Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.4387

World J Gastroenterol 2009 September 21; 15(35): 4387-4391
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Cytokeratin-18 fragments and biomarkers of the metabolic syndrome in nonalcoholic steatohepatitis

Yusuf Yilmaz, Alla Eldeen Kedrah, Osman Ozdogan

Yusuf Yilmaz, Alla Eldeen Kedrah, Osman Ozdogan, Department of Gastroenterology, Marmara University, School of Medicine, 34662 Altunizade, Istanbul, Turkey
Author contributions: Yilmaz Y conceived the project, suggested the content of the review, wrote the draft, and co-wrote the final version; Kedrah AE wrote the draft and co-wrote the final version; Ozdogan O performed overall scientific direction and revision.
Correspondence to: Yusuf Yilmaz, MD, Department of Gastroenterology, Marmara University, School of Medicine, 34662 Altunizade, Istanbul,
Turkey. yusufyilmaz@uludag.edu.tr
Telephone: +90-533-4403995 Fax: +90-216-6886681
Received: May 27, 2009 Revised: August 5, 2009
Accepted: August 12, 2009
Published online: September 21, 2009

Abstract

Nonalcoholic fatty liver disease (NAFLD) remains a leading cause of chronic liver disease. In the context of NAFLD, the presence of nonalcoholic steatohepatitis (NASH) portends an adverse prognosis with greater risk of liver fibrosis and cirrhosis. Although liver biopsy is the keystone of patient management in NAFLD, it is also increasingly clear that such evaluation has its limitations. The availability of biochemical markers of NAFLD and NASH has tremendous potential to radically alter management strategies for these conditions, as well as to monitor disease activity. Our article provides an overview of biomarker discovery and selection in the setting of NAFLD and highlights future directions in the field.

Key words: Nonalcoholic steatohepatitis; Nonalcoholic fatty liver disease; Biomarkers; Liver biopsy

Peer reviewer: Kenichi Ikejima, MD, PhD, Department of Gastroenterology, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, 113-8421, Japan

Yilmaz Y, Kedrah AE, Ozdogan O. Cytokeratin-18 fragments and biomarkers of the metabolic syndrome in nonalcoholic steatohepatitis. *World J Gastroenterol* 2009; 15(35): 4387-4391 Available from: URL: http://www.wjgnet.com/1007-9327/15/4387.asp DOI: http://dx.doi.org/10.3748/wjg.15.4387

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease and its incidence is rising worldwide[1-3]. The term NAFLD is used to describe a wide spectrum of fatty liver changes ranging from simple steatosis to nonalcoholic steatohepatitis (NASH)[4]. Although simple steatosis usually follows a benign course, steatohepatitis is prone to progress to hepatic fibrosis and cirrhosis leading to excess morbidity and mortality[5,6]. In this context, early identification of patients with NASH prior to the onset of advanced fibrosis would be helpful in guiding aggressive intervention. Liver biopsy remains the gold standard for obtaining an accurate diagnosis of NASH, as well as for differentiating this condition from simple steatosis. Unfortunately, biopsy is a costly and invasive diagnostic procedure, possibly subject to inter- and intra-observer variability[7,8]. Thus it is becoming increasingly clear that although liver biopsy is the keystone of patient diagnosis and management in the setting of NAFLD, such evaluation has its limitations. Clinicians should therefore use additional tools to aid clinical assessment and to enhance their ability to identify the patient at risk for NASH and liver fibrosis. Biomarker use is one such tool to better identify subjects with NASH in the context of NAFLD, and hopefully to effectively prognosticate patients with this condition.

