NON-INVASIVE BIOMARKERS OF NON-ALCOHOLIC STEATOHEPATITIS AND LIVER FIBROSIS

The dramatic increase in the prevalence of non-alcoholic steatohepatitis (NASH) world-wide[1-2] and the invasive nature of the liver biopsy as a diagnostic procedure, has necessitated the development of new non-invasive biomarkers.

Although liver biopsy is still considered the gold standard method for diagnosing NASH, the dramatic rise in NASH prevalence means that the resources needed to perform liver biopsies on every patient would be enormous and impractical. The biopsy procedure is invasive for the patient and may be associated with clinical complications such as trauma and bleeding. Additionally, it presents several limitations in terms of accuracy, reproducibility and poor diagnostic performance since a small sample of the liver parenchyma may not be representative of the pathology in the rest of the liver tissue.[3-4] Therefore, the development and validation of non-invasive biomarkers, has become a main focus of interest for screening, stratifying severity and monitoring progression of NASH and liver fibrosis in recent years.

Plasma biomarkers of inflammation, fibrosis, apoptosis and oxidative stress associated with the pathophysiological processes in NASH have been studied and validated in recent years.[5-7] One of the main determinants of NASH prognosis is the identification of fibrosis stage and rate of progression.[8-10] In this sense, the classification of no/minimal fibrosis, significant fibrosis, severe fibrosis or cirrhosis is very useful for selecting patients for treatment studies and monitoring progression/regression of the disease. Current non-invasive methods for assessing fibrosis range from biomarker assays to advanced imaging techniques such as transient elastography.

ABOUT MEDPACE CENTRAL LABORATORIES

Medpace provides customized, high quality central laboratory services to pharmaceutical and biotech clinical development industries. Our four wholly-owned laboratories offer full-service support to seven continents for phase I-IV studies. We have extensive experience from small and simple clinical trials to those that are large, global, and complex.

Our wholly-owned global laboratory facilities, standardized testing platforms, comprehensive test menu, and stellar project management teams allow Medpace to set up fully customized projects for our clients. Combined with Medpace Clinical Research Organization expertise, we provide a fully integrated solution for your clinical development needs.

MEDPACE CENTRAL LABORATORIES WITH BIOMARKER SERVICES SUPPORTING NASH STUDIES

With laboratories in the US, Europe, China and Singapore, Medpace Central Laboratories has the global reach and capabilities to conduct NASH studies in concert with Medpace CRO or as standalone service.

Our test menu includes many validated biomarkers associated with NASH: cytokeratin-18 fragments (M30 and M65), apolipoprotein A1, apolipoprotein B, leptin, adiponectin, resistin, free fatty acids, ghrelin, hsCRP, interleukin-6, and tumor necrosis factor-alpha as well as validated assays used in NASH fibrosis scores such as Fibrotest/FibroMax and ELF (Enhanced Liver Fibrosis). In addition, Medpace Central Laboratories can validate new biomarkers quickly and efficiently — typically in an industry leading 10-12 weeks.

MEDPACE AND NAFLD/NASH

As a global CRO with extensive experience in NAFLD/NASH, Medpace's therapeutically aligned project teams serve as an extension of your team — providing additional medical, regulatory and operational expertise to your study.

- Over eight years supporting NAFLD/NASH clinical trials
- In-house physicians, imaging specialists, and operational teams with relevant and recent successful execution of NASH studies with different endpoints including liver pathology
- Wholly-owned central lab with validated NASH soluble inflammation and fibrosis biomarkers
- Well established relationships with KOLs and high producing quality sites
- US and global experience that spans 14 countries, 1,300 subjects and 150 sites
- Wholly-owned imaging core lab to support NAFLD/NASH studies, ensuring imaging components such as MRE, MRI, PDFF, MRS and LMS are seamlessly integrated into the complex structure of the overall trial
- Established processes and relationships for central pathology assessments
- Hands-on regulatory affairs to guide you through the fastest path to commercial success

Medpace continues to expand on its highly recognized leadership position in the area of NAFLD/NASH clinical research.

