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# Higher levels of serum uric acid influences hepatic damage in patients with non-alcoholic fatty liver disease (NAFLD)

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## ABSTRACT

**Background:** recent evidence suggests a causal link between serum uric acid and the metabolic syndrome, diabetes mellitus, arterial hypertension, and renal and cardiac

disease. Uric acid is an endogenous danger signal and activator of the inflammasome, and has been independently associated with an increased risk of cirrhosis.

**Aim and methods:** six hundred and thirty-four patients from the nation-wide HEPAMET registry with biopsy-proven NAFLD (53% NASH) were analyzed to determine whether hyperuricemia is related with advanced liver damage in patients with non-alcoholic fatty liver disease (NAFLD). Patients were divided into three groups according to the tertile levels of serum uric acid and gender.

**Results:** the cohort was composed of 50% females, with a mean age of 49 years (range 19-80). Patients in the top third of serum uric acid levels were older ($p = 0.017$); they had a higher body mass index ($p < 0.01$), arterial blood pressure ($p = 0.05$), triglyceridemia ($p = 0.012$), serum creatinine ($p < 0.001$) and total cholesterol (0.016) and lower HDL-cholesterol (0.004). According to the univariate analysis, the variables associated with patients in the top third were more advanced steatosis ($p = 0.02$), liver fibrosis (F2-F4 *vs* F0-1; $p = 0.011$), NASH ($p = 0.002$) and NAS score ($p = 0.05$). According to the multivariate logistic regression analysis, the top third of uric acid level was independently associated with steatosis (adjusted hazard ratio 1.7; CI 95%: 1.05-2.8) and NASH (adjusted hazard ratio 1.8; CI 95%: 1.08-3.0) but not with advanced fibrosis (F2-F4) (adjusted hazard ratio 1.09; CI 95%: 0.63-1.87).

**Conclusion:** higher levels of serum uric acid were independently associated with hepatocellular steatosis and NASH in a cohort of patients with NAFLD. Serum uric acid levels warrants further evaluation as a component of the current non-invasive NAFLD scores of histopathological damage.

**Key words:** Serum uric acid. NAFLD. NASH.

## INTRODUCTION

Traditionally, hyperuricemia was thought to be a component of the metabolic syndrome (MetS) secondary to insulin resistance (1). Conversely, fructose-induced hyperuricemia inhibits endothelial production of nitric oxide (NO) that is involved in glucose uptake by tissues. As a result, hyperuricemia might be one of the causal mechanisms of insulin resistance (2). Allopurinol or benzobromarone administration

prevents many features of MetS, which supports the pathogenic role of uric acid in this syndrome (2). Furthermore, uric acid might be a key factor in cardiovascular risk of MetS, as it inhibits the acetylcholine-mediated vasodilation. Recent evidence suggests a direct causal link between hyperuricemia, diabetes mellitus, cardiovascular disease and renal disease (3-6).

Uric acid has antioxidant capacity at the extracellular level, thus circulating levels might attenuate oxidative stress of MetS (7,8). However, once inside the smooth muscle, endothelial cells or adipocytes may have detrimental effects (9,10) such as platelet aggregation (11), NO inhibition (12) and inflammation (13). Overall, these findings support the concept that hyperuricemia is not a secondary phenomenon and may play a key role in the pathogenesis and progression of MetS (14-19). Furthermore, Afzali et al. examined data from the National Health and Nutrition Examination Survey (NHANES) and found that individuals in the top third of uricemia levels had a higher risk of cirrhosis-associated hospitalization or death (20).

Very few studies have evaluated the potential relationship between hyperuricemia and the clinical and histological severity of non-alcoholic fatty liver disease (NAFLD). In this study, the association between serum uric acid (SUA) and the severity of liver damage was determined in a Spanish cohort with biopsy-proven NAFLD (21).