The development of NASH biomarkers can be theoretically achieved *via* two different strategies: the first strategy can be defined as "knowledge-based" (deductive method based on the current knowledge of NASH pathophysiology), while the second one is more "unbiased" (inductive strategy). The "knowledge-based" approach relies on a direct understanding of the pathophysiological processes that underlie the development of NASH as well as the evolution of its sequelae. It may consist of biochemical assays aiming to assess attractive novel candidate markers informed by the biology of the disease process. For instance, the understanding of the role played by hepatocyte apoptosis[9] and insulin resistance[10,11] in the pathobiology of liver injury has enabled the development of promising biomarkers of NASH, such as caspase-cleaved cytokeratin 18 fragments or numerous different adipokines. On the other hand, the "unbiased" approach involves the use of modern techniques including proteomics, metabolomics, and

bioinformatics that have allowed unbiased investigations of numerous putative markers that may be informative with regard to the various stages of NAFLD, including overt NASH and its sequelae[12].

This article provides an overview of biomarker discovery and selection in the setting of NASH starting with some "knowledge-based" biomarkers. The list of biochemical markers provided in this review is not intended to be exhaustive; rather, a brief summary of some key biomarkers is provided.

BIOMARKERS OF HEPATOCYTE APOPTOSIS

To illustrate the opportunities and challenges related to the use of "knowledge-based" biomarkers of NASH, let us consider as an example biomarkers of hepatocyte apoptosis. Growing evidence has now accrued that hepatocellular apoptosis plays a central role in NAFLD progression[9]. Interestingly, data from animal and human studies have suggested that apoptosis is prominent in NASH but not in simple steatosis[13]. Cytokeratin 18 (CK-18) is the major intermediate filament protein in the liver and one of the most prominent substrates of caspases during hepatocyte apoptosis. Apoptotic cell death of hepatocytes is associated with release of caspase-cleaved CK-18 fragments into the bloodstream[14], and several studies have demonstrated elevation of these molecules in the context of NAFLD. A pilot study by Wieckowska *et al*[14] was the first to measure the levels of caspase-generated CK-18 fragments in patients with NAFLD. Results showed that caspase-cleaved CK-18 fragments were significantly higher in NAFLD patients than in controls, and that levels of these molecules correlated with the presence of liver fibrosis[14]. In line with these results, the usefulness of CK-18 fragments for the diagnosis of NAFLD was subsequently confirmed in obese patients[15], as well as in pediatric populations[16]. Interestingly, caspase-generated CK-18 fragments released in NAFLD also serve as an indicator of hepatic inflammation. The increased apoptic rate as a consequence of the hepatic inflammatory response is reflected by elevation of serum CK-18 fragments that may therefore distinguish NASH from simple steatosis[17]. These results have been further confirmed; even in NAFLD patients with normal aminotransferase levels[18].

Although CK-18 fragments can be used to identify patients with NASH and these molecules are currently being incorporated into multimarker schemes[15], their routine use in patients with suspected NAFLD depends on the answers to several questions: Are the sensitivity and specificity of these tests similar to those obtained with liver biopsy? Where is the test being performed (Gastroenterology Department, Physician's Office, Outpatient Unit)? What discrimination limits should be used? Is the test being performed for diagnosis or for prognosis? Answers to these questions are part of the scientific evaluation process that is critical for assessing whether the information gained from CK-18 fragments is worth its cost to the healthcare system. Such answers require the performance of large studies to evaluate the relationship of caspase-cleaved CK-18 fragments with histological phenotypes of interest in the liver and, when applicable, the conduction of clinical trials to relate caspase-cleaved CK-18 fragments to disease risk and to therapeutic responses in patients with NAFLD.

BIOMARKERS OF THE METABOLIC SYNDROME

Although the pathogenesis of NAFLD is clearly multifactorial, this condition is currently considered as the hepatic manifestation of the metabolic syndrome. Comprehensive assessments of the role played by insulin resistance in the pathogenesis of NAFLD have also been published recently[19-21]. The present review will not attempt to replicate these texts. On the other hand, we aim to provide here a concise overview of biomarkers of the metabolic syndrome (leptin, adiponectin, resistin, soluble RAGE) in the setting of NAFLD, including a display of the evidence linking them to NASH.

