

Our work corroborates previous studies showing age-related decline in hepatic function, extends the understanding of the liver status and the role of adipokines, especially adiponectin, in its regulation in the elderly patients with HF, and presents novel findings of pathophysiological and clinical importance.

### Liver markers and related parameters in patients with hip fracture according to age

We found that in older HF patients the prevalence of abnormally elevated ALT, GGT, ALP or bilirubin on admission was relatively low (<10%, ranging between 1.7% and 9.9%) and comparable with or even lower than that reported in population-based studies,<sup>17, 71-75</sup> as well as in a cohort of orthopaedic patients.<sup>59</sup>

In our study, ageing was closely linked with decline in GGT, ALT and leptin concentrations and increasing adiponectin and PTH levels, both in correlation analyses and when young old (<75 years) patients were compared to the old-old (>85 years); adiponectin, leptin and PTH concentrations were significantly higher in females than in males. These findings are consistent with many, but not all<sup>21, 76, 77</sup> previous reports that serum GGT<sup>5, 9</sup> and ALT<sup>6, 8-10, 73, 78-81</sup> activities (within reference range as well as elevated) are negatively associated with age. We observe neither a decline in serum albumin, nor an increase in bilirubin with increasing age as it was reported by some authors.<sup>9</sup> No gender dependence in the mean values of liver markers was found, although lower GGT levels (≤30U/L) were about 2-times prevalent in females; this is in agreement with previous studies that men had significantly higher concentrations of GGT than women.<sup>5, 21, 23</sup> Our data are in agreement with earlier studies showing age-related increase of circulating adiponectin<sup>82-86</sup> and decrease of leptin

levels,<sup>87,88</sup> and with the well-recognized fact that both these adipokines are higher in women than in men. In previous reports, as in ours, PTH levels were significantly associated with age and higher in women than in men.<sup>89-94</sup> However, such influence of age and gender on PTH was not observed in a recent study of 35-65 year old healthy adults.<sup>95</sup> These discrepancies might be due, at least partially, to the higher mean age in our cohort.

### Liver markers and comorbidities

The high burden of pre-existing comorbidities at time of HF and its important affect on outcomes are well known, but the role and impact of liver function status on comorbidities has not yet been examined in the setting of HF. In our study, higher GGT levels (>30U/L), as might be expected, were associated with CAD, DM, and excess alcohol consumption, while lower ALT levels ( $\leq$ 20U/L) were associated with dementia; total bilirubin levels above 20 $\mu$ mol/L were associated with CAD, cervical (vs. trochanteric) HF type and alcohol overuse, and albumin concentrations above 33g/L were associated with CAD. These findings may indicate that even in the absence of overt liver disease hepatic functions are implicated in the pathogenesis of age-related disorders rather than merely be bystanders of ageing.

Our findings are consistent with numerous studies and meta-analysis showing the association between serum GGT levels (within normal limits) and CAD,<sup>21-23, 46, 47, 77, 96-102</sup> DM,<sup>25, 47, 103, 104</sup> alcohol consumption,<sup>32, 47, 105</sup> cardiovascular and all-cause mortality in older adults,<sup>14, 18, 24, 105, 106</sup> and the contribution of GGT to the pathogenesis of atherosclerotic plaques.<sup>46, 107, 108</sup> Our data are also consistent with reports that ALT is poorly linked to CVD,<sup>35, 36</sup> and GGT (but not ALT), which is regarded as less specific for liver injury than ALT, is significantly associated with insulin resistance in non-diabetic subjects.<sup>109</sup> Of note, results of our multivariate analysis demonstrated that DM and alcohol overuse were significant determinants of higher GGT (both as a continuous or categorical variable), but presence of CVD was not an independent predictor of GGT, suggesting that shared factors influence the pathogenetic relationship between the liver and CVD. The association of lower ALT levels with dementia is plausible given that ageing is a multidimensional process and age-related decrease in ALT, a highly specific liver enzyme restricted to the cytosolic component of hepatocytes,<sup>110</sup> may signify a decrease in the mass and function of the normal liver<sup>6, 7</sup> in parallel with progressive decline of other vital functions.

