Table 2: Clinical and biochemical characteristics of 209 subjects with NAFLD divided by serum cytokeratin-18 tertiles.

| | Cytokeratin-18 tertiles | | | $P$ |
|---|---|---|---|---|
| | I | II | III | |
| Males (%) | 68.1 | 63.0 | 62.7 | ns |
| Age (yrs) | 53.8 ± 12.9 | 54.7 ± 10.9 | 54.2 ± 12.3 | ns |
| Body mass index (kg/m$^2$) | 27.2 ± 3.9 | 31.6 ± 3.3 | 36.1 ± 5.4 | <0.001 |
| Urinary 8-iso-PGF2$\alpha$ (pg/mg creatinine) | 616.7 ± 122.9 | 727.5 ± 82.1 | 800.9 ± 82.1 | <0.001 |
| HOMA-IR | 2.8 (1.9/4.0) | 3.2 (2.4/5.7) | 5.3 (3.4/7.3) | <0.001 |
| Total cholesterol (mg/dL) | 201.4 ± 38.4 | 196.9 ± 38.5 | 201.4 ± 41.5 | ns |
| HDL (mg/dL) | 50 (43/61) | 47 (38/54) | 43 (39/51) | <0.05 |
| Triglycerides (mg/dL) | 125.5 (85.5/160.3) | 138 (102.0/180.5) | 165.0 (125.0/206.0) | <0.01 |
| $\gamma$-GT (IU/L) | 23 (16.8/36.5) | 27 (17.5/44.0) | 30 (18/53) | ns |
| ALT (IU/L) | 31 (22.8/40) | 28 (20/41.5) | 26 (20/40) | ns |
| AST (IU/L) | 22 (18/28) | 21 (17/26.5) | 21 (17/28) | ns |
| Adiponectin (ng/mL) | 12 (10.4/14.1) | 7.5 (5.5/10.5) | 5 (4/7) | <0.001 |
| sNOX2-dp (pg/mL) | 49 (40/58.3) | 60 (50/66) | 67 (64/71) | <0.001 |
| Hamaguchi score | 3.5 ± 1.3 | 4.0 ± 1.3 | 4.3 ± 1.3 | <0.01 |
| Metabolic syndrome (%) | 49.4 | 69.0 | 84.8 | <0.001 |
| Diabetes (%) | 20.3 | 30.1 | 43.3 | <0.05 |

Table 3: Correlations between serum cytokeratin-18 and some clinical and metabolic characteristics.

| | Cytokeratin-18 | |
|---|---|---|
| | $r$ | $P$ |
| Age (yrs) | 0.031 | ns |
| BMI (kg/m$^2$) | 0.577 | <0.001 |
| Waist circumference | 0.601 | <0.001 |
| HOMA-IR | 0.191 | <0.01 |
| Fasting blood glucose (mg/dL) | 0.216 | <0.01 |
| Total cholesterol (mg/dL) | 0.036 | ns |
| HDL cholesterol (mg/dL) | −0.150 | <0.05 |
| Triglycerides (mg/dL) | 0.100 | ns |
| $\gamma$-GT (IU/l) | 0.137 | <0.05 |
| AST (IU/l) | −0.078 | ns |
| ALT (UI/l) | −0.039 | ns |
| Serum ferritin (mg/dL) | −0.106 | ns |
| Serum albumin (mg/dL) | −0.207 | <0.01 |
| Adiponectin | −0.455 | <0.001 |
| Urinary 8-iso-PGF2$\alpha$ (pg/mg creatinine) | 0.607 | <0.001 |
| sNOX2-dp (pg/mL) | 0.451 | <0.001 |
| Hamaguchi score | 0.194 | <0.001 |
| Spleen diameter | 0.190 | <0.05 |
| NAFLD Fibrosis score | 0.299 | <0.001 |
| MetS score | 0.377 | <0.001 |

for every 50 U/L increase) [26]. Moreover, CK-18 fragment levels were validated as noninvasive biomarkers for NASH also in a multicenter study performed in a large, diverse population of patients with biopsy-proven NAFLD [7].

Consistent with this theory, we found a significant correlation between CK-18 serum levels and NFS, an accurate, noninvasive scoring system based on routinely measured and readily available clinical and laboratory data, that identifies advanced liver fibrosis in patients with NAFLD [27]. Recently, NFS has been validated for predicting death or liver complications in NAFLD patients over long-term follow-up [28]. To our knowledge, this is the first time that the association between serum CK-18 and NFS has been described.

The finding of a strong independent positive association of two reliable markers of oxidative stress with a marker of hepatocyte apoptosis is consistent with the "two-hit" theory based on the prominent role of oxidative stress as a major player triggering the progression of steatosis to NASH. In fact, according to the "two hits hypothesis," the development of NASH requires "two hits" to become manifested. The first one is represented by the development of steatosis, while the second hit is induced by a disbalance between oxidative stress and antioxidant systems, leading to cell injury and inflammation (i.e., steatohepatitis) and lipid peroxidation. In keeping with this theory, hepatocyte apoptosis is likely to be considered a component of the second hit. Accordingly, a working model in which apoptosis and formation of reactive oxygen species are caspase dependent [9], with final release of CK-18 fragments, has been proposed. Indeed, cell repair, inflammation, regeneration, and fibrosis typical of NASH may be triggered by hepatocyte apoptosis. A link between hepatocyte apoptosis and liver fibrogenesis is supported by both experimental and human studies [8].

High serum CK-18 values were associated with high HOMA-IR, high fasting blood glucose and triglycerides, and low HDL cholesterol, that is, the metabolic features of MetS, whose prevalence in patients belonging to the top CK-18 tertile reached 85%.