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CLINICAL STUDIES

Accuracy of b-GGT fraction for the diagnosis of non-alcoholic fatty liver disease

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Keywords
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Abbreviations
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis HCV related; gGluAMC, gamma-glutamyl-7-amido-4-methylcoumarin; GGT, gamma-glutamyltransferase; HS, healthy subjects; MW, molecular weight; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; ROC-AUC, area under ROC curve; ROC, receiver operating characteristics curve analysis.

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Abstract

Background: Serum gamma-glutamyltransferase (GGT) activity is a sensitive but non-specific marker of non-alcoholic fatty liver disease (NAFLD). Recently, four GGT fractions (big-, medium-, small-, free-GGT) were described in humans. *Aim:* We aimed to investigate whether a specific GGT fraction pattern is associated with NAFLD. *Methods:* Gamma-glutamyltransferase fractions were determined in patients with NAFLD ($n = 90$), and compared with those in control subjects ($n = 70$), and chronic hepatitis C (CHC, $n = 45$) age and gender matched. *Results:* Total GGT was elevated in NAFLD as compared to controls (median, 25°–75°percentile: 39.4, 20.0–82.0 U/L vs. 18.4, 13.2–24.9 U/L respectively, $P < 0.001$). All fractions were higher in NAFLD than in controls ($P < 0.001$). The b-GGT showed the highest diagnostic accuracy for NAFLD diagnosis [area under ROC curve (ROC-AUC): 0.85; cut-off 2.6 U/L, sensitivity 74%, specificity 81%]. Also subjects with CHC showed increased GGT (41.5, 21.9–84.5 U/L, $P < 0.001$ vs. controls, P = n.s. vs. NAFLD), as well as m-, s-, and f-GGT, while b-GGT did not show any significant increase (P = n.s. vs. HS, $P < 0.001$ vs. NAFLD). In subjects with CHC, s-GGT showed the best diagnostic value (ROC-AUC: 0.853; cut-off 14.1 U/L, sensitivity 73%, specificity 90%). Serum GGT did not show any value in the differential diagnosis between NAFLD and CHC (ROC-AUC 0.507, P = n.s.), while b-GGT/s-GGT ratio showed the highest diagnostic accuracy for distinguishing NAFLD and CHC (ROC-AUC: 0.93; cut-off value 0.16, sensitivity 82%, specificity 90%). *Conclusions:* b-GGT increases in NAFLD, but not in CHC. GGT fraction analysis might help in improving the sensitivity and specificity of the diagnosis of NAFLD and other liver dysfunctions.

Non-alcoholic fatty liver disease (NAFLD) is emerging as a major cause of liver disease in Western countries (25% of the adult populations) encompassing a spectrum of pathologic variants including simple steatosis as well as non-alcoholic steatohepatitis (3–6%) (1, 2).

Non-alcoholic fatty liver disease may evolve into advanced fibrosis, cirrhosis, end-stage liver disease, hepatocarcinoma (1), it is now recognized as the hepatic manifestation of the metabolic syndrome being associated with insulin resistance, type 2 diabetes and atherogenic dyslipidaemia (3), and increased risk for cardiovascular events (4).

The lack of accurate humoral biomarkers represents a major obstacle to the diagnosis and prevention of NAFLD, which relies mainly on ultrasonography, because of the risks and costs associated with liver biopsy. In fact, although NAFLD is associated with increased level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides, none of these markers have high specificity for the disease (5, 6).

Serum gamma-glutamyltransferase (GGT) activity is a sensitive marker of liver dysfunction, but its specificity is modest, as its value increases with common conditions causing liver dysfunction (7), such as steatosis

(8, 9) and viral hepatitis (10). GGT value has been already included in diagnostic algorithm (i.e. the Fatty Liver Index, FLI) to test the presence of steatosis and its specificity was high if considered with other markers, but low if considered alone (11).

