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## **M30 Apoptosense ELISA and M65 ELISA**

### ***Reference List***

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# **Non-alcoholic fatty liver disease & Non-alcoholic Steatohepatitis**

*NAFLD & NASH*

# NAFLD and NASH

*Non-alcoholic fatty liver disease and Non-alcoholic steatohepatitis*

## Dvorak, 2014

<b>Title of the Publication</b>	<i>Use of Non-Invasive Parameters of Non-Alcoholic Steatohepatitis and Liver Fibrosis in Daily Practice – An Exploratory Case-Control Study</i>
<b>First and last Author</b>	Dvorak K.; Bruha R.
<b>Journal</b>	PLoS One. 9(10):e111551 (2014)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Observational, case-control study
<b>Patients</b>	A total of 112 patients with NAFLD were included in the prospective study. The patient population was recruited from all consecutive patients referred to the 4th Department of Internal Medicine of the General University Hospital in Prague between 2010 and 2013, and in whom a diagnosis of NAFLD had been confirmed.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), M65 (K18), AST/ALT, c-Glutamyltransferase (GGT) <b>Histopathology</b> Brunt score, NAFLD Activity Score (NAS) according to Kleiner
<b>Principal Findings</b>	<p>As expected, compared to NAFLD/NASH patients, significantly lower serum glucose, adiponectin, insulin levels, and cytokeratin-18 fragments M30 and M65 levels were observed in healthy controls.</p> <p>Serum concentrations of cytokeratin-18 fragments M30 and M65 were significantly higher in patients with NASH, compared to patients with simple steatosis. The sensitivity and specificity of cytokeratin-18 fragments for discrimination between patients with and without NASH were calculated and ROC plotted. The most significant parameter for this discrimination was serum concentration of M65 (sensitivity 80%, specificity 82%, with a cut-off value of 750 U/L). The AUROC for M30 was 0.85; for M65 it was 0.89. No other parameter had a similar sensitivity and specificity.</p>
<b>Conclusions</b>	In our study, the relevance of cytokeratin-18 fragments was even higher - the AUROC for discrimination of NASH from simple steatosis in our patients was 0.89 for M65, and 0.85 for M30. Also, the sensitivity and specificity of cytokeratin-18 fragments in the discrimination between simple steatosis and NASH was higher (75% sensitivity and 81% specificity for M30; 80% sensitivity and 82% sensitivity for M65). Based on our results, the assessment of M65, with a cut-off value 750 U/l, is the best simple non-invasive method with which to diagnose NASH. Our results also support the suggestion for the use of M30/M65 in the screening of NASH, as the differences between patients with NASH and controls were statistically significant.
<b>Recommendations</b>	<p>In patients with NAFLD, non-invasive serum parameters with a high accuracy can differentiate those patients with NASH and/or advanced fibrosis from those with simple steatosis. A substantial portion of those patients not indicated for liver biopsy might have undiagnosed advanced fibrosis.</p> <p>The most promising non-invasive parameter of NASH seems to be the examination of circulating levels of cytokeratin-18, a biomarker of hepatocyte necrosis and apoptosis.</p>

## Aida, 2014

<b>Title of the Publication</b>	<i>Serum cytokeratin 18 fragment level as a noninvasive biomarker for non-alcoholic fatty liver disease</i>
<b>First and last Author</b>	Aida Y.; Aizawa Y.
<b>Journal</b>	Int J Clin Exp Med. 7:4191–4198 (2014)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Single centre, observational
<b>Patients</b>	A total of 116 NAFLD patients admitted to the Jikei University Katsushika Medical Center (Tokyo, Japan) between January 2010 and December 2013 for liver biopsies were enrolled. Sex (41 male/75 female), age (27–82).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), Low-density Lipoprotein-cholesterol (LDL-C) and Insulin resistance (HOMA-IR) <b>Histopathology</b> Brunt score, NAFLD Activity Score (NAS) according to Kleiner
<b>Principal Findings</b>	Serum CK18-F levels showed a positive correlation with histologic steatosis ( $p = 0.271$ , $P = 0.0033$ ), inflammation ( $p = 0.353$ , $P = 0.0005$ ), ballooning ( $p = 0.372$ , $P = 0.0001$ ), and the total NAS ( $p = 0.474$ , $P = 2.68 \times 10^{-7}$ ). The serum CK18-F level was significantly lower for NAFL ( $NAS \leq 2$ ) than for borderline NASH ( $NAS$ of 3–4) or definite NASH ( $NAS \geq 5$ ) ( $P = 0.0294$ , $P = 1.163 \times 10^{-5}$ , respectively). The serum CK18-F level was significantly higher for definite NASH than for borderline NASH ( $P = 0.0002$ ). The area under the receiver operating characteristic curve of serum CK18-F to predict the presence of NAFL and definite NASH was 0.762 and 0.757, respectively. The optimal cut-off point of serum CK18-F for NAFL and definite NASH was 230 and 270 U/L, respectively. The sensitivity, specificity, positive predict value, and negative predict value of serum CK18-F for NAFL were 0.89, 0.65, 0.34, and 0.97, and those for definite NASH were 0.64, 0.76, 0.72, and 0.67, respectively. Accuracies of diagnosis for both NAFL and definite NASH were 0.70.
<b>Conclusions</b>	Serum CK18-F could be a clinically useful biomarker to discriminate between NAFL and NASH.
<b>Recommendations</b>	This study established the use of serum CK18-F as a noninvasive biomarker for differentiating between NAFL and NASH. By combining the serum CK18-F level with other noninvasive markers, higher-precision prognosis prediction for NAFLD may be attained.

**Reis, 2014**

<b>Title of the Publication</b>	<i>(Cleaved) CK18 serum and tissue expression levels differentiate acute HCV reinfection from acute rejection in liver allografts</i>
<b>First and last Author</b>	Reis H.; Baba H. A.
<b>Journal</b>	Liver Int. 35:905-913 (2014)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Single centre, observational
<b>Patients</b>	The studied cohort consisted of 22 patients who received Orthotopic liver transplantation (OLT) for HCV-related cirrhosis, i.e. 11x chronic HCV reinfection, 11x acute HCV reinfection, and of 16 with OLT for diverse causes (excluding HCV), i.e. 16x acute rejection HCV negative patients. As a control group, patients diagnosed with NAFL (19) and NASH (19) were included. All patients received liver biopsy either for establishment of the diagnosis (NAFL, NASH) or post-OLT because of deterioration of graft function or liver damage tests (HCV reinfection, rejection) and blood.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> Serum M30 (ccK18) (M30S), serum M65 (K18) (M65S), ALT/AST and M30-immunohistochemistry (M30H)</p> <p><b>Histopathology</b> Acute rejection was scored adherent to Banff-criteria. For differential diagnosis of NAFL vs. NASH a NAFLD activity score (NAS) was calculated.</p>
<b>Principal Findings</b>	<p>In the total cohort, M30S and M65S as well as M30H were significantly differing (all <math>P &lt; 0.0001</math>; Table 1) with highest values in acute HCV reinfection, lowest in NAFL and intermediate in NASH, chronic HCV reinfection and acute rejection. In contrast, M30S/M65S-ratio values were highest in chronic HCV reinfection, followed by acute rejection, NASH and NAFL and lowest in acute HCV reinfection (<math>P = 0.019</math>).</p> <p>In the differentiation of NAFL and NASH, again M30S and M65S as well as M30H were found to be significantly differing (all <math>P &lt; 0.0001</math>; Table 1) with lower values in NAFL. The median values for NAFL were 185 U/L for M30S and 353 U/L for M65S. For NASH, the median values were 562 U/L for M30S and 965 U/L for M65S.</p> <p>M30S also correlated with M30S/M65-serum ratio (<math>P &lt; 0.0001</math>), while M65S and M30H did not. Higher extent of hepatocellular steatosis was additionally significantly correlated with M30S, M65S and M30H (all <math>P &lt; 0.0001</math>).</p>
<b>Conclusions</b>	In further analyses, the study found consistent with published data both M30S and M65S to be considerably higher in NASH compared to NAFL (each $P < 0.0001$ ). The serological data are confirmed by a four-fold increase in median level of hepatocellular apoptosis detected by M30H from NAFL to NASH ( $P < 0.0001$ , Figs. 1A/B and 2C) and also by the correlation of M30-serum and tissue derived data ( $P = 0.017$ ). These results further support the usefulness of M30 and M65 as non-invasive markers in the differential diagnosis of steatohepatitis.

**El Bassat, 2014**

<b>Title of the Publication</b>	<i>Apoptotic and anti-apoptotic seromarkers for assessment of disease severity of non-alcoholic steatohepatitis</i>
<b>First and last Author</b>	El Bassat H.; Abo Ryia M. H.
<b>Journal</b>	Arab J Gastroenterol. 15: 6-11 (2014)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Single centre, observational
<b>Patients</b>	This study was carried out on 80 patients who had bright liver in ultrasound examination with or without abnormal liver enzyme and 15 non-hepatic persons as control. The patients were divided based on histological examination of liver biopsy into three groups. Group I included 40 patients with NASH, group II had 40 patients with non-alcoholic fatty liver disease (NAFLD) non-NASH and group III had 15 non-hepatic subjects as control.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), ALT/AST, S. Fas, s. Fas ligand and Bcl-2 <b>Histopathology</b> Brunt score, NAFLD Activity Score (NAS) according to Kleiner
<b>Principal Findings</b>	There was a significant increase in the serum level of the K18 fragment in the NASH group ( $1568.6 \pm 329$ U/L) compared to the NAFLD group ( $8113.6 \pm 32.8$ U/L) and control group ( $103.4 \pm 41.5$ U/L) ( $F = 528.021$ , $p < 0.000$ ).  There was positive correlation between K18 fragment and histopathological features, especially with lobular inflammation ( $r = 0.738$ , $p < 0.000$ ) and ballooning ( $r = 0.544$ , $p < 0.013$ ).
<b>Conclusions</b>	The significant increase in the CK-18 fragment level was noticed in the NASH group compared to both the NAFLD and control group of this study. The study also demonstrated positive correlation between the K18 fragment and histological features of NASH, mainly lobular inflammation and hepatocyte ballooning.



**Shen, 2012**

<b>Title of the Publication</b>	<i>Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers</i>
<b>First and last Author</b>	Shen J.; Wong V. W-S.
<b>Journal</b>	J Hepatol. 56:1363-70 (2012)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Prospective, longitudinal single-centre study.
<b>Patients</b>	Biopsy-proven NAFLD patients (n=146, non-NASH: n=64, NASH: n=82) with repeated liver biopsies at month 36, healthy controls (n=74). NAFLD patients with diabetes (n=70), non-NASH (n=26), NASH (n=44).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), AFABP and FGF21</p> <p><b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.</p>
<b>Principal Findings</b>	The median ccK18 level was 355U/L in NAFLD patients and 103U/L in controls ( $p<0.001$ ). ccK18 had the highest accuracy of all biomarkers tested in detecting NAFLD with the optimal cut-off level of 180U/L with a 84% sensitivity and 92% specificity in detecting NAFLD. ccK18 level had positive correlation with BMI $r=0.26$ ; ( $p=0.002$ ), fasting glucose $r=0.28$ ; ( $p=0.001$ ), steatosis grade $r=0.38$ ; ( $p<0.001$ ), lobular inflammation $r=0.25$ ; ( $p=0.002$ ), ballooning $r=0.31$ ; ( $p<0.001$ ), NAS $r=0.37$ ; ( $p<0.001$ ) and fibrosis $r=0.43$ ; ( $p<0.001$ ). By multiple linear regression, higher steatosis grade ( $p=0.006$ ), more severe lobular inflammation ( $p=0.043$ ), and fibrosis stage $\geq F2$ ( $p=0.002$ ) remained independently associated with ccK18.
<b>Conclusions</b>	Serum levels of ccK18 and FGF21 were significantly higher in NASH patients. The median ccK18 levels in NAFLD patients with non-NASH and NASH were 263U/L and 418U/L, respectively ( $p<0.001$ ). The AUROC for ccK18 to diagnose NAFLD was 0.91 and for NASH 0.70 (0.83 when all non-NASH and controls were included). Overall, ccK18 had the highest accuracy in detecting NASH at the optimal cut-off level of 338U/L.
<b>Recommendations</b>	ccK18 (M30) is the most accurate biomarker for NAFLD and NASH. A two-step approach using ccK18 and FGF21 further improves the accuracy in diagnosing NASH.

