CLINICAL STUDIES

GALAXY

GUT-AND-LIVER AXIS IN ALCOHOLIC LIVER FIBROSIS

GRANT NUMBER 668031

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1. AIMS

Deliverable 1.1: Included first 300 patients in the cohort study GALA-ALD: Gut-and-liver axis in alcoholic liver fibrosis (M0-12) (OUH)

Three hundred patients with prior or ongoing alcohol overuse included in the study. Inclusion visit consists of same-day extensive phenotyping with demographical data, quality of life questionnaires, alcohol history, medical history, nutritional parameters such as bioimpedance and hand-grip-strength, liver routine blood samples, abdominal ultrasonography, and liver biopsy with liver histology. Additionally we sample tissue for biobank: blood, feces, urine, liver, and perform state-of-the-art non-invasive fibrosis testing using current methods: Ultrasound elastography, Enhanced Liver Fibrosis (ELF) test, apoptosis and necrosis cytokeratin-18 serum markers M30 and M65.

2. RESULTS

***Results M12:* 300 patients included.**

The research database at Odense University Hospital contains full datasets on 300 patients included in the study.

The biobank at the Odense University Hospital contains blood plasma and serum from 298 patients included in the study, since blood samples for two participants were accidentally mishandled by hospital porters and could not be restored. Urine sampling is complete. Fecal sampling is missing in approximately 5% of patients due to patient non-compliance. The biobank also contains liver samples from approximately 70% of included patients since biobanking is not always possible for the liver tissue due to small samples specimen.

We have shipped liver samples to GALAXY partners BRFA and SDC.

We have shipped fecal samples to GALAXY partners UCPH and SDC.

We have shipped serum samples to GALAXY partners NB.

***Results M12:* 2. Existing markers of alcoholic liver fibrosis.**

We have used the intermediate analysis to evaluate the performance of the existing, commercially available serum fibrosis marker ELF (Enhanced Liver Fibrosis test). The publication will be submitted to a scientific journal by end of January 2017.

Participants 289 patients with ongoing or prior alcohol overuse; 128 from the primary care with a 6% prevalence of advanced fibrosis and 161 from the secondary care with a prevalence of 36%.

Interventions The ELF test combines three direct markers of liver fibrosis. We compared the ELF test with six indirect fibrosis indices available from routine biochemistry and liver stiffness measurements by ultrasound elastography.

Main outcome measure Diagnosis of advanced fibrosis at a predefined ELF cut-off of 10.5. Secondary outcomes included diagnosis of significant fibrosis and cirrhosis, correlation of ELF with health-related quality of life and whether there was a diagnostic yield of combining ELF with liver stiffness measurements.

Results ELF above 10.5 correctly classified 73% (51/66) of patients with advanced fibrosis and 93% (204/223) without advanced fibrosis. The diagnostic accuracy was similar in primary and secondary healthcare patients (areas under the receiver operating characteristics curve: 0.89 and 0.90, P=0.917). In primary care, ELF below 10.5 had a 98% negative predictive value. Higher ELF predicted lower physical health-related quality of life measured by Short-Form 36 and the Chronic Liver Disease Questionnaire. A diagnostic strategy with sequential use of an indirect index, ELF and elastography correctly classified 94% of patients.

Conclusions The Enhanced Liver Fibrosis test diagnoses advanced alcoholic liver fibrosis with excellent

discrimination and calibration and predicts health-related quality of life. Advanced fibrosis can be ruled out in primary healthcare by ELF below 10.5.

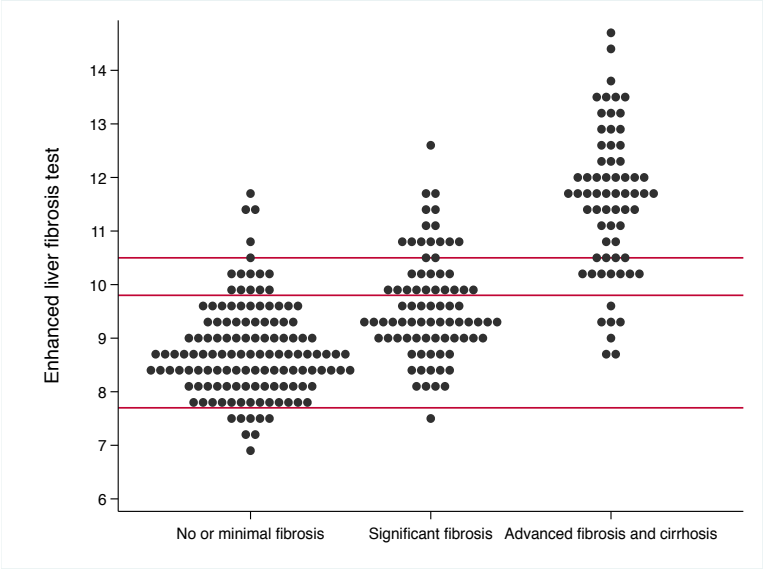

Figure 1: Distribution of the Enhanced Liver Fibrosis test according to fibrosis stage: None or minimal fibrosis (F0-1), significant fibrosis (F2) and advanced fibrosis and cirrhosis (F3-4). The red horisontal lines represent Enhanced Liver Fibrosis test cut-offs evaluated in this paper: 7.7, 9.8 and 10.5.

