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The triglycerides and glucose (TyG) index: a new marker associated with nonalcoholic steatohepatitis (NASH) in obese patients

Benjamin Rivière ^a, Audrey Jausset* ^b, Valérie Macioce* ^b, Stéphanie Faure ^c, Nicolas Builles ^d, Patrick Lefebvre ^e, Philippe Géraud ^f, Marie-Christine Picot ^{b,f}, Sandra Rebuffat ^g, Eric Renard ^{e,f}, Valérie Paradis ^h, Marie-Dominique Servais ⁱ, Nathalie de Preville ⁱ, David Nocca ^j, COMET study group (see Appendix; supplementary materials associated with this article on line), Anne-Dominique Lajoix[†] ^g, Georges-Philippe Pageaux[†] ^c, Florence Galtier ^{e,f}

*,[†] These authors equally contributed to this work

^a Pathology Department, CHU Montpellier, Univ Montpellier, 80 Avenue Augustin Fliche, 34295 Montpellier Cedex 5, France

^b Clinical research and epidemiology unit, CHU Montpellier, Univ Montpellier, 39 avenue Charles Flahault, 34295 Montpellier, France

^c Hepato-gastroenterology department, CHU Montpellier, Univ Montpellier, 34295 Montpellier, France

^d Biological Resources Center; Tissue Bank, CHU Montpellier, Univ Montpellier, 80 Avenue Augustin Fliche, 34295 Montpellier Cedex 5, France

^e Endocrinology Department, CHU Montpellier, Univ Montpellier, 371 Av du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France

^f Clinical Investigation Center 1411, INSERM, CHU Montpellier, Univ Montpellier, 80 Avenue Augustin Fliche, 34295 Montpellier Cedex 5, France

^g Biocommunication in Cardio-Metabolism (BC2M), University of Montpellier, 15 avenue Charles Flahault, 34093 Montpellier cedex 5, France

^h DHU UNITY, Pathology Department, Hôpital Beaujon, AP-HP, Clichy, France

ⁱ Servier, 50 rue Carnot, 92284 Suresnes Cedex, France

^j Department of Digestive Surgery, CHU Montpellier, Univ Montpellier, 80 Avenue Augustin Fliche, 34295 Montpellier Cedex 5, France

Corresponding author: Florence GALTIER, f-galtier@chu-montpellier.fr

Centre d'Investigation Clinique, Hôpital St Eloi, 80 Avenue Augustin Fliche

34295 Montpellier Cedex 5

Tel: 04 67 33 23 29

GSM: 07 88 01 43 43

Fax: 04 67 33 23 35

Conflict of interest statement

The authors declare that they have no competing interests

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Abstract

Aim. - Diagnosis of nonalcoholic steatohepatitis (NASH) relies on liver biopsy. Noninvasive tools would be useful to target patients to refer for a biopsy. We aimed to determine the diagnostic value of the triglycerides and glucose (TyG) index, an insulin-resistance indicator, to predict NASH.

Methods. - Our study included grade II-III obese patients aged 18-65 years undergoing bariatric surgery and included in the COMET (COllection of MEtabolic Tissues) biobank (NCT02861781). Liver biopsies performed during bariatric surgery were collected from the biobank along with blood derivatives. Biopsies were analysed according to the steatosis, activity and fibrosis (SAF) scoring system to diagnose NASH, nonalcoholic fatty liver disease (NAFLD), and fibrosis. Logistic regression models were performed to identify factors predicting NASH, NAFLD, and fibrosis.

Results. - Of 238 analysed subjects (mean age 43 ± 12 years, 33.6% men), 29% had type 2 diabetes. Steatosis was present in 67.2%, while NASH and advanced fibrosis (stage F3) were diagnosed in 18.1% and 2.9% respectively. TyG index was independently associated with NASH (odds ratio (OR): 4.7 [95% confidence interval: 2.3;9.5] $P < 0.0001$), NAFLD (OR: 2.0 [1.1;3.7] $P = 0.03$) and stages 2-3 fibrosis (OR: 4.0 [1.5;10.8] $P = 0.007$). NASH was also predicted by gamma-glutamyl transferase (GGT) with an area under the ROC curve: 0.79 [0.71;0.87] $P = 0.04$ for GGT and TyG index combined.

Conclusion. - In our cohort of severely obese patients, TyG index, when associated with GGT level, exhibited high diagnostic performance to predict NASH. Although validation in larger populations is needed, this result may be of considerable clinical value to predict need for liver biopsy.

