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White paper

UTILITY OF CASPASE-CLEAVED KERATIN 18 TO DIAGNOSE NASH IN PATIENTS WITH OBESITY

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INTRODUCTION

In treating patients with nonalcoholic steatohepatitis (NASH), an ongoing challenge is the paucity of adequately performing noninvasive biomarkers, necessitating the use of a liver biopsy to obtain diagnosis[1]. The M30® antibody detecting caspase-cleaved keratin 18 (ccK18) has emerged as a promising circulating biomarker of liver injury, showing utility to identify patients with NASH[2]. In addition, a ccK18-incorporating composite score, MACK-3 (ccK18, AST and HoMa), was specifically developed to predict fibrotic NASH [3,4], which is the primary inclusion criterion in ongoing clinical trials studying pharmacotherapies for NASH[3, 4]. Although NASH and its complications are especially prevalent in patients with severe obesity and type 2 diabetes[5], few data exist on biomarker performance within this demographic. Here, we studied the applicability of ccK18 circulating fragments to diagnose NASH or fibrotic NASH in a representative obese cohort.

METHODS

We recruited 354 patients aged 25 to 71 years undergoing a liver biopsy during laparoscopic bariatric surgery at the Helsinki University Hospital (Helsinki, Finland). Patients with primary liver diseases other than nonalcoholic fatty liver disease (NAFLD) or significant alcohol consumption (males >30 g/day; females >20 g/day) were excluded. A week before the liver biopsy, the patients underwent clinical examination and blood sampling. Plasma concentrations of ccK18 fragments were determined using the M30® antibody (M30 Apoptosense® ccK18 kit [ELISA]; VLVbio, Nacka, Sweden). We calculated MACK-3, the Fibrosis-4 index (FIB-4), and the NAFLD Fibrosis Score (NFS) using their respective formulae[4,6,7]. Histopathological features of NAFLD were assessed using the NASH Clinical Research Network grading and definitions[8]. Fibrotic NASH was defined as NASH with a NAFLD Activity Score (NAS) ≥4 and concomitant ≥F2 fibrosis stage[3].

RESULTS

Patient characteristics

The mean age of the patients was 51±10 years and the mean BMI 40.4±7.2 kg/m². Type 2 diabetes was diagnosed in 43% of the patients. In liver histology, NASH was present in 15%, fibrotic NASH in 6%, and advanced liver fibrosis (stage F3–F4) in 7% of the patients.

Associations between ccK18, liver histology, and liver enzymes

Concentrations of ccK18 correlated significantly with the full histological spectrum of NAFLD, including steatosis, ballooning, inflammation, fibrosis, and NAS (Figure 1). A linear and positive association was present between ccK18 and NAS, but median concentrations decreased at fibrosis stage F4 (Figure 1). In a multiple linear regression analysis, ccK18 lost its association with fibrosis stage after adjusting for NAS, confirming its role primarily as a biomarker of liver injury associated with NASH (data not shown). Moreover, ccK18 correlated significantly with plasma ALT (r_s=0.63, P<0.001) and AST (r_s=0.60, P<0.001).