In patients with chronic viral hepatitis C (CHC) (10), GGT elevation has been correlated with more severe hepatic fibrosis (12, 13). In fact its value has been included in a multimarker algorithm, named FibroTest, recently proposed in CHC patients as surrogate index of fibrosis (14), as a valid alternative to liver biopsy, especially to monitor disease progression (15, 16).

A new method based on molecular-size exclusion chromatography, followed by a GGT-specific post-column reaction, allowed to identify and quantify, in healthy subjects (HS), four plasma GGT fractions with high sensitivity, specificity and reproducibility (17, 18). These fractions, named big-GGT (b-GGT), medium-GGT (m-GGT), small-GGT (s-GGT) and free-GGT (f-GGT) showed different molecular weight, i.e. 2000, 1000, 250 and 70 kDa respectively (17). We have previously shown that in HS f-GGT is the most abundant fraction, while b-GGT showed the highest degree of correlation with established cardiovascular risk factors, such as level of serum triglycerides, LDL-cholesterol, C-reactive protein, diastolic blood pressure (19). Interestingly b-GGT has been found in atherosclerotic plaques together with products deriving from the pro-oxidant reactions catalysed by the enzyme, suggesting that only b-GGT, rather than total serum GGT itself, is responsible of the association between serum GGT and cardiovascular disease in population and clinical studies (20).

This study was aimed to test the hypothesis that a specific GGT fraction pattern might be associated with NAFLD, in order to improve its diagnosis and contribute to understanding the relationship between NAFLD, the so-called metabolic syndrome, and the risk of cardiovascular disease.

Patients and methods

Patient selection

Ninety consecutive patients diagnosed with NAFLD, and 45 consecutive patients with CHC were enrolled in the study at the Department of Internal Medicine of the University of Pisa. Seventy HS were selected out of 256 blood donors already characterized and studied for the determination of fractional GGT reference values (18), to obtain a cohort matched for age and gender with NAFLD and CHC patients. The baseline characteristics of patient cohorts and HS are presented in Table 1.

Non-alcoholic fatty liver disease was diagnosed on the basis of liver ultrasonography, after thorough exclusion of other sources of liver disease. In subjects with anti-HCV antibodies, viral infection was confirmed by HCV-RNA assessment. The alcohol consumption was assessed

Table 1. Baseline characteristics of patients with non-alcoholic fatty liver disease (NAFLD), chronic viral hepatitis C (CHC) and healthy subjects (HS)

	NAFLD (n = 90)	CHC (n = 45)	HS (n = 70)	P NAFLD vs. CHC	P NAFLD vs. HS	P CHC vs. HS
N males (%)	90 (72)	45 (49)	70 (70)	<0.05	n.s.	n.s.
Age, years	56.8 (12.4)	62.3 (13.7)	56.6 (6.5)	<0.05	n.s.	<0.001
BMI, kg/m^2	29.4 (6.1)	25.7 (3.5)	25.2 (2.8)	<0.01	<0.001	n.s.
Diabetes, *n* (%)*	18 (20.0)	1 (2.2)	0	<0.05	<0.001	n.s.
Hypertension, *n* (%)*	44 (48.8)	3 (6.7)	0	<0.001	<0.001	n.s.
Glucose, mg/dl	101.0 (29.1)	92.0 (19.1)	97.8 (9.6)	n.s.	n.s.	n.s.
Creatinine, mg/dl	1.1 (0.5)	0.8 (0.2)	0.9 (0.2)	n.s.	n.s.	n.s.
eGFR, ml/min/1.73 m^2	153.2 (53.0)	80.2 (23.3)	93.4 (22.1)	n.s.	<0.001	<0.001
Total cholesterol, mg/dl	218.8 (56.9)	170.1 (31.3)	199.4 (34.2)	<0.001	<0.05	<0.01
HDL cholesterol, mg/dl	45.0 (14.8)	53.1 (16.3)	57.0 (13.7)	<0.05	<0.001	n.s.
LDL cholesterol, mg/dl	142.8 (48.8)	100.6 (28.9)	129.4 (34.0)	<0.001	n.s.	<0.01
Triglycerides†, mg/dl	167.8 (104.3)	101.2 (47.6)	83.5 (28.2)	<0.001	<0.001	n.s.
AST, U/L	28.9 (30.4)	59.0 (41.2)	18.9 (4.4)	<0.001	n.s.	<0.001
ALT, U/L	40.7 (43.1)	57.8 (44.7)	18.5 (5.9)	<0.05	<0.001	<0.001
Total bilirubin, mg/dl	0.75 (0.29)	0.82 (0.54)	0.85 (0.46)	n.s.	n.s.	n.s.
Direct bilirubin, mg/dl	0.16 (0.09)	0.29 (0.21)	0.13 (0.05)	<0.01	n.s.	<0.001
Haemoglobin, g/dl	9.8 (16.8)	13.3 (1.6)	14.9 (1.1)	<0.01	n.s.	<0.01
Leucocytes, x10^9 L	6.7 (1.6)	5.2 (1.5)	6.1 (1.4)	<0.001	<0.05	<0.05

