REVIEW ARTICLE

Novel circulating biomarkers for non-alcoholic fatty liver disease: a systematic review[†]

Running title: Circulating biomarkers of NAFLD

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[†]This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jcp.25779]

Received 23 December 2016; Accepted 5 January 2017

Journal of Cellular Physiology

DOI 10.1002/jcp.25779

Abstract

Currently, a liver biopsy remains the only reliable way to precisely diagnose non-alcoholic fatty liver disease (NAFLD) and establish the severity of liver injury, presence of fibrosis and architecture remodeling. However, the cost and the intrinsic invasive procedure of a liver biopsy rules it out as a gold standard diagnostic test, and the imaging test are not the best choice due to the price, and currently is being refined. The lack of a biomarker of NAFLD pushes to develop this new line of research. The aim of the present systematic review is to clarify and update all the NAFLD biomarkers described in the literature until recently. We highlight α-ketoglutarate and CK18-F as currently the best potential biomarker of NAFLD. However, due to methodological differences, we propose the implementation of international, multicenter, multiethnic studies with larger population size and biopsy proven NAFLD diagnosis to analyze and compare α-ketoglutarate and CK18-F as potential biomarkers of the silent evolution of NAFLD.

Keywords: Non-alcoholic fatty liver disease; Biomarker; Diagnosis; α-ketoglutarate and CK18-F

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1. Introduction

It is well known the increasing incidence and prevalence of obesity worldwide (Caballero, 2007). Results from the National Health and Nutritional Examination Survey (NHANES) estimates 33.9% of U.S adults are overweight, 35.1% are obese and 6.4% are extremely obese (Anonymous). In the WHO European Region the results not differ, over 50% of both men and women were overweight, and roughly 23% of women and 20% of men were obese (Anonymous). Obesity comorbidities have been mainly studied, among them, nonalcoholic fatty liver disease (NAFLD) plays a key role (Korenblat et al., 2008; Ruhl and Everhart, 2003). NAFLD is described as a fat accumulation within the hepatocytes in form of triglycerides. More than 5% of steatotic hepatocytes in a liver tissue section is considered the minimum criteria for the diagnosis of NAFLD (Tiniakos et al., 2010). NAFLD is no longer considered a benign condition (Froguel and Boutin, 2001; Korenblat et al., 2008; Magi et al., 2013). It is a silent disease with his potential malignant evolution to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, liver failure and hepatocarcinoma (Tiniakos et al., 2010).

Epidemiology shows that the prevalence of NAFLD is 80-90% in obese adults (Bellentani et al., 2010), approximately 30-40% of NAFLD patients develop NASH, and hepatic fibrosis occurs in 40-50% of these patients (Byrne and Targher, 2015). Nowadays, the relation between NAFLD and NASH is being discussed. Even though both seem part of the same spectrum disease, we consider them as two different histological entities. Steatosis in NAFLD is usually macrovesicuLar, consisting on a simple large intracytoplasmic droplet or smaller well defined droplets displacing the nucleus to the cell periphery. However, *Ludwig* et a. first described NASH and the diagnose include also hepatocyte injury in form of ballooning and lobular

necroinflammation typically located in the azinar zone 3 (Abd El-Kader and El-Den Ashmawy, 2015; Brunt and Tiniakos, 2010; Ludwig et al., 1980; Tiniakos et al., 2010).

Currently a liver biopsy remains the only reliable way to precisely diagnose NAFLD and establish the severity of liver injury, presence of fibrosis and architecture remodeling. It also provides important information regarding prognosis as well as response to therapeutic interventions. Nevertheless, it is an invasive technique, with several risks and diagnostic limitations such as sampling error, inter- and intra-observer variability or histological interpretation (Sumida et al., 2014).

The combination of noninvasive clinically available laboratory and imaging tests may help in the diagnostic evaluation of a patient with suspected NAFLD (Wieckowska and Feldstein, 2008). Oppositely, the cost and the intrinsic invasive procedure of a liver biopsy ruled it out as a gold standard diagnostic test, and the imaging test are not the best choice due to the price, and currently is being refined (Sharma et al., 2009).

Several studies have shown that ordinary liver biomarkers such as levels of alanine aminotransferase (ALT) are not correlated with necroinflamatory activity and fibrosis in patients with known NAFLD (Brunt and Tiniakos, 2010). The lack of a biomarker of NAFLD pushes to develop this new line of research.