DIAGNOSING AND MONITORING NASH

Inflammation Biomarkers

General markers of inflammation such as TNF-α, interleukin(IL) 6, high sensitivity C-reactive protein, monocyte chemoattractant protein-1 (MCP-1), and ferritin, have been reported to be elevated in patients with NASH.[11-12] Adipokines and other cytokines have been shown to correlate well with the presence and severity of the disease. In particular, high plasma levels of tumor necrosis factor α (TNF-α) and low levels of adiponectin are found in NASH patients and are associated with a greater degree of liver damage.[13-16] Adiponectin modifies insulin receptor function and influences hepatocellular free acid metabolism. Circulating levels of adiponectin are negatively associated with insulin resistance, type 2 diabetes and dyslipidemia. Recent findings from a meta-analysis[17] showed that adiponectin is a biomarker of non-alcoholic fatty liver disease (NAFLD) progression to NASH. Adiponectin - leptin ratio in combination with insulin resistance index gave an area under the receiver operating characteristic (AUROC) curve of 0.82 for prediction of disease.[18] Resistin was reported[19] to correlate well with NASH severity in a study of 91 patients. IL 6 and IL8 have also been studied and found to have an AUROC of 0.8 for the prediction of NASH.[20, 21] The ghrelin-ghrelin O-acyltransferase (GOAT) system has recently been found to play a crucial role in the development of steatosis and its progression to NASH. Furthermore, a significant correlation has been described between ghrelin expression and NASH.

Oxidative Stress Biomarkers

Oxidative stress is an important mechanism in the pathogenesis of NASH. Malonaldehyde, thiobarbituric acid reactive substances (TBARS) and oxidized low density lipoprotein (LDL) have been associated with the oxidative stress process in patients with NASH.[22-24]

Apoptosis Biomarkers

Markers of apoptosis have been shown to be very useful in differentiating simple steatosis from NASH.[25] One of the most promising biomarkers associated with the degree of hepatocyte apoptosis is cytokeratin-18 (CK-18). Activation of caspase 3 results in cleavage of CK18 and circulating levels of CK18 fragments have been investigated and associated extensively with the diagnosis of NASH. Levels of CK-18-M30 fragments have been reported to correlate well with the severity of NASH and with histological progression of the disease.[26-29] A few studies suggested that CK-18 fragments may have a better performance for the diagnosis of NASH when combined with other tests (e.g. computed tomography (CT) scan, fibroblast growth factor 21, etc.).[30] CK18-M65 levels (antibodies which recognize uncleaved CK18) have been also reported to be useful for detecting NASH.[31]

Biomarkers of Fibrosis

The severity and progression of liver fibrosis plays a key role for predicting diseased outcomes and for making therapeutic decisions in NASH patients.[33-35] In this sense, the development of non-invasive markers of hepatic fibrosis is extremely valuable to identify NASH patients that may progress to cirrhosis. On the other hand liver biopsies have shown poor diagnostic performance for assessing hepatic fibrosis.[32]

Several non-invasive methods have been used to measure the degree of extracellular matrix (ECM) turnover as predictors of fibrosis in patients with NASH. Constituents of the extracellular matrix represent a more direct method of assessing fibrogenic activity, as a dynamic process rather than a static one.

Combinations of both clinical markers and ECM turnover are used as NASH fibrosis scores and include the FibroTest and the Enhanced Liver Fibrosis test (ELF). Fibrotest is an algorithm of 13 markers derived from regression analysis including haptoglobin, α 2-macroglobulin, apolipoprotein A1, bilirubin, γ-glutamyl transpeptidase, age and gender.[35, 36] The use of Fibrotest in NAFLD has shown a sensitivity of 92% and a specificity of 98% for advanced fibrosis detection.[41]

The ELF test provides a single score by combining, in an algorithm, the quantitative measurements of three direct serum markers, including hyaluronic acid (HA: a glycosaminoglycan that is produced by hepatic stellate cells), amino-terminal pro-peptide of type III pro-collagen (PIIINP: a marker of early fibrogenesis and inflammation), and tissue inhibitor of metalloproteinase 1 (TIMP-1: which is the circulating inhibitor of matrix metalloproteinases (MMP) enzymes that can enhance fibrogenesis). Studies have confirmed that the ELF test can accurately determine the degree of liver fibrosis[37-39] with a good discrimination of the different fibrosis stages (mild moderate, severe liver fibrosis and cirrhosis) showing a sensitivity of 0.90 and a specificity of 0.63 for identifying severe fibrosis.[40] Therefore, the ELF test may be useful to evaluate the impact of treatment targeted to the underlying causes, and in the development of new treatments, such as anti-fibrotic drugs.