# NAFLD

Non-alcoholic fatty liver disease

## Ergelen, 2015

<b>Title of the Publication</b>	<i>Measurements of serum procollagen-III peptide and M30 do not improve the diagnostic accuracy of transient elastography for the detection of hepatic fibrosis in patients with nonalcoholic fatty liver disease.</i>
<b>First and last Author</b>	Ergelen R.; Yilmaz Y.
<b>Journal</b>	Eur J Gastroenterol Hepatol . 56:1363-70 (2015)
<b>Disease</b>	NAFLD (fibrosis)
<b>Type of Study</b>	Single-centre study, prospective.
<b>Patients</b>	The study sample consisted of 87 patients with NAFLD (43 men and 44 women, mean age: 45.8 ± 9.0 years) who were consecutively enrolled at the Department of Gastroenterology, Marmara University School of Medicine, Istanbul, Turkey. All patients were of Turkish descent. Potential candidates for liver biopsy included (a) patients with a persistent (>6 months) increase in transaminases and steatosis on ultrasound; (b) patients with normal transaminases presenting with hepatomegaly and/or splenomegaly, and (c) patients with normal transaminases but persistently increased γ-glutamyl transferase. All participants were required to have absent-to-low alcohol consumption (i.e. <30 g/day in men and < 20 g/ day in women). Patients with viral B and C hepatitis, Wilson's disease, α1-antitrypsin deficiency, autoimmune hepatitis, genetic hemochromatosis, and use of steatogenic drugs were excluded.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), Procollagen-III-peptide (PIIP), Liver Stiffness Measurement (LSM) by Transient Elastography (TE).</p> <p><b>Histopathology</b> NAFLD histologic activity score (NAS)</p>
<b>Principal Findings</b>	The median M30 level in the study participants was 119.7 U/l (range: 59.1, 1007.3 U/l). In multivariate analyses, serum M30 was an independent predictor of both significant ( $\beta=0.158$ ; $P=0.027$ ) and advanced fibrosis ( $\beta=0.004$ ; $P=0.006$ ). At an optimal cutoff value of 243.04 U/l, serum M30 had an AUROC of 0.747 (95% CI=0.599–0.895) for the prediction of significant fibrosis ( $P<0.001$ ). Moreover, serum M30 had an AUROC of 0.895 (95% CI=0.808–0.991) for the prediction of advanced fibrosis at an optimal cutoff value of 262.2 U/l ( $P<0.001$ ).
<b>Conclusions</b>	This study was carried out to determine whether the diagnostic performance of TE could be improved by measurements of serum PIIP and M30 levels in patients with NAFLD. The main results are as follows: (a) PIIP did not show any significant association with hepatic fibrosis; (b) both TE and serum M30 levels were similarly accurate for the noninvasive diagnosis of both significant and advanced hepatic fibrosis in NAFLD; and (c) the combined evaluation of TE and serum M30 levels did not improve the overall diagnostic accuracy significantly compared with either test alone.
<b>Recommendations</b>	Both TE and serum M30 levels are accurate for the noninvasive diagnosis of fibrosis in NAFLD. However, their combination did not improve the overall diagnostic accuracy.

**Feldstein, 2013**

<b>Title of the Publication</b>	<i>Serum Cytokeratin-18 Fragment Levels Are Useful Biomarkers for Nonalcoholic Steatohepatitis in Children</i>
<b>First and last Author</b>	Feldstein A. E.; Nobili V.
<b>Journal</b>	Am J Gastroenterol. 108:1526-31 (2013)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Single centre, observational, prospective
<b>Patients</b>	Clinically confirmed NAFLD children (n=201) of which had biopsy NASH (n=140), and not NASH (n=61). Inclusion criteria were persistently elevated serum aminotransferase levels, diffusely hyperechogenic liver on ultrasonography suggestive of fatty liver, and biopsy consistent with the diagnosis of NAFLD.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18)</p> <p><b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.</p>
<b>Principal Findings</b>	ccK18 plasma levels were significantly higher in patients with NASH (NAS $4.4 \pm 1.3$ ) compared with those with not-NASH (NAS $2.0 \pm 0.68$ ) median: 322U/L, 164U/L, respectively; ( $p < 0.001$ ). This association remained significant even after adjusting for multiple confounders including waist circumference percentile, Metabolic Syndrome (MetS), international normalized ratio, and triglyceride level. There was a significant association between ccK18 levels and histology (all $p < 0.001$ ) which was very strong for NAS ( $r = 0.92$ ), strong for steatosis grade ( $r = 0.76$ ) and ballooning ( $r = 0.74$ ), moderate for lobular inflammation ( $r = 0.45$ ), and fibrosis ( $r = 0.41$ ), and weak for portal inflammation ( $r = 0.32$ ). Furthermore, there was a progressive increase in ccK18 levels according to the grade of the histological feature of NAS and the stage of fibrosis.
<b>Conclusions</b>	AUROC for NASH diagnosis for ccK18 was 0.93 with a cut-off value of 233U/L giving a sensitivity of 85% and specificity of 86.9% with a positive predictive value (PPV) of 93.7% and a negative predictive value (NPV) of 71.6%. Using a cut-off value for ccK18 level of 218U/L maximized the sensitivity and negative predictive value (90.7% and 78%, respectively) and, therefore, can be used to rule out the presence of NASH. On the other hand, a cut-off value of 268U/L maximized the specificity and positive predictive value (95.1% and 97%, respectively) and can be used to rule in the diagnosis of NASH. ccK18 levels were significantly higher in patients with fibrosis (F1–3) on biopsy compared with those with no fibrosis (F0) 304U/L versus 210U/L ( $p < 0.001$ ). MetS did not affect the association between ccK18 and NASH. The only MetS component found to be associated with ccK18 level was obesity: median 281U/L for obese compared with 215U/L in non-obese children ( $p = 0.011$ ).
<b>Recommendations</b>	Determination of plasma ccK18 (M30) levels is an accurate biomarker for the presence of NASH within the spectrum of NAFLD in children. As in adults, ccK18 may become one of the most promising single non-invasive test for diagnosing NASH in children.

**Shen, 2012**

<b>Title of the Publication</b>	<i>Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers</i>
<b>First and last Author</b>	Shen J.; Wong V. W-S.
<b>Journal</b>	J Hepatol. 56:1363-70 (2012)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Prospective, longitudinal single-centre study.
<b>Patients</b>	Biopsy-proven NAFLD patients (n=146, non-NASH: n=64, NASH: n=82) with repeated liver biopsies at month 36, healthy controls (n=74). NAFLD patients with diabetes (n=70), non-NASH (n=26), NASH (n=44).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), AFABP and FGF21</p> <p><b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.</p>
<b>Principal Findings</b>	The median ccK18 level was 355U/L in NAFLD patients and 103U/L in controls ( $p<0.001$ ). ccK18 had the highest accuracy of all biomarkers tested in detecting NAFLD with the optimal cut-off level of 180U/L with a 84% sensitivity and 92% specificity in detecting NAFLD. ccK18 level had positive correlation with BMI $r=0.26$ ; ( $p=0.002$ ), fasting glucose $r=0.28$ ; ( $p=0.001$ ), steatosis grade $r=0.38$ ; ( $p<0.001$ ), lobular inflammation $r=0.25$ ; ( $p=0.002$ ), ballooning $r=0.31$ ; ( $p<0.001$ ), NAS $r=0.37$ ; ( $p<0.001$ ) and fibrosis $r=0.43$ ; ( $p<0.001$ ). By multiple linear regression, higher steatosis grade ( $p=0.006$ ), more severe lobular inflammation ( $p=0.043$ ), and fibrosis stage $\geq F2$ ( $p=0.002$ ) remained independently associated with ccK18.
<b>Conclusions</b>	Serum levels of ccK18 and FGF21 were significantly higher in NASH patients. The median ccK18 levels in NAFLD patients with non-NASH and NASH were 263U/L and 418U/L, respectively ( $p<0.001$ ). The AUROC for ccK18 to diagnose NAFLD was 0.91 and for NASH 0.70 (0.83 when all non-NASH and controls were included). Overall, ccK18 had the highest accuracy in detecting NASH at the optimal cut-off level of 338U/L.
<b>Recommendations</b>	ccK18 (M30) is the most accurate biomarker for NAFLD and NASH. A two-step approach using ccK18 and FGF21 further improves the accuracy in diagnosing NASH.

**Fitzpatrick, 2010**

<b>Title of the Publication</b>	<i>Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD</i>
<b>First and last Author</b>	Fitzpatrick E.; Dhavan A.
<b>Journal</b>	J Pediatr Gastroenterol Nutr. 51:500-6 (2010)
<b>Disease</b>	NAFLD
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	Children with biopsy-proven NAFLD (n=45), of which showed no/minimal fibrosis (<F2) (n=22) and significant fibrosis (≥F2) (n=23); and age-matched healthy controls (n=13).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), HA, leptin, high-sensitivity CRP and adiponectin. <b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.
<b>Principal Findings</b>	ccK18 levels were significantly higher in patients with NAFLD versus controls, median 288U/L versus 172U/L (p<0.001), and in those with NASH (NAS ≥5), median 347U/L versus simple steatosis (NAFLD activity score <3), median 191U/L (p=0.006) and not-NASH (NAS≤2). Significant fibrosis (≥F2) could be differentiated from no/minimal fibrosis (<F2), median 393U/L versus 243U/L (p=0.03).
<b>Conclusions</b>	The AUROC for predicting significant or severe fibrosis (score ≥F2) was 0.66 with a cut-off value of 200U/L with a sensitivity of 83% and specificity of 40%. AUROC for NASH diagnosis for ccK18 was 0.85 with a cut-off value of 207U/L giving a sensitivity of 84% and specificity of 88% with a positive predictive value (PPV) of 90% and a negative predictive value (NPV) of 80%.
<b>Recommendations</b>	Serum biomarkers, especially ccK18 (M30) are useful in stratifying disease severity in paediatric NAFLD.

# NASH

*Non-alcoholic steatohepatitis*

## Rahman, 2015

<b>Title of the Publication</b>	<i>Association of Serum Cytokeratin-18 Fragment Concentration in Patients with Different Types of Nonalcoholic Fatty Liver Disease</i>
<b>First and last Author</b>	Rahman T.; Ahmed A. N. N.
<b>Journal</b>	Gastroenterol Hepatol Open Access. 2(2): 00037 (2015)
<b>Disease</b>	NASH
<b>Type of Study</b>	Cross sectional
<b>Patients</b>	Patients with NAFLD admitted in the Department of Hepatology in BSMMU. Total NAFLD patients (40) were divided into three groups according to the NAFLD activity score (NAS); Non-NASH (n=8), borderline NASH (n=18) and NASH (n=14).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18)</p> <p><b>Histopathology</b> NAFLD Activity Score (NAS); NASH Clinical Research Network histological scoring system</p>
<b>Principal Findings</b>	<p>The Receiver-operating characteristic (ROC) curve analysis of CK18 suggested that a cutoff value of 150 U/L has the sensitivity (85.7%) and specificity (80.8%) for detecting patients with NASH with an area under the curve (AUC) of 0.918 (95% CI: 0.828-1.007), (P&lt;0.05).</p> <p>CK 18 shows 85.71% sensitivity, 80.80% specificity, 66.67% positive predictive value, 90.91% negative predictive value and 80% accuracy in the diagnosis of NASH.</p>
<b>Conclusions</b>	Serum cytokeratin-18 fragment concentration has statistically significant positive relationship with different types of NAFLD patients. Besides this, CK-18 fragment concentration has significantly high sensitivity, specificity, negative predictive value and accuracy in assessing NASH. Serum CK-18 fragment can be used for the assessment of NASH in NAFLD patients. As the sensitivity of serum CK-18 fragment level was high, it can be used as a good screening tool for NASH which may help physicians to narrow down the patient's population before biopsy. Serum CK-18 fragment can also be helpful for regular monitoring of the patients to see the disease progression and response to treatment. It might reduce the need for repeated liver biopsy of the patients and biopsy related hazards as well.

**Chen, 2014**

**Title of the Publication** *Serum cytokeratin-18 in the diagnosis of non-alcoholic steatohepatitis: A meta-analysis*

**First and last Author** Chen J.; Jiang J.

**Journal** Hepatology research. 44:854-862 (2014)

**Disease** NASH

**Type of Study** Meta-analysis, Literature study

**Patients** 10 studies, with in total 838 patients.  
9 studies included CK-18 fragments, called the M30 group.  
5 studies included total CK-18, called the M65 group.

**Methodology****Data sources**

Studies published in English-language were identified by a literature search of the PubMed, Ovid, Medline and Cochrane Library databases up to January 2012 with the following key words: "nonalcoholic fatty liver disease" or "nonalcoholic steatohepatitis" plus "Cytokeratin- 18". In addition, a manual literature search was conducted using the references of original manuscripts and reviews to identify additional studies, if necessary and meta-analyses were performed. All searches were conducted independently by two investigators. The results were compared, and any questions or discrepancies were resolved through iteration and consensus.

**Statistical analysis**

All included patients were classified into two groups: group M30 (M30 antigen was assessed) and group M65 (M65 antigen was assessed). The diagnostic threshold was the same as the reference standard in each included study. The two-by-two tables were constructed according to the data reported or calculated from the articles. The individual study results were presented graphically by plotting the estimates of sensitivity and specificity, and their 95% confidence intervals (95% CI), in paired forest plots. A DerSimonian-Laird random effects model was used to compute the pooled estimates of SEN, SPE, positive likelihood ratio, negative likelihood ratio and diagnostic odds ratio (DOR), and a summary receiver operating characteristic (SROC) curve was constructed. The SROC curve represents the overall diagnostic performance of the test, and the change in diagnostic accuracy according to changes in the cut-off value.