In our cohort, contrary to some previous studies, higher bilirubin levels were associated with CAD. Low bilirubin levels have been reported to be associ-

ated with CAD,<sup>111</sup> its severity,<sup>112</sup> increased carotid intima-media thickness,<sup>113</sup> carotid atherosclerosis,<sup>114</sup> peripheral arterial disease,<sup>115</sup> and total mortality,<sup>116</sup> while higher levels or mild to moderate elevations were associated with a protective effect against coronary microvascular dysfunction,<sup>117</sup> reduced cardiovascular events<sup>118</sup> and stroke,<sup>119-121</sup> as well as CAD in subjects with Gilbert syndrome.<sup>122</sup> On the other hand, total bilirubin has been shown to be a strong and independent predictor of morbidity and mortality in patients with heart failure,<sup>123-126</sup> acute myocardial infarction,<sup>127, 128</sup> and after heart valve surgery.<sup>129</sup>

Reports on the relationship between serum albumin level and atherosclerotic CVD are also conflicting, demonstrating a negative,<sup>111, 130</sup> positive,<sup>131-133</sup> or no association.<sup>134, 135</sup> A recent study showed that higher albumin levels were associated with insulin resistance, but did not predict the development of diabetes.<sup>136</sup> In our cohort, the odds ratio for presence of CAD was 3.1 in patients with serum albumin above 33g/L. A possible explanation for the associations between higher bilirubin levels and/or normal albuminaemia with CVD is that individuals with lower bilirubin or albumin levels have already died by the time of HF. In the current as many previous studies<sup>61-68, 137</sup> hypoalbuminaemia was strongly associated with mortality. Therefore, the high-normal bilirubin and/or albumin levels in our older HF patients with CAD are not contradictory to observations of negative correlations between serum albumin and/or bilirubin levels and cardiovascular risk factors, as well as CAD.

The abovementioned conflicting findings regarding the associations between liver markers and comorbidities might be attributable to different characteristics of the study populations (age and sex distribution, alcohol consumption and smoking habits, obesity and insulin resistance, presence of metabolic syndrome or DM) and emphasise the complexity of the underlying mechanisms indicating the importance of an integrated approach to interpretation.

### Correlations: hepatic markers and adipokines, PTH and vitamin D

To make matters even more difficult to the interpretation of results, the literature data on the inter-correlations between liver function markers, as well as on correlations between these markers and adipokines, and between adipokines and 25(OH) D and PTH in different pathologies are controversial.

In our study, GGT activity and other hepatic markers exhibited a bidirectional relationship: a positive with ALT, ALP and bilirubin and a negative with albumin. These observations are in line with many, but not all,<sup>120</sup> reports showing significant positive



correlations between GGT and ALT,<sup>5, 8, 138, 139</sup> ALP<sup>140</sup> and bilirubin.<sup>141</sup> These bidirectional links indicate that GGT activity represents an integral component of liver function.

The adipocytokines adiponectin, leptin and resistin, three best-studied pleiotropic hormones involved in a number of physiological and pathophysiological processes from energy homeostasis to inflammation and immunity, have been implicated in hepatic dysfunction.<sup>70, 142-150</sup> However, in our cohort we were not able to demonstrate an association between leptin or resistin and liver markers, except higher mean resistin levels in patients with GGT>30 U/L.

In contrast, a main novelty of this study is a significant positive correlation between serum adiponectin, the most abundant adipocytokine, and GGT activity. Yet it should be noted that the robustness of adiponectin as an independent determinant of higher GGT become obvious after adjusting for important confounders (Table 5). Our results are in contrast with those observed in patients with type 2 DM,<sup>151, 152</sup> visceral obesity,<sup>153</sup> non-alcoholic hepatic steatosis (NASH),<sup>154, 155</sup> as well as in healthy (often overweight and obese) individuals,<sup>96, 156-159</sup> and Japanese male workers,<sup>160</sup> in all of which adiponectin levels were negatively correlated with GGT. Although a significant association between ALT and adiponectin was observed in young healthy men,<sup>161</sup> in the majority of previous studies, as in ours, ALT and ALP levels were not associated with adiponectin.<sup>153, 155, 159, 162</sup> Why adiponectin is more strongly linked to GGT than to ALT and other liver markers is not entirely clear. Although serum GGT is predominantly secreted by the liver, it is present and active on the surface of most cell types where it plays an important role in glutathione metabolism; GGT may also capture extra-hepatic processes relevant to ageing.