## PATIENTS AND METHODS

The nationwide HEPAMET registry includes a prospective follow-up of patients with biopsy-proven NAFLD (21). In this study, a retrospective observational analysis of 634 patients from ten Spanish institutions was performed. Patients included in the registry met at least one of the following criteria: a liver biopsy with proven non-alcoholic steatohepatitis (NASH) or hepatocellular steatosis. Exclusion criteria included secondary causes of NAFLD/NASH in the setting of chronic liver diseases, such as chronic viral hepatitis B or C or an alcohol consumption higher than 20 g/day in females or 30 g/day in males. In addition, autoimmune hepatitis or primary biliary cholangitis (PBC), primary hemochromatosis, Wilson disease, a deficit of α1-antitrypsin or a recent history of drugs that could induce hepatocellular steatosis were exclusion criteria for the HEPAMET registry. A separate analysis by gender was

performed, as hyperuricemia is defined as > 5.5 mg/dl in females and 6.5 mg/dl in males. The cohort was divided into three tertiles according to uricemia (dependent variable). The cut-off for the first (T1) and second tertiles (T2) in females were 4.5 mg/dl and 5.6 mg/dl, respectively and 5.6 mg/dl and 6.8 mg/dl in males, respectively.

The study was performed according to the guidelines of the Declaration of Helsinki and the local Ethics Committee (HUFA) approved all procedures involving patients. Patient data were coded in order to anonymize cases.

**Aims**

The primary objectives were to determine whether higher levels of SUA (top tertile) were associated with a higher grade of hepatic steatosis, necro-inflammation and fibrosis in patients with NAFLD and also liver-related survival (death or liver transplantation).

Secondary goals were to explore a potential association between high SUA and components of the metabolic syndrome such as glycaemia, triglyceridemia, serum HDL and LDL/cholesterol, the homeostasis model for insulin resistance (HOMA-IR) and arterial hypertension.

**Histopathological evaluation**

Liver biopsies were evaluated by the local pathologist at each participating center and were evaluated according to the NAS score (21). Hepatocellular steatosis was scored as follows: < 5% grade 0, 5-33% grade 1, > 33%-66% grade 2 and > 66% grade 3. Lobular inflammation was graded as 0 if no inflammation foci were observed, grade 1 if there were less than 2 foci per 200 times amplification field, grade 2 if there were 2-4 foci/200x field and grade 3 if > 4 foci/200x field were observed. Hepatocellular ballooning is a histological marker of hepatocellular death. This was graded as 1 when a few ballooning cells were present or 2 if there were many cells with prominent ballooning. The stage of liver fibrosis was as follows: 0 if no fibrosis was observed, 1 if perisinusoidal or portal fibrosis was observed, 2 if perisinusoidal and portal/periportal fibrosis was observed, 3 if bridging fibrosis was present and 4 if cirrhosis was already present. Advanced liver fibrosis was considered when patients had stage F2 to F4

fibrosis.

**Variables**

The independent variables analyzed included the following: age, gender, body mass index (BMI), serum glucose, HOMA-IR, serum cholesterol, LDL and HDL-cholesterol, serum triglycerides, INR, serum albumin, total bilirubin and treatment with serum modifying uricemia drugs (xanthine-oxidase inhibitors, thiazides or loop diuretics, low dose salicylates, benzobromarone, probenecid or sulfinpyrazone), NAFLD score, NAS histological score and its components (20) and the occurrence of events (liver-associated death or liver transplantation).

**Statistical analysis**

Statistical analysis was performed using the SPSS v17 software. The cut-off points were 4.5 and 5.6 mg/ml in females and 6.8 mg/dl in males. Quantitative variables were expressed as the mean ± SD and the median and interquartile range, depending on the type of distribution. Absolute and relative frequencies were used for qualitative data. A univariate analysis was used to assess the clinical differences in SUA tertiles and the relationship with dependent variables. The Chi-square test or Fisher's exact test were used for qualitative variables and one-way ANOVA or the non-parametric Kruskall-Wallis test to study differences in the distribution of quantitative variables.

Univariate and multivariate logistic regression models were adjusted for potential confounding factors in order to explain the potential association of SUA and disease progression. Univariate and multivariate lineal models were adjusted to analyze the effect of SUA on the NAS and NAFLD scores. p values of $p \leq 0.05$ were considered as significant. All significant variables according to the univariate analysis were introduced into the logistic multivariate analysis.

**RESULTS**

Data from 634 patients was collected and 317 cases were female. Patients in the top third tended to be older, had arterial hypertension, hypertriglyceridemia and BMI, lower LDL-cholesterol and higher serum creatinine (Table 1). Survival analysis was not

possible as there were insufficient events (eight deaths and one liver transplantation).

