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Apoptosis and diagnosis of nonalcoholic steatohepatitis

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An apoptosis panel for nonalcoholic steatohepatitis diagnosis.

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Nonalcoholic fatty liver disease (NAFLD) is a wide-spectrum disease entity from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis. After Ludwig et al[1] introduced the name of NASH firstly in 1980, the concerns about NASH have been explored intensively. The majority of cryptogenic cirrhosis diagnosed in the past presume the progressed state of NASH. Approximately 90% of abnormal liver function without specific causes such as virus, alcohol, toxic and genetic factor presumes NAFLD.[2] For diagnosis of NAFLD, several causes which can induce hepatic steatosis should be excluded such as protein-calorie malnutrition, starvation, total parenteral nutrition, rapid weight loss, amiodarone, methotraxate, valproic acid, glucocorticoid, calcium-channel blocker, tamoxifen, tetracycline, and aspirin.[2] Although the prevalence of NAFLD is different among countries and races, about 10-20% of general population may have NAFLD.[2]

Simple hepatic steatosis is usually thought as a non-progressive condition,[3] but about 5% of hepatic steatosis can progress to NASH.[4] In contrast to hepatic steatosis, approximately 30% of NASH shows the progression of fibrosis within 5 years;[5] about 20% of NASH progresses to cirrhosis during lifetime.[6] Once cirrhosis is developed, the risk of complications of cirrhosis is increased such as ascites, variceal bleeding, hepatic encephalopathy and development of hepatocellular carcinoma.[7] NASH should be discriminated from simple hepatic steatosis because of the above reasons. Liver biopsy is still gold standard for differentiating between steatosis and NASH. The pathologic findings of NASH are macrovesicular steatosis, lobular inflammation, Mallory bodies, ballooning degeneration, and perisinusoidal/perivenular fibrosis. Using these parameters, Brunt et al[8] introduced necro-inflammatory grade and fibrosis score system for NASH in 1999. Recently, NASH Clinical Research Network introduced new system in which the degrees of steatosis, lobular inflammation, hepatocyte ballooning and Mallory bodies are scored as NAFLD activity score (NAS).[9] NASH can be differentiated from hepatic steatosis with NAS system. However, liver biopsy is an invasive procedure with significant complication like bleeding. Several non-invasive biomarkers for diagnosis of NASH have been investigated recently. Palekar et al[10] investigated the model for predicting NASH using 8-epi-PGF2α, TGF-β, hyaluronic acid and adiponectin (sensitivity: 73.7%, specificity: 65.7%, positive predictive value: 68.2%, negative predictive value: 68.2%). Poynard et al[11] introduced the model, Steatotest for predicting over 30% steatosis using alanine aminotransferase (ALT), a2-macroglobulin, apolipoprotein A-I, haptoglobin, total bilirubin, γ-glutamyl transpeptidase (GGT), cholesterol, triglycerides, glucose, age, gender and body mass index (BMI) (sensitivity: 90%, specificity: 90%, negative predictive value: 93%, positive predictive value: 63%). FibroTest was introduced for predicting

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Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NAS, NAFLD activity score; ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; BMI, body mass index; AST, aspartate aminotransferase; CK-18, cytokeratin 18; sFas, soluble Fas; AUC, area under curve

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advanced fibrosis (F3, F4) using α2-macroglobulin, apolipoprotein A-I, haptoglobin, total bilirubin, GGT and ALT (positive predictive value: 73%, negative predictive value: 90%).[12] Similarly, NAFLD fibrosis score was investigated for predicting advanced fibrosis using age, hyperglycemia, BMI, platelet count, albumin and aspartate aminotransferase (AST)/ ALT ratio (positive predictive value: 82%, negative predictive value: 93%).[13] Several inflammatory mediators are increased in NASH compared to simple hepatic steatosis such as tumor necrosis factor (TNF)-α, interleukin-6 (IL-6), CC-chemokine ligand 2 (CCL-2), and hyaluronic acid.[14-16]

Deregulation of hepatocyte apoptosis is an important mechanism of liver injury and NASH development.[17-19] Fas has a critical role in apoptosis pathway as a death receptor member of the TNF receptor family. Expression of this membrane protein is increased in NASH patients.[17] Accumulation of free fatty acids in hepatocytes results in up-regulation of Fas in the cell membrane which increases the sensitivity to Fas mediated apoptosis.[20] At the level of mitochondria, apoptosis pathway induces permiabilization of the mitochondrial outer membrane and release of multiple protein from the mitochondrial inner membrane space to cytoplasm.[21] This results in activation of effector caspases (mainly caspase 3) which will cleave substrates in cytoplasm like cytokeratin 18 (CK-18), the major intermediate filament protein of liver, inducing the specific morphologic change of apoptosis.[21] There are several reports showing that the quantification of this caspase generated CK-18 fragments is useful for diagnosis of NASH.[22-25]

In subject study, they investigated the apoptosis panel for non-invasive diagnosis of NASH using these two biomarkers, serum CK-18 fragments and soluble Fas (sFas). Compared to each single biomarker, the combination of two biomarkers, serum CK-18 fragments and sFas showed significantly higher area under curve (AUC) (0.86 in CK-18, 0.86 in sFas and 0.93 in CK-18 with sFas), sensitivity (88%), and specificity (89%) for diagnosis of NASH. The high AUC in the validation group (0.79) indicates the reproducibility of this apoptosis panel. This apoptosis panel for NASH diagnosis is non-invasive, simple, reproducible and reliable method which is needed in real clinical situation. Not only for diagnosis but also for assessment of treatment response and prognosis, this apoptosis panel with serum CK-18 fragments and sFas could be used after proper researches are done.

In conclusion, this diagnostic apoptosis panel including serum CK-18 fragments and sFas for diagnosis of NASH is useful test in clinical practice for noninvasive diagnosis of NASH as a simple, easy to measure, reproducible and reliable method. Further studies are needed to validate the accuracy of this apoptosis panel for NASH diagnosis in each race and country. We need to investigate whether this apoptosis panel could be used for assessment of treatment response and predicting prognosis of NASH.

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