Leptin is a peptide hormone, mainly produced by adipocytes, that plays a central role in the regulation of body weight[22]. In human liver, leptin has been shown to attenuate a number of insulin-induced activities ultimately resulting in insulin resistance[23]. Furthermore, a proinflammatory and profibrogenic activity of leptin has been reported[24]. Since leptin has been linked to metabolic abnormalities and insulin resistance, its potential role in NAFLD has been the focus of much investigation. Compared with controls, significantly higher levels of leptin have been observed in patients with NAFLD[25,26] and in those with NASH[27,28]. However, there are inconsistencies in published literature, with some authors showing an unaltered level of leptin in the setting of NAFLD[29]. In addition, serum leptin levels showed no correlation with liver fibrosis[30].

Adiponectin is an adipose tissue-specific protein whose receptors are expressed in several cell types including hepatocytes[31]. Main functions of this molecule comprise of the downregulation of inflammatory processes, the promotion of lipolysis, and the prevention of lipid accumulation[32]. Adiponectin is known to exert antifibrogenic and antiestrogenic effects in the liver[33]. Hypoadiponectinemia has been suggested as contributing to insulin resistance and the metabolic syndrome[34], and several studies have reported decreased adiponectin levels in patients with liver steatosis as compared with controls[35-37]. Of note, some authors have also demonstrated that lower adiponectin levels are associated with more extensive necroinflammation in the setting of NAFLD and that they may contribute to the development of NASH[38,39]. However, controversy surrounds the role of decreased adiponectin level as a reliable laboratory marker of NASH[40].

Resistin is a 10 kDa protein of 94 amino acids highly expressed in the adipose tissue. It is a major determinant of hepatic insulin resistance induced by high-fat diet in animal models[41]. Although human data regarding the role of this adipokine in insulin sensitivity and the metabolic syndrome are controversial[42], preliminary evidence seems to suggest a potential role of this adipokine in the pathogenesis of NAFLD. Pagano *et al*[43] have initially shown that patients with NAFLD are characterized by higher serum resistin levels in association with the NASH score, an index that takes into account necrosis, inflammation, and fibrosis in liver biopsies. However, no correlation was found between insulin resistance and hepatic steatosis score[43]. These findings were recently replicated by Jiang *et al*[44], who showed that serum resistin levels were significantly elevated in patients with NAFLD compared to controls. In contrast, Cho *et al*[40] failed to show altered levels of this molecule in Korean male patients.

Besides classical adipokines, levels of soluble receptor for advanced glycation endproducts (sRAGE) have been recently linked to insulin resistance and several components of the metabolic syndrome[45]. Recently, Yilmaz *et al*[46] have investigated concentrations of sRAGE across the spectrum of NAFLD. Levels of sRAGE were significantly lower in patients with definite NASH and borderline NASH compared to controls. Interestingly, levels of sRAGE were significantly and inversely correlated with serum aminotransferase, indicating that lower concentrations of sRAGE are associated with the most severe forms of NAFLD[46].

Altogether, our investigations indicate that a flurry of case-control studies relating biomarkers of the metabolic syndrome to NAFLD and NASH have been conducted in the past decade. Most of the available data are on adipokines, and much less is known about soluble RAGE. Of note, most studies to date also have been carried out in groups of people of Caucasian ancestry, and there are few data on African populations or African Americans. Furthermore, matching of cases and controls limits comparisons across sex and age demographics. Finally, the use of different assays across the studies also makes it difficult to define cut-off points. Additional larger studies of more diverse populations, including a full range of potential confounding variables, would be helpful at this juncture.