*Chi-squared test.

†Statistical analysis performed on ln-transformed data.

Data are reported as means (SD). AST, aspartate amino transferase; ALT, alanine amino transferase; BMI, body mass index; eGFR, estimated glomerula filtration rate; GGT, gamma-glutamyltransferase. eGFR has been estimated by MDRD formula; LDL cholesterol has been estimated by the Friedewald formula. Statistical analysis: one-way ANOVA followed by Tukey's multiple comparison test. n.s., not significant.

by a direct interview of the participants to establish the amount and the modality of alcohol consumption. According to World Health Organisation recommendations concerning hazardous alcohol consumption, subjects assuming more than 45 g/day (men) or 30 g/day (women) were excluded from the study (21).

The Institutional Ethics Committee approved the study and all subjects gave informed consent.

Laboratory analyses

Standard assay of all blood tests including serum GGT were simultaneously performed according to the standard clinical laboratory procedures by automated analysers (Beckman Synchron CX 9- PRO analyser, Beckman Coulter, Brea, CA, USA; Abbott Cell-Dyn Sapphire for blood cell count, Abbott Laboratories, Libertyville, IL, USA). Estimated glomerular filtration rate (eGFR) was estimated by the MDRD (Modification of Diet in Renal Disease) formula [eGFR ml/min/1.73 $m^2 = 186.3 \times \text{Creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if woman)]; LDL cholesterol was calculated using the Friedewald formula [LDL-C = total cholesterol−HDL cholesterol−(triglycerides/5)].

Gamma-glutamyltransferase fraction analysis

Analysis of total and fractional GGT was performed, as previously described (17, 18), on plasma-ethylenediamine-tetra-acetic acid samples using a fast protein liquid chromatography system (AKTA purifier; GE Healthcare Europe, Milan, Italy) equipped with a gel-filtration column (Superose 6 HR 10/300 GL; GE Healthcare Europe) and a fluorescence detector (Jasco FP-2020; Jasco Europe, Lecco, Italy). Separation of fractional GGT was obtained by gel-filtration chromatography and the enzymatic activity was quantified by post-column injection of the fluorescent substrate for GGT, gamma-glutamyl-7-amido-4-methylcoumarin (gGluAMC). Enzymatic reaction, in the presence of gGluAMC 0.030 mmol/L and glycylglycine 4.5 mmol/L, proceeded for 4.5 min in a reaction coil (PFA, 2.6 mL) kept at the 37°C in a water bath. The fluorescence detector operating at excitation/emission wavelengths of 380/440 nm detected the AMC signal; the intensity of the fluorescence signal was expressed in arbitrary fluorescence units. Under this reaction conditions, area under curve (AUC) is proportional to GGT activity. Fractional GGT activity was quantified as previously described (18). The overall cost per sample for fractional GGT analysis is 4 euro (5.56$), including cost of consumables 2.62 euro (3.64$)/sample.

