Impact of baseline serum ferritin on survival of patients with chronic hepatitis C after a 15 years follow-up

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**Background and aims:** Increased serum ferritin (SF) and liver iron are commonly observed in chronic viral hepatits C (CHC). A correlation with severity of fibrosis, response to antiviral therapy and incidence of hepatocellular carcinoma (HCC) have been suggested in cross-sectional studies. The real impact is still debated. The aim of this study was to investigate the impact of baseline SF on long-term survival.

**Methods:** Patients were selected from a single-center cohort of 4293 CHC(+) consecutive patients. Survival at the date of 15/09/2013 was obtained from death certificate for 3361 patients. Inclusion criteria were HCV-ARN positivity and availability of SF at baseline. Liver fibrosis was determined using liver biopsy (n=959) and/or non-invasive tests (elastography of fibrotest®). Survival was studied according to SF level: group1 (SF < 100µg/L); group2 (SF=100-300µg/L); group3 (SF > 300µg/L).

A Cox model was built to take into account age, gender, therapy and baseline stage fibrosis.

**Results:** 1451 patients were included (mean age 43.5±13.7 years; male 59.8%; fibrosis n=1173 F0/F1=60.2%, F2=16.8%, F3=10%, F4=13%); median SF=171µg/L [IQR 80-343]; median follow-up=14.7 years[9.5-18.7]). The overall survival (261 deaths and 55 HCC) was 83.5% after 15 years of follow-up. The survival was lower in group3 than in group1/2 for patients with F0/F1/F2 (p< 0.0001) but not for patients with F3/F4 fibrosis score (p=0.42). In multivariate analysis, when fibrosis and age were introduced in Cox model, SF was no longer associated with survival.

**Conclusions:** The link between SF and mortality, ascertained in univariate analysis for F ≤ 2 fibrosis score disappeared in multivariate model suggesting absence of direct influence of iron in survival of CHC patients.
Profiling new biomarkers for the diagnosis of drug induced liver necrosis. A pilot study assessing performance of cleaved or full-length cytokeratin 18, and micro-rna-122

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**Background and aims:** A new performant DILI-biomarker should be either more specific or more sensitive than ALT. We aim to assess the "necroticoinflammatory activity" specificity of cytokeratin-18 cleaved (CK18-cleaved), full (CK18-full) and microRNA-122 (miR-122), vs ALT according to presence of steatosis or fibrosis.

**Methods:** 283 patients were included: 7 DILI, 31 healthy-volunteers, 110 NAFLD, 35 ALD, 35 CHC, 32 CHB, and 33 PSC; 279 had reliable blood-tests of activity (ActiTest), fibrosis (FibroTest), steatosis (SteatoTest), and 79 NAFLD had blindly centralized SAF scores: activity-SAF, steatosis-SAF and fibrosis-SAF in contemporaneous biopsies.

**Results:** In univariate analysis [Spearman(S)], candidates were associated with histological and blood-tests of activity, but also with steatosis/fibrosis (all S>0.28 for histology P< 0.01 and >0.49 for blood-tests P< 0.0001), which underlined the risk of low specificity. In multivariate models [multivariate regression-coefficient (MRC 95%CI; P-value)], CK18-cleaved/CK18-full had similar profiles, with strong associations with activity-SAF [0.09(0.02-0.17) P=0.01/0.10(0.03-0.17) P=0.009], and ActiTest [0.69 (0.51-0.87)/0.72 (0.55-0.89);both P< 0.0001] adjusted on steatosis/fibrosis. Associations CK18-cleaved/CK18-full with steatosis-SAF/SteatoTest were strongly reduced when adjusted on activity-SAF/fibrosis-SAF but remained significant for steatosis-SAF/SteatoTest, [0.12(0.03-0.20) P=0.007/0.28(0.15-0.41) P< 0.0001]. Associations CK18-cleaved/CK18-full with fibrosis-SAF/FibroTest were no more significant when adjusted on activity-SAF/steatosis-SAF, miR-122 profile was different with lower associations with SAF/blood-tests (R2=0.28 vs 0.41 for CK18-cleaved and 0.52 for CK18-full). Association with activity-SAF persisted [MRC=0.14(7-20)P=0.0001] after fibrosis-SAF or steatosis-SAF adjustment (0.11 (0.04-0.18) P=0.002) but not with both. Same conclusions were obtained using biopsy or FibroTest-ActiTest-SteatoTest.