More importantly, while in DM, metabolic syndrome and NASH adiponectin levels are reduced and aminotransferase activities increased, in advanced liver disease, paradoxically, adiponectin levels are elevated and positively correlate with markers of liver cell injury, including GGT.<sup>163-165</sup> The contrasting findings in the relationship between adiponectin and liver markers in these diseases may reflect different underlying mechanisms. For example, the strong association between adiponectin and insulin resistance in the first group of conditions<sup>166, 167</sup> was not observed in cirrhotic patients;<sup>164</sup> in subjects with normal adiponectin and leptin levels liver enzyme activities did not reflect insulin resistance.<sup>168</sup> It has been suggested that the inverse association between adiponectin and insulin could be a function of suppressed adiponectin secretion by hyperinsulinemia,<sup>169</sup> although in other

studies higher adiponectin levels predicted lower incidence of diabetes independent of prevalent insulin resistance and glycemic status.<sup>170, 171</sup> Possible explanations for these conflicting associations should also include other factors influencing adiponectin levels, such as adipose mass, hepatic<sup>163</sup> and renal<sup>172</sup> catabolism, natriuretic peptides, which directly stimulate adiponectin production,<sup>22</sup> and adiponectin resistance.<sup>173</sup>

The complex pathophysiological role of adiponectin is further reflected in contradictory reports on the relationship between adiponectin levels and mortality in animal and human studies.<sup>174-181</sup>

All these discrepancies should be interpreted in the context of known adiponectin "paradoxes":<sup>182-185</sup> 1) inverse association of circulating adiponectin level with body weight, obesity/visceral fat percentage; 2) metabolically beneficial effects of the hormone (anti-atherogenic, anti-inflammatory, insulin sensitizing, anti-fibrinogenic and anti-apoptotic properties in the liver and other organs) well-documented in experimental and human studies, low serum adiponectin concentrations in non-alcoholic fatty liver disease, atherosclerotic CVD (CAD, stroke, peripheral artery disease), hypertension, DM, metabolic syndrome, and cancers (prostate, colon, gastric, breast, leukemia) in contrast with increased adiponectin levels in advanced liver disease, including NASH-related cirrhosis, as well as in high-risk CVD patients, subjects with chronic heart failure, kidney, pulmonary and connective tissue diseases, preeclampsia, and in critically ill; 3) its favorable (protective) associations with DM, metabolic syndrome and CVD in middle-aged cohorts and the opposite (increased risk of CVD, cardiovascular outcomes and all-cause mortality) for older populations; 4) a U-shaped relationship with CVD, particularly CAD, and mortality in older adults,<sup>186</sup> although the oldest-old individuals<sup>83, 187, 188</sup> and centenarians<sup>173</sup> have higher adiponectin levels than younger subjects. It appears, therefore, that in different pathologies and age groups adiponectin may be regulated in opposite directions.

In the present study, multivariate analysis showed that in older HF patients adiponectin is correlated positively with GGT in contrast to the inverse association reported in other cohorts, including healthy persons and, especially, patients with obesity-associated chronic diseases. The fact that adiponectin was significantly higher in patients with GGT>30U/L, and GGT levels (after controlling for confounding factors) were bidirectionally inversely correlated with albumin (synthetic liver function), and positively with other hepatic markers (ALT, ALP, bilirubin), but did not predict serum adiponectin concentration, indicates the important regulatory role

of adiponectin on serum GGT activity.