The aim of the present systematic review is to clarify and update all the NAFLD biomarkers described in the literature until recently. We assume no genetic differences in the background of our patients (Bellentani et al., 2010); consequently we have done the systematic review focused on serum biomarkers.

2. Methodology and Search Strategy

The basis of methodology and search strategy was built following the PRISMA-P statement (Moher et al., 2015).

2.1. Search Strategy

The search was performed in three electronic databases: PubMed, Scopus and The Cochrane Library. Article selection was accomplished in May 2015, and no date limit was applied to articles selected. Studies included were restricted to those on humans and to English-language journals. Search terms were combined using Boolean operations. The search terms included: (NAFLD OR non-alcoholic fatty liver disease) AND biomarker.

2.2. Selection Criteria

All the manuscripts related with NAFLD and non-invasive diagnostic methods were selected. The selection focused on the serum biomarkers and hepatic steatosis. The articles selected must base the diagnostic of NAFLD on liver biopsy. Other pathologies associated or steatohepatitis were excluded.

The exclusion criteria were a sample size of <100 patients, non-human studies, imaging test or genetics studies, pediatric population, case reports, editorials and systematic reviews. No country distinctions were applied.

Initially, the referenced articles were manually screened independently by two investigators of the team. The first screening selection was based on the article's title and a second screening on the abstract. All duplicate publications were omitted.

2.3 Data Extraction

Data that were extracted included: year of publication, country of origin, sample size, type of study and the biomarker studied. Main conclusions were summarized, and missing data were obtained directly from the respective corresponding authors. In some cases, these data were not forthcoming, and the studies were deleted from our analysis.

3. Results

3.1. Identification of Studies

The initial electronic search of the above-mentioned databases identified 1178 articles (Figure 1). After exclusion of duplicates (n = 592) and with 503 contributions considered irrelevant based on the titles, the selection was reduced to 89 full-text articles. Subsequently, studies (n = 75) with missing data or without compliance with inclusion or exclusion criteria were discarded. Finally, 14 manuscripts were included in this systematic review (Aida et al., 2014; Charlton et al., 2008; Eren et al., 2012; Li et al., 2010; Maleki et al., 2014; Rodriguez-Gallego et al., 2015; Targher et al., 2006; Targher et al., 2007; Tsutsui et al., 2010; Yilmaz et al., 2012; Yilmaz et al., 2011a; Yilmaz et al., 2011b; Yilmaz et al., 2010; Zimmermann et al., 2011). The process of selection is depicted in Figure 1.

3.2. Study Characteristics

The overall study characteristics are displayed in Table 1. All of the articles were observational studies focusing on the NAFLD's biomarkers. Most of them were cohort studies, cross-sectional and case-control studies were also included. They were published between 2006 and 2014 and included a total of 2982 participants. The largest

manuscript is from Denmark, no publications from South America or Africa has been found. There is a vast variability in the biomarkers analyzed, from the 14 articles selected only 2 studied a mutual molecule: cytokeratin 18. The diagnosis of NAFLD has been limited to those articles that performed a liver biopsy.

3.3. Biomarkers and NAFLD

The biomarkers included in the present systematic review come from very diverse pathophysiological mechanisms. There are different kinds of molecules (immunoglobulins, ketones, growth factors, hormones, inflammation markers) all involved in NALFD, but from varied approaches. There are molecules involved in metabolomics disorders (α-ketoglutarate, DHEA, D3 [25(OH)D], FGF-19 and FGF-21), inflation process (IgA, Galectin-3, hs-CRP, Osteoprotegerin and Adiponectin), cell apoptosis (Cytokeratine-18 and Syndecan-1) and even immune system (ZAG).

The first molecule analyzed was Adiponectin by Targher et al. (Targher et al., 2006). They highlight a significant reduction the plasma levels of Adiponectin in NAFLD patients. Among NAFLD patients, low Adiponectin levels independently predicted hepatic steatosis (no fibrosis) and necroinflammation, even after adjustment for age, sex, BMI, HOMAR-IR score and metabolic syndrome components. No cut-off level for diagnose NAFLD is given.

The same group studied D3 [25(OH)D] (Targher et al., 2007). In this case they objectivize a inversely association between liver histology and D3 [25(OH)D] levels (the severer liver histology proven is related with the lower D3 [25(OH)D] levels). They found D3 [25(OH)D] is decreased in NAFLD and even more in NASH patients, which correlates with the inversely association.