**Conclusions:** DILI-candidates such as cleaved/full-length-cytokeratin-18 and miR-122 are highly correlated with activity, but also associated with steatosis and fibrosis. Specificity of such candidates must be assessed and compared in appropriate controls before qualification.
Longitudinal validation of biomarkers of FibroMax panel, as surrogates for dynamic histological changes on liver biopsy (LB) in NALFD patients

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**Background and aims**: No longitudinal studies used repeated LB to evaluate surrogate markers (FibroMaxTM) during longitudinal follow-up (FU), in NALFD-patients. (PlosOne2010)

**Aim**: Evaluate in NALFD-patients:
1) Dynamics of liver Steatotest estimates according to LB;
2) The impact on fibrosis of presumed steatosis (Steatotest) and NAS (Nashtest) using two repeated FibroMax and histological fibrosis.

**Methods**: Patients from previously published study (JHepatol2013) with persistent NALFD, selected with 2-repeated LB and FibroMax (panel including Fibrotest/Steatotest/Nashtest) were included. Statistics used Student t-test and Log-rank tests (Hazard Ratios).

**Results**: N=53pts were pre-included with repeated LB and 42pts had repeated FibroMax [delay-to-LB 0yrs(0.5-2.7); 24%males; age=53yrs(34-72), BMI=30kg/m2(21.6-38.5), type-2 diabetes 36%]. LB advanced fibrosis (AF) (SAF fibrosis score) 13(31%), LB-steatosis >10%, >30% and >60%; 100%, 57% and 24%, respectively; Fibrotest AF (score>=0.48) 12(29%)pts; Steatotest presumed steatosis >5% and >30% 96% and 81%, respectively. The median(range) FU was 2.3yrs(1.0-7.3).

Evolution of steatosis: N=9pts LB-progression [mean(se) Steatotest evolution : 0.70(0.04) vs 0.76(0.03), p=0.03]; N=8pts stable LB-steatosis [Steatotest 0.66(0.08) vs 0.68(0.08), p=0.03]; N=25pts had LB-regression [Steatotest 0.68(0.03) vs 0.61(0.03), p=0.03]. During follow-up 7/42pts progressed fibrosis stage classification on LB. According to Steatotest [follow-up=2.5(0.3)yrs] the HR(95%CI) to progress at least one fibrosis histological stage (SAF-fibrosis score) was 24.97 (0.03-20899) in subjects with Nashtest scores predicting NASH (Kleiner-score, Hepatology 2005).

**Conclusions**: This study confirmed the accuracy of Steatotest to reflect dynamic histological changes in NALFD-patients. In subjects with Steatotest progression, hazard ratio to develop fibrosis was higher in subjects with Nashtest predicting NAS (Kleiner-score, Hepatology 2005).
Effect of ursodeoxycholic acid or ursodeoxycholic acid combined with Losartan for treatment of non-alcoholic steatohepatitis

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**Background and aims:** The innate immune system, including angiotensin-II (AT-II) and cytokines play important roles in the progression of liver fibrosis development. The cross talk between AT-II and cytokines has not been elucidated yet. Aim of the study was to elucidate the effect of AT-II type 1 receptor blocker losartan on the liver fibrosis development, especially in conjunction with the interaction of serum cytokines and AT-II in non-alcoholic steatohepatitis (NASH) patients.

**Methods:** 58 NASH patients were randomly assigned to receive ursodeoxycholic acid (UDCA) at a dose of 30 mg/kg a day (n=28) or UDCA combined with losartan at a dose of 50 mg once a day (n=30) for 6 months. Serum tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) levels were determined using an ELISA in 58 patients with NASH and 25 healthy volunteers. In every subject serum cytokines levels, 13C-methacetin breath test (13C-MBT) and FibroTest was applied before treatment and after 6 months.

**Results:** The losartan group showed a significant decline in serum TNF-α (from 34.22 ± 0.31 to 15.36 ± 0.12 pg/ml (mean ± SD), P = 0.003) and IL-6 (from 27.06 ± 0.17 to 10.13 ± 0.07pg/ml, P = 0.005) and a significant increase in functioning hepatocyte mass (13C-MBT results) (P = 0.018), and a significant reduction in liver fibrosis (P = 0.026) evaluated by FibroTest, while these parameters were less changed in the UDCA group.