MISCELLANEOUS "KNOWLEDGE-BASED" BIOMARKERS

By using the "knowledge-based" approach, numerous other potential biomarkers of NAFLD and NASH have been explored in pilot cross-sectional studies. Angiopoietin-like protein 3 (ANGPTL3) is a liver-derived plasma protein that modulates plasma triglyceride clearance[47], which has been recently investigated in the setting of NAFLD[48]. Levels of ANGPTL3 have been found to be higher in patients with NASH than in those with simple fatty liver. Nonetheless, no association with histological staging and pathological characteristics of NAFLD was seen[48]. Pentraxin 3 (PTX3) is an acute-phase reactant that reflects the tissue inflammatory response[49]. It has been recently demonstrated that plasma PTX3 levels may differentiate NASH patients from non-NASH subjects, and that higher plasma PTX3 levels are associated with severe stages of hepatic fibrosis[50]. However, it is unknown whether any of these biochemical markers are involved in the causal chain of NAFLD progression, mediating the effects of other risk factors (e.g. insulin resistance or inflammation) or whether they merely reflect the presence of NAFLD. Additional basic and clinical research will likely shed more light on these issues.

PROTEOMIC APPROACHES

Proteomic approaches to the identification of NASH biomarkers rely principally on the unbiased comparative analysis of protein expression in normal and diseased liver tissues to identify aberrantly expressed proteins that may represent new biomarkers, as well as direct serum protein profiling[12]. Proteomics methodologies include the isolation, identification, and quantification of proteins in biosamples by adsorption of proteins to activated surfaces (matrix-assisted laser desorption-ionization technology), or *via* peptide ionization procedures and mass spectroscopy. Mass spectrometry can yield a comprehensive profile of peptides and proteins in biosamples without the need for initial protein separation, thereby facilitating biomarker identification with reduced sample requirements and a high throughput[12].

Only two studies to date have examined the NAFLD proteome. Younossi *et al*[51] investigated, by means of surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF), serum protein profiles of different subtypes of NAFLD and identified twelve significantly different protein peaks across the study groups. Using a similar SELDI-TOF technique, Trak-Smayra *et al*[52] have searched for serum markers of steatosis and NASH in obese nonalcoholic patient candidates for bariatric surgery. The authors identified two peaks of 7558.4 and 7924.2 m/z that may distinguish between NASH patients and controls. Such peaks were identified as being the double charged ions of hemoglobin-alpha and hemoglobin-beta. There were also three peaks, the intensity of which increased significantly according to severity of liver lesions (steatosis and NASH), but no association was seen with either liver function tests or metabolic parameters[52].

Although further research of the NAFLD/NASH proteome is certainly needed, the global analysis of protein expression represents an important paradigm shift from the traditional single-molecule approach to the evaluation of protein networks. The future availability of rapid, high-throughput analytical platforms are likely to facilitate molecular phenotyping of different subtypes (simple fatty liver, borderline NASH, definitive NASH) in the spectrum of NAFLD.

CONCLUSION

The need to carry out a biopsy to distinguish NASH from simple steatosis has impeded research to develop strategies for interventions to treat steatohepatitis. In this context, the overall expectation of a NASH biomarker is to enhance the ability of the clinician to optimally manage the patient without the use of liver biopsy[53]. Theoretically, biomarkers may provide a powerful approach in the understanding of the spectrum of NAFLD, with potential applications in several areas including screening, diagnosis, prognosis, and therapeutic monitoring. Advances in proteomics, metabolomics, and bioinformatics have revolutionized unbiased investigations of several biomarkers that may be informative with regard to the various stages of NAFLD, including NASH and its potential sequelae (advanced fibrosis, hepatocellular carcinoma, end-stage liver disease). Obviously, a crucial prerequisite for the clinical use of biomarkers is elucidation of analytical features, standardization of analytical methods, assessment of performance characteristics, and demonstration of cost-effectiveness. A new biomarker of NASH will be of clinical value only if it is reproducibly obtained in a standardized fashion, it is easy to interpret by clinicians, and if it has high sensitivity and high specificity for identifying NASH, and for distinguishing this condition from benign simple fatty liver. Establishing the prognostic utility of a biomarker in the setting of NAFLD to identify patients that may progress to NASH and fibrosis is more challenging because it requires a prospective design, and serial liver biopsies are presently the gold standard. Although there is evidence to suggest that currently available biomarkers (and their combination) have an increasing ability to distinguish "case" (NASH) from "noncase" (not-NASH) in cross-sectional studies, the step ahead in this field is to differentiate by the use of a biochemical marker "those who will develop fibrosis and end-stage liver disease" from "those who will not" in longitudinal investigations. Hopefully, the ongoing research in NASH biomarker development will also mandate a systematic organization of data that may facilitate the online sharing of biomarker metadata among researchers. Over the next years, technological advances will likely facilitate the use of multimarker profiling to replace the gold standard for the diagnosis of NASH, liver biopsy, with a "biomarker biopsy" [54].