The age-related increase in adiponectin and its positive association with GGT in our study were independent of other liver parameters (ALT, ALP, bilirubin and albumin), leptin (a sensitive marker of adiposity negatively associated with adiponectin) and resistin (a biomarker strongly associated with an inflammatory response) levels, 25(OH)D, PTH, eGFR, alcohol consumption, presence of DM or CVD, although some of these factors were also independent contributors to higher GGT levels (Table 4). In other words, the origin of higher serum GGT activity is multifactorial (eight factors accounted for 54.3% of GGT variance), and the elevated adiponectin concentration and its relation to GGT are part of and reflect the complex homeostatic dysregulation(-s) that accompany ageing. Consistent with this hypothesis, are our findings demonstrating that PTH, which also increases with age, correlated positively with adiponectin and in the multivariate analysis was an independent determinant of adiponectin (but not of leptin or resistin). Of note, despite the marked prevalence of vitamin D insufficiency in our cohort, with its associated increase in PTH, 25(OH)D levels were not independent contributors of adiponectin, indicating a specific affect of higher PTH levels on production and/or release of adiponectin by adipocytes. Our results are in line with observations that 25(OH)D is not associated with adiponectin in nondiabetic obese adults,<sup>229</sup> while PTH is independently associated with adiponectin in patients with heart failure.<sup>189</sup> However, in patients with primary hyperparathyroidism adiponectin concentrations were found to be normal<sup>190</sup> or reduced,<sup>191, 192</sup> did not changed or reversed by parathyroidectomy,<sup>190, 192</sup> and were not associated with PTH levels.<sup>193</sup> The complex multifactorial origin of GGT activity is further suggested by our observation of its significant correlation with TSH (positive) and T4 (negative); thyroid hormones are known to play an important role in oxidative stress balance.<sup>194</sup>

The positive correlation between PTH and adiponectin has not been previously documented in HF patients, and it may explain, at least partially, the adiponectin-GGT "paradox": although in general with ageing GGT activity decreases, adiponectin levels, mediated in part by elevated PTH, rise resulting in higher GGT levels. We hypothesise that PTH elevation may partly contribute to higher adiponectin concentration, which is clearly related to higher GGT.

### **Towards an integrated and unifying hypothesis**

Our data in line with numerous previous publications showed that decline in liver functions, increase in adiponectin and PTH are interconnected universal phenomena associated with human ageing.

Age-related rise in serum adiponectin and PTH concentrations are positively correlated, but age and adiponectin are paradoxically compatible with hepatic function, especially with GGT activity. In parallel with adiponectin elevation GGT activity increases indicating that the hormonal effect of adiponectin takes precedence over age-related suppression in the enzyme activity. The serum GGT activity reflects the integrated response of these opposite effects.

These observations raised two key questions: 1) is there a special common cause that underlies the metabolic changes occurring with advancing age, although each change results from an interplay of numerous independent mechanisms, and 2) is higher serum adiponectin concentration associated with GGT elevation, as in our case, an adoptive/compensatory response or a harmful effect.

The exact answers remain largely unknown, and any attempt to adequately explain the observations should include at least two fundamental mechanisms: homeostasis and oxidative stress. Several lines of evidence suggest close but complex interactions between oxidative stress and GGT, albumin, bilirubin, adiponectin and PTH (these factors may act as causes and consequences of oxidative stress). Oxidative stress, an imbalance between the production and inactivation of reactive oxygen species in favour of oxidants accumulation, is widely accepted as an important mechanism associated with human ageing and its adverse effects.<sup>195-199</sup> Hepatic aging is associated with greater oxidative stress and cell apoptosis.<sup>200-202</sup> GGT, a membrane-bound enzyme, plays a pivotal role in the intracellular antioxidant defence being involved in the gamma-glutamyl cycle by which extracellular glutathione is transported into cells. Depletion of intracellular glutathione, a principal intracellular antioxidant,<sup>203</sup> in response to oxidative stress results in an increase in GGT so that the metabolic homeostasis are maintained. Serum GGT activity is inversely associated with the concentration of serum antioxidants.<sup>204</sup> Serum GGT within its normal range is recognized as a sensitive marker of oxidative stress.<sup>30, 141, 203-207</sup> However, in physiological conditions, GGT may also act as a pro-oxidant,<sup>203, 204, 208</sup> generating reactive oxygen species,<sup>209, 210</sup> which could exceed the capacity of the antioxidant system and induce cellular oxidative stress damage.