**Conclusions:** This study shows that ursodeoxycholic acid combined with losartan may exert beneficial effects in NASH patients.
Coffee does not prevent development of NAFLD but may delay fibrosis progression: a prospective cohort study in the general population

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Background and aims: Studies suggest that coffee consumption reduces the risk for type 2 diabetes, cirrhosis, hepatocellular carcinoma and possibly of NAFLD. However, conclusive data to support coffee's protective role in NAFLD is lacking. We tested the association between coffee consumption and NAFLD in a prospective general population cohort.

Methods: The analysis was performed both in a cross sectional manner (n=347, 31% NAFLD) and in a prospective manner in a sub-cohort without NAFLD at baseline followed for 7 years (n=147, 19% incident NAFLD). NAFLD was diagnosed with abdominal ultrasound and SteatoTest. FibroTest was used to assess fibrosis. A detailed structured questionnaire on coffee consumption was administered during a face-to-face interview.

Results: NAFLD patients did not differ from controls in their coffee consumption. Subjects with high coffee consumption (of $\geq 3$ cups/day) did not differ from those with lower consumption in steatosis assessed by Hepato-Renal index and SteatoTest, or fibrosis assessed by FibroTest or liver enzymes. In a multivariate analysis, adjusting for age, gender, smoking, sugar consumption and physical activity no association was demonstrated between high coffee consumption and NAFLD (OR=0.95, 0.60-1.52, 95%CI). In the prospective analysis, no association was demonstrated between high coffee consumption and new onset of NAFLD (OR=1.02, 0.45-2.35). High coffee consumption was significantly associated with a lower rate of advanced fibrosis (10.4% vs. 26.3%, P=0.047). Adjusting for the variables mentioned above the association become borderline significant (OR=0.54, 0.27-1.08, P=0.083).

Conclusions: No association was demonstrated between coffee consumption and NAFLD, but a protective affect from fibrosis is suggested.
Presence of hepatic steatosis as per non-invasive biomarkers overestimates fibrosis stages based on liver stiffness measurement (LSM) by transient elastography in type-2 diabetic (T2D) patients

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**Background and aims:** LSM and FibroTest (FT) are validated non-invasive methods to assess liver fibrosis. Necro-inflammatory activity influences LSM overestimating fibrosis. We aimed to evaluate the impact of hepatic steatosis on LSM in T2D patients.

**Methods:** T2D patients without liver disease that had been screened for fibrosis with FT were reinvestigated using FT and LSM after a 7-year median delay. Patients with minor fibrosis (FT < 0.48; F0F1) at baseline and without progression to advanced fibrosis (AF) on repeated FT were included. Exclusion criteria were the presence of AF by FT (≥0.48; F2F3F4) or activity (ActiTest ≥ 0.17; A1A2A3). Patients staged minor fibrosis by repeated FT but with AF on LSM (≥7.1 kPa) at the end of follow-up were considered as supposed LSM overestimation. Severe steatosis (>32% hepatocytes) was estimated by SteatoTest (≥ 0.69), Fatty Liver Index (FLI) ≥ 60, Hepatic Steatosis Index (HSI) ≥ 36 or Controlled Attenuated Parameter (CAP) ≥ 283 dB/m. LSM applicability was defined as IQR/LSM-ratio < 30%, > 60% success rate and ≥ 10 valid measures.

**Results:** 102 patients were pre-included (55% male, 62 years, BMI 27.6 Kg/m²; ALT 23 (10-59), 35% insulin treated). After exclusion of non-applicable LSM by both M and XL probes (7%), 95 patients were analyzed. Patients with supposed LSM overestimation compared to those without had higher; median [IQR]: BMI 32.0 [28.4-43.4] vs. 26.6 [24.4-35.3] Kg/m²; waist circumference 109 [103-134] vs. 100 [93-119] cm; thoracic fold 24.4 [19.5-31.5] vs. 18.8 [16.5-27] mm; SteatoTest 0.64 [0.52-0.84] vs. 0.48 [0.32-0.83]; FLI 92 [61-99] vs. 60 [27-93]; and HSI 46 [40-53] vs. 38 [34-49], all p < 0.001. In a multivariate analysis [OR (95% CI)], severe steatosis as estimated by SteatoTest [5.2 (1.5-22.6); p = 0.01], FLI [5.8 (1.2-23.6); p = 0.03], HSI [6.4 (1.2-35.0); p = 0.03] or CAP [7.9 (1.4-45.0); p = 0.02] were associated with supposed LSM overestimation.