Charlton et al. analyzed DHEA (Charlton et al., 2008). They couldn't find significant differences between healthy patients and NAFLD. Nevertheless, they found low circulating sulfated DHEA (DHEA-S) levels are strongly associated with advanced fibrosis stage. In order to determine the specificity of this discovery, they measured DHEA-S levels in a cohort of patients with primary biliary cirrhosis and primary sclerosing cholangitis, in this group DHEA-S levels were not significantly predictive of severity of disease.

Tsutsui et al. (Tsutsui et al., 2010) and Aida et al. (Aida et al., 2014) both describe the relation among cytokeratin 18 fragment (CK18-F) and NAFLD. There is no bias comparing these two studies because both come from the same country, have very similar samples sizes and methodologically both designed the same kind of study (cohort study and the diagnose of NAFLD was based on liver biopsy). In first place, Tsutsui et al. found CK18-F significantly positive correlated with degree of steatosis, lobular inflammation, and ballooning, and showed stronger positive correlation with histologic activity score than serum aspartate and alanine aminotransferase. Secondly, Aida et al. agreed with Tsutsui's results and went one step further. They found very similar results and propose a cut-off level for diagnose NAFLD and differ it from NASH. The optimal cut-off point of serum CK18-F for NAFLD was 230 U/L and for definite NASH was 270 U/L. The sensitivity of NAFLD cut-off level was 0.89, the specificity 0.65, positive predict value 0.34 and negative predict value 0.97. The sensitivity of NASH cut-off level was 0.64, the specificity 0.76, positive predict value 0.72 and negative predict value 0.67. Accuracies of diagnosis for both NAFLD and definite NASH were 0.70.

The next one molecule studied was FGF21 by Li et al. (Li et al., 2010). Their study indicated that serum FGF21 levels were significantly increased in NAFLD

patients and were positively correlated with intrahepatic triglycerides. These finding suggest that FGF21 could serve as a potential biomarker of NAFLD. They also studied FGF21 mRNA expression, which concentrations were positively correlated with intrahepatic triglyceride, and protein levels in liver tissues, which were elevated as well.

The group of Yilmaz et al. studied five different molecules, which none of them could demonstrate a key role as a biomarker of NAFLD (Eren et al., 2012; Yilmaz et al., 2012; Yilmaz et al., 2011a; Yilmaz et al., 2011b; Yilmaz et al., 2010). The first one analyzed was Osteoprotegrin (Yilmaz et al., 2010), their results showed that concentrations of Osteoprotegrin are significantly lower in NASH patients but not in those subjects with NAFLD. The next molecule studied was ZAG (Yilmaz et al., 2011b); in this case the serum ZAG concentrations did not differ in patients with NAFLD compared with healthy controls. They found, among patients with NAFLD, serum ZAG concentrations were significantly higher in males and in those with metabolic syndrome. Galectin-3 was also studied (Yilmaz et al., 2011a), among patients with NAFLD they describe elevated serum Galectin-3 levels correlated significantly with BMI. However, no association between NAFLD patients and healthy controls could be proved. The next particle studied was FGF19 (Eren et al., 2012), their results demonstrates that serum levels of FGF19 are significantly lower than in healthy controls. Nevertheless, this association was very slight and the authors suggest that FGF19 might be potentially involved in the pathophysiology of NAFLD but does not seem to be a key biomarker. The more recently molecule studied by this group is Syndecan-1 (Yilmaz et al., 2012). They prove that serum levels of Syndecan-1 are significantly higher in patients with biopsy-proven NAFLD than in controls. However, they did not find evidence of a significant association of both the hepatic expression and

serum levels of Syndecan-1 with any of the histological features, consequently, it particle may play in the pathophysiology of NAFLD but does not seem to be a key biomarker.

Hs-CRP was analyzed by Zimmermann et al., they performed study with the largest sample size among the studies included in the present systematic review (Zimmermann et al., 2011). In this case, Zimmermann et al. prove that high levels of hs-CRP are a strong predictor of NAFLD. They describe a strongly association between BMI and hs-CRP, as well as a positive association between degree of steatosis and hs-CRP was also observed. This effect remained significant after adjusting for BMI, lobular inflammation, hepatocyte ballooning and fibrosis. They are the first group to introduce the term that the accumulation of fat (either in the adipose tissue or as liver steatosis) leads to increased hs-CRP levels in obese patients, consequently hs-CRP may be a marker of steatosis but not of NASH in obese patients.