**Principal Findings**

In the M30 group (caspase-cleaved CK18, termed M30 antigen), pooled sensitivity was 0.83 and specificity was 0.71. AUROC was 0.8445.  
In the M65 group (uncleaved CK-18, termed M65 antigen), pooled sensitivity was 0.77 and specificity was 0.71. AUROC was 0.8170.

**Conclusions**

Both CK-18 fragments and total CK-18 have a clinically meaningful benefit in noninvasive diagnosing of NASH in patients with NAFLD, though total CK-18 showed relatively low diagnostic accuracy compared with CK-18 fragments. CK-18 fragments may be a useful biomarker for screening rather than identifying NASH.



**Feldstein, 2013**

<b>Title of the Publication</b>	<i>Serum Cytokeratin-18 Fragment Levels Are Useful Biomarkers for Nonalcoholic Steatohepatitis in Children</i>
<b>First and last Author</b>	Feldstein A. E.; Nobili V.
<b>Journal</b>	Am J Gastroenterol. 108:1526-31 (2013)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Single centre, observational, prospective
<b>Patients</b>	Clinically confirmed NAFLD children (n=201) of which had biopsy NASH (n=140), and not NASH (n=61). Inclusion criteria were persistently elevated serum aminotransferase levels, diffusely hyperechogenic liver on ultrasonography suggestive of fatty liver, and biopsy consistent with the diagnosis of NAFLD.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18)</p> <p><b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.</p>
<b>Principal Findings</b>	ccK18 plasma levels were significantly higher in patients with NASH (NAS $4.4 \pm 1.3$ ) compared with those with not-NASH (NAS $2.0 \pm 0.68$ ) median: 322U/L, 164U/L, respectively; ( $p < 0.001$ ). This association remained significant even after adjusting for multiple confounders including waist circumference percentile, Metabolic Syndrome (MetS), international normalized ratio, and triglyceride level. There was a significant association between ccK18 levels and histology (all $p < 0.001$ ) which was very strong for NAS ( $r = 0.92$ ), strong for steatosis grade ( $r = 0.76$ ) and ballooning ( $r = 0.74$ ), moderate for lobular inflammation ( $r = 0.45$ ), and fibrosis ( $r = 0.41$ ), and weak for portal inflammation ( $r = 0.32$ ). Furthermore, there was a progressive increase in ccK18 levels according to the grade of the histological feature of NAS and the stage of fibrosis.
<b>Conclusions</b>	AUROC for NASH diagnosis for ccK18 was 0.93 with a cut-off value of 233U/L giving a sensitivity of 85% and specificity of 86.9% with a positive predictive value (PPV) of 93.7% and a negative predictive value (NPV) of 71.6%. Using a cut-off value for ccK18 level of 218U/L maximized the sensitivity and negative predictive value (90.7% and 78%, respectively) and, therefore, can be used to rule out the presence of NASH. On the other hand, a cut-off value of 268U/L maximized the specificity and positive predictive value (95.1% and 97%, respectively) and can be used to rule in the diagnosis of NASH. ccK18 levels were significantly higher in patients with fibrosis (F1–3) on biopsy compared with those with no fibrosis (F0) 304U/L versus 210U/L ( $p < 0.001$ ). MetS did not affect the association between ccK18 and NASH. The only MetS component found to be associated with ccK18 level was obesity: median 281U/L for obese compared with 215U/L in non-obese children ( $p = 0.011$ ).
<b>Recommendations</b>	Determination of plasma ccK18 (M30) levels is an accurate biomarker for the presence of NASH within the spectrum of NAFLD in children. As in adults, ccK18 may become one of the most promising single non-invasive test for diagnosing NASH in children.



**Cao, 2013**

<b>Title of the Publication</b>	<i>Cytokeratin 18, Alanine Aminotransferase, Platelets and Triglycerides Predict the Presence of Nonalcoholic Steatohepatitis</i>
<b>First and last Author</b>	Cao W.; Wang Y.
<b>Journal</b>	PLoS One. (8):e82092 (2013)
<b>Disease</b>	NASH
<b>Type of Study</b>	Single centre, observational
<b>Patients</b>	NAFLD patients (n=95), non-NASH (n=51) and definitive NASH (n=44).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b></p> <p>Body mass index (BMI), waist-on-hip ratio (WHR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), <math>\gamma</math>-glutamyl transpeptidase (<math>\gamma</math>-GT), platelets, uric acid (UA), hs-C-reactive protein (hs-CRP), triglycerides (TG), albumin (ALB), and M30 (ccK18).</p> <p><b>Histopathology</b></p> <p>Brunt score, NAFLD Activity Score (NAS) according to Kleiner</p>
<b>Principal Findings</b>	<p>Median ccK18 levels were significantly higher in patients with NASH: 372.9U/L than that in the non-NASH group: 248.1U/L, (<math>p&lt;0.001</math>)</p> <p>ccK18 levels showed a significant positive correlation with steatosis severity <math>r=0.492</math> (<math>p&lt;0.001</math>), ballooning <math>r=0.211</math>, (<math>p&lt;0.041</math>), lobular inflammation <math>r=0.346</math> (<math>p=0.001</math>), and fibrosis stage <math>r=0.407</math>, <math>p&lt;0.001</math>). ALT (<math>p=0.007</math>), platelets (<math>p=0.040</math>), ccK18 (M30) (<math>p=0.011</math>), or TG (<math>p=0.019</math>) were independent variables in patients with NASH.</p> <p>The AUROC curves were 0.81 for ALT, 0.63 for platelets, 0.89 for ccK18, and 0.71 for TG for the prediction of NASH. ccK18 levels showed a significant positive correlation with ALT <math>r=0.639</math>, (<math>p&lt;0.001</math>) and TG <math>r=0.390</math>, (<math>p&lt;0.001</math>). No correlation was found with platelets (<math>p=0.645</math>).</p>
<b>Conclusions</b>	<p>A new <b>Definitive NASH</b> model was established through logistic regression in the NAFLD patients. The equation of this model was: <math>-12.764 + 0.075 \times \text{ALT (U/L)} + 0.013 \times \text{platelets (}\times 10^9/\text{L)} + 0.012 \times \text{ccK18 (M30) (U/L)} + 0.006 \times \text{TG (mg/dL)}</math>.</p> <p>The AUROC curve for the prediction of NASH was 0.92. A cut-off value of 0.361, with a sensitivity of 89% and a specificity of 86%, has a positive predictive value of 89% and a negative predictive value of 89%.</p>

**Jain, 2013**

<b>Title of the Publication</b>	<i>Serum keratin fragment 18 (CK18) levels significantly predict changes in liver histology in children and adolescents with nonalcoholic fatty liver disease (NAFLD): Results from the TONIC trial</i>
<b>First and last Author</b>	Jain A. K.; Chalasani N. P.
<b>Journal</b>	AASLD Presentation and Abstract #114 (2013)
<b>Disease</b>	NASH
<b>Type of Study</b>	Randomized, double-blind, double-dummy, placebo-controlled clinical trial conducted at 10 university clinical research centers with biopsy-confirmed NAFLD (n=173) conducted between September 2005 and March 2010. Interventions were daily dosing of 800 IU of vitamin E (n=58), 1000 mg of metformin (n=57), or placebo (n=58) for 96 weeks.
<b>Patients</b>	NAFLD patients (n=173), not-NASH (n=100) and NASH (n=73). NASH in the Vitamin E: (n=27) Metformin: (n=24) and Placebo: (n=22) with the following scores: steatosis: 2.3/2.1/2.1; lobular inflammation: 1.6/1.6/1.7; ballooning: 1.0/0.8/0.8 and a NAFLD activity score (NAS) 4.8/4.5/4.6, respectively.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18). Serum ccK18 levels were measured at baseline and weeks 24, 48 and 96 in 127 out of 147 children who had both baseline and week 96 liver biopsies as part of the TONIC (Treatment of NAFLD in Children) trial (JAMA 2011;305;1659-1668). Changes in serum ccK18 across treatment groups as well as the relationship between changes in serum ccK18 and liver histology over the 96 week trial duration were assessed. <b>Histopathology</b> Brunt score, NAFLD Activity Score (NAS) according to Kleiner
<b>Principal Findings</b>	There was a strong relationship between changes in serum ccK18 and changes in liver histology, irrespective of the treatment assignment. Children who met the primary histological end point, compared to those who did not, had a greater decrease in ccK18 at w24: $\Delta$ -134 versus $\Delta$ 19U/L; (p=0.09), at w48: $\Delta$ -194 versus $\Delta$ -45U/L; (p=0.004) and at w96: $\Delta$ -206 versus $\Delta$ -2U/L; (p<0.001). Similarly, changes in serum ccK18 were significantly associated with resolution of NASH at w96. Mean change at w96 in responders $\Delta$ -202U/L versus non-responders $\Delta$ +16U/L (p<0.001). By logistic regression, after controlling for treatment group and baseline ccK18, change in serum ccK18 levels strongly predicted changes in all histological components of NAFLD and fibrosis and changes in ALT over the 96w treatment
<b>Conclusions</b>	Changes in serum ccK18 (M30) levels predict changes in liver histology in NAFLD. This suggests that ccK18 is a potentially useful biomarker for predicting histological improvement in children and adolescents with NAFLD.

**Ghaemi, 2013**

<b>Title of the Publication</b>	<i>How Much Weight Loss is Effective on Nonalcoholic Fatty Liver Disease?</i>
<b>First and last Author</b>	Ghaemi A.; Fakheri H.
<b>Journal</b>	Hepat Mon. 13:e15227 (2013)
<b>Disease</b>	NASH
<b>Type of Study</b>	Single centre, interventional (diet), pilot study
<b>Patients</b>	<p>The diagnosis of NAFLD was established in obese (body mass Index (BMI) <math>&gt;30\text{kg/m}^2</math>) adults (<math>n=44</math>) by the presence of steatosis of the liver on ultrasound associated with a persistent increase in alanine aminotransferase (ALT), and aspartate aminotransferase (AST) of at least 1.5x ULN and BMI between 25 and <math>40\text{kg/m}^2</math>.</p> <p>No histological differentiation between NASH and non-NASH has been performed. Patients were all tested negative for HBS-Ag, HBc-Ab, HCV antibody, anti-nuclear antibody (ANA), anti-mitochondrial antibody (AMA), anti-smooth muscle antibody (ASMA), anti-liver-kidney-microsomal antibody (anti-LKM), ceruloplasmin and ferritin to rule out the presence of other liver diseases. Other exclusion criteria were: alcohol consumption, patient receiving hepatotoxic and insulin-sensitizing medication, cigarette smoking, weight reduction surgery within the past year, or used weight loss medication or program in the previous 3 months.</p>
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b></p> <p>Adult patients suspected to have NAFLD (<math>n=44</math>) were subjected to a diet including a 500 to 1000 kcal per day intake reduction as 30% fat, 15% protein, and 55% carbohydrate for six months. At the end of follow up, patients were classified as adherent (<math>n=25</math>) or non-adherent to treatment (<math>n=19</math>) according to a weight loss of <math>\geq 5\%</math>, or <math>&lt; 5\%</math> of initial body weight, respectively. Anthropometric parameters, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), lipid profile, malondialdehyde (MDA), TNF-<math>\alpha</math>, IL-6, cck18 were measured at baseline and at the end of the study.</p>
<b>Principal Findings</b>	<p>Baseline cck18 (M30) in both treatment groups were similar with 460.3U/L (adherent) and 404.9U/L (non-adherent); (<math>p=0.181</math>), respectively. However, there was a significant relationship between changes in serum cck18 and weight loss. Adults who met the primary end point, compared to those who did not, had a significant decrease in serum mean cck18 after 6 months compared to baseline: 460.3U/L: 370.7U/L; (<math>p=0.003</math>) compared to 404.9U/L at baseline and 469U/L (<math>p=0.295</math>).</p>

**Shen, 2012**

<b>Title of the Publication</b>	<i>Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers</i>
<b>First and last Author</b>	Shen J.; Wong V. W-S.
<b>Journal</b>	J Hepatol. 56:1363-70 (2012)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Prospective, longitudinal single-centre study.
<b>Patients</b>	Biopsy-proven NAFLD patients (n=146, non-NASH: n=64, NASH: n=82) with repeated liver biopsies at month 36, healthy controls (n=74). NAFLD patients with diabetes (n=70), non-NASH (n=26), NASH (n=44).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), AFABP and FGF21</p> <p><b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.</p>
<b>Principal Findings</b>	The median ccK18 level was 355U/L in NAFLD patients and 103U/L in controls ( $p<0.001$ ). ccK18 had the highest accuracy of all biomarkers tested in detecting NAFLD with the optimal cut-off level of 180U/L with a 84% sensitivity and 92% specificity in detecting NAFLD. ccK18 level had positive correlation with BMI $r=0.26$ ; ( $p=0.002$ ), fasting glucose $r=0.28$ ; ( $p=0.001$ ), steatosis grade $r=0.38$ ; ( $p<0.001$ ), lobular inflammation $r=0.25$ ; ( $p=0.002$ ), ballooning $r=0.31$ ; ( $p<0.001$ ), NAS $r=0.37$ ; ( $p<0.001$ ) and fibrosis $r=0.43$ ; ( $p<0.001$ ). By multiple linear regression, higher steatosis grade ( $p=0.006$ ), more severe lobular inflammation ( $p=0.043$ ), and fibrosis stage $\geq F2$ ( $p=0.002$ ) remained independently associated with ccK18.
<b>Conclusions</b>	Serum levels of ccK18 and FGF21 were significantly higher in NASH patients. The median ccK18 levels in NAFLD patients with non-NASH and NASH were 263U/L and 418U/L, respectively ( $p<0.001$ ). The AUROC for ccK18 to diagnose NAFLD was 0.91 and for NASH 0.70 (0.83 when all non-NASH and controls were included). Overall, ccK18 had the highest accuracy in detecting NASH at the optimal cut-off level of 338U/L.
<b>Recommendations</b>	ccK18 (M30) is the most accurate biomarker for NAFLD and NASH. A two-step approach using ccK18 and FGF21 further improves the accuracy in diagnosing NASH.