As summarized in table 2, patients in the top third had more NASH, grade 2-3 hepatocellular steatosis, stage 2-4 of fibrosis and higher NAFLD score values. The NAFLD score was higher in the top third than in the second third (p = 0.05), but not for the first third (Table 2). In addition, patients in the top third had a higher NAS histological score than those in the first third (Table 2). In contrast, there was no association between the parameters of hepatocellular necro-inflammation such as hepatocellular ballooning and lobular or portal inflammation and SUA. In addition, SUA did not correlate with serum markers of systemic inflammation such as the C relative protein (CRP) (n = 310; r = 0.081; p = 0.155).

The multivariate analysis was adjusted for age, gender, arterial hypertension and serum creatinine. Patients in the top third of SUA more frequently had hepatocellular steatosis (grade 2-3 *vs* 0-1) than patients in the second tertile (adjusted hazard ratio 1.892; CI 95%: 1.153-3.1; p = 0.012) and those in the first tertile (adjusted hazard ratio 1.723; CI 95%: 1.051-2.826; p = 0.031). In addition, patients in the top third more frequently had NASH than those in the first tertile (adjusted hazard ratio 1.8; CI 95%: 1.077-3.). However, there was no association with advanced fibrosis (F2-F4) (Table 3).

Twenty-four patients were taking allopurinol (3.81%) and 56, thiazides (8.89%). Those who received allopurinol had a lower rate of grade 2-3 hepatocellular steatosis than those receiving thiazides (41.67% [n = 5] *vs* 64.1% [n = 50]). They also had a lower rate of fibrosis (33.3% [n = 4] *vs* 45.57% [n = 36]), although these differences did not reach statistical significance.

## DISCUSSION

In this cohort, patients in the top third of SUA were older and more frequently had components of the metabolic syndrome such as arterial hypertension, hypertriglyceridemia, BMI and lower HDL-cholesterol. Older patients may have a longer NAFLD evolution and a lower glomerular filtration rate, which explains the association between NASH and SUA. However, the association between higher SUA levels with hepatocellular steatosis and NASH was maintained when the analysis was adjusted for age, gender, renal function and arterial hypertension.

SUA has been independently related to NAFLD in large cross-sectional studies. However, a NAFLD diagnosis was established by abdominal ultrasound (22) and the impact of hyperuricemia on histopathology could not be established in these studies. Thus, the influence of SUA on liver histopathology has been scarcely explored. A recent Italian study addressed the association of uric acid and NASH and the authors found that HOMA index, female gender and SUA were independently associated with NASH (23). In the present cohort, there were no differences among the SUA groups with regard to the presence of diabetes, glycemia and HOMA-IR. In this regard, a recent large cross-sectional study found that more non-diabetic patients in the top quartile of serum SUA had NAFLD in comparison to the lower quartile, independently of metabolic syndrome (24). This suggests that some metabolic routes, other than insulin resistance and diabetes, may underlie NAFLD pathogenesis. No correlation between SUA levels and the HOMA-index was found, although there was an independent association between serum SUA and hepatocellular steatosis. As previously mentioned, other mechanisms may induce steatosis. Recently, mitochondrial oxidative stress and *de novo* lipogenesis induced by uric acid was found in *in vitro* and *in vivo* studies in hepatic cells and murine liver tissue. This suggests that uric acid directly stimulates DNL and promotes hepatic inflammatory cell infiltration (25).

The activation of the inflammasome appears to be important in chronic liver diseases (26,27) and is thought to play a role in NASH pathogenesis. In addition to saturated fatty acids, other compounds such as uric acid may act as danger-associated molecular patterns (DAMPs) (28,29). These compounds may act synergistically with the gut microbiota-derived pathogen-associated molecular patterns (PAMPs). Both are delivered to the liver via the portal circulation and may activate the hepatic inflammasome by triggering the expression of some proinflammatory cytokines and stimulating apoptosis via caspase-1 activation (30). Some studies have found an association between lobular and portal inflammation and higher levels of SUA (31). However, we did not find an association between hepatocellular necro-inflammation parameters such as hepatocellular ballooning, lobular or portal inflammation and SUA. In addition, SUA did not correlate with serum markers of systemic inflammation such

as the C relative protein (CRP). In addition to NASH, steatosis was the only variable associated with higher levels of uric acid. This suggests that the association of higher SUA levels with NASH may occur via the steatosis pathway.