REFERENCES

1 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231
2 **Sass DA**, Chang P, Chopra KB. Nonalcoholic fatty liver disease: a clinical review. *Dig Dis Sci* 2005; **50**: 171-180
3 **Torres DM**, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. *Gastroenterology* 2008; **134**: 1682-1698
4 **Erickson SK**. Nonalcoholic fatty liver disease. *J Lipid Res* 2009; **50** Suppl: S412-S416
5 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121
6 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873
7 **Wieckowska A**, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. *Semin Liver Dis* 2008; **28**: 386-395
8 **Wieckowska A**, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. *Hepatology* 2007; **46**: 582-589
9 **Feldstein AE**, Gores GJ. Apoptosis in alcoholic and nonalcoholic steatohepatitis. *Front Biosci* 2005; **10**: 3093-3099
10 **Tilg H**, Moschen AR. Insulin resistance, inflammation, and non-alcoholic fatty liver disease. *Trends Endocrinol Metab* 2008; **19**: 371-379
11 **Utzschneider KM**, Kahn SE. Review: The role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2006; **91**: 4753-4761
12 **Baranova A**, Liotta L, Petricoin E, Younossi ZM. The role of genomics and proteomics: technologies in studying non-alcoholic fatty liver disease. *Clin Liver Dis* 2007; **11**: 209-220, xi
13 **Feldstein AE**, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, Gores GJ. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. *Gastroenterology* 2003; **125**: 437-443
14 **Wieckowska A**, Zein NN, Yerian LM, Lopez AR, McCullough AJ, Feldstein AE. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. *Hepatology* 2006; **44**: 27-33
15 **Younossi ZM**, Jarrar M, Nugent C, Randhawa M, Afendy M, Stepanova M, Rafiq N, Goodman Z, Chandhoke V, Baranova A. A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH). *Obes Surg* 2008; **18**: 1430-1437
16 **Vos MB**, Barve S, Joshi-Barve S, Carew JD, Whitington PF, McClain CJ. Cytokeratin 18, a marker of cell death, is increased in children with suspected nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2008; **47**: 481-485
17 **Yilmaz Y**, Dolar E, Ulukaya E, Akgoz S, Keskin M, Kiyici M, Aker S, Yilmaztepe A, Gurel S, Gulten M, Nak SG. Soluble forms of extracellular cytokeratin 18 may differentiate simple steatosis from nonalcoholic steatohepatitis. *World J Gastroenterol* 2007; **13**: 837-844
18 **Yilmaz Y**, Ulukaya E, Dolar E. Serum M30 levels: a potential biomarker of severe liver disease in nonalcoholic fatty liver disease and normal aminotransferase levels. *Hepatology* 2009; **49**: 697; author reply 697
19 **Kim CH**, Younossi ZM. Nonalcoholic fatty liver disease: a manifestation of the metabolic syndrome. *Cleve Clin J Med* 2008; **75**: 721-728
20 **Jiang J**, Torok N. Nonalcoholic steatohepatitis and the metabolic syndrome. *Metab Syndr Relat Disord* 2008; **6**: 1-7
21 **Marchesini G**, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. *Curr Opin Lipidol* 2005; **16**: 421-427
22 **Margetic S**, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord* 2002; **26**: 1407-1433
23 **Cohen B**, Novick D, Rubinstein M. Modulation of insulin activities by leptin. *Science* 1996; **274**: 1185-1188
24 **Potter JJ**, Womack L, Mezey E, Anania FA. Transdifferentiation of rat hepatic stellate cells results in leptin expression. *Biochem Biophys Res Commun* 1998; **244**: 178-182
25 **Chitturi S**, Farrell G, Frost L, Kriketos A, Lin R, Fung C, Liddle C, Samarasinghe D, George J. Serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 2002; **36**: 403-409
26 **Huang XD**, Fan Y, Zhang H, Wang P, Yuan JP, Li MJ, Zhan XY. Serum leptin and soluble leptin receptor in non-alcoholic fatty liver disease. *World J Gastroenterol* 2008; **14**: 2888-2893
27 **Yalniz M**, Bahcecioglu IH, Ataseven H, Ustundag B, Ilhan F, Poyrazoglu OK, Erensoy A. Serum adipokine and ghrelin