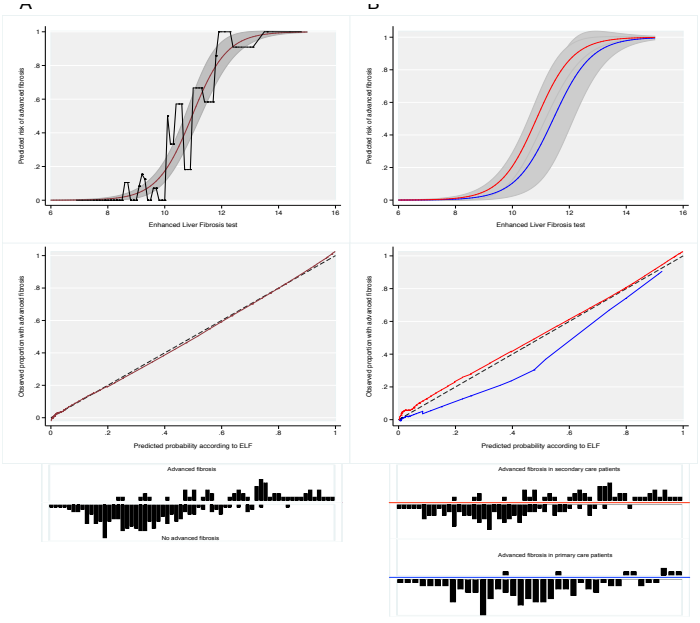

Figure 2: Prediction and calibration plots for the Enhanced Liver Fibrosis test (ELF) to diagnose advanced liver fibrosis, overall and in primary versus secondary health care.
(A) Overall cohort. The upper graph is predicted probabilities of advanced fibrosis according to ELF. The maroon line is the average predictions, with 95% confidence intervals in dark gray. The black line is the observed prevalence of advanced fibrosis for 30 centiles of ELF values.
The lower graph is the observed/expected calibration plot for ELF. The black dashed line represents perfect calibration, with 100% agreement between predicted probability of advanced fibrosis on the X-axis and observed proportion with advanced fibrosis on the Y-axis. The maroon line represents the smoothed regression line of predicted versus observed probabilities. The histogram below the graph shows count frequencies of observed advanced fibrosis (above the line) and no advanced fibrosis (below the line) according to the predicted probabilities on the X-axis.
(B) Subgroups according to recruitment from primary care (blue lines) and secondary care (red lines).

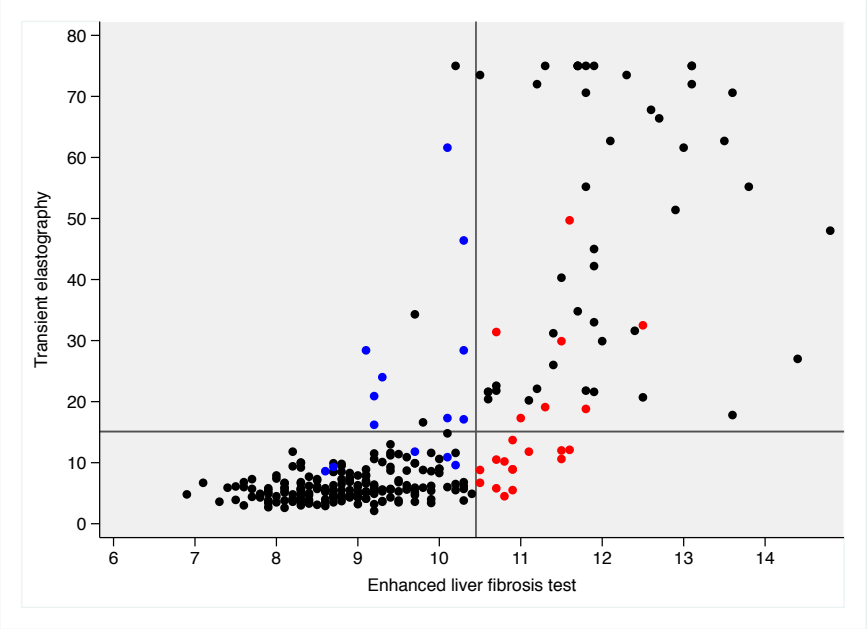

Figure 3: Correlation between Enhanced Liver Fibrosis (ELF) and transient elastography (TE) measurements. The black dots represent correct classifications with the ELF test. The red dots represent ELF false positives (to the right of the vertical 10.5 cut-off line). Of 21 false positives with ELF, 14 were true negative with TE (below the horizontal 15 kPa line). The blue dots represent ELF false negatives (left of the 10.5 line). Of 14 false negatives with ELF, nine were true positive with TE (above the 15 kPa line).