## Grigorescu, 2012

<b>Title of the Publication</b>	<i>A novel pathophysiological-based panel of biomarkers for the diagnosis of nonalcoholic steatohepatitis</i>
<b>First and last Author</b>	Grigorescu M.; Serban A.
<b>Journal</b>	J. Physiol. Pharmacol. 63:347-53 (2012).
<b>Disease</b>	NASH
<b>Type of Study</b>	Single centre, observational
<b>Patients</b>	NAFLD patients (n=79), not-NASH (n=20) and definitive NASH (n=59). The patients with NASH had significantly higher degrees of steatosis and inflammation. None of the not-NASH patients had ballooning or fibrosis.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> Adiponectin, Interleukin-6 (IL-6), and M65 (total K18).</p> <p><b>Histopathology</b> According to this scoring system NAS is the unweighted score of steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2). The stage of fibrosis was assessed using a four-point scale: 0= no fibrosis; 1= mild/moderate zone 3 perisinusoidal fibrosis or portal/periportal fibrosis only; 2=perisinusoidal and portal/periportal fibrosis; 3=bridging fibrosis and 4=cirrhosis. According to the NAS patients were classified as not-NASH (NAS ≤2), borderline NASH (NAS 3-4) and definite NASH (NAS ≥5).</p>
<b>Principal Findings</b>	<p>Adiponectin, IL-6, apoptosis+necrosis=total K18 (M65) were significantly higher in patients with NASH versus not-NASH patients. Median K18 levels were significantly higher in patients with NASH: 682.4U/L than that in the non-NASH group: 341.6U/L; (p&lt;0.001).</p> <p>K18 levels showed a significant positive correlation with steatosis severity r=0.403; (p=0.0015), ballooning r=0.270; (p&lt;0.045), lobular inflammation r=0.330; (p=0.011), and NAS r=0.482; (p&lt;0.0001). Adiponectin (p=0.05), IL-6 (p=0.04), or K18 (M65) (p=0.01) were independent variables in patients with NASH.</p>
<b>Conclusions</b>	<p>In multivariate analysis three markers were independently predictors of NASH: adiponectin, IL-6 and M65 levels. In decreasing order, the independent predictors of NASH (NAS≥5) were M65 with an AUROC of 0.791, IL-6 with an AUROC of 0.727 and adiponectin with an AUROC of 0.709. The combination of two biomarkers yielded an AUROC of 0.828 for M65 and IL-6, 0.841 for adiponectin and M65 and 0.852 for adiponectin and IL-6. The best value was obtained by triple combination: adiponectin, M65 and IL-6 with an AUROC of 0.903, Sp=85.7% (PPV=94.2%) and Se=84.5% (NPV=66.7%).</p> <p>In conclusion, a novel pathophysiological - based panel of biomarkers combining total K18 (M65), IL-6 and adiponectin may be useful to predict NASH.</p>

**Malik, 2009**

<b>Title of the Publication</b>	<i>The clinical utility of biomarkers and the nonalcoholic steatohepatitis CRN liver biopsy scoring system in patients with nonalcoholic fatty liver disease</i>
<b>First and last Author</b>	Malik R.; Afdhai N.
<b>Journal</b>	J Gastroenterol Hepatol. 24:564-8 (2009)
<b>Disease</b>	NASH
<b>Type of Study</b>	Prospective study, single centre, observational
<b>Patients</b>	NAFLD patients (n=95): simple steatosis with NAS score 1-3, (n=35), and NAS score 4-8 for NASH (n=60)
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), hyaluronic acid, TIMP-1, and YKL 40, blood samples taken within 6 months after biopsy. <b>Histopathology</b> NAFLD histologic activity (NAS) and metavir score
<b>Principal Findings</b>	ccK18 levels were significantly higher in the NASH group compared to the simple steatosis cohort 394U/L and 194U/L; (p<0.05). There were no differences between groups in the levels of the surrogate fibrosis markers HA, TIMP 1 and YKL 40.
<b>Conclusions</b>	ccK18 performed the best in analysis at identifying NASH yielding an AUCROC of 0.80, with a cut-off value of 300U/L giving a PPV of 81% and NPV of 85%.
<b>Recommendations</b>	ccK18 (M30) is the only biomarker studied that can identify NASH. Additionally, liver biopsy should be performed in all high risk patients, including those with the following risk factors: advanced age (>45 years), high BMI (>35), type 2 diabetes, elevated ALT (>x2.5ULN) and ccK18 >300U/L to determine the standardised NAS score. A high NAS score will identify patients at high risk of disease progression who will require a therapeutic intervention.

**Feldstein, 2009**

<b>Title of the Publication</b>	<i>Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study</i>
<b>First and last Author</b>	Feldstein A. E.; McCullough A. J.
<b>Journal</b>	Hepatology. 50:1072-8 (2009)
<b>Disease</b>	NASH
<b>Type of Study</b>	Multicentre validation study, observational
<b>Patients</b>	All subjects (n=139), not NASH (n=44), borderline NASH (n=26), definitive NASH (n=69)
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), blood samples taken within 3 months of the liver biopsy. <b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.
<b>Principal Findings</b>	Histology was assessed centrally by study pathologists. ccK18 were markedly increased in patients with NASH (NAS $\geq 5$ ) versus those without NASH (NAS $\leq 2$ ) and borderline diagnosis (NAS 3-4) with medians: 335U/L, 194U/L, 200U/L, respectively; ( $p < 0.001$ ).
<b>Conclusions</b>	On multivariate regression analysis, ccK18 remain an independent predictor of NASH after adjusting for variables associated with ccK18 or NASH on univariate analysis (fibrosis, ALT, AST, age, biopsy length). The AUROC for NASH diagnosis was 0.83. The odds of having fibrosis on liver biopsy increase with increasing plasma ccK18 levels ( $p < 0.001$ ).
<b>Recommendations</b>	Determination of ccK18 (M30) in blood samples predicts histological NASH and severity of disease in a large, diverse population of patients with biopsy-proven NAFLD, supporting the potential usefulness of this test in clinical practice.

**Younossi, 2008**

<b>Title of the Publication</b>	<i>A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH)</i>
<b>First and last Author</b>	Younossi Z.M.; Baranova A.
<b>Journal</b>	Obes Surg. 18:1430-7 (2008)
<b>Disease</b>	NASH
<b>Type of Study</b>	Training and validation study, single centre, observational
<b>Patients</b>	Morbidly obese patients with liver biopsies (n=101) divided into training (n=69) of which had NASH (n=22), simple steatosis (SS) (n=15), and BMI-matched controls whose liver biopsy did not show NAFLD (n=32). The randomly recruited patient validation group (n=32) showed histological NASH (n=21) and SS (n=11).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), M65 (total K18), adiponectin, resistin, insulin, glucose, TNF-alpha, IL-6, and IL-8.</p> <p><b>Histopathology</b> The degree of steatosis was graded as the percentage of tissue occupied by fat vacuoles as: 0: none, 1: ≤5%, 2: &gt;5–33%, 3: &gt;33–66%, 4: &gt;66%. Other histological features evaluated included portal inflammation, lymphoplasmacytic lobular inflammation, polymorphonuclear lobular inflammation, Kupffer cell hypertrophy, apoptotic bodies, focal parenchymal necrosis, glycogen nuclei, hepatocellular ballooning, and Mallory bodies. These histological features were graded as follows: 0=none, 1=mild or few, 2=moderate, and 3=marked or many. Portal fibrosis and interlobular pericellular fibrosis were graded as follows: 0=none, 1=mild, 2=moderate, and 3=marked. Each liver biopsy was assigned to one of four diagnostic categories: no fatty liver disease present (controls), SS, steatosis with nonspecific inflammation (excluded), or NASH. NASH was identified when, in addition to steatosis, the pathologist identified one of the following features: (1) prominent hepatocellular ballooning with associated lobular inflammation, (2) Mallory bodies, or (3) perisinusoidal fibrosis.</p>
<b>Principal Findings</b>	Patients with NASH had ( $p<0.02$ ) higher levels of ccK18 compared to SS and controls (307U/L, 127U/L, 137U/L). The differences in the levels of apoptosis were highly significant ( $p<0.001$ ) when NAFLD patients were subdivided according to their HOMA scores (high HOMA scores (n=15) vs middle and low HOMA scores, (n=22).



**Wieckowska, 2006**

<b>Title of the Publication</b>	<i>In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease</i>
<b>First and last Author</b>	Wieckowska A.; Feldstein A. E.
<b>Journal</b>	Hepatology. 44:27-33 (2006)
<b>Disease</b>	NASH
<b>Type of Study</b>	Pilot study, single centre, observational, prospective
<b>Patients</b>	Clinically suspected NAFLD patients (n=39) of which had normal biopsy (n=10), SS (n=8) and NASH (n=21); and healthy controls (n=35).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18) <b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.
<b>Principal Findings</b>	ccK18 plasma levels were strikingly higher in patients with definitive NASH (NAS $\geq 5$ ) compared with those with not-NASH (simple steatosis) (NAS $\leq 2$ ) or normal biopsies (NAS 0) with medians: 766U/L, 202U/L, 215U/L, respectively; ( $p < 0.001$ ). ccK18 levels showed a weak correlation with body mass index: $r = 0.36$ ; ( $p = 0.024$ ) and stage of fibrosis: $r = 0.55$ ; ( $p < 0.001$ ) but not with age, serum AST/ALT ratio, AST, or ALT. They did not differ significantly according to the presence or absence of history of diabetes, dyslipidemia, or hypertension.
<b>Conclusions</b>	A cut-off value of 395 U/L calculated using the ROC approach showed a specificity of 99.9%, a sensitivity of 85.7%, and positive and negative predictive values of 99.9% and 85.7%, respectively, for the diagnosis of NASH.
<b>Recommendations</b>	Determination of plasma ccK18 (M30) is a strong and independent predictor of NASH and a noninvasive diagnostic means of determining histological disease severity in patients with NAFLD.