Statistical analysis

Statistical analysis was conducted by one-way ANOVA analysis followed by Tukey's multiple comparison test. Total, b-, m- and s-GGT, as well as b/s ratio and triglyceride values were ln-transformed to reduce the distribution skewness. Proportion of diabetes and hypertension among the three groups was compared by the chi-squared test.

Receiver operating characteristics curve analysis (ROC) was performed to establish the diagnostic power of total and fractional GGT and of the b-GGT/s-GGT ratio values. In fact, ROC analysis provided the AUC values, which define the increment in predictive power between variables, as well as corresponding sensitivity, specificity, positive and negative predictive values and the optimum cut-off values (at the point of ROC corresponding to maximal sum of specificity and sensitivity). ROC analysis and the comparison between ROC curves were performed with MEDCALC 11.5 analysis software on the basis of the De Long method (22).

Results

Patients' characteristics

No difference was found among patients with NAFLD, CHC and controls as concerns the level of serum glucose, creatinine and total bilirubin. As expected, patients with NAFLD showed significantly higher level of BMI ($P < 0.001$), total cholesterol ($P < 0.05$), triglycerides ($P < 0.001$), ALT ($P < 0.001$), and lower values of HDL cholesterol ($P < 0.001$), as compared to HS (Table 1). As compared to NAFLD, CHC patients showed higher values of HDL cholesterol, AST and ALT, and lower LDL cholesterol and triglycerides.

Serum GGT values in NAFLD and CHC were very similar and significantly higher than in HS ($P < 0.001$) (Table 2).

Fractional gamma-glutamyltransferase analysis

The b-GGT fraction in NAFLD patients was higher than in CHC patients ($P < 0.001$) and HS ($P < 0.001$) (Table 2). m-GGT, s-GGT and f-GGT fractions were all significantly higher in NAFLD ($P < 0.001$) and CHC ($P < 0.05$, <0.001, <0.01, respectively) as compared to HS (Table 2), while no difference was seen between NAFLD and CHC patients. Because of the concomitant increase of b-GGT and s-GGT in NAFLD, the b/s ratio in NAFLD was not significantly different than that in HS, but significantly higher than in CHC ($P < 0.001$).

Diagnostic value of fractional gamma-glutamyltransferase: receiver operating characteristic curve analysis

In ROC analysis, b-GGT showed the greatest accuracy for the NAFLD diagnosis among fractions (cut-off: 2.6 U/L; $P = 0.02$ vs. s-GGT, $P < 0.001$ vs. m-GGT, f-GGT), while s-GGT showed a higher accuracy for the diagnosis of CHC (cut-off: 14.1 U/L; $P < 0.0001$ vs. all the other fractions) (Tables 3 and 4).

The b/s ratio showed at a same time the highest diagnostic accuracy for CHC (cut-off: 0.16; $P < 0.0001$ vs.

Table 2. Total and fractional gamma-glutamyltransferase (GGT) activity (U/L) in plasma of patients with non-alcoholic liver disease (NAFLD), chronic viral hepatitis C (CHC) and healthy subjects (HS)

	NAFLD (n = 90)	CHC (n = 45)	HS (n = 70)	P NAFLD vs. CHC	P NAFLD vs. HS	P CHC vs. HS
Total GGT*	39.4 (20.0–82.0)	41.5 (21.9–84.5)	18.4 (13.2–24.9)	n.s.	<0.001	<0.001
b-GGT*	5.1 (2.5–14.9)	2.1 (1.2–5.3)	1.7 (1.0–2.4)	<0.001	<0.001	n.s.
m-GGT*	1.7 (0.6–3.7)	1.2 (0.4–3.7)	0.6 (0.4–0.9)	n.s.	<0.001	<0.05
s-GGT*	14.9 (5.9–43.5)	22.9 (10.0–59.6)	4.7 (2.9–8.9)	n.s.	<0.001	<0.001
f-GGT	14.2 (10.3–18.6)	13.8 (9.3–16.9)	9.9 (8.4–12.2)	n.s.	<0.001	<0.01
b/s ratio*	0.37 (0.24–0.51)	0.10 (0.07–0.15)	0.32 (0.22–0.48)	<0.001	n.s.	<0.001