levels in nonalcoholic steatohepatitis. *Mediators Inflamm* 2006; **2006**: 34295
28 **Uygun A**, Kadayifci A, Yesilova Z, Erdil A, Yaman H, Saka M, Deveci MS, Bagci S, Gulsen M, Karaeren N, Dagalp K. Serum leptin levels in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2000; **95**: 3584-3589
29 **Nakao K**, Nakata K, Ohtsubo N, Maeda M, Moriuchi T, Ichikawa T, Hamasaki K, Kato Y, Eguchi K, Yukawa K, Ishii N. Association between nonalcoholic fatty liver, markers of obesity, and serum leptin level in young adults. *Am J Gastroenterol* 2002; **97**: 1796-1801
30 **Angulo P**, Alba LM, Petrovic LM, Adams LA, Lindor KD, Jensen MD. Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease. *J Hepatol* 2004; **41**: 943-949
31 **Lu JY**, Huang KC, Chang LC, Huang YS, Chi YC, Su TC, Chen CL, Yang WS. Adiponectin: a biomarker of obesity-induced insulin resistance in adipose tissue and beyond. *J Biomed Sci* 2008; **15**: 565-576
32 **Kadowaki T**, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 2006; **116**: 1784-1792
33 **Xu A**, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003; **112**: 91-100
34 **Gil-Campos M**, Cañete RR, Gil A. Adiponectin, the missing link in insulin resistance and obesity. *Clin Nutr* 2004; **23**: 963-974
35 **Targher G**, Bertolini L, Scala L, Poli F, Zenari L, Falezza G. Decreased plasma adiponectin concentrations are closely associated with nonalcoholic hepatic steatosis in obese individuals. *Clin Endocrinol* (Oxf) 2004; **61**: 700-703
36 **Aygun C**, Senturk O, Hulagu S, Uraz S, Celebi A, Konduk T, Mutlu B, Canturk Z. Serum levels of hepatoprotective peptide adiponectin in non-alcoholic fatty liver disease. *Eur J Gastroenterol Hepatol* 2006; **18**: 175-180
37 **Yoon D**, Lee SH, Park HS, Lee JH, Park JS, Cho KH, Kim SM. Hypoadiponectinemia and insulin resistance are associated with nonalcoholic fatty liver disease. *J Korean Med Sci* 2005; **20**: 421-426
38 **Arvaniti VA**, Thomopoulos KC, Tsamandas A, Makri M, Psyrogiannis A, Vafiadis G, Asimakopoulos S, Labropoulou-Karatza C. Serum adiponectin levels in different types of non alcoholic liver disease. Correlation with steatosis, necroinflammation and fibrosis. *Acta Gastroenterol Belg* 2008; **71**: 355-60
39 **Lemoine M**, Ratziu V, Kim M, Maachi M, Wendum D, Paye F, Bastard JP, Poupon R, Housset C, Capeau J, Serfaty L. Serum adipokine levels predictive of liver injury in non-alcoholic fatty liver disease. *Liver Int* 2009; 1431-1438
40 **Cho YK**, Lee WY, Oh SY, Park JH, Kim HJ, Park DI, Sohn CI, Jeon WK, Kim BI, Kim SW, Oh KW, Yun EJ, Oh ES. Factors affecting the serum levels of adipokines in Korean male patients with nonalcoholic fatty liver disease. *Hepatogastroenterology* 2007; **54**: 1512-1516
41 **McTernan PG**, Kusminski CM, Kumar S. Resistin. *Curr Opin Lipidol* 2006; **17**: 170-175