Imaging Techniques

Magnetic resonance imaging-estimated proton density fat fraction (MRI-PDFF) is the imaging technique with the best accuracy and typically used in NASH clinical research settings. This imaging technique is useful to determine hepatic fat content.[96-99] Other imaging techniques for the evaluation of hepatic fibrosis are magnetic resonance elastography[42] and ultrasound-based transient elastography.[43] The rationale for these methods is that the collagen deposition associated with fibrosis produces a lattice-like framework that imparts stiffness to the pressure compliance of the liver. Transient elastography (TE) has been well validated showing overall a good accuracy and an excellent diagnostic capability across different liver diseases for cirrhosis.[44]

Emerging Biomarkers

In the past years, a great effort has been made for the identification and validation of novel biomarkers to assess NASH using high-throughput analysis based on genomics, proteomics, and metabolomics.

Several studies have shown differences in the expression of a panel of genes when comparing NASH patients to controls[45] or patients with other cirrhosis etiologies. Some of the upregulated gene sets found in NASH included those responsible for the platelet derived growth factor, hepatic nuclear factor 3 and the smad4 pathways.[46]

One of the polymorphisms showing stronger association with NASH is patatin-like phospholipase domain-containing 3 (PNPLA3) rs738409. It has been observed that individuals with the rs738409 polymorphism presented a significantly increased risk for developing NASH.[61]

There is a different protein expression profile in the serum of NASH patients vs normal and simple steatosis patients.[47,48] Some of the proteins that showed increased expression in NASH disease include lumican, (a keratan sulphate proteoglycan involved in collagen cross-linking and epithelial-mesenchymal transition[49,50] and hemoglobin.[51]

Changes in glycosylation in serum can be particularly useful as biomarkers of liver dysfunction, since most glycoproteins are made in the liver. In addition, the asialoglycoprotein receptor and the mannose/O-linked betaN-acetylglucosamine receptor in liver are important in clearing aberrantly glycosylated proteins from the serum. Thus, the N-glycome profile will reflect any changes in the liver and can be very valuable biomarker of NASH disease. The GlycoCirrhotest,[52] the GlycoFibrotest,[53] and the GlycoHCC test[54] are tests that are associated with the detection of cirrhosis, fibrosis and hepatocellular carcinoma respectively, based on differences in N-glycome patterns.

Circulating microRNAs (miRNAs) are very stable in the blood and can be easily quantitated, constituting promising clinically-useful biomarkers of liver injury in the NASH disease. miR-122 is the most abundant miRNA in the adult human liver and its expression is strongly decreased in NASH patients compared to healthy individuals.[55,56] Several studies have been described an association between miR-122 and advanced fibrosis in the liver.[57] The expression of miR-21 in the liver has been shown to progressively increase with progression of NAFLD disease.[58] Some studies reported a correlation between histological results from NASH patients and miR-16 circulating levels.[59] miR-34a has been found to regulate cell death, oxidative stress and metabolism in the liver and correlates with NAFLD histologic severity.[56,60]

REFERENCES

1. Sanyal AJ. NASH: a global health problem. Hepatol Res.2011;41(7):670–4.

2. Williams CD, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology. 2011;140(1):124–31.

3. Wieckowska A, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. Semin Liver Dis. 2008;28(4):386–95.

4. Ratziu V, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128(7):1898–906.

5. Wieckowska A, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. Hepatology 2007; 46: 582-589.

6. Yoneda M, Yoneda M, Mawatari H, Fujita K, et al. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). Dig Liver Dis 2008; 40: 371-378.

7. Mandato C, Lucariello S, Licenziati MR, Franzese A, Spagnuolo MI, Ficarella R, Pacilio M, Amitrano M, Capuano G, Meli R, Vajro P. Metabolic, hormonal, oxidative, and inflammatory factors in pediatric obesity-related liver disease. J Pediatr 2005; 147: 62-66.

8. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999; 116:1413-1419

9. 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.

10. Angulo P. Long-term mortality in nonalcoholic fatty liver disease: is liver histology of any prognostic significance? Hepatology 2010; 51: 373-375

11. Targher G. Relationship between high-sensitivity C-reactive protein levels and liver histology in subjects with non-alcoholic fatty liver disease. J Hepatol 2006; 45: 879-881.

12. Hui JM, Farrell GC, Kench JG, George J. High sensitivity C-reactive protein values do not reliably predict the severity of histological changes in NAFLD. Hepatology 2004; 39: 1458-1459.

13. Jarrar MH, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, Fang Y, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2008; 27: 412-421

14. Haukeland JW, Damås JK, Konopski Z, Løberg EM, Haaland T, Goverud I, Torjesen PA, Birkeland K, Bjøro K, Aukrust P. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated levels of CCL2. J Hepatol 2006; 44: 1167-1174

15. Manco M, Marcellini M, Giannone G, Nobili V. Correlation of serum TNF-alpha levels and histologic liver injury scores in pediatric nonalcoholic fatty liver disease. Am J Clin Pathol 2007; 127: 954-960

16. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology 2004; 40: 46-54

17. Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: A systematic review and meta-analysis. Metabolism. 2011;60:313–26.