Key words; Fibrosis; Morbid obesity; NAFLD; NASH, TyG

Introduction

According to the World Health Organization (WHO), overweight and obesity affected more than 1.9 billion and 650 million adults in the world, respectively, in 2016 [1]. Paralleling the obesity epidemic, nonalcoholic fatty liver disease (NAFLD, defined by an accumulation of triglycerides in more than 5% of hepatocytes) is currently the most common chronic liver disease, currently affecting both developed and developing countries with a global prevalence of 25% [2, 3]. NAFLD may progress to more severe liver disease, namely nonalcoholic steatohepatitis (NASH), which associates lipid accumulation with cellular damages (ballooning, necrosis, and inflammation), and different degrees of fibrosis. Although less prevalent than NAFLD (1.5 to 6.5% in the general population) [1, 2], NASH must be recognised early as liver inflammation can trigger more severe hepatic complications such as fibrinogenesis, cirrhosis and finally hepatocarcinoma. In France, the general-population NASH-Co cohort showed that 18.2% of individuals had NAFLD, and among them, that 2.6% had advanced fibrosis [4].

Both NAFLD and fibrosis can be screened by noninvasive tests. For NAFLD, fatty liver index [5] and ultrasound examination can be used. Several methods have been developed for fibrosis: fibrosis-4 index (FIB-4) uses alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), platelet counts and age of patient to evaluate the risk of severe hepatic fibrosis [6]; Fibrotest [7] is more indicative of the level of fibrosis; transient elastography using FibroScan™ [8]. However, NASH diagnosis relies solely on histopathological analysis of a liver biopsy. For this reason, a noninvasive tool is obviously needed to select candidates for liver biopsy. This issue is of increasing concern, and several potential biomarkers have been explored [9, 10]. Routine liver tests, e.g. ALAT and gamma-glutamyl transferase (GGT) do not correlate with necro-inflammatory activity and fibrosis in patients with NAFLD [11]. Sophisticated molecular methods involving a combination of proteic and nucleic biomarkers like NIS4™ technology [12], and breathing measures following intravenous injection of ¹³C-methacetin [13], are emerging, but cannot be used for routine screening. Thus, to date, no noninvasive biomarker has been validated to diagnose NASH in clinical practice.

As insulin resistance and NASH share many common features, insulin sensitivity indices may be related to the severity of liver damage. However, most insulin resistance scores, such as HOMA [14], QUICKI [15] and MATSUDA [16], are based on fasting plasma insulin, an assay that has several limitations in clinical practice: *i*) plasma insulin level varies with the duration of the fasting period; *ii*) haemolysis-related interferences are common; *iii*) it is not a good indicator in patients with long-term

diabetes and impaired insulin secretion. More recently, the triglycerides and glucose (TyG) index has been proposed as an alternative method to evaluate insulin resistance. TyG index is calculated using fasting plasma glucose and serum triglycerides [17], which are inexpensive and routine biomarkers. TyG index was first shown to be correlated with a glucose clamp evaluation of insulin sensitivity in a population that included patients with type 2 diabetes mellitus (T2DM) [18] and has since been used in several other populations, including children and adolescents [17, 19]. Interestingly, TyG index has been shown to predict both prevalence and incidence of NAFLD in the general population [20-22], and was already suggested as effective in screening for NAFLD and NASH in a small group of asymptomatic women [23].

All patients undergoing bariatric surgery are at-risk patients for NAFLD and NASH, and are evaluated for glucose and lipid profiles, as well as liver function, before surgery. Using these routine parameters would be extremely useful in clinical practice to identify patients who need a liver biopsy. Therefore, the main objective of this study was to determine whether insulin-resistance indices, and particularly TyG index, could be of diagnostic value in screening for patients who would require further hepatic investigations.

Material and methods

Biobank

Our study was carried out in grade II-III obese patients (body mass index [BMI] ≥ 35 kg/m²) undergoing bariatric surgery, and included in the COMET (Collection of METabolic Tissues) biobank (<https://cometbiobank.com/>). COMET is a project initiated in 2015 aiming at investigating factors associated with metabolic complications of obesity. The biobank contains blood derivatives and insulin-sensitive tissue samples collected from 270 patients stratified according to metabolic status, i.e. insulin sensitivity (HOMA-IR [14] < 3), insulin resistance without diabetes [24] (HOMA-IR ≥ 3), and T2DM [24]. In each metabolic category, patients are stratified by age (18-29, 30-39, 40-49 and 50-64 years). Recruitment stratification was planned as follows: patients with diabetes (n=90); patients with insulin resistance (n=80, including 20 patients in each age category 18-29, 30-39, 40-49, 50-64); patients with normal insulin sensitivity (n=100, including 20 patients in each age category 18-29, 30-

39, 40-49, 50-64 as well as 20 patients aged 18-29 years with a history of diabetes in a 1st degree relative).

COMET is in compliance with the French regulations and approved by the National Agency for the Safety of Medicines and Health Products and by the Committee for the Protection of Persons (Sud Méditerranée I Ethics Committee). It has been declared on clinicaltrials.gov (NCT02861781). Data management, sampling procedures and samples preservation were managed with a Quality Assurance system guaranteeing the high quality and reliability of the data and biological samples used in this study. The biobank is located at the Biological Resource Center (BRC) of the University Hospital of Montpellier, France (BB-0033-00031).