Total and fractional GGT data are presented as median (25th–75th percentile).
*One-way ANOVA followed by Tukey's multiple comparison test performed on ln-transformed data.
n.s., not significant.

Table 3. Results of the receiver operating characteristic curve analysis

	HS (N = 70) vs. NAFLD (N = 90)	HS (N = 70) vs. CHC (N = 45)	NAFLD (N = 90) vs. CHC (N = 46)
Total GGT	0.807* (0.737–0.865)	0.803* (0.718–0.871)	$0.507^{n.s.}$ (0.419–0.694)
b-GGT	0.850* (0.785–0.901)	$0.601^{n.s.}$ (0.506–0.692)	0.743* (0.661–0.814)
m-GGT	0.743* (0.688–0.809)	0.662‡ (0.568–0.748)	$0.554^{n.s.}$ (0.466–0.640)
s-GGT	0.788* (0.716–0.848)	0.853* (0.774–0.912)	$0.573^{n.s.}$ (0.485–0.658)
f-GGT	0.726* (0.650–0.793)	0.689† (0.596–0.772)	$0.557^{n.s.}$ (0.469–0.643)
b/s ratio	$0.555^{n.s.}$ (0.474–0.633)	0.912* (0.845–0.957)	0.931* (0.874–0.967)
AST	0.722* (0.644–0.790)	0.899* (0.827–0.948)	0.796* (0.716–0.862)
ALT	0.793* (0.721–0.853)	0.879* (0.804–0.933)	0.657‡ (0.568–0.738)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, patients with chronic hepatitis HCV related; NAFLD, patients with non-alcoholic fatty liver disease; n.s., not significant; ROC-AUC, area under receiver operating characteristic curve.
Data are ROC-AUC (95% CI).
*$P < 0.0001$;
†$P < 0.001$;
‡$P < 0.01$.

Table 4. Diagnostic values for total and fractional gamma-glutamyltransferase (GGT) for the diagnosis of non-alcoholic fatty liver disease (NAFLD) and chronic viral hepatitis C (CHC)

	Cut-off	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV%	NPV%
NAFLD vs. CHC					
Total-GGT*	13.3 U/L	11.1 (3.7–24.1)	98.9 (94.0–100.0)	83.3	69.0
b-GGT	1.8 U/L	44.4 (29.6–60.0)	92.2 (84.6–96.8)	74.1	76.9
m-GGT*	0.5 U/L	26.7 (14.6–41.9)	86.7 (77.9–92.9)	55.0	70.3
s-GGT	14.7 U/L	71.1 (55.7–83.6)	50.0 (39.3–60.7)	41.6	77.6
f-GGT*	17.3 U/L	86.7 (73.2–94.9)	30.0 (20.8–40.6)	38.2	81.8
b/s ratio	0.15	80.0 (65.4–90.4)	92.2 (84.6–96.8)	83.7	90.2
HS vs. NAFLD					
Total GGT	26.2 U/L	66.7 (55.9–76.3)	82.7 (72.0–90.8)	83.3	65.9
b-GGT	2.6 U/L	74.44 (64.2–83.1)	81.4 (79.3–89.7)	83.7	71.2
m-GGT	1.7 U/L	51.1 (40.3–61.8)	94.3 (86.0–98.4)	92.0	60.0
s-GGT	17.5 U/L	48.9 (38.2–59.7)	95.7 (88.0–99.1)	93.6	59.3
f-GGT	13.9 U/L	52.2 (41.1–62.9)	88.6 (78.7–94.9)	85.5	59.0
b/s ratio*	0.37	50.0 (39.3–60.7)	65.7 (53.4–76.6)	65.2	50.2
HS vs. CHC					
Total GGT	26.2 U/L	71.1 (55.7–83.6)	82.9 (72.0–90.8)	72.7	81.7
b-GGT*	2.7 U/L	44.4 (29.6–60.0)	82.86 (72.0–90.8)	62.5	69.9
m-GGT	1.9 U/L	42.2 (27.7–57.8)	97.1 (90.1–99.7)	90.5	72.3
s-GGT	14.1 U/L	73.3 (58.1–85.4)	90.0 (80.5–95.9)	82.5	84.0
f-GGT	14.3 U/L	46.7 (31.7–62.1)	90.0 (80.5–95.9)	75.0	72.4
b/s ratio	0.16	82.2 (67.9–92.0)	90.0 (80.5–95.9)	84.1	88.7