18. 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 nonalcoholic fatty liver disease. Liver Int 2009; 29: 1431-1438.

19. 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.

20. Abiru S, Migita K, Maeda Y, Daikoku M, Ito M, Ohata K, Nagaoka S, Matsumoto T, Takii Y, Kusumoto K, Nakamura M, Komori A, Yano K, Yatsuhashi H, Eguchi K, Ishibashi H. Serum cytokine and soluble cytokine receptor levels in patients with non-alcoholic steatohepatitis. Liver Int 2006; 26: 39-45.

21. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. Am J Gastroenterol 2008; 103: 1372-1379.

22. Yesilova Z, Yaman H, Oktenli C, Ozcan A, Uygun A, Cakir E, Sanisoglu SY, Erdil A, Ates Y, Aslan M, Musabak U, Erbil MK, Karaeren N, Dagalp K. Systemic markers of lipid peroxidation and antioxidants in patients with nonalcoholic Fatty liver disease. Am J Gastroenterol 2005; 100: 850-855

23. Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2004; 99: 1497-1502.

24. Fromenty B, Robin MA, Igoudjil A, Mansouri A, Pessayre D. The ins and outs of mitochondrial dysfunction in NASH. Diabetes Metab 2004; 30: 121-138.

25. 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.

26. 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.

27. Diab DL, Yerian L, Schauer P, Kashyap SR, Lopez R, Hazen SL, Feldstein AE. Cytokeratin 18 fragment levels as a noninvasive biomarker for nonalcoholic steatohepatitis in bariatric surgery patients. Clin Gastroenterol Hepatol 2008; 6: 1249-1254.

28. Younossi ZM, Page S, Rafiq N, Birerdinc A, Stepanova M, Hossain N, Afendy A, Younoszai Z, Goodman Z, Baranova A. A biomarker panel for non-alcoholic steatohepatitis (NASH) and NASH-related fibrosis. Obes Surg 2011; 21:431-439.

29. Feldstein AE, Alkhouri N, De Vito R, Alisi A, Lopez R, Nobili V. Serum cytokeratin-18 fragment levels are useful biomarkers for nonalcoholic steatohepatitis in children. Am J Gastroenterol 2013; 108: 1526-1531.

30. Pearce SG, Thosani NC, Pan JJ. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. Biomark Res. 2013;1(1):7.

31. Shen J, Chan HL, Wong GL, Chan AW, Choi PC, Chan HY, Chim AM, Yeung DK, Yu J, Chu WC, Wong VW. Assessment of non-alcoholic fatty liver disease using serum total cell death and apoptosis markers. Aliment Pharmacol Ther 2012; 36: 1057-1066.

32. Ratziu V, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128(7):1898–906.

33. Gholam PM, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. Am J Gastroenterol 2007; 102: 399-408 [PMID: 17311652

34. Palekar NA, Naus R, Larson SP, Ward J, Harrison SA. Clinicalm model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. Liver Int 2006; 26: 151-156.

35. Poynard T, Ratziu V, Charlotte F, Messous D, Munteanu M, Imbert-Bismut F, Massard J, Bonyhay L, Tahiri M, Thabut D, Cadranel JF, Le Bail B, de Ledinghen V. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcohol steato hepatitis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol 2006; 6: 34.

36. Colli A, Cocciolo M, Mumoli N, Cattalini N, Fraquelli M, Conte D. Hepatic artery resistance in alcoholic liver disease. Hepatology 1998; 28: 1182-1186.

37. Younossi ZM, et al. A biomarker panel for non-alcoholic steatohepatitis (NASH) and NASH-related fibrosis. Obes Surg. 2011;21(4):431–9.

38. Ratziu V, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128(7):1898–906.

39. Angulo P, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45(4):846–54.

40. Guechot, J., Trocme, C.et al (2012). 'Independent validation of the Enhanced Liver Fibrosis (ELF) score in the ANRS HC EP 23 Fibrostar cohort of patients with chronic hepatitis C'. Clin Chem Lab Med, 50 (4), 693-9.

41. Vlad R, Julien M, Frederic C, Djamila M, Françoise I, Luninita B, Mohamed T, Mona M, Dominique T, Jean François C, Brigitte L, Victor de L,7 Thierry P, and the LIDO Study Group and the CYTOL study group. Diagnostic value of biochemical markers (FibroTest-FibroSURE) for the prediction of liver fibrosis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol. 2006; 6: 6.42. Huwart L, et al. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. Gastroenterology. 2008;135(1):32–40.