There was a significant association in the univariate analysis between higher levels of SUA and more advanced fibrosis (F2-F4). However, this association was not observed in the multivariate analysis and only 7% (n = 40) of patients in this cohort had stage F4 of fibrosis. Uric acid has been associated with cardiovascular risk (32), which was associated with hepatocellular steatosis and NASH, but not with advanced fibrosis in this cohort. This is consistent with the idea that the cardiovascular events determine the outcome in NASH but not in advanced fibrosis (33).

NASH pathogenesis is very complex and heterogeneous (30), with an important genetic background as well as the metabolic syndrome and obesity. The PNPLA3 gene variant is the most extensively validated genetic factor associated with steatosis, fibrosis, NAFLD progression and HCC across different ethnic groups (34). In fact, some PNPLA3 polymorphisms may influence the response to NASH therapy (35).

Xu et al. showed that uricemic lowering agents (allopurinol and benzobromarone) attenuated hepatic steatosis in a Mongolian gerbil model (36). However, there were few patients in this study that received treatment with SUA modifying drugs, which precluded a comparative analysis. Atorvastatin has been recently suggested as a useful drug in NASH, which is due in part to its hypouricemic effect (37). In addition, a noninvasive score that included SUA has been proposed for NAFLD screening (38).

There were some limitations in this study. There was a missing values rate of 35% for some variables such as HOMA-IR and waist circumference, which may have prevented some results from reaching statistical significance. In addition, the histopathological study was not centralized. Therefore, inter-observer variation might have introduced some bias.

In summary, high levels of uric acid in patients with NAFLD are significantly and independently associated with hepatocellular steatosis and NASH but not with necro-inflammation and fibrosis stage in this cohort of biopsy proven NAFLD patients. This suggests a contributory role of SUA to NASH that is not mediated through inflammatory mechanisms. In addition, the incorporation of SUA to the current non-

invasive scores deserves further evaluation.

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**Table 1. Demographic, clinical and biochemical features of the cohort**

| | | Total | URIC ACID (tertiles) | | | p-value |
|---|---|---|---|---|---|---|
| | | | T1 | T2 | T3 | |
| | | n = 634 | 221 (35%) | 202 (32%) | 209 (33.1%) | |
| Gender | Female | 317 (50%) | 106 (48%) | 105 (52%) | 104 (49.8%) | 0.711 |
| Age | Mean ± SD | 49.6 ± 12.7 | 47.9 ± 12.2 | 49.7 ± 12.6 | 51.4 ± 18.4 | 0.017 |
| | Range | 14.8-79.9 | 19-75.2 | 20-78.3 | 79.9-41.7 | |
| BMI (kg/m$^2$) | Mean ± SD | 35.4 ± 9.4 | 36.4 ± 10 | 33 ± 8.2 | 36.6 ± 19.5 | < 0.001 |
| | Range | 18.2-76.3 | 20.8-76.3 | 18.2-57.1 | 64.5-29.4 | |
| DM-2 | | 140 (27.56%) | 44 (24.04%) | 48 (28.24%) | 48 (30.97%) | 0.355 |
| Hypertension | | 195 (38.54%) | 63 (34.81%) | 60 (35.29%) | 72 (46.45%) | 0.052 |
| Hypercholesterolemia | | 235 (46.91%) | 80 (44.2%) | 83 (49.11%) | 72 (47.68%) | 0.638 |
| Hypertriglyceridemia | | 199 (40.37%) | 57 (32.76%) | 69 (40.59%) | 73 (48.99%) | 0.012 |
| AST (U/l) | Median (p25-p75) | 36 (26-52) | 33 (24.4-49) | 36 (27-53) | 38.9 (28.8-56) | 0.084 |
| | Range | 7-414 | 11-265 | 11-414 | 7-288 | |
| ALT (U/l) | Median (p25-p75) | 55 (35.5-85) | 52 (31-77.3) | 61 (38-90) | 55 (39-86) | 0.068 |
| | Range | 8.8-860 | 8.8-269 | 10.6-860 | 10-328 | |
| Bilirubin (mg/dl) | Mean ± SD | 0.8 ± 0.6 | 0.7 ± 0.6 | 0.7 ± 0.5 | 0.8 ± 0.7 | 0.329 |
| | Range | 0.1-7 | 0.2-6.7 | 0.1-5 | 0.2-7 | |
| Albumin (g/dl) | Mean ± SD | 4.3 ± 0.4 | 4.4 ± 0.4 | 4.3 ± 0.4 | 4.3 ± 0.4 | 0.526 |
| | Range | 2.3-5.3 | 2.7-5.3 | 3.5-5.2 | 2.3-5.2 | |
| Creatinine (mg/dl) n = 430 | Mean ± SD | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.92 ± 0.5 | < 0.001 |
| | Range | 0.4-3 | 0.4-1.4 | 0.4-1.3 | 0.5-3 | |
| Glucose (mg/dl) | Mean ± SD | 110.9 ± 38 | 110.7 ± 38.4 | 111 ± 37.4 | 110.4 ± 37.5 | 0.990 |
| | Range | 53-359 | 53-268 | 67-359 | 62-330 | |