*ROC-AUC was not significantly different from 0.5, see Table 3. HS, healthy subjects; NPV, negative predictive value; PV, positive predictive value.

b-GGT, m-GGT, f-GGT, $p = 0.060$ vs. s-GGT) and for the differential diagnosis between NAFLD and CHC (cut-off: 0.15; $P < 0.001$ vs. all GGT fractions) (Tables 3 and 4).

Although also ALT showed a high ROC-AUC (Table 3) for the diagnosis of NAFLD and CHC, it showed a diagnostic power significantly lower than b/s ratio ($P < 0.0001$) in the differential diagnosis between the two conditions.

Discussion

The main finding of this study is that patients with NAFLD and CHC display different GGT fraction patterns, despite similar total GGT activity values.

Among all the GGT fractions, b-GGT provided the best specificity and sensitivity for the diagnosis of NAFLD, with an accuracy equivalent or even greater to those provided by available diagnostic algorithms such as SteatoTest (ROC-AUC 0.79, SE 0.03) (23) or FLI (ROC-AUC 0.85, 95% CI 0.81–0.88) (11). Interestingly, these algorithms include total serum GGT activity and it is likely that replacing GGT with b-GGT would increase their sensitivity and specificity.

In subjects with CHC, b-GGT did not show any significant change, while s-GGT showed a prominent increase, thus suggesting that the ratio between these fractions was a key aspect of the disease-associated GGT fraction pattern. For this reason, although the study was not originally designed to test the diagnostic value of the ratios between GGT fractions, the diagnostic value of the b/s ratio was tested, and indeed showed the highest specificity for distinguishing between CHC and NAFLD, as well as for the diagnosis of CHC as compared to total GGT and all individual fractions. All other ratios between the remaining fractions were also tested, but their ROC-AUCs were significantly lower (not shown).

Although an obvious consideration is that the diagnosis of CHC relies on virological tests, rather than on serum enzymes, these findings for the first time open the perspective of a positive diagnosis of NAFLD that might be helpful as a screening test or to perform large population studies on the prevalence of NAFLD and related diseases.

The precise nature of GGT fractions has not yet been established, and at present it is not possible to speculate on the possible reasons conducting to different GGT fraction patterns in NAFLD and CHC. The fact that in NAFLD the increase of serum GGT occurs through a proportional increase of b-GGT and s-GGT, while in viral hepatitis occurs through a prominent increase of s-GGT suggests that GGT fraction pattern specificity might depend on its ability to reflect the different extents of inflammatory, structural and functional derangement in liver disease.

On the basis of the present results, further studies on larger population will show if GGT fraction analysis might also be used to identify subjects with NAFLD progressing towards parenchymal inflammation and fibrosis, which might experience a progressive decrease of the b/s ratio.