The oxidative stress responses involve also other potent antioxidants, namely albumin,<sup>211</sup> the major protein in plasma, which accounts 80% of thiol's antioxidant effect in the body,<sup>212, 213</sup> bilirubin, which protects cells from a 10 000-fold excess of oxidants through rapid regeneration of bilirubin by biliverdin reductase,<sup>119, 214</sup> and adiponectin. In animal models<sup>215, 216</sup> and in humans,<sup>217, 218</sup> including the elderly,<sup>219</sup> adi-

ponectin inhibits oxidative stress, but oxidative stress suppresses adiponectin production and its powerful protective antioxidant properties.<sup>220</sup> Importantly, adiponectin is also involved in apoptosis, an evolutionary conserved controlled-death program, which ensures proper regulation of the size and quality of cell populations in tissues.<sup>221</sup> PTH may affect oxidative stress directly or by intracellular calcium accumulation.<sup>222, 223</sup> In other words, GGT, albumin, bilirubin, adiponectin and PTH, all of which play a critical role in homeostasis, may be elicited by oxidative stress and/or may have both protective and promoting effects on oxidative stress. The interplay and high degree of complexity of aforementioned and other factors involved in oxidative stress indicate the role of regulatory feedback mechanisms in different conditions. Normally, the oxidative stress responses maintain metabolic homeostasis and are beneficial for adaptation and survival, while dysregulation in the feedback processes may cause the vicious cycle(-s) of oxidants overproduction resulting in aging and aging-related diseases.<sup>224</sup> Despite considerable gaps in our knowledge, emerging data suggests that adiponectin and oxidative stress can function in both a defence and harmful manner.

In regard to our observations, age-related decrease of GGT activity may reflect the decline not only in liver function, but in the whole antioxidant defence system (including the decrease in the antioxidant effects of albumin as one of its components), and the increase in serum GGT level may be a compensatory response to oxidative stress, a recognised hallmark of ageing and chronic diseases. This response includes increases in PTH and adiponectin. The former is at least in part responsible for the adiponectin elevation, while the later enhances the anti-oxidant potential in the cells by increasing GGT and promotes apoptosis.<sup>225</sup> In this way, it can be hypothesized that in our patients, higher GGT activity which is positively associated with both adiponectin and bilirubin (also a potent antioxidant) levels, reflects a compensatory response to oxidative stress. However, in the setting of advanced age and co-morbidities this response is unable fully counter-regulate the oxidative damage and prevent its progression if oxidative stress increases, as in the perioperative period. Indeed, in our cohort, both GGT and adiponectin above the median levels were independently and synergistically associated with prolonged LOS, but not with in-hospital mortality, whereas even mild decrease in albumin concentration, an important extracellular antioxidant, demonstrated a strong relation to fatal outcome. These complex interactions are summarised in Figure 3.



**Figure 3. Diagram illustrating complex interplay between ageing, oxidative stress, parathyroid hormone (PTH), adiponectin and gamma-glutamyltransferase (GGT) and other hepatic markers and their relation to short-term outcomes in older patients with hip fracture.** LOS, length of hospital stay. In advanced age, GGT, albumin, bilirubin, adiponectin and PTH, all of which are influenced by oxidative stress, may exert both protective and promoting effects on oxidant formation, affect each other, and contribute to poor outcomes. Excessive PTH along with different other age-related factors drives adiponectin levels higher, and such elevations increase GGT activity which reflects an integrative adaptive/compensatory or pathological response (homeostatic regulation or dysregulation). In HF patients, both GGT and adiponectin concentrations above the median levels are independent markers of prolonged LOS and contribute synergistically to this outcome, hypoalbuminaemia is associated with in-hospital death, and hyperparathyroidism is an independent predictor of both fatal outcome and prolonged hospital stay.