# Diabetes

## Miyasato, 2014

<b>Title of the Publication</b>	<i>The cytokeratin-18 fragment level as a biomarker of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus</i>
<b>First and last Author</b>	Miyasato M.; Hanafusa, T.
<b>Journal</b>	Clinica Chimica Acta. 433:184-189 (2014)
<b>Disease</b>	NAFLD and type 2 diabetes mellitus
<b>Type of Study</b>	Cross-sectional study, longitudinal study
<b>Patients</b>	The study was divided into 2 parts. In the first study, which was cross-sectional in design, patients with T2DM (n = 200, mean age: $63.8 \pm 12.0$ y, 58.5% men) and control subjects (n = 58, age: $49.2 \pm 9.0$ y, 41.4% men) were enrolled. The T2DM patients and nondiabetic controls were subdivided into groups with or without NAFLD (DM/NAFLD, DM/nonNAFLD, nonDM/NAFLD and nonDM/nonNAFLD, respectively) based on ultrasonographic findings. In the next study, which was longitudinal, we evaluated the change ( $\Delta$ ) in the CK-18 concentration after three months in type 2 diabetic patients with NAFLD (n = 40, mean age: $62.5 \pm 11.4$ y, 52.5% men) who were randomly selected from among the 137 DM/NAFLD patients.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> Triglycerides, total cholesterol, high-density lipoprotein cholesterol, AST/ALT, total protein, albumin, hemoglobin A1c, M30 (ccK18)
<b>Principal Findings</b>	<p>The median [IQR] concentration of serum CK-18 was 158.4 U/I [107.1–291.9] in the DM/NAFLD group, 96.1 U/I [74.1–142.6] in the DM/nonNAFLD group, 172.4 U/I [130.4–278.8] in the nonDM/NAFLD group and 120.4 U/I [97.5–158.1] in the nonDM/ nonNAFLD group. The serum CK-18 concentrations were significantly higher in the NAFLD group than in the nonNAFLD group among both the diabetic patients (p = 0.0001) and nondiabetic controls (p = 0.004). The serum CK-18 concentration was found to have the greatest correlation with the score for diagnosing NAFLD compared to the other seven metabolic parameters.</p> <p>Among the T2DM patients, the area under the ROC curve (AUROC) analysis indicated that the CK-18 concentration was the best serum predictor of NAFLD (0.75, 95% CI 0.67–0.81 compared with 0.73, 95% CI 0.65–0.79 for ALT, and 0.62, 95% CI 0.54–0.70 for AST). The best cut-off point predicting NAFLD was 180.93 U/I, with a sensitivity of 44% and a specificity of 97%.</p>
<b>Conclusions</b>	<p>1) the CK-18 concentrations were increased in the NAFLD subjects and did not differ between the diabetic and nondiabetic subjects; 2) the CK-18 concentration was identified to be the best independent determinant of NAFLD in the T2DM patients; 3) in the T2DM patients, the CK-18 concentration showed a significant positive correlation with the ultrasonography score; 4) the CK-18 concentration was found to be positively correlated with the AST and ALT concentrations in the T2DM patients; and 5) a reduction in the concentration of CK-18 was found to be significantly associated with a reduction in bodyweight in the T2DM patients with NAFLD.</p> <p>In the general population, CK-18 has been established to be one of the most accurate parameters for diagnosing NAFLD/NASH. Likewise, our study revealed that the CK-18 concentration is a diagnostic biomarker for NAFLD among T2DM patients.</p>

# Children

## Feldstein, 2013

<b>Title of the Publication</b>	<i>Serum Cytokeratin-18 Fragment Levels Are Useful Biomarkers for Nonalcoholic Steatohepatitis in Children</i>
<b>First and last Author</b>	Feldstein A. E.; Nobili V.
<b>Journal</b>	Am J Gastroenterol. 108:1526-31 (2013)
<b>Disease</b>	NAFLD and NASH in Children
<b>Type of Study</b>	Single centre, observational, prospective
<b>Patients</b>	Clinically confirmed NAFLD children (n=201) of which had biopsy NASH (n=140), and not NASH (n=61). Inclusion criteria were persistently elevated serum aminotransferase levels, diffusely hyperechogenic liver on ultrasonography suggestive of fatty liver, and biopsy consistent with the diagnosis of NAFLD.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18)</p> <p><b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.</p>
<b>Principal Findings</b>	ccK18 plasma levels were significantly higher in patients with NASH (NAS $4.4 \pm 1.3$ ) compared with those with not-NASH (NAS $2.0 \pm 0.68$ ) median: 322U/L, 164U/L, respectively; ( $p < 0.001$ ). This association remained significant even after adjusting for multiple confounders including waist circumference percentile, Metabolic Syndrome (MetS), international normalized ratio, and triglyceride level. There was a significant association between ccK18 levels and histology (all $p < 0.001$ ) which was very strong for NAS ( $r = 0.92$ ), strong for steatosis grade ( $r = 0.76$ ) and ballooning ( $r = 0.74$ ), moderate for lobular inflammation ( $r = 0.45$ ), and fibrosis ( $r = 0.41$ ), and weak for portal inflammation ( $r = 0.32$ ). Furthermore, there was a progressive increase in ccK18 levels according to the grade of the histological feature of NAS and the stage of fibrosis.
<b>Conclusions</b>	AUROC for NASH diagnosis for ccK18 was 0.93 with a cut-off value of 233U/L giving a sensitivity of 85% and specificity of 86.9% with a positive predictive value (PPV) of 93.7% and a negative predictive value (NPV) of 71.6%. Using a cut-off value for ccK18 level of 218U/L maximized the sensitivity and negative predictive value (90.7% and 78%, respectively) and, therefore, can be used to rule out the presence of NASH. On the other hand, a cut-off value of 268U/L maximized the specificity and positive predictive value (95.1% and 97%, respectively) and can be used to rule in the diagnosis of NASH. ccK18 levels were significantly higher in patients with fibrosis (F1–3) on biopsy compared with those with no fibrosis (F0) 304U/L versus 210U/L ( $p < 0.001$ ). MetS did not affect the association between ccK18 and NASH. The only MetS component found to be associated with ccK18 level was obesity: median 281U/L for obese compared with 215U/L in non-obese children ( $p = 0.011$ ).
<b>Recommendations</b>	Determination of plasma ccK18 (M30) levels is an accurate biomarker for the presence of NASH within the spectrum of NAFLD in children. As in adults, ccK18 may become one of the most promising single non-invasive test for diagnosing NASH in children.

**Fitzpatrick, 2010**

<b>Title of the Publication</b>	<i>Serum levels of CK18 M30 and leptin are useful predictors of steatohepatitis and fibrosis in paediatric NAFLD</i>
<b>First and last Author</b>	Fitzpatrick E.; Dhavan A.
<b>Journal</b>	J Pediatr Gastroenterol Nutr. 51:500-6 (2010)
<b>Disease</b>	NAFLD
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	Children with biopsy-proven NAFLD (n=45), of which showed no/minimal fibrosis (<F2) (n=22) and significant fibrosis (≥F2) (n=23); and age-matched healthy controls (n=13).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), HA, leptin, high-sensitivity CRP and adiponectin. <b>Histopathology</b> NAFLD histologic activity score (NAS), fibrosis according to Kleiner score.
<b>Principal Findings</b>	ccK18 levels were significantly higher in patients with NAFLD versus controls, median 288U/L versus 172U/L (p<0.001), and in those with NASH (NAS ≥5), median 347U/L versus simple steatosis (NAFLD activity score <3), median 191U/L (p=0.006) and not-NASH (NAS≤2). Significant fibrosis (≥F2) could be differentiated from no/minimal fibrosis (<F2), median 393U/L versus 243U/L (p=0.03).
<b>Conclusions</b>	The AUROC for predicting significant or severe fibrosis (score ≥F2) was 0.66 with a cut-off value of 200U/L with a sensitivity of 83% and specificity of 40%. AUROC for NASH diagnosis for ccK18 was 0.85 with a cut-off value of 207U/L giving a sensitivity of 84% and specificity of 88% with a positive predictive value (PPV) of 90% and a negative predictive value (NPV) of 80%.
<b>Recommendations</b>	Serum biomarkers, especially ccK18 (M30) are useful in stratifying disease severity in paediatric NAFLD.

**Vos, 2008**

<b>Title of the Publication</b>	<i>Cytokeratin 18, a marker of cell death, is increased in children with suspected nonalcoholic fatty liver disease</i>
<b>First and last Author</b>	Vos M. B.; McLain CJ.
<b>Journal</b>	J Pediatr Gastroenterol Nutr. 47:481-5 (2008)
<b>Disease</b>	NAFLD
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	Children (n=62): normal weight (n=28), obese (n=14), suspected NAFLD (n=20)
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M65 (total K18), fasting glucose, fasting insulin, and tumor necrosis factor-alpha.
<b>Principal Findings</b>	<p>The study demonstrates that plasma CK18 levels are elevated in children with a clinical diagnosis of NAFLD relative to normal weight children and overweight children without NAFLD. This suggests that plasma CK18 may have potential as a serological marker of NAFLD in children.</p> <p>CK18 levels were significantly elevated in the children with suspected NAFLD compared with obese controls and normal weight controls with median: 424U/L compared with 243U/L and 214U/L respectively; (p&lt;0.001).</p>
<b>Conclusions</b>	In multiple logistic regression analysis, CK18 (M65) was the best single predictor of suspected NAFLD.

# Health Economics

## Zhang, 2015

<b>Title of the Publication</b>	<i>Cost-utility analysis of nonalcoholic steatohepatitis screening.</i>
<b>First and last Author</b>	Zhang, E.; Tang A.
<b>Journal</b>	Eur Radiol. 25(11):3282-3294 (2015)
<b>Disease</b>	NASH
<b>Type of Study</b>	Cost-utility analysis
<b>Patients</b>	From a health-care system perspective, a decisional Markov model was developed to estimate the expected lifetime costs and quality adjusted life-years (QALYs) associated with screening strategies for NASH. This model was constructed to mirror the natural history of NAFLD disease progression through the histopathological continuum of simple steatosis, NASH, fibrosis stages, and cirrhosis. To address our research aims, we ran the simulation for a general population and for high-risk populations, either with obesity or type 2 diabetes.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), NAFLD fibrosis score, Ultrasound transient elastography, ARFI.</p> <p><b>Cost-utility analysis</b> We performed a cost-utility analysis of annual noninvasive screening strategies using third-party payer perspective in a general population in comparison to screening a high-risk obese or diabetic population. Screening algorithms involved well-studied techniques, including NAFLD fibrosis score, transient elastography (TE), and acoustic radiation force impulse (ARFI) imaging for detecting advanced fibrosis (<math>\geq F3</math>); and plasma cytokeratin (CK)-18 for NASH detection. Liver biopsy and magnetic resonance elastography (MRE) were compared as confirmation methods. Canadian dollar (CAD or C\$) costs were adjusted for inflation and discounted at 5 %. Incremental cost-effectiveness ratio (ICER) of <math>\leq C\\$ 50,000</math> was considered cost-effective.</p>
<b>Principal Findings</b>	Compared with no screening, screening with NAFLD fibrosis score/TE/Cytokeratin-18 algorithm with MRE as confirmation for advanced fibrosis had an ICER of C\$ 26,143 per quality-adjusted life year (QALY) gained. Screening in high-risk obese or diabetic populations was more cost-effective, with an ICER of C\$ 9,051 and C\$ 7,991 per quality-adjusted life-year (QALY) gained, respectively. Liver biopsy confirmation was not found to be cost-effective.
<b>Conclusions</b>	In summary, our cost-utility model suggests that NASH screening is cost effective with noninvasive screening methods for steatohepatitis and advanced fibrosis. Furthermore, screening in high-risk populations of obese or type 2 diabetes patients is more cost-effective than in a general Western population.

# Alcoholic Steatohepatitis

*ASH*

**Lavallard, 2011**

<b>Title of the Publication</b>	<i>Serum markers of hepatocyte death and apoptosis are non-invasive biomarkers of severe fibrosis in patients with alcoholic liver disease</i>
<b>First and last Author</b>	Lavallard V.J.; Gual P.
<b>Journal</b>	PLoS One. 6:e17599 (2011)
<b>Disease</b>	ASH
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	Heavy alcoholics (n=143), of which showed according to liver biopsy mild (n=85) or severe fibrosis (n=58).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18) and M65 (total K18)</p> <p><b>Histopathology</b> Histopathological features were evaluated: grade of steatosis (0: &lt;5%; 1: 5%–30%; 2: 30%–60%; 3: &gt;60%); hepatocellular ballooning (0, none; 1, few balloon cells; 2: many cells/prominent ballooning); mega-mitochondria (0: none to rare; 1: many); Mallory's hyaline (0: none to rare; 1: many) and fibrosis stage (From 0: none, to 4: cirrhosis). Grading of hepatic activity according to Orrego score.</p>
<b>Principal Findings</b>	<p>Serum levels of markers of total hepatocyte death (K18) (M65), apoptosis (ccK18) (M30) and necrosis (total K18-ccK18) were lower in patients with mild fibrosis (&lt;F3) compared to patients with severe fibrosis (≥F3) K18: 669U/L and 1,392U/L (<math>p&lt;0.000001</math>); ccK18: 381U/L and K18-ccK18: 658U/L (<math>p&lt;0.000001</math>).</p> <p>These markers strongly correlated with Mallory-Denk bodies, hepatocyte ballooning, fibrosis and with hepatic TNF<math>\alpha</math> and TGF<math>\beta</math>.</p>
<b>Conclusions</b>	Elevated levels of serum hepatocyte death and apoptotic markers were independent risk factors in predicting severe fibrosis in a model combining alkaline phosphatase, bilirubin, prothrombin index, hyaluronate, hepatocyte death and apoptotic markers. The level of markers of overall hepatocyte death (total K18) and apoptosis (ccK18) had an AUROC that predicted severe fibrosis of 0.84 and 0.76, respectively. Using a cut-off point of 790U/L for total K18 (M65) predicted ≥ F3 with a sensitivity of 84%, a specificity of 71% with a Positive and Negative Predictive Value of 67.1% and 87.1%, respectively.
<b>Recommendations</b>	Death of hepatocytes can be easily evaluated with ccK18 and correlate with severe fibrosis in heavy alcohol drinkers. ccK18 could be useful to rapidly evaluate liver injuries and the efficacy of therapies.