The use of GGT fractional analysis might also contributes to the understanding of the complex epidemiological association between serum GGT, NAFLD and cardiovascular disease (9). In fact, during the last decade, large population studies have shown that serum GGT (independently from alcohol consumption) is one of the strongest predictors of metabolic syndrome (4, 24), diabetes (25, 26) as well as final events (myocardial infarction and stroke) associated with the atherosclerotic disease (27–29), thus opening new perspectives as concerns the association between liver damage, metabolism and atherosclerosis. We showed that GGT activity, in the presence of trace iron and glutathione, its physiological substrate, catalyses the production of free radicals and reactive oxygen species, thus promoting LDL oxidation *in vitro* (30), and we found that human atherosclerotic plaques contain GGT activity in correspondence of the oxidized LDL and $CD68^+$ foam cells (31, 32). The GGT extracted from human carotid plaques was found to correspond in part to the human serum b-GGT fraction (20). The fact that b-GGT, which has a potential atherogenic role (19, 20), was found in this study to increase specifically in NAFLD, a condition strictly associated with metabolic syndrome, iperinsulinemia, and dyslipidemia (3), suggests that NAFLD act as cardiovascular risk factor by promoting b-GGT accumulation and GGT-related oxidative events within the plaque. Thus, results of this study might help to define a new pathway connecting dysmetabolism with cardiovascular disease.

In conclusion, GGT fraction analysis revealed, in NAFLD, a GGT fraction pattern characterized by increased levels of all GGT fractions, including b-GGT, which did not increase in subjects with HCV, and similar levels of serum GGT. Extensive investigation on the diagnostic value of GGT fractions might provide a novel diagnostic tool for liver diseases; understanding the nature, properties, and pathophysiological variations of GGT fraction pattern might allow a better understanding of the pathogenesis of the diseases associated with increased GGT.