Patients

Patient recruitment for COMET biobank started on 2 February 2016. Inclusion criteria were an indication for bariatric surgery according to current recommendations (grade III obesity or grade II obesity with one or more significant comorbid conditions), age 18-64 years, negative serology for human immunodeficiency virus, hepatitis B virus and hepatitis C virus. Exclusion criteria were diabetes other than T2DM, use of immunosuppressive or anti-inflammatory drugs, previous bariatric surgery except laparoscopic adjustable gastric banding, history of chronic inflammatory disease or neoplasia and alcohol misuse, defined as either mean alcohol intake over three glasses/day or addiction to alcohol. All consecutive patients eligible for bariatric surgery were invited to participate in the protocol, until completion of each age/metabolic category. They were informed about the study, gave written consent to study procedures and use of their biospecimen and associated data in research programs and received a copy of the signed document. Medical history, lifestyle habits (including tobacco and alcohol consumption) and current treatments were recorded. From data extracted on 9 May 2021, 264 patients were included in COMET, and 238 patients with complete biochemistry and liver biopsy results were included in the present analysis ([Fig. 1](#)).

Sampling procedures

Patients attended the surgery department the day immediately before their surgical procedure, and weight and height were recorded. The day of surgery, after an overnight fast, blood was drawn immediately before induction of anaesthesia, to measure plasma glucose, plasma insulin, total and

HDL-cholesterol, triglycerides, liver tests and blood cell count. Patients then underwent laparoscopic sleeve gastrectomy or bypass, during which biopsies from several insulin-sensitive tissues (liver, muscle, subcutaneous tissue and visceral fat) were obtained. Liver samples were wedge biopsies taken from segment 2 or 3 of the left lobe. Blood samples were also collected 3 months and 12 months after surgery.

Analyses

Liver biopsies were immediately immersed in formaldehyde solution (4%) before being sent to the pathology department. Then, after optimal fixation, biopsies were embedded in paraffin after dehydration. Microtome 4 μ m sections were cut, deparaffined and stained with haematoxylin/eosin and Sirius red.

Biopsies were evaluated by two independent senior histologists familiar with liver diseases, according to the SAF scoring system [25] as follows: steatosis score (S) was graded from 0 to 3 (S0: < 5%; S1: 5%-33%, mild; S2: 34%-66%, moderate; S3: > 67%, marked); activity (A) was graded from 0 to 4, by the unweighted addition of hepatocyte ballooning (0-2) and lobular inflammation (0-2); fibrosis (F) was graded from 0 to 4 (F0: none; F1: perisinusoidal or portal fibrosis; F2: perisinusoidal and periportal fibrosis without bridging; F3: bridging fibrosis; F4: cirrhosis) [25]. As surgical biopsies are taken from the subcapsular region of the liver, fibrosis stages from 2 to 4 were considered as clinically significant. NAFLD was defined as no alcohol misuse, steatosis affecting at least 5% of hepatocytes (steatosis score S1 to S3), absence of steatohepatitis [26]. Metabolic syndrome was defined according to modified NCEP-ATP III criteria [27].

The minimal criteria for the diagnosis of NASH include the presence of > 5% macrovesicular steatosis, inflammation, and liver cell ballooning, typically with a predominantly centrilobular (acinar zone 3) distribution in adults.

TyG index was calculated as $[\text{Ln}(\text{fasting triglycerides})(\text{mg/dl}) * \text{fasting glucose}(\text{mg/dl})/2]$ [17].

Statistical methods

Categorical variables are presented as numbers (percentages), and quantitative variables as means with standard deviation (SD). Demographic, clinical and biological variables associated with each outcome (presence of NAFLD, NASH, fibrosis) were compared using logistic regression models. In

order to determine which factors were independently associated with each outcome, non-collinear factors associated in univariate analysis at $P < 0.20$ were proposed in a multivariable logistic regression model. A forward selection had been applied using the Akaike information criterion (AIC).

To control for bias, the relationship between the level of the TyG index and NASH was then analysed using an unconditional regression logistic model and a conditional regression logistic model (each NASH patient was matched with two non-NASH patients on NAFLD and fibrosis stage using the SAS macro %GMATCH (Division of Biostatistics, Mayo Clinic). Nine patients with NASH could not be matched with non-NASH patients.

To compare the diagnostic performance of biological parameters, receiver-operating characteristic (ROC) analyses were performed. The areas under the ROC curves (AUROC) were compared to investigate whether one of the biological parameters yielded significant advantages over the others using the DeLong method [28]. The specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) for NASH were calculated (95% CI). As NASH prevalence was biased in our population due to our stratified recruitment, PPV and NPV were calculated using literature data for NASH prevalence (25-37% [29-31]). The best possible cut-off point was defined by two different methods: *i*) threshold set as the highest Youden Index [(specificity + sensitivity) – 1]; *ii*) a threshold defined favouring sensitivity while maintaining an acceptable rate of well-classified patients. Therefore, sensitivity and specificity were given for both thresholds, and PPV and NPV values were given for both thresholds and for each value of NASH prevalence considered according to the literature.