# Chronic Liver Disease

*CLD*

**Denk, 2014**

<b>Title of the Publication</b>	Soluble intracellular adhesion molecule, M30 and M65 as serum markers of disease activity and prognosis in cholestatic liver diseases.
<b>First and last Author</b>	Denk G.; Rust C.
<b>Journal</b>	Hepatol Res. Dec;44(13):1286-1298 (2014)
<b>Disease</b>	CLD
<b>Type of Study</b>	Pilot study, observational
<b>Patients</b>	Sera of 33 patients with autoimmune hepatitis (AIH), of 52 patients with primary biliary cirrhosis (PBC), 37 patients with primary sclerosing cholangitis (PSC) and of 20 healthy volunteers as controls.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> Distinct cell death markers were quantified: M30 (ccK18), M65 (K18), soluble intracellular adhesion molecule [sICAM], macrophage migration inhibitory factor [MIF], soluble Fas [sFas], plasminogen activator inhibitor 1 [PAI-1] and DNase.
<b>Principal Findings</b>	In comparison with healthy controls, the apoptotic markers sFas, sICAM (only in PSC patients), M30 and the cell death marker M65 were substantially elevated in sera of patients with immune-mediated liver diseases, whereas DNase activity was reduced. Interestingly, patients with advanced PSC presented with higher levels of sICAM, M30 and M65 than patients with mild PSC. Regression analysis revealed correlations between serum levels of sICAM, M30 and M65 with the Mayo Risk Score for PSC, and of M65 with the Mayo Risk Score for PBC.
<b>Conclusions</b>	Concentrations of the serum markers of apoptosis sFas and M30 and of the marker of total cell death M65 are elevated in patients with immune-mediated liver diseases, whereas activity of DNase is reduced. In patients with PSC, sICAM, M30 and M65 may serve as indicators for disease activity and prognosis.

## Joka, 2012

<b>Title of the Publication</b>	<i>Prospective Biopsy-Controlled Evaluation of Cell Death Biomarkers for Prediction of Liver Fibrosis and Nonalcoholic Steatohepatitis</i>
<b>First and last Author</b>	Joka E.; Bantel H.
<b>Journal</b>	Hepatology 40:651-655 (2012).
<b>Disease</b>	CLD
<b>Type of Study</b>	Validation study, prospective, single centre, observational
<b>Patients</b>	Chronic liver diseases (CLD) (n=121) and healthy controls (n=18) and "real-life cohort" consisting of blood donors (n=200). Etiologies for CLD viral hepatitis (n=66), Wilson's disease, (n=4), NAFLD/NASH, (n=22), unknown origin: (n=14).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b></p> <p>M30 (ccK18), M65 ELISA ("Classic") and M65 EpiDeath (M65ED) (both total K18) In 107 of 121 patients transient elastography was performed using the FibroScan.</p> <p><b>Histopathology</b></p> <p>The fibrosis stage (F1-F6) was assessed by liver biopsy according to Ishak (n=121). The diagnosis of NAFL (n=10) versus NASH (n=12) was based on the NAFLD activity score (NAS) as the sum of steatosis, lobular inflammation, and hepatocellularballooning scores was assessed according to Kleiner.</p>
<b>Principal Findings</b>	<p>K18 cell death biomarkers were useful to discriminate between different stages of fibrosis, including patients with low F0-F1 (n=79), moderate F2-F4, (n=31) or high F5-F6 (n=11) fibrosis.</p> <p>All three K18 biomarkers discriminated significantly (<math>p&lt;0.01</math>) between patients with different fibrosis stages and healthy control individuals (M30: mean 111.9U/L, M65 (Classic): mean 234.5U/L, M65ED: mean 96.8U/L; n=18) or individuals from the real-life cohort (M30: mean 128.2U/L; M65: mean 288.4U/L; M65ED mean 100.1U/L; n=200). Whereas the M30 assay could significantly (<math>p&lt;0.01</math>) discriminate between low (mean 174.1U/L) or moderate (mean 199.1U/L) and high fibrosis stages (mean 346.5 U/L), M65 ELISA, allowed the significant (<math>p&lt;0.05</math>) differentiation between low (mean 503.2U/L) and moderate (mean 635.2U/L) fibrosis as well as between moderate and high (mean 988.0U/L) fibrosis. Similar results between moderate (mean 549.6U/L) and high (mean 1145.5U/L) fibrosis stages were found with the M65ED ELISA. All 3 cell death markers were able to discriminate (<math>p&lt;0.01</math>) between minimal steatosis: &lt;10% hepatocytes with fat droplets; (M30: mean 189.3U/L; M65: mean 528.9U/L; M65ED: mean 500.6U/L) and healthy individuals. Whereas the M30 marker did not significantly differentiate between minimal (mean 189.3U/L) and higher (mean 205.3U/L) grades of steatosis (&gt;10%), both M65 assays showed significant (<math>p&lt;0.01</math>) differences between minimal (M65: mean 528.9U/L; M65ED: mean 500.6U/L), and higher steatosis (M65: mean 650.3U/L and M65ED: mean 557.7U/L).</p>
<b>Conclusions</b>	All K18 biomarkers (ccK18 and total K18) can detect liver fibrosis and steatosis compared to healthy individuals and a "real-life" blood donor control cohort. Compared to ccK18 a better diagnostic performance was found for the total K18 assays, especially M65ED for both the detection of clinically relevant fibrosis ( $\geq F2$ ) and steatosis (>10%).

**Yagmur, 2007**

<b>Title of the Publication</b>	<i>Elevated apoptosis-associated cytokeratin 18 fragments (CK18Asp386) in serum of patients with chronic liver diseases indicate hepatic and biliary inflammation</i>
<b>First and last Author</b>	Yagmur E.; Tacke F.
<b>Journal</b>	Clin Biochem. 40:651-5 (2007)
<b>Disease</b>	CLD
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	Chronic liver diseases (CLD) (n=76) and healthy controls (n=62). Etiologies for CLD cHBV and/or cHCV (n=18), alcohol or cryptogenic (n=20), biliary or autoimmune (primary biliary cirrhosis, primary sclerosing cholangitis, secondary biliary cirrhosis and autoimmune hepatitis (n=23) and other origin (e.g., malignant tumours, liver cysts, hereditary metabolic disorders (n=15). Additional 15 hepatocellular carcinoma patients were recruited to the 6/76 HCC patients.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18) <b>Histopathology</b> CLD patients with liver biopsy (n=46) assessed for hepatic necroinflammation, cholangitis, cholestasis, steatosis and fibrosis/cirrhosis
<b>Principal Findings</b>	Median ccK18 levels were significantly elevated in CLD patients: 296U/L compared with healthy controls: 153U/L; (p<0.001) and increased with disease severity (Child-Pugh or MELD score). ccK18 levels correlated with aminotransferase activities and parameters indicating cholestasis such as bile acids. Highest serum ccK18 was associated with histologically confirmed severe intrahepatic cholestasis median: 599U/L or biliary duct inflammation median: 648U/L. Furthermore, in contrast to patients with liver cirrhosis, presence of hepatocellular carcinoma was associated with elevated ccK18 in patients without cirrhosis.
<b>Conclusions</b>	ccK18 (M30) serum levels were elevated in CLD and correlate with hepatic inflammation as well as cholangitis and cholestasis.

# **Acute Liver Failure & Drug-Induced Liver Injury**

*ALF & DILI*

# ALF

Acute Liver failure

## Bechmann, 2010

<b>Title of the Publication</b>	<i>Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury</i>
<b>First and last Author</b>	Bechmann L.P.; Canbay A.
<b>Journal</b>	J Hepatol. 53:639-47 (2010)
<b>Disease</b>	ALF
<b>Type of Study</b>	Prospective study, single centre, observational
<b>Patients</b>	Longitudinal monitoring of ALF patients (n=68), of which spontaneously recovered (n=50), were transplanted (n=8) or died (n=10). Control groups: cHCV (n=68), healthy controls (n=20). Acute HBV infection was the most frequent single cause of ALF (n=13) followed by drug toxicity (DILI) (n=21), of which were acetaminophen (APAP) intoxication (n=9) and congestive heart failure (n=9).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), M65 (total K18), GST, subtype alpha
<b>Principal Findings</b>	International normalized ratio (INR) at date of admission alone had a sensitivity (83.3%) and specificity (73.6%) comparable to M65 AUC: 0.80 (p<0.001) for both INR and total K18. Although, both apoptotic (ccK18) and total K18 (including necrosis) cell death were highly elevated, ccK18 had limited prognostic value at date of admission. In contrast, total serum K18 had a prognostic value for spontaneous recovered in ALF at the time of its peak value as well as at admission. According to the ROC curve, total K18 at an optimal cut-off value of 12,316U/L showed a high sensitivity (83.3%) and specificity (72.9%; AUC: 0.82; (p<0.001).
<b>Conclusions</b>	MELD score showed a strong correlation with the clinical outcome (cut-off: 25.5; on admission: AUC: 0.808; (p<0.001) as well as at the time point with maximal total K18 (highest K18 AUC: 0.885 (p<0.001). Substitution of bilirubin with total K18 (M65) while retaining all other factors of the MELD score significantly increased its prognostic value at the date of patient admission as well as at the date of the highest K18 levels (cut-off: 53.5; on admission: AUC: 0.87 (p<0.001); highest K18 AUC: 0.94 (p<0.001)). In the modified MELD for ALF ( <b>M-MELD score</b> ) total bilirubin was replaced by M65: $M-MELD = 10 \times (0.957 \times \ln_{Creatinine} [mg/dl] + 0.378 \times \ln_{M65} [U/\mu l] + 1.12 \times \ln_{INR} + 0.643)$ and displayed improved sensitivity on admission (MELD: 76.5%, M-MELD: 82.4%) and at the maximum M65 (total K18) concentration (MELD: 76.9%, M-MELD: 92.3%) and better specificity on admission (MELD: 76.2%, M-MELD: 82.4%).
<b>Recommendations</b>	Although total K18 may not be useful as a single marker, it may be a valuable test to be included e.g. in the MELD score or in any further scoring system.

**Rutherford, 2007**

<b>Title of the Publication</b>	<i>Serum apoptosis markers in acute liver failure: a pilot study</i>
<b>First and last Author</b>	Rutherford A.; Chung R. T.
<b>Journal</b>	Clin Gastroenterol Hepatol. 5:1477-83 (2007)
<b>Disease</b>	ALF
<b>Type of Study</b>	Pilot study, multicentre, observational
<b>Patients</b>	Longitudinal monitoring of ALF patients (n=67), of which were survivors without liver transplant (n=23), were transplanted (n=22) or died (n=27). Control groups: cHCV (n=68), healthy controls (n=20). ALF was caused by acetaminophen (APAP) intoxication (n=17), viral hepatitis (n=16), Drug-induced liver injury (DILI) (n=12), Wilson's disease (n=6) undetermined (n=16).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), HGF, sFas, TNF-alpha, IL-6.</p> <p><b>Histopathology</b> M30-CytoDEATH™ (PEVIVA) monoclonal antibody for apoptotic hepatocytes ALF (n=12), cHCV (n=6)</p>
<b>Principal Findings</b>	Median ccK18 levels were significantly higher in all ALF patients (1,686U/L) than in healthy controls (105U/L) or chronic HCV patients (288U/L) ( $p<0.0001$ ) and differed among different etiologies of ALF: acetaminophen (1,217U/L), viral hepatitis (2,345U/L), DILI (1,441 U/L), Wilson's disease (5,392 U/L), and undetermined 1,991 U/L), but these differences were not statistically significant, given the small sample sizes. Median ccK18 levels were considerably higher in patients who were transplanted and/or died (2,183U/L) ( $p=0.026$ ).
<b>Conclusions</b>	TNF-alpha, HGF, IL-6, and ccK18 were significantly elevated in ALF. High levels of sFas and HGF might help to confirm a diagnosis of drug-induced liver injury or acetaminophen-related ALF. Higher levels of ccK18 are associated with poor clinical outcomes in ALF.
<b>Recommendations</b>	All patients with NAFLD should undergo periodic assessment for ccK18 and lifestyle modification.