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| | | | | | | |
|---|---|---|---|---|---|---|
| HOMA-IR<br><br>n = 442 | Median (p25-p75) | 3.1 (1.86-5.23) | 2.9 (1.9-4.8) | 3.3 (1.8-5.6) | 3.4 (2-5.3) | 0.441 |
| | Range | 0.05-22.93 | 0.4-22.3 | 0.1-20.7 | 0.4-23 | |
| Total cholesterol (mg/dl) | Mean ± SD | 191.1 ± 46 | 185.3 ± 45 | 197.5 ± 45.9 | 191.3 ± 46.7 | 0.026 |
| | Range | 50-368 | 92-368 | 67-364 | 50-348 | |
| HDL cholesterol (mg/dl) | Mean ± SD | 52.6 ± 21.5 | 55.6 ± 22.6 | 53.7 ± 22.9 | 48.3 ± 4 | 0.004 |
| | Range | 4-167 | 20-167 | 7-159 | 118-36 | |
| LDL-cholesterol (mg/dl) | Mean ± SD | 115.73 ± 37.72 | 111.49 ± 35.39 | 120.28 ± 35.64 | 115.86 ± 16 | 0.093 |
| | | 16-298 | 42-237 | 21-238 | 298-91 | |
| Triglycerides (ml/dl) | Mean ± SD | 159.37 ± 89.38 | 153.35 ± 99.3 | 154.67 ± 80.27 | 170.27 ± 38 | 0.102 |
| | Range | 17-971 | 17-971 | 32-536 | 453-106 | |
| INR (n = 363) | Mean ± SD | 1.02 ± 0.1 | 1.03 ± 0.08 | 1.01 ± 0.12 | 1.02 ± 0.8 | 0.647 |
| | Range | 0.8-1.97 | 0.89-1.31 | 0.8-1.97 | 1.46-0.97 | |
| Platelet count (K/l)<br><br>n = 499 | Mean ± SD | 249.4 ± 73.38 | 250.08 ± 77.39 | 248.99 ± 68.16 | 249.03 ± 64 | 0.988 |
| | Range | 64-592 | 97-568 | 90-456 | 592-196.75 | |

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**Table 2. Univariate analysis of uric acid according to tertiles and histopathological components of NAS score, stage of fibrosis and NAFLD score**