43. Yoneda M, et al. Transient elastography in patients with nonalcoholic fatty liver disease (NAFLD). Gut. 2007;56(9):1330–1.

44. Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008; 134: 960-974 for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008; 134: 960-974

45. Younossi ZM, Gorreta F, Ong JP, Schlauch K, Del Giacco L, Elariny H, Van Meter A, Younoszai A, Goodman Z, Baranova et al. Hepatic gene expression in patients with obesity-related non-alcoholic steatohepatitis. Liver Int 2005; 25: 760-771.

46. Sreekumar R, Rosado B, Rasmussen D, Charlton M. Hepatic gene expression in histologically progressive nonalcoholic steatohepatitis. Hepatology 2003; 38: 244-251.

47. 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.

48. 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.

49. Bieback K, Kern S, Klüter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells 2004; 22: 625-634

50. Allen KJ, Mifsud NA, Williamson R, Bertolino P, Hardikar W. Cell-mediated rejection results in allograft loss after liver cell transplantation. Liver Transpl 2008; 14: 688-694.

51. Yu C, Xu C, Xu L, Yu J, Miao M, Li Y. Serum proteomic analysis revealed diagnostic value of hemoglobin for nonalcoholic fatty liver disease. J Hepatol 2012; 56: 241-247.

52. Callewaert N, Van Vlierberghe H, Van Hecke A, Laroy W, Delanghe J, Contreras R. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. Nat Med 2004; 10: 429-434.

53. Vanderschaeghe D, Laroy W, Sablon E, Halfon P, Van Hecke A, Delanghe J, Callewaert N. GlycoFibroTest is a highly performant liver fibrosis biomarker derived from DNA sequencer-based serum protein glycomics. Mol Cell Proteomics 2009; 8: 986-994.

54. Liu XE, Desmyter L, Gao CF, Laroy W, Dewaele S, Vanhooren V, Wang L, Zhuang H, Callewaert N, Libert C, Contreras R, Chen C. N-glycomic changes in hepatocellular carcinoma patients with liver cirrhosis induced by hepatitis B virus. Hepatology 2007; 46: 1426-1435

55. Pirola C.J., Fernandez Gianotti T., Castano G.O., Mallardi P., San Martino J., Mora Gonzalez Lopez Ledesma M., Flichman D., Mirshahi F., Sanyal A.J., Sookoian S. Circulating microRNA signature in non-alcoholic fatty liver disease: From serum non-coding RNAs to liver histology and disease pathogenesis. Gut. 2015;64:800–812.

56. Cheung O., Puri P., Eicken C., Contos M.J., Mirshahi F., Maher J.W., Kellum J.M., Min H., Luketic V.A., Sanyal A.J. Nonalcoholic steatohepatitis is associated with altered hepatic microRNA expression. Hepatology. 2008;48:1810–1820.

57. Halasz T., Horvath G., Par G., Werling K., Kiss A., Schaff Z., Lendvai G. miR-122 negatively correlates with liver fibrosis as detected by histology and fibroscan. World J. Gastroenterol. 2015;21:7814–7823.

58. Rodrigues P.M., Afonso M.B., Simão A.L., Caridade M., Carvalho C.C., Trindade A., Duarte A., Borralho P.M., Machado M.V., Cortez-Pinto H., et al. miR-21 inhibition and FXR activation synergistically ameilorate disease pathogenesis in a mouse model of NAFLD. J. Hepatol. 2015;62:S704.

59. Sharma H., Estep M., Birerdinc A., Afendy A., Moazzez A., Elariny H., Goodman Z., Chandhoke V., Baranova A., Younossi Z.M. Expression of genes for microRNA-processing enzymes is altered in advanced non-alcoholic fatty liver disease. J. Gastroenterol. Hepatol. 2013;28: 1410–1415.

60. Castro R.E., Ferreira D.M., Afonso M.B., Borralho P.M., Machado M.V., Cortez-Pinto H., Rodrigues C.M. miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. J. Hepatol. 2013;58:119–125.

61. Renfan Xu, Anyu Tao, Shasha Zhang, Youbin Deng, and Guangzhi Chen. Association Between Patatin-Like Phospholipase Domain Containing 3 Gene (PNPLA3) Polymorphisms and Nonalcoholic Fatty Liver Disease: A HuGE Review and Meta-Analysis. Sci Rep. 2015; 5: 9284.