The significance level was set at $P < 0.05$. Analyses were performed using SAS (version 9.4; SAS Inc., Cary, North Carolina).

Results

Population characteristics

Among the 238 analysed subjects, mean age was 43 years, 34% were men and 29% had T2DM. Although this cohort is constituted of grade II-grade III obese subjects, 33% of them displayed no NAFLD. Only 18% experienced NASH and 3% severe fibrosis (stage 3 or 4). [Table I](#) details the main characteristics of the population.

Associations between liver disorders and clinical/biological characteristics

Univariate associations between patient characteristics and NAFLD, NASH and fibrosis are described in [Tables II, III and IV](#), respectively. T2DM was associated with all 3 types of liver disorders, while age was positively associated only with NAFLD and fibrosis, and male sex with NAFLD. Among biological parameters, higher blood glucose, triglycerides, liver tests (ASAT, ALAT, GGT) and TyG index were also associated with the three types of liver disorders.

After adjustment for potential confounding factors (multivariable models), TyG index remained independently associated with NASH (odds ratio (OR): 4.7 [95% confidence interval 2.3;9.5] $P < 0.0001$, [Table III](#)), stages 2-3 fibrosis (OR: 4.0 [1.5;10.8] $P = 0.007$, [Table IV](#)) and NAFLD (OR: 2.0 [1.1; 3.7] $P = 0.03$, [Table II](#)).

TyG index and prediction of NASH

As only GGT and TyG index were significantly associated with NASH in the multivariable model, ROC curves were constructed and showed that combining TyG index with GGT gave higher sensitivity and specificity than using GGT alone ([Fig. 2](#)). AUCs were 0.71 [0.62;0.80] for GGT alone and 0.79 [0.71;0.87] for GGT and TyG index ($P = 0.04$). The best cut-off value to discriminate NASH from non-NASH patients is presented in [Table V](#) along with predictive values, according to the different hypotheses taken. The maximum value of the Youden index selected a TyG threshold at 9.2, with 0.63 [0.47;0.77] sensitivity, 0.76 [0.69;0.82] specificity, PPV ranging from 0.46 to 0.60 and NPV ranging from 0.78 to 0.86 depending on NASH prevalence. When choosing to favour sensitivity, the TyG cut-off was 9.0, with 0.72 [0.56-0.85] sensitivity, 0.63 [0.55-0.69] specificity and PPV ranging from 0.39 to 0.53 and NPV ranging from 0.79 to 0.87 depending on NASH prevalence.

Finally, we investigated the relation between TyG index and NASH, and the impact of fibrosis stage and steatosis class, in the subpopulation of patients with NAFLD. TyG index was higher in patients with NASH, (9.34 ± 0.56 vs 8.88 ± 0.54 , raw OR 4.5 [2.2;9.1], $P < 0.0001$). When NASH patients were matched with non-NASH patients for steatosis class and stage of fibrosis, the raw OR of the TyG index remained significant (OR 2.3 [1.0;5.0], $P = 0.04$).

Discussion

In our cohort of obese patients, TyG index seems to be consistently associated with liver disorders and particularly NASH. This is of considerable practical value in clinical practice, as physicians could predict which patients would need a liver biopsy and specific follow-up in this high-risk population.

In our sample, 67% of patients undergoing bariatric surgery had > 5% steatosis. This rate is in line with previous studies reporting NAFLD in 63% to 93% of morbidly obese patients [29-32], while the worldwide NAFLD prevalence is around 25% [33]. NASH was histologically diagnosed in 18% of our population, corresponding to 27% of our patients with steatosis, which seems consistent with the estimation that about 20% of patients with NAFLD have NASH [34]. Nevertheless, other studies carried out in bariatric surgery patients reported NASH in up to 25 to 37% of patients [29-31]. The COMET population is stratified by age with an over-representation of 18-30-year-olds, and no subjects over 65. Therefore, the prevalence of NASH may be lower than what is found in other studies. Another characteristic of our population is a high BMI (mean 42 ± 5 kg/m²) and low alcohol consumption in all subjects, which may explain why the two latter variables were not significant predictors of liver disorders.