42 **Kusminski CM**, McTernan PG, Kumar S. Role of resistin in obesity, insulin resistance and Type II diabetes. *Clin Sci* (Lond) 2005; **109**: 243-256
43 **Pagano C**, Soardo G, Pilon C, Milocco C, Basan L, Milan G, Donnini D, Faggian D, Mussap M, Plebani M, Avellini C, Federspil G, Sechi LA, Vettor R. Increased serum resistin in nonalcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. *J Clin Endocrinol Metab* 2006; **91**: 1081-1086
44 **Jiang LL**, Li L, Hong XF, Li YM, Zhang BL. Patients with nonalcoholic fatty liver disease display increased serum resistin levels and decreased adiponectin levels. *Eur J Gastroenterol Hepatol* 2009; **21**: 662-666
45 **Geroldi D**, Falcone C, Emanuele E. Soluble receptor for advanced glycation end products: from disease marker to potential therapeutic target. *Curr Med Chem* 2006; **13**: 1971-1978
46 **Yilmaz Y**, Ulukaya E, Gul OO, Arabul M, Gul CB, Atug O, Oral AY, Aker S, Dolar E. Decreased plasma levels of soluble receptor for advanced glycation endproducts (sRAGE) in patients with nonalcoholic fatty liver disease. *Clin Biochem* 2009; **42**: 802-807
47 **Li C**. A tale of two angiopoietin-like proteins. *Curr Opin Lipidol* 2007; **18**: 597-599
48 **Yilmaz Y**, Ulukaya E, Atug O, Dolar E. Serum concentrations of human angiopoietin-like protein 3 in patients with nonalcoholic fatty liver disease: association with insulin resistance. *Eur J Gastroenterol Hepatol* 2009; Epub ahead of print
49 **Manfredi AA**, Rovere-Querini P, Bottazzi B, Garlanda C, Mantovani A. Pentraxins, humoral innate immunity and tissue injury. *Curr Opin Immunol* 2008; **20**: 538-544
50 **Yoneda M**, Uchiyama T, Kato S, Endo H, Fujita K, Yoneda K, Mawatari H, Iida H, Takahashi H, Kirikoshi H, Inamori M, Nozaki Y, Kobayashi N, Kubota K, Saito S, Maeyama S, Sagara M, Aburatani H, Kodama T, Nakajima A. Plasma Pentraxin3 is a novel marker for nonalcoholic steatohepatitis (NASH). *BMC Gastroenterol* 2008; **8**: 53
51 **Younossi ZM**, Baranova A, Ziegler K, Del Giacco L, Schlauch K, Born TL, Elariny H, Gorreta F, VanMeter A, Younoszai A, Ong JP, Goodman Z, Chandhoke V. A genomic and proteomic study of the spectrum of nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 665-674
52 **Trak-Smayra V**, Dargere D, Noun R, Albuquerque M, Yaghi C, Gannagé-Yared MH, Bedossa P, Paradis V. Serum proteomic profiling of obese patients: correlation with liver pathology and evolution after bariatric surgery. *Gut* 2009; **58**: 825-832
53 **Emanuele E**. Is biopsy always necessary? Toward a clinico-laboratory approach for diagnosing nonalcoholic steatohepatitis in obesity. *Hepatology* 2008; **48**: 2086-2087; author reply 2087
54 **Yilmaz Y**, Ulukaya E, Dolar E. A "biomarker biopsy" for the diagnosis of NASH: promises from CK-18 fragments. *Obes Surg* 2008; **18**: 1507-1508; author reply 1509-1510

S- Editor Li LF **L- Editor** Logan S **E- Editor** Zheng XM