Rodiguez-Gallego et al. mapped the circulating metabolome and highlight α-ketoglutarate as a surrogate biomarker of NAFLD (Rodriguez-Gallego et al., 2015). They conducted a case-control study with patients undergoing bariatric surgery. All patients with steatohepatitis, fibrosis and hepatocyte injury in these patients were histologically ruled out. They found plasma α-ketoglutarate levels were significantly increased in obese patients compared with lean controls. They calculated, with 95% confidence, that the true area under the curve of the reported receiver operator characteristic curve for plasma α-ketoglutarate ranged from 0.9 to 0.96 with a specificity of 0.93 at a fixed sensitivity of 0.8. Plasma α-ketoglutarate levels may

distinguish lean controls form obese patients with a 'predictive accurancy' of 100% and predict obese patients with or without NAFLD better than commonly used biomarkers.

The most recently analyzed molecule was IgA, it was studied by Maleki et al (Maleki et al., 2014). They found the extent of liver fibrosis correlated positively with IgA levels. However, no significant correlation was found between steatosis grade and serum IgA levels.

To summarize, only α-ketoglutarate, cytokeratin-18, hs-CRP, FGF-21, D3 [25(OH)D] and adiponectine molecules has shown significant association with NAFLD and could be a key biomarker. The unique two molecules that a cut-off level or an area under ROC is proposed are α-ketoglutarate and cytokeratin-18.

4. Discussion

The incidence and prevalence of obesity is increasing global until reach pandemic proportions (Caballero, 2007). The obesity comorbidities are not isolated of this phenomenon and research on this topic is flourishing. Ten years history supports the investigation on NAFLD biomarkers to identify which obese patients have liver injury. Many molecules have been analyzed from many different approaches, always based on physiopathology of NAFLD.

The genetic background of NAFLD is confusing (Anstee and Day, 2013); nevertheless, there is more consensus in pathogenesis. NAFLD is characterized by the accumulation of triglycerides, which are formed from the esterification of free fatty acids (FFA) and glycerol within the hepatocyte (Dowman et al., 2010; Tiniakos et al., 2010). Donnelly et al. demonstrated that approximately 60% of liver triglyceride

content derived from FFA influx from adipose tissue, 26% from *de novo* lipogenesis, and 15% from diet (Donnelly et al., 2005). Hepatic fat accumulation can occur as a result of increased fat synthesis, increased fat delivery, decreased fat export, and/or decreased fat oxidation (Dowman et al., 2010). In addition, insulin plays a very important role due to its ability to suppress adipose tissue lipolysis. However, in situations of insulin resistance, such as NAFLD, this suppression is impaired resulting in an increased efflux of FFA from adipose tissue (Lewis et al., 2002). Progression to steatohepatitis and fibrosis depends on additional factors such as FFAs, inflammatory cytokines and adipokines, oxidative stress and mitochondrial dysfunction in a complex interplay with genetic predisposition (Dowman et al., 2010).

In the present systematic review only six molecules (α-ketoglutarate, cytokeratin-18, hs-CRP, FGF-21, D3 [25(OH)D] and adiponectin) have proven significant differences between NAFLD patients versus controls (Aida et al., 2014; Eren et al., 2012; Rodriguez-Gallego et al., 2015; Targher et al., 2006; Targher et al., 2007; Tsutsui et al., 2010; Zimmermann et al., 2011). Among them, Rodríguez-Gallego et al. and Aida et al. performed the most accurate analyze, providing the area under ROC curve or proposing a cut-off level of α-ketoglutarate and cytokeratin-18 respectively (Aida et al., 2014; Rodriguez-Gallego et al., 2015). Interestingly, these two molecules come from different pathogenic ways.

On one hand, α-ketoglutarate is a product of the citric acid cycle (McKenna and Rae, 2015). The citric acid cycle is the most important metabolic pathway for the energy supply in the organism, and an alteration on it reflects a defective liver function. Specifically, Rodríguez-Gallego et al. found the levels of branched-chain keto acids (3-methyl-2-oxobutyrate, 3-methyl-2-oxovalerate and 4-methyl-2-oxopentanoate) elevated in the plasma from patients with steatosis. They suggest steatosis sequesters fatty acids

from β-oxidation in liver cells using an alternative energy source, because a significant decreased levels of 3-hydroxybutayrate and a significant increase in the concentration of plasma α-ketoglutarate and succinylcarnitine was proven in patients with steatosis. They identified α-ketoglutarate as a diagnostic biomarker with an area under the ROC curve ranged from 0.90 to 0.96 with a specificity of 0.93 at a fixed sensitivity of 0.8 (Rodriguez-Gallego et al., 2015).