The TyG index was strongly associated with NASH, with an adjusted OR of 4.7 [2.3;13.7]. This association remained significant in the subgroup of patients with NAFLD, and when patients were matched for steatosis stage and fibrosis, ruling out a confounding role that these two factors might have played in the relationship between TyG index and NASH. This result is in line with the conclusions of a recent review highlighting that insulin resistance is a nearly universal feature of NASH, with both impaired glycaemia and dyslipidaemia [3]. The TyG index was previously evaluated as a steatosis marker, and was found to be poorly specific and unable to discriminate between mild, moderate and severe steatosis. However, NASH was not considered in this analysis [35]. In our sample, sensitivity analyses show that both components of the TyG index (i.e. glycaemia and triglycerides) contribute to predict NASH. The TyG index had the best predictive value when associated with GGT level, with an AUROC of 0.79. The importance of GGT as a marker of insulin resistance was previously addressed in a population of patients with a diversity of hepatic diseases (familial heterozygous hypobetalipoproteinaemia, NAFLD, hepatitis C and healthy controls without steatosis), and it was shown that steatosis and GGT predicted insulin resistance [36]. Insulin

resistance and liver enzymes have already been used in models attempting to predict NASH from noninvasive biomarkers. The HAIR score based on hypertension, ALAT and HOMA exhibited 80% sensitivity and 89% specificity to diagnose NASH in morbidly obese patients undergoing bariatric surgery, with an AUROC of 0.90 [37]. Another study carried out in adults with T2DM and NAFLD found that NASH was predicted by white race, BMI, waist, ALAT, ASAT, albumin, HbA1c, HOMA-IR and ferritin [38]. All these scores are based on plasma insulin value, which could limit their use. The LiMAX® liver test had good sensitivity (85.2%) and specificity (82.9%) to distinguish between definite NASH and not NASH in morbidly obese patients, with an AUROC of 0.86 [13], but cannot be used in routine care. Concerning the TyG index, very few studies have explored its interest to predict NASH. It was found effective in a small sample of asymptomatic women [23], whereas Cazzo et al. found a non-significant association in obese adults [39]. In the latter study, all patients underwent a weight loss program prior to surgery, which may have impacted the triglycerides levels and therefore the TyG value. In addition, the TyG index was considered as a binary variable, which may explain why no association with NASH was observed in that analysis. The TyG index has several advantages. First, it is highly related to insulin resistance [18] and would even perform better than HOMA-IR in predicting NAFLD features [20, 40], without requiring the dosage of plasma insulin. In the primary care setting, these two parameters are easily obtained. Using a combined index, giving a single value, is easier than considering triglycerides and glucose separately. In such a primary care setting, if our threshold of 9.2 were used to decide on specialist referral, 46 to 60% of these referred patients would actually have NASH. A slightly lower threshold (9.0) would improve sensitivity, but with only 39 to 53% of patients really having NASH. This remains a considerable improvement over the current situation, where no screening index is available. The TyG index has little variability provided that an overnight fast is performed, and is not influenced by the most widely used lipid-lowering drugs, i.e statins.

We found that NAFLD was mainly predicted by age, ASAT level and TyG index. However, the need for blood or clinical biomarkers is less stringent than for NASH or fibrosis as NAFLD is a non-severe disorder and may resolve with weight loss following bariatric surgery [32]. Moreover, hepatic volume can be estimated with imaging. Both cross-sectional and longitudinal studies reported that the TyG index can predict NAFLD, with a threshold of 8.5 to 8.7 consistent between studies [20-22]. These findings were observed in large samples of 4 to 10 thousand subjects with a wide range of age and BMIs and mostly healthy people. A cross-sectional analysis conducted in Chinese adults also

found that TyG index was positively related to the severity of hepatic steatosis, with an OR of 4.65 [3.42;6.31] for severe NAFLD as compared with no NAFLD [20].

The TyG index was an independent predictor of fibrosis, with a 4-fold increased risk of stage 2-3 fibrosis for each 1-unit increase in TyG index (OR 4.0 [1.5;10.8]. The AUROC of the model including TyG, GGT, platelet count and age was high (0.900). In a previous cross-sectional study, Guo et al. found that TyG index was independently associated with fibrosis but was not a reliable predictor, with an AUROC of only 0.59 [20]. Nevertheless, several reliable scores can predict fibrosis. In particular, FIB-4 index, NAFLD fibrosis score and APRI (aspartate aminotransferase to platelet ratio index)–are highly sensitive to predict fibrosis and exclude advanced fibrosis in clinical practice, and also to predict mortality [34, 41, 42]. Thus, despite an association with fibrosis, we believe that the main value of the TyG score relies on its independent association with NASH, allowing earlier referral of patients to specialised care and implementation of preventive measures.

Our study has several limitations. Our results observed in a sample of 238 severely obese patients need to be replicated in a validation cohort to confirm the diagnostic value of the TyG index, and to test a score combining the TyG index and other significantly linked parameters. Our sample size is too small to perform subgroups analysis, such as sex-specific and menopausal status-specific data or analysis in patients with diabetes. In addition, the low prevalence of severe fibrosis precludes conclusions about the factors predicting this condition. Patients with treated diabetes took their treatments the day before surgery, which might have altered fasting parameters; however, this would be the same situation if this score were to be used in clinical practice, thus our results would remain relevant in routine practice. Another limitation is that our findings cannot be extrapolated to the non-obese population. However, our work has high internal validity as our analysis was based on a cohort with a wide range of available variables and no missing data. In addition, the quality assurance management system of our data and sample management ensured the reliability of our results.