# ALF & DILI

Acute Liver Failure & Drug-Induced Liver Injury

## Rutherford, 2012

<b>Title of the Publication</b>	<i>Development of an accurate index for predicting outcomes of patients with acute liver failure</i>
<b>First and last Author</b>	Rutherford A.; Chung R. T.
<b>Journal</b>	Gastroenterology. 143:1237-43 (2012)
<b>Disease</b>	ALF/ DILI (APAP)
<b>Type of Study</b>	Prospective, clinical, multicentre, training and validation study, observational
<b>Patients</b>	Total ALF cohort (n=500). Longitudinal monitoring of patients with ALF Derivation set (n=250), ALF patients, randomly selected; survivors (n=125) without liver transplant or transplanted or died (n=125). Validation set (n=250) ALF patients, randomly selected: survivors without liver transplant (n=122) or transplanted or died (n=128).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b></p> <p>ALFSG Index: M30 (ccK18) and M65 (total K18), international normalized ratio (INR); serum pH; body mass index; levels of creatinine, bilirubin, phosphorus, arterial ammonia, and lactate; and log(10) M30 and log(10) M65 were measured on 3 of the first 4 days following admission. Logistic regression was used to determine whether the following factors, measured on day 1, were associated with liver transplantation or death: age, etiology; coma grade; INR; serum pH; body mass index; levels of creatinine, bilirubin, phosphorus, arterial ammonia, and lactate; and log(10) M30 and log(10) M65.</p>
<b>Principal Findings</b>	<p><b>ALFSG</b> is composed of <b>entry levels of bilirubin, INR and log10 ccK18 (M30)</b> as continuous variables combined with <b>coma grade</b> as a categorical variable with coma grade I as a the reference group. <b>Phosphorus</b> was used as a categorical variable at a threshold of <math>\geq 3.7</math> versus <math>&lt;3.7</math>. ALFSG Index identified patients who would require liver transplant (LT) or die with 85.6% sensitivity and 64.7% specificity in the training set (n=250). With an AUROC of 0.822 this novel index better identified patients most likely to require LT or die better compared to King's College Criteria (KCC) AUROC: 0.65 or MELD AUROC: 0.70 (<math>p=0.002</math> and <math>p&lt;0.001</math>), respectively. In the validation set (n=250) ALFSG index had an AUROC: 0.839 compared to KCC: 0.684 and MELD: 0.717 in predicting liver transplant or death for patients with KCC (<math>p=0.003</math>) or MELD (<math>p=0.0005</math>).</p>
<b>Conclusions</b>	ALFSG Index in acetaminophen (APAP) ALF (DILI) (80.6% sensitivity, 78.1% specificity), non-APAP ALF (84.7% sensitivity, 59.2% specificity), and in the group overall (83.4% sensitivity, 69% specificity) was very similar, and superior to KCC in APAP ALF (DILI) (73.8% sensitivity, 65.5% specificity), non-APAP ALF (57.8% sensitivity, 81.7% specificity), and overall (62.8% sensitivity, 74.1% specificity).
<b>Recommendations</b>	The ALFSG-index would require only a simple 1-time ccK18 (M30) measurement early in admission of a patient with ALF. Given its simplicity and reproducibility, this assay could be performed at the same time as other standard serologic tests in critically ill patients.



# DILI

## Drug-Induced Liver Injury

### Antoine, 2014

<b>Title of the Publication</b>	<i>Stratification of paracetamol overdose patients using new toxicity biomarkers: current candidates and future challenges</i>
<b>First and last Author</b>	Antoine D.; Dear J.
<b>Journal</b>	Expert Rev. Clin. Pharmacol. 7(2): 181-189
<b>Disease</b>	DILI (APAP)
<b>Type of Study</b>	Review
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> K18 (M65), ccK18 (M30), miRNA-122, HMGB-1

In this study, the writers aimed at providing a perspective on the application of these mechanistic biomarkers to the deeper understanding of paracetamol hepatotoxicity in clinical and preclinical studies.

#### Principal Findings

The study demonstrated, through the quantification of different molecular forms of K18, that both necrosis (85%) and apoptosis (15%) of hepatocytes are important features of paracetamol-induced liver injury in humans and are quantitatively consistent with preclinical studies. A recent report has independently replicated our observation that the character and time course of cell death can be quantified via K18 isoforms present in human blood following paracetamol overdose. Necrosis-related K18 significantly correlated with ALT activity ( $R^2 = 0.60$ ,  $p < 0.0001$ ) and patients who either died/required a liver transplant or reached KCC had higher circulating levels of necrosis K18 and a lower percentage of total cell death attributed to apoptosis. These findings have been supported by other research groups who have demonstrated that K18, alongside currently used biomarkers, adds significant prognostic value when incorporated into mathematical models following acute liver failure of mixed etiologies.

#### Conclusions

K18 provides unique information regarding the balance between necrosis and apoptosis, which correlates with patient outcome and susceptibility to paracetamol-induced liver injury.

MiR-122, HMGB1 and K18 have been shown to represent sensitive biomarkers of paracetamol-induced hepatotoxicity (with respect to the onset of injury) and prognosis in clinical studies. These biomarkers can potentially be used to rule in or out the presence of liver injury more confidently than currently used indicators.

## Thulin, 2013

<b>Title of the Publication</b>	<i>Keratin-18 and microRNA-122 complement alanine aminotransferase as novel safety biomarkers for drug-induced liver injury in two human cohorts</i>
<b>First and last Author</b>	Thulin P.; Schuppe-Koistinen I.
<b>Journal</b>	Liver Int. 2013 [Epub ahead of print]
<b>Disease</b>	DILI
<b>Type of Study</b>	Pilot study, single centre
<b>Patients</b>	<p><i>APAP study:</i> Healthy volunteers (n=30) received 4g of acetaminophen per day for 7 days divided in groups who showed ALT elevations larger than 2 times their own baseline value (n=15) and where ALT levels never increased more than 1.5 times from baseline levels (n=15).</p> <p><i>HIV/TB study:</i> Patients infected with HIV and/or TB (n=76) were treated with HAART and/or anti-TB heparin as potentially hepatotoxic drugs and were followed up for 12 weeks after the commencement of treatment. Patients with ALT values exceeding three times their own baseline levels at any time point during the study period (n=38) were available for analysis. These patients were matched against patients from the same treatment groups that did not show strong ALT elevations (n=38).</p> <p><i>Exercise:</i> To assess whether the novel biomarkers were affected by damage to a non-hepatic tissue, plasma from healthy individuals (n=12) was collected before and after heavy exercise. Compared to ALT, AST and CK (as a control marker) levels, which were highly elevated following the muscular injury, neither M65 nor M30 levels changed.</p>
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b></p> <p>M30 (ccK18), M65 EpiDeath (total K18), microRNA-122 (miR-122), glutamate dehydrogenase (GLDH) and alpha-fetoprotein (AFP).</p>
<b>Principal Findings</b>	In the acetaminophen study, total K18 marker was more sensitive in a temporal sense than ALT as it increased significantly already at day 7 compared to ALT, which did not reach significance until day 8. The maximal fold increase levels of Total K18 could be detected earlier and was also larger than for ALT. Total K18 levels dropped towards baseline when therapeutic doses of acetaminophen were no longer administered whereas ALT-levels remained elevated in the study.
<b>Conclusions</b>	Total K18 as measured by M65 EpiDeath and miR-122 are biomarkers competing with ALT in terms of sensitivity, as defined as an earlier indicator of liver injury, and additional mechanistic information is gained by ccK18 measurement.
<b>Recommendations</b>	The occurrence of asymptomatic elevations of liver function tests during clinical trials in drug development occur frequently and may not be drug-related but reflect other factors, such as exercise and diet. Total K18 as a complement to ALT would help to interpret early signals relating to liver safety concerns arising from undesirable elevations in ALT in clinical studies, making it a valuable tool to exclude DILI when ALT is not released from the liver or as a relevant DILI signal when other reasons for ALT elevation can be excluded.

## Harrill, 2012

<b>Title of the Publication</b>	<i>The Effects of Heparins on the Liver: Application of Mechanistic Serum Biomarkers in a Randomized Study in Healthy Volunteers</i>
<b>First and last Author</b>	Harrill AH.; Watkins PB.
<b>Journal</b>	Clin Pharmacol Ther. 92:214 (2012)
<b>Disease</b>	Drug-Induced Liver Injury
<b>Type of Study</b>	Pilot study, randomized, single centre
<b>Patients</b>	Healthy volunteers (n=48) received unfractionated heparin, and the following heparin therapeutics: dalteparin, enoxaparin, or adomiparin twice daily for 4 consecutive days plus the morning of the fifth day (a total of nine doses) as a supporting safety study for the therapeutic use of heparins in acute coronary syndrome.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), M65 (total K18), serum sorbitol dehydrogenase (SDH), glutamate dehydrogenase (GLDH), miR-122, high-mobility group box-1 protein (including the acetylated form) and DNA.
<b>Principal Findings</b>	Treatment-emergent transient elevations in serum ALT and AST values above the upper limits of normal were observed in 94% and 90% of the subjects, respectively. During treatments, the values generally began to rise by day 3, reaching their peaks on day 7, 2 days after the end of the heparin course. Episodes of grade 3 increases in ALT (exceeding five times the upper limits of normal) were reported in 11 subjects (23%) across all the treatment groups. No grade 4 elevations were noted; however, in one subject treated with dalteparin, the peak ALT value was 13 times the upper limit of normal. The duration of the ALT elevation outside the reference range varied from ~2 days to 33 days. None of the subjects experienced clinical signs of liver injury.
<b>Conclusions</b>	The time course of aminotransferase elevations was consistent across the various heparin treatment arms, beginning at 3 days after the initiation of dosing in all groups, attaining peak values 24–48 h after cessation of dosing and generally returning to normal values within 1 week of discontinuing treatment. The elevations in serum ALT and AST were asymptomatic and not associated with increases in bilirubin that would suggest impairment of liver function.
<b>Recommendations</b>	The magnitude of the elevations observed in some subjects would prompt concern regarding liver safety in most clinical settings and might be sufficient to initiate an extensive liver evaluation. In a phase I clinical trial of a new drug, such elevations might prompt discontinuation of development. Given that heparins have not been reported to cause clinically important liver injury, it has been suggested that the ALT and AST elevations in this setting may not reflect liver injury. In contrast, ccK18 (hepatic apoptosis) represented the only serum biomarker that was not elevated under circumstances when other (liver-)safety biomarkers tested were substantially elevated (AST, ALT). These (apoptosis) biomarker assays will be useful in clinical trials, where the interpretation of data obtained in terms of liver injury may be complex and ccK18 be useful to identify only relevant liver safety concerns.

# Acute-on-chronic Liver Failure

**Adebayo, 2015**

<b>Title of the Publication</b>	<i>Mechanism of cell death in acute-on-chronic liver failure: a clinico-pathologic-biomarker study.</i>
<b>First and last Author</b>	Adebayo D.; Jalan R.
<b>Journal</b>	Liver Int. 2015 Apr 16. (Epub ahead of print)
<b>Disease</b>	Acute-on-chronic Liver failure,
<b>Type of Study</b>	k
<b>Patients</b>	Twenty-seven patients with acute decompensation of liver disease were divided into two groups: no-ACLF (n = 11) or ACLF (n=16). Healthy controls (n = 8) and acute liver failure (ALF) patients (n = 10) were also enrolled.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), M65 (K18), TNF-<math>\alpha</math>, IL-18 levels, Caspase 3 and circulating gDNA.</p> <p><b>Histopathology</b> Scoring of steatosis, alcoholic steatohepatitis and ductal cholestasis according to Mookerjee et al</p>
<b>Principal Findings</b>	<p>The mean M30 level was <math>128.3 \pm 20.9</math> U/L in healthy controls; <math>1575 \pm 192.5</math> in ALF patients, <math>440 \pm 42</math> U/L in no ACLF patients and <math>1761 \pm 207.6</math> U/L in ACLF patients. Compared with healthy individuals, the M30 value was significantly elevated in ACLF patients (<math>P = 0.0001</math>). M30 levels were significantly greater in the ACLF patients compared with the no ACLF patients (<math>P = 0.0001</math>). There was no significant difference in M30 levels between the ACLF and ALF patients.</p> <p>The mean M65 level was <math>104 \pm 2357</math> U/L in healthy controls, <math>36\,498 \pm 14\,843</math> U/L in ALF patients, <math>5607 \pm 1572</math> U/L in no ACLF patients and <math>9811 \pm 1914</math> U/L in ACLF patients. M65 level was higher in ALF compared with ACLF patients (<math>P = 0.002</math>). However, there was no significant difference between the no ACLF and ACLF patients.</p> <p>The apoptotic index (M30/M65 ratio) was 1.39 (range: 0.96–2.90), in the healthy controls. In patients with ALF, the apoptotic index was 0.13 (range: 0.09–0.30) compared to those with ACLF 0.28 (range: 0.08–0.71). The apoptotic index was significantly higher in the ACLF patients compared with the ALF patients (<math>P = 0.03</math>).</p> <p>Significant correlation was observed between the M30 levels and the number of organ failures observed in patients with acute decompensation of cirrhosis (<math>r^2 = 0.78</math>; <math>P &lt; 0.0001</math>). The area under the curve for prediction of mortality was 0.88. Using an M30 cut-off value of 1312 had a sensitivity of 83% and a specificity of 72% in the prediction of mortality.</p>
<b>Conclusions</b>	<p>The values of M30 increased in a linear manner with the number of organ failures in the ACLF patients. The results of this study suggest that hepatocyte apoptosis is the predominant mode of cell death in ACLF, which can be identified in the peripheral blood.</p> <p>Compared with healthy individuals and no ACLF patients, the M30 value was significantly elevated in ACLF patients.</p>