### Liver markers and short-term outcomes

Our data shows that in older HF patients liver markers (usually within the normal ranges) on admission are associated with a continuum of risk for poor outcomes. Of clinical interest, GGT, albumin and adiponectin levels analysed as categorical variables (given the practical convenience of cut-off values), independently of known risk factors, may help to identify subjects at increased risk of prolonged hospital stay (LOS>20 days) and in-hospital death. Patients with GGT>30U/L and albumin<33g/L were near two times as likely to have a LOS>20 days, and more than 3 times as likely to have died during hospitalisation, respectively, compared to those without such conditions. The calculated sensitivity and specificity of serum GGT>30U/L for discriminating those with LOS>20 days were 48.9% and 65.8%, respectively, and of albumin <33g/L for prediction in-hospital death were 42.9% and 78.9%, respectively; serum albumin<33g/L on admission had a NPV of 96.5%. The risk of LOS>20 days also doubled in subjects with adiponectin>17.14 ng/L (median level). Furthermore, joint effects of GGT>30U/L and adiponectin>17.14 ng/ml raised the odds of LOS>20 days by 4.7-fold, demonstrating synergism; the specificity of two markers in combination for predicting LOS>20 days was 83.2%. These two biomarkers, although interrelated, are associated with different pathogenetic processes, and, not surprisingly, when

measured in parallel are more informative for predicting LOS.

Our observations are in line with previous reports that low albumin is of prognostic value on mortality in HF patients.<sup>61, 62, 65, 66, 69, 137, 226-228</sup> Our data are also concordant with clinical observations that demonstrate that serum GGT, a robust predictor of mortality due to CVD in younger individuals, did not predict mortality in individuals aged 70 years or more.<sup>69</sup> The prognostic value of GGT and/or adiponectin in HF patients has not been reported previously. Of note, in contrast to the present multivariate analyses, most of the previous prognostic studies in patients with HF examined only few liver parameters (mainly albumin) and often did not control for possible confounders, clinical and laboratory.<sup>67</sup>

The reason(-s) for prolonged LOS among patients with higher GGT and/or higher adiponectin remains unclear. The finding that only GGT and not ALT (a more specific marker of hepatic function), ALP, bilirubin or albumin was associated with LOS in adjusted models may suggest that the GGT -- LOS association reflects not only the liver status but the increased oxidative stress.

The additional (albeit moderate) prognostic information to HF outcomes provided by liver markers, accurate and not expensive laboratory tests, could be helpful in early identification and appropriate treatment of this frail population, and, consequently, improve outcomes, especially prolonged LOS. Currently, HF's represent about two-thirds of all hospital days due to fracture and account for more hospital days than any other musculoskeletal injury.<sup>228</sup>

### Limitations and strength of the study

Several limitations of this study should be considered. First, it was a cross-sectional analysis and, therefore, the cause-effect relationship could not be determined. Second, we measured serum GGT activity and total adiponectin, but the GGT fractions and high-molecular weight adiponectin, shown in some, but not all,<sup>185</sup> studies to be more informative, were not assessed. Third, potential effects of confounding from variables not represented in our models (e.g. medication used) cannot be excluded. Fourth, as the cohort was mainly Caucasian our findings may not be generalizable to other ethnic groups.

The strengths of this study are its prospective design, and the amount of obtained variables. Although multiple comparisons may potentiate the significance of multicollinearity phenomena in multivariate regression analysis, the variance inflation factor in all models presented in Tables 4 and 5 was less than 1.3, indicating that the amount of multicollinearity was not significant.

## Conclusions

The present study of older persons with hip fracture demonstrates that biochemical parameters of liver function even within its normal range in the majority of patients are relevant to comorbidities and outcomes, documents the regulatory role of adiponectin (but not leptin or resistin) as an independent contributor to GGT activity, and shows that serum GGT (>30 IU/L) and albumin (<33g/L) levels on admission may be helpful in predicting prolonged hospital stay and in-hospital death, respectively.

## Authors' contributions

Both LF and AF were involved in the design of the study, data collection and analyses, manuscript preparation and review, WS performed statistical analysis, PS was involved in drafting and review of the manuscript. All authors approved the final version of the article, including the authorship list.

## Conflicts of interest

The authors report no conflicts of interest in this work.

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