| | | Total | T1 | T2 | T3 | p-value |
|---|---|---|---|---|---|---|
| | | n = 634 | 221 (34.97%) | 202 (31.96%) | 209 (33.07%) | |
| NASH | | 270 (53.15%) | 81 (44.26%) | 91 (53.22%) | 98 (63.64%) | 0.002 |
| Steatosis (NAS) | Grade 2-3 | 319 (50.72%) | 99 (45.21%) | 93 (46.27%) | 126 (60.87%) | 0.002 |
| Ballooning (NAS) | None | 224 (35.84%) | 68 (31.19%) | 72 (36%) | 82 (40%) | 0.156 |
| | Few balloon cells | 313 (50.08%) | 123 (56.42%) | 100 (50%) | 90 (43.9%) | |
| | Many cells/prominent ballooning | 88 (14.08%) | 27 (12.39%) | 28 (14%) | 33 (16.1%) | |
| Lobular inflammation (NAS) | < 2 foci/200x | 182 (29.07%) | 64 (29.36%) | 63 (31.5%) | 54 (26.21%) | 0.64 |
| | 2-4 foci/200x | 323 (51.6%) | 117 (53.67%) | 98 (49%) | 108 (52.43%) | |
| | > foci/200x | 121 (19.33%) | 37 (16.97%) | 39 (19.5%) | 44 (21.36%) | |
| Portal inflammation | More than minimal | 84 (16.8%) | 30 (16.67%) | 28 (16.67%) | 26 (17.11%) | 0.993 |
| Stage of fibrosis | F2-4 | 185 (29.27%) | 61 (27.6%) | 47 (23.38%) | 76 (36.54%) | 0.011 |
| NAS score | Mean ± SD | 3.34 ± 1.71 | 3.21 ± 1.82 | 3.27 ± 1.82 | 3.59 ± 1.67 | 0.069 |
| NAFLD score (n = 456) | Mean ± SD | -1.45 ± 1.7 | -1.47 ± 1.8 | -1.69 ± 1.68 | -1.21 ± 1.58 | 0.05 |
| | Range | -7.77 to 3.95 | -5.65 to 3.9 | -6.52 to 3.95 | -7.77 to 2.37 | |

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**Table 3. Unadjusted and adjusted multivariate analysis of uric acid according to tertiles and histopathological components**

| | | *Unadjusted* | | | | *Adjusted by age, arterial hypertension and creatinine* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sig. | Exp (B) | 95% CI for EXP (B) | | Sig. | Exp (B) | 95% CI for EXP (B) | |
| Stage of fibrosis F2-4 | T2/T1 | 0.322 | 0.8 | 0.52 | 1.24 | 0.136 | 0.66 | 0.38 | 1.14 |
| | T3/T1 | 0.048 | 1.51 | 1 | 2.27 | 0.753 | 1.09 | 0.64 | 1.87 |
| | T3/T2 | 0.004 | 1.89 | 1.23 | 2.9 | 0.076 | 1.65 | 0.95 | 2.87 |
| NASH | T2/T1 | 0.093 | 1.43 | 0.94 | 2.18 | 0.530 | 1.17 | 0.72 | 1.90 |
| | T3/T1 | < 0.001 | | 1.42 | 3.42 | 0.025 | 1.80 | 1.08 | 3.00 |
| | T3/T2 | 0.058 | 1.54 | 0.99 | 2.4 | 0.101 | 1.54 | 0.92 | 2.57 |
| Steatosis grade 2-3 | T2/T1 | 0.827 | 1.04 | 0.71 | 1.53 | 0.697 | 0.91 | 0.57 | 1.46 |
| | T3/T1 | 0.001 | 1.89 | 1.28 | 2.77 | 0.031 | 1.72 | 1.05 | 2.83 |
| | T3/T2 | 0.003 | 1.81 | 1.22 | 2.68 | 0.012 | 1.89 | 1.15 | 3.10 |

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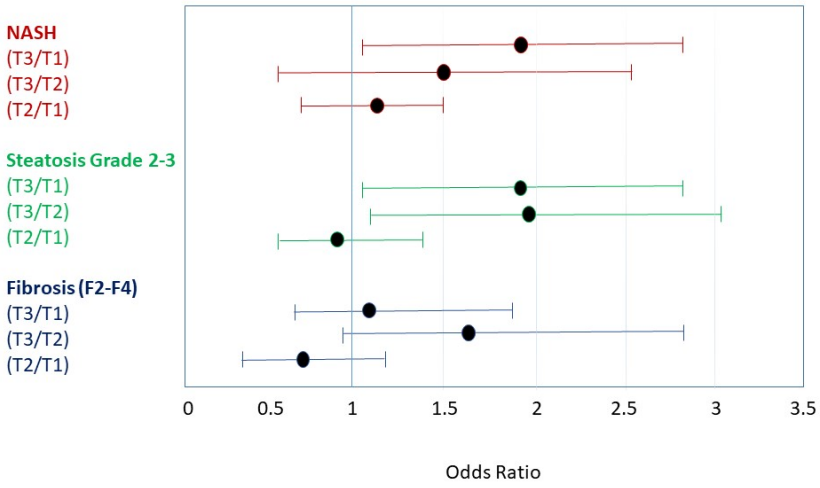


Fig. 1. Adjusted odds ratio (OR) of uric acid according to the tertiles and histopathological components.