**Conclusions:** The presence of hepatic steatosis in type 2 diabetic patients could overestimate liver fibrosis by liver stiffness measurement.
Applicability riteria for real-time Shearwave elastography by Aixplorer compared to transient elastography by Fibroscan and to FibroTest

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Background and aims: App of non-invasive methods, liver stiffness measurements (LSM) by Fibroscan and Fibrotest, are defined after excluding failures and unreliable results. (Castera 2010, Poynard 2010). RT-SWE by Aixplorer is a new 3D-elastography coupled with a B-mode imaging. To better identify applicability criteria for SWE in terms of positioning and acquisition procedure.

Methods: We used standard-AUROCs and the strength of concordance with validated methods, LSM and Fibrotest. Each patient had 3-SWE regions of interest (ROI) studied [right lobe (RL)] with 3 measures within each: central-zone (Q-boxC), zone displayed in blue-color (Q-boxB), and in red-color (Q-boxR).

Results: 189 prospective-patients were pre-included with App-FT; 166(87.8%) had concomitant App-LSM: 57%males, age=53yrs, BMI=25kg/m2, ALT=40IU/L; 30% advanced fibrosis (AF) as per Fibrotest. Q-boxC and the mean of three C-measurements correlated with Fibrotest ($p< 0.05$). Q-boxB correlated with Fibrotest ($p< 0.05$) unlike to Q-boxR ($p=NS$). Taking Fibrotest as standard, the best AUROCs were obtained for Q-boxC (0.73) and Q-boxB (0.74), but not different from the mean of 3 Q-boxC(0.71) and Q-boxB (0.69). Using the mean of 3 Q-boxC (-B) does not improve diagnosis provided by QboxC(-B) alone ($p=NS$). SWE with a ratio SD/mean-SWE >60% versus < 60% were less correlated to LSM (Spearman correlation-coefficient=0.11, $p=0.55vs0.37$, $p< 0.0001$) and to Fibrotest (SCC=0.14, $p=0.46vs0.33$, $p< 0.001$).

Conclusions: A single measurement in the central-zone or blue-zone of the ROI has the best accuracy similar to the mean of 3 measurements. ROI zones displayed in red have to be avoided as they are poorly correlated with validated fibrosis markers. SD should be included among quality criteria for SWE-reliability.
Real-Time shear-wave elastography performances in chronic hepatitis C patients compared to liver stiffness measurement and FibroTest with liver biopsy as reference

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**Background and aims**: Liver fibrosis is a predictor of disease progression and treatment response in CHC. SWE is a new three-dimensional elastography for fibrosis evaluation. The purpose of this study is to evaluate SWE accuracy compared to that of LSM and Fibrotest with liver biopsy as reference.

**Methods**: Consecutive CHC-patients had undergone LB scoring METAVIR (from F0 no-fibrosis to F4-cirrhosis) and SWE (Super-Sonic Imagine S.A.), LSM M-probe (Echosens) and Fibrotest (BioPredictive), within the period not exceeding 12-weeks from LB-date. Three SWE-measurements were performed in the 5th,6th and 7th liver segments by a radiologist. Statistical analysis used standard-AUROCs and Obuchowki-method.

**Results**: 99 CHC-patients were assessed and 93 included with four not missing applicable fibrosis-estimators. Main characteristics: 63% males, age 38yrs(21-63); fibrosis stages prevalences(n): F0F1-65.6%(61), F2F3-24.7%(23), F4-9.7%(9) and 37.9%(35) important necroinflammatory activity. 5th liver segment SWE-measurements was used for analysis as there was no difference with those in the 6th,7th segment or their mean. Standard-AUROCs(95%CI) for SWE, LSM and Fibrotest were, respectively: advanced fibrosis (F2F3F4) 0.899(0.789-0.954;p=NS vsLSM and vsFibrotest), 0.942(0.860-0.977;p=0.02 vsFibrotest) and 0.840(0.724-0.910); for cirrhosis 0.971(0.912-0.990;p=NS vsLSM and vsFibrotest), 0.979(0.929-0.994;p=0.03 vsFibrotest), 0.867(0.705-0.943). Overall accuracies [AUROCs(SE)] by Obuchowski method were: for SWE 0.917(0.015p=0.37 vsLSM and p=0.14 vsFibrotest), LSM 0.903(0.016) and Fibrotest 0.867(0.020). Sensitivity analyses performed in 53 patients with LB sample length >=10mm found no differences between AUROCs neither for F2F3F4 nor for cirrhosis (all p=NS).

**Conclusions**: SWE had similar performances to those of LSM and Fibrotest for advanced fibrosis and cirrhosis. One SWE-measurement had accuracy not different of the mean of three liver segments SWE-measurements.