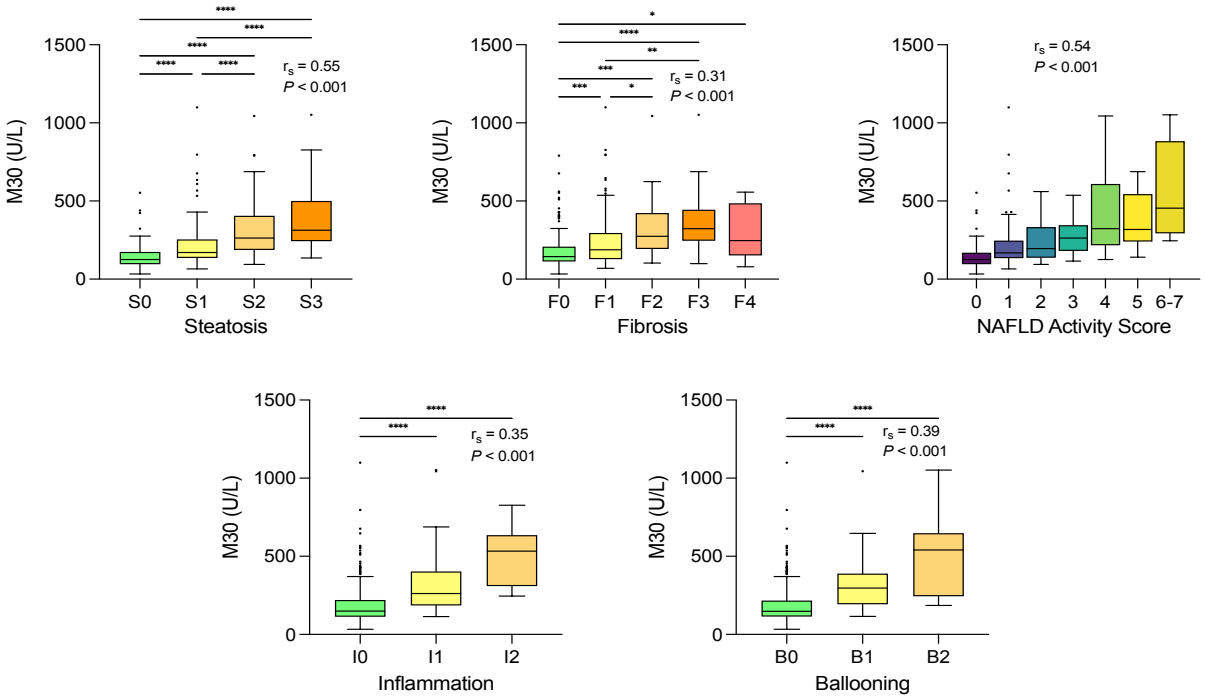

Figure 1. Associations between concentrations of ccK18 M30 and liver histology. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

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ccK18 as a predictor of NASH and fibrotic NASH
The ccK18-incorporating MACK-3 score had the highest area under the receiver operating characteristic (AUROC) for NASH (0.84) and fibrotic NASH (0.88) (Table 1, Figure 2). The ccK18-based biomarkers (M30® and MACK-3) had significantly higher AUROCs to diagnose NASH as compared to FIB-4, NFS, ALT, and AST (P<0.05 for all comparisons, DeLong's test), and a significantly higher AUROC to diagnose fibrotic NASH as compared to NFS and ALT (P<0.05).

Table 1. Diagnostic accuracy of the biomarkers (n=354).

Biomarker	NASH AUROC (95% CI)	Fibrotic NASH* AUROC (95% CI)
ccK18	0.84 (0.79–0.89)	0.88 (0.83–0.93)
MACK-3	0.87 (0.82–0.92)	0.89 (0.84–0.95)
FIB-4	0.67 (0.59–0.75)	0.80 (0.70–0.89)
NFS	0.59 (0.50–0.67)	0.63 (0.52–0.74)
ALT	0.73 (0.65–0.80)	0.77 (0.66–0.87)
AST	0.79 (0.72–0.85)	0.83 (0.73–0.92)

AUROC, area under the receiver operating characteristic; CI, confidence interval. *Defined as NASH + NAS≥4 + F≥2.

CONCLUSIONS
Plasma concentrations of ccK18 associate with the full histological spectrum of NASH. In patients with severe obesity, ccK18 and the related MACK-3 score have an adequate discriminatory ability to identify NASH or fibrotic NASH.

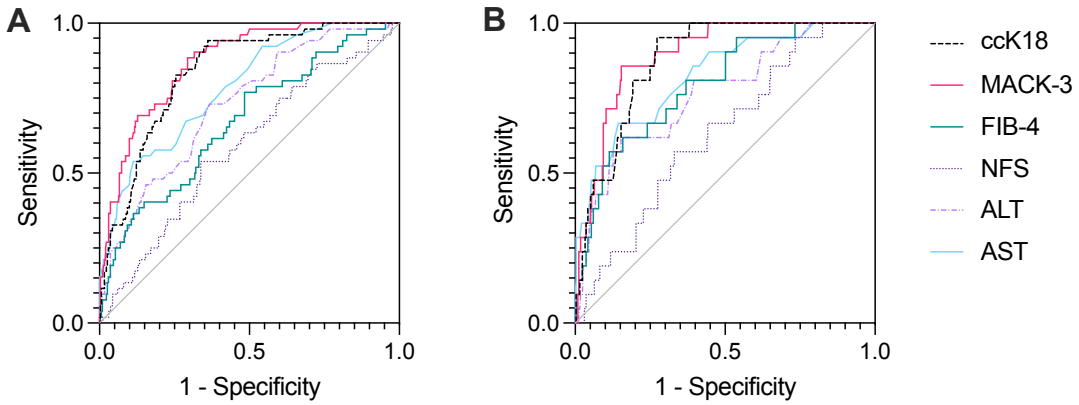

Figure 2. Receiver operating characteristic curves for the studied biomarkers to identify (A) NASH or (B) Fibrotic NASH, defined as NASH + NAS≥4 + F≥2.

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