Conclusions

In conclusion, the TyG index, when associated with GGT level, seems to exhibit high diagnostic performance to predict NASH in severely obese patients. This may be of clinical importance to identify patients who should be referred for liver biopsy. The conditions of blood collection remain to be determined to measure reliable blood glucose and triglycerides levels and optimise the TyG index

predictive value. The TyG index, although related to NAFLD and fibrosis, seemed of limited value to predict them. These results need to be validated in a larger sample of morbidly obese patients, and then among overweight and type I obesity populations.

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Figure legends

Figure 1: Flow chart of patients included in the analysis.

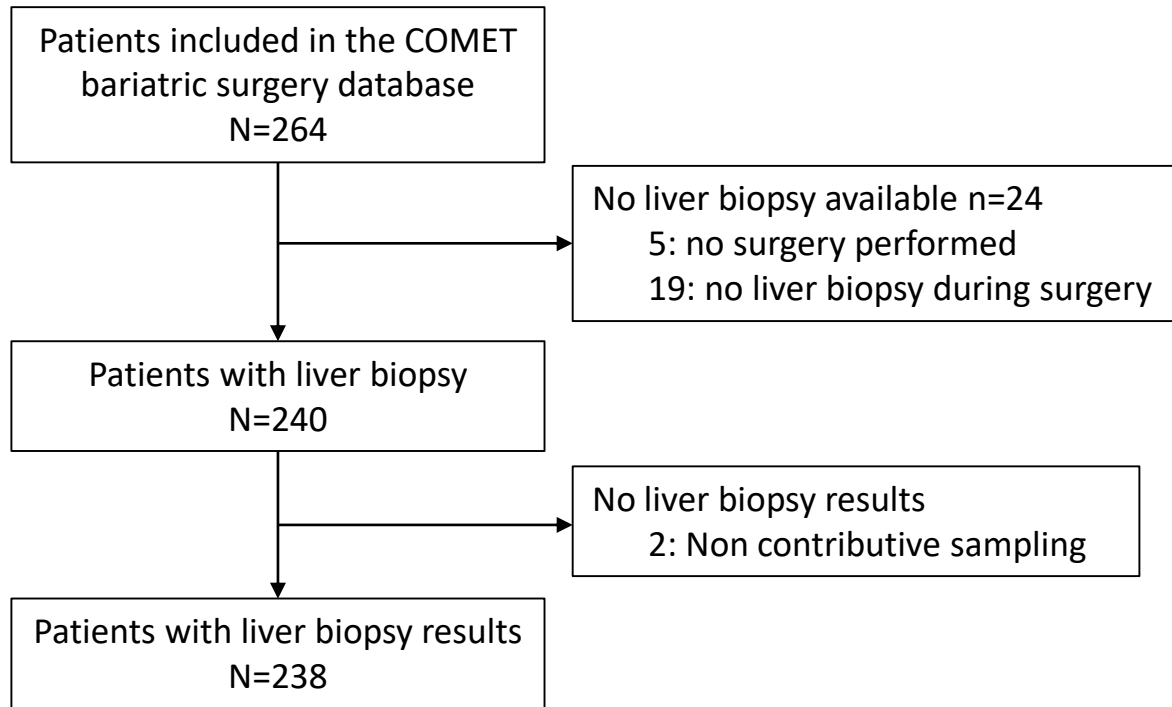
This flow diagram illustrates the sample size and reasons for not having a liver biopsy result available after inclusion in the COMET study.

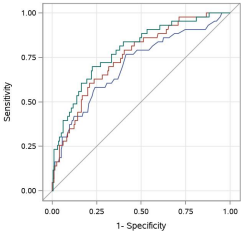
Figure 2: ROC curves for NASH comparing the predictive value of the final model (including TyG index and GGT) with models including each variable alone.

GGT (AUC 0.71 95% CI [0.62;0.80]) vs GGT+Tyg (AUC 0.79 95% CI [0.71;0.87]): $P = 0.04$

GGT (AUC 0.71 95% CI [0.62;0.80]) vs TyG (AUC 0.75 95% CI [0.67 ;0.83]): $P = 0.38$

GGT+Tyg (AUC 0.79 95% CI [0.71;0.87]) vs TyG (AUC 0.75 95% CI [0.67 ;0.83]): $P = 0.06$





ROC curve

- Model GGT alone (AUC= 0.7065)
- Model TyG alone (AUC= 0.7524)
- Model GGT+TyG (AUC= 0.7902)