On the other hand, cytokeratin 18 fragment (CK18-F) is the major intermediate filament protein in the liver, and its cleavage by caspases is a characteristic of liver apoptotic cells (Danial and Korsmeyer, 2004). Since CK18-F is generated mainly by caspase 3, which is reportedly activated in a NASH liver, it is conceivable that CK18-F is increased in NASH patients (Feldstein et al., 2003). Previous articles have described CK18-F as useful biomarker for differentiating NASH from NAFLD (Malik et al., 2009; Tsutsui et al., 2010; Wieckowska et al., 2006). Aida et al. more precisely define the optimal cut-off points of serum CK18-F for NAFLD and definite NASH at 230 U/L and 270 U/L respectively. Serum CK18-F (<230 U/L) performed well as a screening test for NAFLD giving its high sensitivity (0.89) and high negative predictive value (0.97), while it had low specificity (0.65) and low positive predictive value (0.34). Oppositely, serum CK18-F (>270 U/L) did not perform well as a screening test for definite NASH (sensitivity 0.64, negative predictive value 0.67) (Aida et al., 2014).

We have to be aware of the demographic differences between α-ketoglutarate and CK18-F studies. Caucasian populations composed α-ketoglutarate sample, while Japanese populations composed CK18-F sample. A definite trend is evident that Japanese populations have an elevated risk for comorbidities at a lower body mass index than Caucasian populations (Kojima et al., 2003), which may be due to ethnic-specific condition in body compositions profiles (Consultation, 2004).

The results of all the articles included in this systematic review have special value. This importance lies in methodology, all authors performed a liver biopsy, which is currently the gold standard for diagnose NAFLD (Abd El-Kader and El-Den Ashmawy, 2015).

To summarize, α-ketoglutarate and CK18-F are the two molecules that arise as potential NAFLD biomarkers. However, the small sample size and demographic differences between both studies limit their potential as standard NAFLD biomarkers.

5. Conclusion

This systematic review summarizes the current moment of NAFLD biomarkers research. We highlight α-ketoglutarate and CK18-F as currently the best potential biomarker of NAFLD. However, due to methodological differences, we propose the implementation of international, multicenter, multiethnic study with larger sample size and biopsy proven NAFLD diagnosis to analyze and compare α-ketoglutarate and CK18-F as potential biomarkers of the silent evolution of NAFLD.

Conflict of interests: None.

References

Abd El-Kader SM, El-Den Ashmawy EM. 2015. Non-alcoholic fatty liver disease: The diagnosis and management. World journal of hepatology 7(6):846-858.

Aida Y, Abe H, Tomita Y, Nagano T, Seki N, Sugita T, Itagaki M, Ishiguro H, Sutoh S, Aizawa Y. 2014. Serum cytokeratin 18 fragment level as a noninvasive biomarker for non-alcoholic fatty liver disease. International journal of clinical and experimental medicine 7(11):4191-4198.

Anonymous. http://www.cdc.gov/nchs/data/hestat/obesity_adult_11_12/obesity_adult_11_12.htm .

Anonymous. http://www.euro.who.int/en/health-topics/noncommunicable-diseases/obesity/data-and-statistics.

Anstee QM, Day CP. 2013. The genetics of NAFLD. Nature reviews Gastroenterology & hepatology 10(11):645-655.

Bellentani S, Scaglioni F, Marino M, Bedogni G. 2010. Epidemiology of non-alcoholic fatty liver disease. Digestive diseases 28(1):155-161.

Brunt EM, Tiniakos DG. 2010. Histopathology of nonalcoholic fatty liver disease. World journal of gastroenterology 16(42):5286-5296.

Byrne CD, Targher G. 2015. NAFLD: a multisystem disease. Journal of hepatology 62(1 Suppl):S47-64.

Caballero B. 2007. The global epidemic of obesity: an overview. Epidemiologic reviews 29:1-5.

Charlton M, Angulo P, Chalasani N, Merriman R, Viker K, Charatcharoenwitthaya P, Sanderson S, Gawrieh S, Krishnan A, Lindor K. 2008. Low circulating levels of dehydroepiandrosterone in histologically advanced nonalcoholic fatty liver disease. Hepatology 47(2):484-492.

Consultation WHOE. 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 363(9403):157-163.