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References

1. Bedogni G, Miglioli L, Masutti F *et al.* Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005; **42**: 44–52.

2. Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. *Semin Liver Dis* 2008; **28**: 339–50.
3. De Alwis NM, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. *J Hepatol* 2008; **48**(Suppl.1): S104–12.
4. Lee DS, Evans JC, Robins SJ *et al.* Gamma glutamyltransferase and metabolic syndrome, cardiovascular disease, and mortality risk: the Framingham Heart study. *Arterioscler Thromb Vasc Biol* 2007; **27**: 127–33.
5. Loria P, Adinolfi LE, Bellentani S *et al.* NAFLD Expert Committee of the Associazione Italiana per lo studio del Fegato. Practice guidelines for the diagnosis and management of nonalcoholic fatty liver disease. A decalogue from the Italian Association for the Study of the Liver (AISF) Expert Committee. *Dig Liver Dis* 2010; **42**: 272–82.
6. Fierbinteanu-Braticevici C, Dina I, Petrisor A *et al.* Noninvasive investigations for non alcoholic fatty liver disease and liver fibrosis. *World J Gastroenterol* 2010; **16**: 4784–91.
7. Whitfield JB. Gamma glutamyl transferase. *Crit Rev Clin Lab Sci* 2001; **38**: 263–355.
8. Fraser A, Ebrahim S, Smith GD, Lawlor DA. A comparison of associations of alanine aminotransferase and gamma-glutamyltransferase with fasting glucose, fasting insulin, and glycated hemoglobin in women with and without diabetes. *Hepatology* 2007; **46**: 158–65.
9. Haring R, Wallaschofski H, Nauck M *et al.* Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated serum gamma-glutamyl transpeptidase levels. *Hepatology* 2009; **50**: 1403–11.
10. Itoh S, Nakajima M. Liver gamma-glutamyl-transferase activity in viral liver-disease. *Digestion* 1986; **33**: 121–5.
11. Bedogni G, Bellentani S, Miglioli L *et al.* The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33–39.
12. Hwang SJ, Luo JC, Lai CR *et al.* Clinical, virological and pathological significance of elevated serum gamma-glutamyltranspeptidase in patients with chronic hepatitis C. *Zhonghua Yi Xue Za Zhi (Taipei)* 2000; **63**: 527–35.
13. Vardar R, Vardar E, Demiri S *et al.* Is there any non-invasive marker replace the needle liver biopsy predictive for liver fibrosis, in patients with chronic hepatitis? *Hepatogastroenterology* 2009; **56**: 1459–65.
14. Imbert-Bismut F, Ratziu V, Pieroni L *et al.*; MULTIVIRC Group. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069–75.
15. Shaheen AA, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: a systematic review of diagnostic test accuracy. *Am J Gastroenterol* 2007; **102**: 2589–600.
16. Halfon P, Munteanu M, Poynard T. FibroTest-ActiTest as a non-invasive marker of liver fibrosis. *Gastroenterol Clin Biol* 2008; **32**: 22–39.
17. Franzini M, Bramanti E, Ottaviano V *et al.* A high performance gel filtration chromatography method for gamma-glutamyltransferase fraction analysis. *Anal Biochem* 2008; **374**: 1–6.
18. Franzini M, Ottaviano V, Fierabracci V *et al.* Fractions of plasma gamma-glutamyltransferase in healthy individuals: reference values. *Clin Chim Acta* 2008; **395**: 188–9.
19. Franzini M, Paolicchi A, Fornaciari I *et al.* Cardiovascular risk factors and gamma-glutamyltransferase fractions in healthy individuals. *Clin Chem Lab Med* 2010; **48**: 713–7.
20. Franzini M, Corti A, Martinelli B *et al.* Gamma-glutamyltransferase activity in human atherosclerotic plaques–biochemical similarities with the circulating enzyme. *Atherosclerosis* 2009; **202**: 119–27.
21. Schellenberg F, Schwan R, Mennetrey L *et al.* Dose effect relation between daily ethanol intake in the range 0-70 grams and %CDT value: validation of a cut-off value. *Alcohol Alcohol* 2005; **40**: 531–4.
22. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; **44**: 837–45.
23. Poynard T, Ratziu V, Naveau S *et al.* The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005; **4**: 10–24.
24. Ryu S, Chang Y, Woo HY *et al.* Longitudinal increase in gamma-glutamyltransferase within the reference interval predicts metabolic syndrome in middleaged Korean men. *Metabolism* 2010; **59**: 683–9.
25. Lim JS, Lee DH, Park JY, Jin SH, Jacobs DR Jr. A strong interaction between serum gammaglutamyltransferase and obesity on the risk of prevalent type 2 diabetes: results from the Third National Health and Nutrition Examination Survey. *Clin Chem* 2007; **53**: 1092–8.
26. Fraser A, Harris R, Sattar N *et al.* Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. *Diabetes Care* 2009; **32**: 741–50.
27. Emdin M, Passino C, Michelassi C *et al.* Prognostic value of serum gamma-glutamyl transferase activity after myocardial infarction. *Eur Heart J* 2001; **22**: 1802–7.
28. Ruttmann E, Brant LJ, Concin H *et al.* Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality. An investigation in a cohort of 163,944 Austrian adults. *Circulation* 2005; **112**: 2130–7.
29. Fraser A, Harris R, Sattar N *et al.* Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol intake: analysis of the British Women's Heart and Health Study and Meta-Analysis. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2729–35.
30. Paolicchi A, Minotti G, Tonarelli P *et al.* Gamma-glutamyl transpeptidase-dependent iron reduction and LDL oxidation – a potential mechanism in atherosclerosis. *J Investig Med* 1999; **47**: 151–60.
31. Paolicchi A, Emdin M, Ghliozeni E *et al.* Human atherosclerotic plaques contain gammaglutamyltranspeptidase enzyme activity [Images in cardiovascular medicine]. *Circulation* 2004; **109**: 1440.
32. Emdin M, Passino C, Donato L, Paolicchi A, Pompella A. Serum gamma-glutamyltransferase as a risk factor of ischemic stroke might be independent of alcohol consumption. *Stroke* 2002; **33**: 1163–4.