Table I: Clinical, biological and histological characteristics of the study population (n=238)

	n (%) or mean \pm SD
Age (years)	43 \pm 12
Male sex	80 (34)
Body mass index (kg/m ²)	42.0 \pm 5.0
Type 2 diabetes	66 (29)
Metabolic syndrome	174 (73)
Alcohol consumption pattern	
No alcohol consumption	100 (42)
Low-risk drinking	138 (58)
Misuse	0
Alcohol intake (drinks/day)	0.10 \pm 0.31
Grade of steatosis	
S 0 (< 5%)	78 (33)
S 1 (5-33%)	82 (34)
S 2 (34-66%)	40 (17)
S 3 (> 66%)	38 (16)
Grade of activity (ballooning + inflammation)	
A0 (no activity)	134 (56)
A1 (mild)	59 (25)
A2 (moderate)	29 (12)
A3 (severe)	12 (5)
A4 (very severe)	4 (2)
NASH	43 (18)
Stage of fibrosis	
F0 (none)	170 (71)
F1 (perisinusoidal or portal)	49 (21)
F2 (perisinusoidal and periportal fibrosis without bridging)	12 (5)
F3 (bridging fibrosis)	7 (3)
F4 (cirrhosis)	0
Fasting blood glucose (mmol/l)	5.7 \pm 1.6
Fasting plasma triglycerides (mmol/l)	1.7 \pm 0.9
ASAT (IU/l)	32 \pm 24
ALAT (IU/l)	43 \pm 39
GGT (IU/l)	45 \pm 42
HOMA-IR*	3.6 \pm 3.3
TyG index	8.9 \pm 0.6
Platelet count (10 ⁹ /l)	252 \pm 56

*Calculated in patients without diabetes only (n=170)

Abbreviations: AUROC: area under the receiver-operating characteristic curve; NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; NPV: negative predictive value; PPV: positive predictive value; SAF scoring system: steatosis, activity and fibrosis scoring system; TyG index: Triglycerides and Glucose index.

Table II: Factors associated with NAFLD: univariate and multivariable analyses

	Unit for NAFLD		OR _{crude} (95% CI)	P-value	OR _{adjusted} ⁽¹⁾ (95% CI)	P-value	
	OR	No (n=78)					Yes (n=160)
Age (years)	10	38 ± 12	46 ± 12	1.7 (1.3;2.1)	< 0.001	1.5 (1.1;2.0)	0.004
Male sex (%)		14 (18)	66 (41)	3.2 (1.7;6.2)	< 0.001	1.9 (0.9;3.9)	0.09
Body mass index (kg/m ²)	5	41.4 ± 5.0	42.3 ± 5.0	1.2 (0.9;1.6)	0.15		
Type 2 diabetes (%)		6 (8)	60 (38)	7.2 (3.0;17.6)	< 0.001		
Metabolic syndrome (%)		44 (56)	130 (81)	3.4 (1.8;6.1)	< 0.001		
No alcohol intake (%)		28 (36)	72 (45)	-			
Glucose (mmol/l)	1	5.2 ± 1.2	5.9 ± 1.7	1.4 (1.1; 1.8)	0.004		
Triglycerides (mmol/l)	1	1.5 ± 0.7	1.8 ± 0.9	1.7 (1.2;2.5)	0.007		
ASAT (U/l)	20	22 ± 13	37 ± 27	3.2 (1.9;5.6)	< 0.001	2.7 (1.6;4.7)	<0.001
ALAT (U/l)	20	26 ± 24	52 ± 42	2.5 (1.7;3.6)	< 0.001		
GGT (U/l)	20	32 ± 24	52 ± 47	1.6 (1.2;2.2)	< 0.001		
Platelet count (10 ⁹ /l)	-200	255 ± 52	250 ± 57	1.4 (0.5;3.6)	0.55		
HOMA-IR [#]	1	2.6 ± 1.4	4.3 ± 4.0	1.4 (1.2;1.7)	<0.001		
TyG index	1	8.7 ± 0.5	9.0 ± 0.6	2.7 (1.6;4.6)	<0.001	2.0 (1.1;3.7)	0.03

Values are mean ± SD unless otherwise stated; # evaluated in patients without diabetes only

(1): Variables proposed for the multivariate model were non-collinear factors associated in univariate analysis at $P < 0.20$, that is: sex, age, BMI, ASAT, GGT and TyG