Danial NN, Korsmeyer SJ. 2004. Cell death: critical control points. Cell 116(2):205-219.

Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. 2005. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. The Journal of clinical investigation 115(5):1343-1351.

Dowman JK, Tomlinson JW, Newsome PN. 2010. Pathogenesis of non-alcoholic fatty liver disease. QJM : monthly journal of the Association of Physicians 103(2):71-83.

Eren F, Kurt R, Ermis F, Atug O, Imeryuz N, Yilmaz Y. 2012. Preliminary evidence of a reduced serum level of fibroblast growth factor 19 in patients with biopsy-proven nonalcoholic fatty liver disease. Clinical biochemistry 45(9):655-658.

Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, Gores GJ. 2003. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 125(2):437-443.

Froguel P, Boutin P. 2001. Genetics of pathways regulating body weight in the development of obesity in humans. Experimental biology and medicine 226(11):991-996.

Kojima S, Watanabe N, Numata M, Ogawa T, Matsuzaki S. 2003. Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. Journal of gastroenterology 38(10):954-961.

Korenblat KM, Fabbrini E, Mohammed BS, Klein S. 2008. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology 134(5):1369-1375.

Lewis GF, Carpentier A, Adeli K, Giacca A. 2002. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocrine reviews 23(2):201-229.

Li H, Fang Q, Gao F, Fan J, Zhou J, Wang X, Zhang H, Pan X, Bao Y, Xiang K, Xu A, Jia W. 2010. Fibroblast growth factor 21 levels are increased in nonalcoholic fatty liver disease patients and are correlated with hepatic triglyceride. Journal of hepatology 53(5):934-940.

Ludwig J, Viggiano TR, McGill DB, Oh BJ. 1980. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clinic proceedings 55(7):434-438.

Magi R, Manning S, Yousseif A, Pucci A, Santini F, Karra E, Querci G, Pelosini C, McCarthy MI, Lindgren CM, Batterham RL. 2013. Contribution of 32 GWAS-identified common variants to severe obesity in European adults referred for bariatric surgery. PloS one 8(8):e70735.

Maleki I, Aminafshari MR, Taghvaei T, Hosseini V, Rafiei A, Torabizadeh Z, Barzin M, Orang E. 2014. Serum immunoglobulin A concentration is a reliable biomarker for liver fibrosis in non-alcoholic fatty liver disease. World journal of gastroenterology 20(35):12566-12573.

Malik R, Chang M, Bhaskar K, Nasser I, Curry M, Schuppan D, Byrnes V, Afdhal N. 2009. The clinical utility of biomarkers and the nonalcoholic steatohepatitis CRN liver biopsy scoring system in patients with nonalcoholic fatty liver disease. Journal of gastroenterology and hepatology 24(4):564-568.

McKenna MC, Rae CD. 2015. A new role for alpha-ketoglutarate dehydrogenase complex: regulating metabolism through post-translational modification of other enzymes. Journal of neurochemistry 134(1):3-6.

Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA, Group P-P. 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic reviews 4:1.

Rodriguez-Gallego E, Guirro M, Riera-Borrull M, Hernandez-Aguilera A, Marine-Casado R, Fernandez-Arroyo S, Beltran-Debon R, Sabench F, Hernandez M, del Castillo D, Menendez JA, Camps J, Ras R, Arola L, Joven J. 2015. Mapping of the circulating metabolome reveals alpha-ketoglutarate as a predictor of morbid obesity-associated non-alcoholic fatty liver disease. International journal of obesity 39(2):279-287.

Ruhl CE, Everhart JE. 2003. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. Gastroenterology 124(1):71-79.

Sharma P, Martin DR, Pineda N, Xu Q, Vos M, Anania F, Hu X. 2009. Quantitative analysis of T2-correction in single-voxel magnetic resonance spectroscopy of hepatic lipid fraction. Journal of magnetic resonance imaging : JMRI 29(3):629-635.

Sumida Y, Nakajima A, Itoh Y. 2014. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World journal of gastroenterology 20(2):475-485.

Targher G, Bertolini L, Rodella S, Zoppini G, Scala L, Zenari L, Falezza G. 2006. Associations between plasma adiponectin concentrations and liver histology in patients with nonalcoholic fatty liver disease. Clinical endocrinology 64(6):679-683.

Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G, Arcaro G. 2007. Associations between serum 25-hydroxyvitamin D3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. Nutrition, metabolism, and cardiovascular diseases : NMCD 17(7):517-524.

Tiniakos DG, Vos MB, Brunt EM. 2010. Nonalcoholic fatty liver disease: pathology and pathogenesis. Annual review of pathology 5:145-171.

Tsutsui M, Tanaka N, Kawakubo M, Sheena Y, Horiuchi A, Komatsu M, Nagaya T, Joshita S, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Aoyama T, Tanaka E, Sano K. 2010. Serum fragmented cytokeratin 18 levels reflect the histologic activity score of nonalcoholic fatty liver disease more accurately than serum alanine aminotransferase levels. Journal of clinical gastroenterology 44(6):440-447.

Wieckowska A, Feldstein AE. 2008. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. Seminars in liver disease 28(4):386-395.

Wieckowska A, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. 2006. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology 44(1):27-33.

Yilmaz Y, Eren F, Colak Y, Senates E, Celikel CA, Imeryuz N. 2012. Hepatic expression and serum levels of syndecan 1 (CD138) in patients with nonalcoholic fatty liver disease. Scandinavian journal of gastroenterology 47(12):1488-1493.

Yilmaz Y, Eren F, Kurt R, Yonal O, Polat Z, Senates E, Bacha M, Imeryuz N. 2011a. Serum galectin-3 levels in patients with nonalcoholic fatty liver disease. Clinical biochemistry 44(12):955-958.

Yilmaz Y, Yonal O, Eren F, Kurt R, Celikel CA, Ozdogan O, Imeryuz N, Kalayci C, Avsar E. 2011b. Serum zinc-alpha2-glycoprotein concentrations in patients with non-alcoholic fatty liver disease. Clinical chemistry and laboratory medicine 49(1):93-97.

Yilmaz Y, Yonal O, Kurt R, Oral AY, Eren F, Ozdogan O, Ari F, Celikel CA, Korkmaz S, Ulukaya E, Imeryuz N, Kalayci C, Avsar E. 2010. Serum levels of osteoprotegerin in the spectrum of nonalcoholic fatty liver disease. Scandinavian journal of clinical and laboratory investigation 70(8):541-546.

Zimmermann E, Anty R, Tordjman J, Verrijken A, Gual P, Tran A, Iannelli A, Gugenheim J, Bedossa P, Francque S, Le Marchand-Brustel Y, Clement K, Van Gaal L, Sorensen TI, Jess T. 2011. C-reactive protein levels in relation to various features of non-alcoholic fatty liver disease among obese patients. Journal of hepatology 55(3):660-665.

Figure legend

Figure 1. Flow diagram of the study selection process.

Table 1. Characteristics of the included studies

First Author	Year of publication	Location of the studied population	Type of study	Sample size	Biomarker	Diagnostic method	Reference number
Maleki, I.	2014	Iran	Case-control	104	IgA	Bx	(Maleki et al., 2014)
Rodríguez-Gallego, E.	2014	Spain	Cohort	230	α-ketoglutarate	Bx	(Rodriguez-Gallego et al., 2015)
Aida, Y.	2014	Japan	Cohort	116	Cytokeratin 18 fragment	Bx	(Aida et al., 2014)
Yilmaz, Y.	2012	Turkey	Observational case-control	113	Syndecan-1	Bx	(Yilmaz et al., 2012)
Eren, F.	2012	Turkey	Cohort	165	FGF19	Bx	(Eren et al., 2012)
Yilmaz, Y.	2011	Turkey	Observational case-control	110	Galectin-3	Bx	(Yilmaz et al., 2011a)
Zimmermann, E.	2011	Denmark	Cohort	627	hs-CRP	Bx	(Zimmermann et al., 2011)
Yilmaz, Y.	2011	Turkey	Observational case-control	171	ZAG	Bx	(Yilmaz et al., 2011b)
Yilmaz, Y.	2010	Turkey	Cohort	157	Osteoprotegerin	Bx	(Yilmaz et al., 2010)
Li, H.	2010	China	Cohort	348	FGF21	Bx	(Li et al., 2010)
Tsutsui, M.	2010	Japan	Cohort	118	Cytokeratin 18 fragment	Bx	(Tsutsui et al., 2010)
Charlton, M.	2008	USA	Cohort	483	DHEA	Bx	(Charlton et al., 2008)
Targher, G.	2007	Italy	Observational case-control	120	D_3 [25(OH)D]	Bx	(Targher et al., 2007)
Targher, G.	2006	Italy	Cross-sectional	120	Adiponectin	Bx	(Targher et al., 2006)