Table 3: Factors associated with NASH: univariate and multivariable analyses

	Unit for OR	NASH		OR _{crude} (95% CI)	P-value	OR _{adjusted} ⁽¹⁾ (95% CI)	P-value
		No (n=195)	Yes (n=43)				
Age (years)	10	43 ± 12	45 ± 12	1.2 (0.9;1.6)	0.17		
Male sex (%)		63 (32)	17 (40)	1.4 (0.7;2.7)	0.36		
Body mass index (kg/m ²)	5	42.2 ± 5.1	41.3 ± 4.2	0.8 (0.6;1.2)	0.32		
Type 2 diabetes (%)		42 (22)	24 (56)	4.6 (2.3;9.2)	< 0.001		
Metabolic syndrome (%)		136 (70)	38 (88)	3.3 (1.2;8.8)	0.02		
No alcohol intake (%)		83 (43)	17 (40)	-			
Glucose (mmol/l)	1	5.4 ± 1.3	6.7 ± 2.1	1.5 (1.3;1.9)	< 0.001		
Triglycerides (mmol/l)	1	1.6 ± 0.7	2.2 ± 1.2	2.1 (1.5;3.0)	< 0.001		
ASAT (U/l)	20	29 ± 20	46 ± 35	1.6 (1.2;2.0)	< 0.001		
ALAT (U/l)	20	40 ± 34	61 ± 55	1.3 (1.1;1.5)	0.003		
GGT (U/l)	20	39 ± 32	74 ± 65	1.4 (1.2;1.6)	< 0.001	1.3 (1.1;1.5)	0.002
Platelet count (10 ⁹ /l)	-200	251 ± 55	256 ± 60	0.7 (0.2;2.4)	0.60		
HOMA-IR [#]	1	3.1 ± 2.0	7.8 ± 6.9	1.5 (1.2;1.8)	< 0.001		
TyG index	1	8.8 ± 0.5	9.3 ± 0.6	5.6 (2.9;11.2)	< 0.001	4.7 (2.3;9.5)	< 0.001

Values are mean (SD) unless otherwise stated; # evaluated in patients without diabetes only.

⁽¹⁾: Variables proposed for the multivariate model were non-collinear factors associated in univariate analysis at $P < 0.20$, that is: age, ASAT, GGT and TyG

Table IV: Factors associated with fibrosis: univariate and multivariable analyses

	Unit	Fibrosis			<i>P</i> -value	OR _{adjusted} ⁽¹⁾	<i>P</i> -value
	for OR	Stages (n=219)	0-1Stages (n=19)	2-3OR _{crude} (95% CI)		(95% CI)	
Age (years)	10	42 ± 12	51 ± 10	2.1 (1.3;3.4)	0.004	1.8 (1.0;3.2)	0.05
Male sex (%)		70 (32)	10 (53)	2.4 (0.9;6.1)	0.07		
Body mass index (kg/m ²)	5	42.0 ± 5.0	41.9 ± 4.2	1.0 (0.6;1.6)	0.90		
Type 2 diabetes (%)		51 (23)	15 (79)	12.4 (3.9;38.9)	< 0.001		
Metabolic syndrome (%)		156 (71)	18 (95)	7.3 (1.0;55.6)	0.06		
No alcohol intake (%)		94 (43)	6 (32)	-			
Glucose (mmol/L)	1	5.5 ± 1.4	7.7 ± 2.2	1.8 (1.4;2.3)	< 0.001		
Triglycerides (mmol/l)	1	1.6 ± 0.9	2.1 ± 1.0	1.6 (1.0;2.4)	0.05		
ASAT (U/l)	20	30 ± 23	52 ± 29	1.6 (1.2;2.0)	0.001		
ALAT (U/l)	20	41 ± 37	73 ± 49	1.3 (1.1;1.6)	0.002		
GGT (U/l)	20	41 ± 35	99 ± 71	1.4 (1.2;1.7)	< 0.001	1.4 (1.2;1.7)	< 0.001
Platelet count (10 ⁹ /l)	-200	254 ± 55	223 ± 58	8.5 (1.4;51.2)	0.02	19.3 (1.8;205.5)	0.01
HOMA-IR [#]	1	3.5 ± 3.3	3.5 ± 0.6	1.0 (0.7;1.4)	0.95		
TyG index	1	8.9 ± 0.6	9.5 ± 0.5	6.1 (2.5;14.9)	< 0.001	4.0 (1.5;10.8)	0.007

Values are mean ± SD unless otherwise stated; # evaluated in patients without diabetes only.

(1): Variables proposed for the multivariate model were non-collinear factors associated in univariate analysis at *P* < 0.20, that is: sex, age, ASAT, GGT, platelet and TyG

Table V: Predictive values of the TyG index to predict NASH according to the method used to determine the cut-off and to NASH prevalence.

AUC [95%CI]	Sensitivity [95% CI]	Specificity [95% CI]	Prevalence of NASH	Proportion of well classified patients	PPV [95% CI]	NPV [95% CI]
<i>TyG cut off =9.0 (Better sensitivity)</i>						
0.75 [0.67-0.83]	0.72 [0.56;0.85]	0.63 [0.55;0.69]	25%	0.649	0.39 [0.30;0.49]	0.87 [0.81;0.93]
			31%	0.655	0.46 [0.37;0.56]	0.83 [0.77;0.90]
			37%	0.661	0.53 [0.44;0.62]	0.79 [0.72;0.87]
<i>TyG's cut off =9.2 (Youden value)</i>						
0.75 [0.67-0.83]	0.63 [0.47;0.77]	0.76 [0.69;0.82]	25%	0.726	0.46 [0.36;0.58]	0.86 [0.81;0.91]
			31%	0.718	0.54 [0.43;0.65]	0.82 [0.76;0.88]
			37%	0.710	0.60 [0.51;0.71]	0.78 [0.71;0.84]

PPV positive predictive value, NPV negative predictive value.