Abbreviations: IgA, immunoglobulin A; FGF19, fibroblast growth factor 19; hs-CRP, high sensitivity C-reactive protein; ZAG, zinc-α_2-glycoprotein; FGF21, fibroblast growth factor 21; DHEA, dehydroepiandrosterone; D_3 [25(OH)D], 25-hydroxyvitamin D_3; Bx, hepatic biopsy

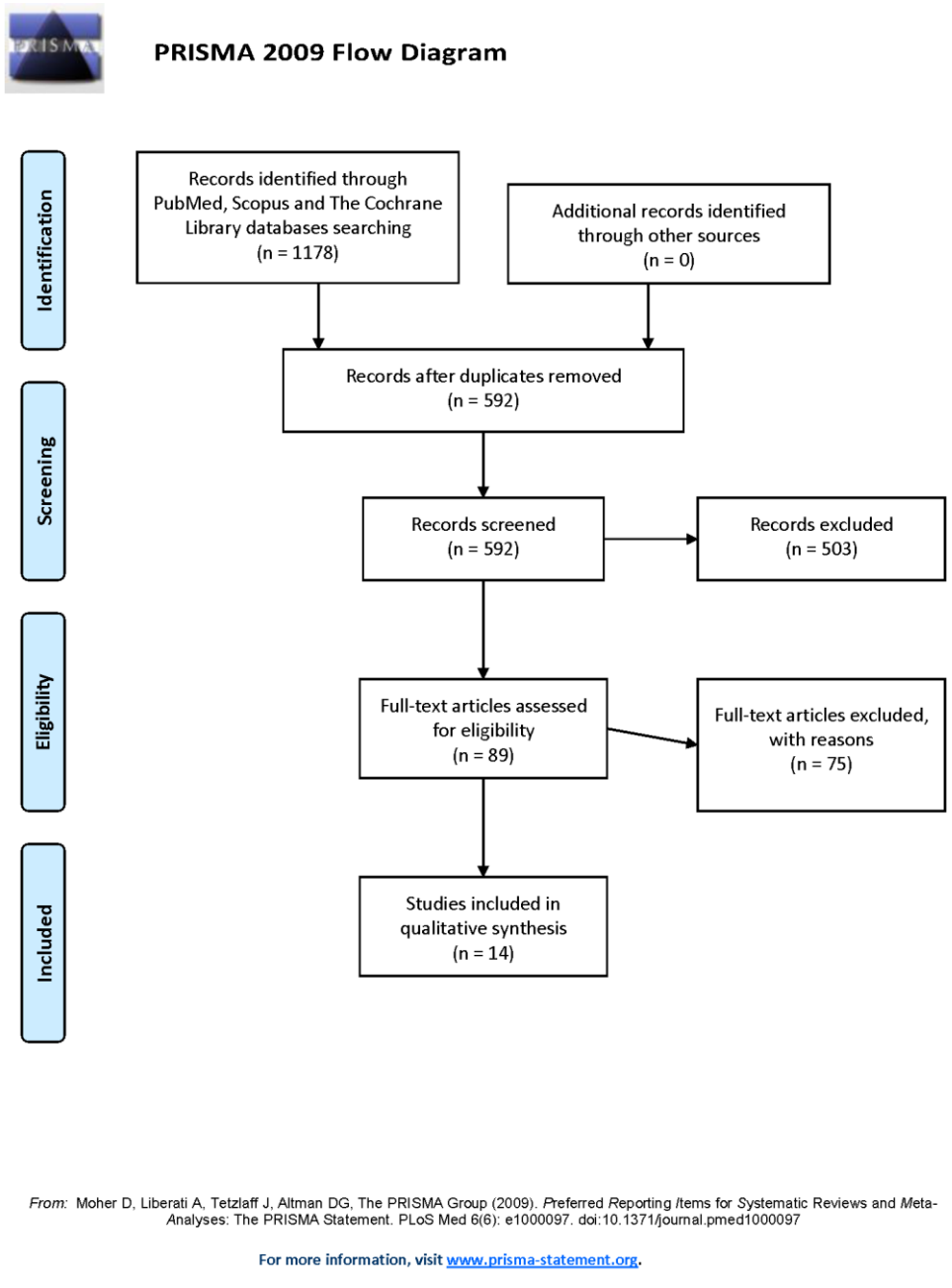

Figure 1