**Zheng, 2014**

<b>Title of the Publication</b>	<i>Prognostic value of M30/M65 for outcome of hepatitis B virus-related acute-on-chronic liver failure</i>
<b>First and last Author</b>	Zheng S.J.; Duan Z.P.
<b>Journal</b>	World J. Gastroenterol. 7;20(9):2403-2411 (2014)
<b>Disease</b>	Acute-on-chronic Liver failure, HBV
<b>Type of Study</b>	Single centre, observational
<b>Patients</b>	M30 and M65 were identified by ELISA in 169 subjects including healthy controls ( $n = 33$ ), chronic hepatitis B (CHB) patients ( $n = 55$ ) and ACLF patients ( $n = 81$ ).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), M65 (K18), ALT/AST, total bilirubin, albumin.</p> <p><b>Histopathology</b> Yes</p>
<b>Principal Findings</b>	<p>ELISA was used to measure M65 and M30 in a chronic hepatitis B (CHB) infection cohort which included healthy controls, CHB and acute-on-chronic liver failure (ACLF) patients. Elevated M65 and M30 differentiated CHB or ACLF patients from healthy controls, and M65 and M30 levels significantly increased gradually as liver disease progressed (for M65: <math>P &lt; 0.001</math> for all between different groups; for M30: control vs CHB, <math>P = 0.072</math>; others: <math>P &lt; 0.001</math> for all). In contrast, the M30/M65 ratio was significantly higher in controls compared with CHB or ACLF patients.</p> <p>The ROC analysis showed that both serum M65 and M30 levels had significant diagnostic value for identifying ACLF patients from CHB patients [M65: cutoff: 1260.30 U/L, AUC = 0.87 (95%CI: 0.78-0.95); sensitivity, 83.6%; specificity, 83.9%; M30: cutoff: 333.35 U/L, AUC= 0.80 (95%CI: 0.68 0.92); sensitivity, 96.7%; specificity, 71.0%)</p> <p>M30/M65 was significantly increased in ACLF patients with spontaneous recovery (<math>P = 0.032</math>), and the AUC of this ratio at the 3-mo survival period was 0.661 (sensitivity: 52.9%) with a high specificity (92.6%) compared with the Model for End-Stage Liver Disease and Child-Pugh scores.</p>
<b>Conclusions</b>	M65 and M30 levels differentiated CHB or ACLF patients from healthy controls, and the levels significantly increased gradually as liver disease progressed. The M30/M65 ratio may be a potential prognostic marker for spontaneous recovery in patients with HBV-related ACLF.

# Hepatitis Virus

# HBV

## Hepatitis B Virus

### Sumer, 2013

<b>Title of the Publication</b>	<i>The Clinical Significance of Serum Apoptotic Cytokeratin 18 Neoepitope M30 (CK-18 M30) and Matrix Metalloproteinase 2 (MMP-2) Levels in Chronic Hepatitis B Patients with Cirrhosis</i>
<b>First and last Author</b>	Sumer S.; Ural O.
<b>Journal</b>	Hepat Mon. 26;13(6):e10106 (2013)
<b>Disease</b>	HBV
<b>Type of Study</b>	Single centre, observational
<b>Patients</b>	cHBV patients (HBeAg-negative) (n=189) and healthy controls (n=51).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18) and Matrix Metalloproteinase 2 (MMP-2) <b>Histopathology</b> cHBV histologic activity according to Knodell and Ishak
<b>Principal Findings</b>	Mean ccK18 serum levels were significantly lower in healthy controls: 168U/L than in cHBV patients: 308U/L ( $p<0.001$ ) and increased with fibrosis stages: 2 (n=77): 259U/L, 3 (n=37): 396U/L, 4 (n=230): 462U/L, 5 (n= 12): 689U/L; ( $p<0.001$ ) whereas in cHBV patients with stage 1 (n=40): no difference could be observed: 168U/L.
<b>Conclusions</b>	ccK18 (M30) levels are higher in cHBV patients compared to healthy controls and are associated with significant hepatic fibrosis, especially cirrhosis. ccK18 is a more sensitive marker than MMP-2 for predicting the histological stage of fibrosis.



## Papatheodoridis, 2008

<b>Title of the Publication</b>	<i>Serum apoptotic caspase activity as a marker of severity in HBeAg-negative chronic hepatitis B virus infection</i>
<b>First and last Author</b>	Papatheodoridis G. V.; Archimandritis A. J.
<b>Journal</b>	Gut. 57:500-6 (2008)
<b>Disease</b>	HBV
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	Treatment-naïve, consecutive HBV patients (total n=115) with inactive carriers (n=52) and chronic hepatitis B patients (n=62) and healthy controls (n=30)
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18) and M65 (total K18)
<b>Principal Findings</b>	ccK18 levels were significantly lower in healthy controls mean: 154U/L than in inactive carriers mean: 172U/L; (p=0.003) and in chronic hepatitis B patients mean: 474U/L; (p<0.001). The AUROC showed excellent diagnostic accuracy 0.87 for differentiating inactive carriers from chronic hepatitis B patients.
<b>Conclusions</b>	A ccK18 (M30) cut-off level of 240U/L gave a sensitivity of 60%, and a specificity and positive predictive value of 100% for chronic hepatitis B diagnosis. ccK18 levels were also lower in inactive carriers than in chronic hepatitis B patients with transiently normal ALT mean: 27U/L; (p=0.001), offering good accuracy for such a differentiation AUROC 0.78. In chronic hepatitis B patients, serum ccK18 correlated positively with ALT/aspartate aminotransferase (AST), viraemia, grading score and their immunohistochemical hepatic expression, and negatively with platelet counts, but not with fibrosis or steatosis severity.

# HCV

Hepatitis C Virus

## Reis, 2014

<b>Title of the Publication</b>	<i>(Cleaved) CK18 serum and tissue expression levels differentiate acute HCV reinfection from acute rejection in liver allografts</i>
<b>First and last Author</b>	Reis H.; Baba H. A.
<b>Journal</b>	Liver Int. 35:905-913 (2014)
<b>Disease</b>	NAFLD and NASH
<b>Type of Study</b>	Single centre, observational
<b>Patients</b>	The studied cohort consisted of 22 patients who received Orthotopic liver transplantation (OLT) for HCV-related cirrhosis, i.e. 11 with chronic HCV reinfection and 11 with acute HCV reinfection, and of 16 with OLT for diverse causes (excluding HCV), i.e. 16 acute rejection HCV negative patients. As a control group, patients diagnosed with NAFL (19) and NASH (19) were included. All patients received liver biopsy either for establishment of the diagnosis (NAFL, NASH) or post-OLT because of deterioration of graft function or liver damage tests (HCV reinfection, rejection) and blood.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> Serum M30 (ccK18) (M30S), serum M65 (K18) (M65S), ALT/AST and M30-immunohistochemistry (M30H)</p> <p><b>Histopathology</b> Acute rejection was scored adherent to Banff-criteria. For differential diagnosis of NAFL vs. NASH a NAFLD activity score (NAS) was calculated.</p>
<b>Principal Findings</b>	<p>In the total cohort, M30S and M65S as well as M30H were significantly differing (all <math>P &lt; 0.0001</math>; Table 1) with highest values in acute HCV reinfection, lowest in NAFL and intermediate in NASH, chronic HCV reinfection and acute rejection. In contrast, M30S/M65S-ratio values were highest in chronic HCV reinfection, followed by acute rejection, NASH and NAFL and lowest in acute HCV reinfection (<math>P = 0.019</math>).</p> <p>Median levels of M30S, M65S and M30H were each time highest in acute HCV reinfection and similarly lower in chronic HCV reinfection and acute rejection. In whole cohort analyses including chronic and acute HCV reinfection and acute rejection, M65S, M30H and the M30S/M65S-ratio but not M30S were able to discriminate the groups.</p>
<b>Conclusions</b>	<p>In the non-invasive differential diagnosis of acute HCV reinfection vs. acute rejection, we found both serum levels of M30 and M65 to be of potential diagnostic value. In addition, tissue expression of cleaved K18 detected by M30H proved to be a possible useful diagnostic tool in the histopathological evaluation of liver biopsies regarding acute (and chronic) HCV reinfection and acute rejection. M65S, however, exhibited the most favourable overall diagnostic characteristics compared to M30-based methods in differential diagnostic implications this group.</p> <p>Median levels of M30S, M65S and M30H were each time highest in acute HCV reinfection and similarly lower in chronic HCV reinfection and acute rejection. In whole cohort analyses including chronic and acute HCV reinfection and acute rejection, M65S, M30H and the M30S/M65S-ratio but not M30S were able to discriminate the groups.</p>

**Jazwinski, 2012**

<b>Title of the Publication</b>	<i>Elevated serum CK18 levels in chronic hepatitis C patients are associated with advanced fibrosis but not steatosis</i>
<b>First and last Author</b>	Jazwinski A.B.; Patel K.
<b>Journal</b>	J Viral Hepat. 19:278-82 (2012)
<b>Disease</b>	HCV
<b>Type of Study</b>	Validation study, single centre, observational, retrospective
<b>Patients</b>	Total cHCV patients (n=267) treatment-naïve, and blood donors with normal ALT levels (n=100) as controls. cHCV patients with fibrosis stage 0 (n=35 or 13%); 1 (n=125 or 47%), 2 (n=42 or 16%), 3 (n=35 or 13%), 4 (n=30 or 11%). Steatosis: 0 (n=130 or 49%), 1 (n=92 or 35%), 2 (n=31 or 12%), 3 (n=14 or 5%). HAI: Inflammation 0–5 (n=79 or 31%), 6–10 (n=110 or 44%), 11–16 (n=62 or 25%).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18) <b>Histopathology</b> METAVIR fibrosis stage (F0–F4), HAI inflammation score, (mild/0=0–5, moderate/1=6–10, severe/2=11–16) and steatosis grade according to the percentage of hepatocytes containing fat droplets (0<3%, 1=3–30%, 2=31–59% and 3=>59%).
<b>Principal Findings</b>	Median ccK18 levels were higher in cHCV patients compared to controls 411 versus 196U/L; (p<0.0001). Fibrosis stage was associated with increasing serum ccK18 levels (p=0.015) and ccK18 levels were higher for F2–F4 versus F0–F1 500 versus 344U/L; (p=0.001). There was no association between ccK18 and increasing steatosis grade 1, 2 or 3 460.7 versus 416.8 versus 508.3U/L; (p=0.35) and presence or absence of steatosis 445.3 versus 365.8U/L; (p=0.075).
<b>Conclusions</b>	Fibrosis stage was independently associated with serum M30 in a multivariable linear regression model (p=0.03). ccK18 levels were higher in cHCV compared to healthy controls and associated with hepatic fibrosis. In this cHCV cohort with low-level grades of steatosis and high levels of inflammation no significant difference in ccK18 M30 levels between cHCV patients with and without steatosis could be observed.

**Sgier, 2010**

<b>Title of the Publication</b>	<i>Effect of antiviral therapy on circulating cytokeratin-18 fragments in patients with chronic hepatitis C</i>
<b>First and last Author</b>	Sgier C.; Dufour J.-F.
<b>Journal</b>	J Viral Hepat. 17:845-50 (2010)
<b>Disease</b>	HCV
<b>Type of Study</b>	Pilot study, multicentre, observational
<b>Patients</b>	Total cHCV patients (n=315) under anti-viral treatment. Patients with sustained response (n=183), non-responders (n=64) and patients who relapsed (n=68).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18)
<b>Principal Findings</b>	Mean levels of circulating ccK18 before therapy were 174U/L for responders, 188U/L for non-responders and 269U/L for patients who relapsed. The values were significantly higher in the relapse group ( $p<0.006$ ). A sustained response was associated with a significant improvement of the plasma levels: 94U/L; ( $p<0.000001$ ), whereas there was no improvement in the non-responder group: 183U/L and in the relapser group: 158U/L.
<b>Conclusions</b>	Successful antiviral therapy resulted in a significant decrease in circulating levels of ccK18 (M30) arguing for a reduction in hepatocellular apoptosis after clearance of the HCV. Baseline cck18 levels were higher in relapsers.

## Valva, 2010

<b>Title of the Publication</b>	<i>Apoptosis Markers Related to Pathogenesis of Pediatric Chronic Hepatitis C Virus Infection: M30 Mirrors the Severity of Steatosis</i>
<b>First and last Author</b>	Valva P.; Preciado M. V.
<b>Journal</b>	Journal of Medical Virology. 82:949-957 (2010)
<b>Disease</b>	Pediatric Chronic HCV
<b>Type of Study</b>	Retrospective study
<b>Patients</b>	Twenty-three paediatric patients with chronic HCV infection who attended the Liver Unit at the Ricardo Gutierrez Children's Hospital, University of Buenos Aires. Median age: 6 years (1–17 years); 65.2% female.
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), AST/ALT,</p> <p><b>Histopathology</b> Knodell scoring system (HAI) to measure necroinflammation and METAVIR to measure fibrosis. Presence of lymphoid follicles as well as of bile duct lesion and grade of steatosis were also evaluated.</p>
<b>Principal Findings</b>	<p>HCV patients with different grades of disease activity revealed significantly elevated serum M30 levels [median: 122.15 UL-1 (86.68–794.58)]. In contrast, healthy controls disclosed low M30 levels in serum [median: 81.44 UL-1 (41.17–129.30)], (<math>P &lt; 0.0001</math>). Cut-off value corresponding to the highest accuracy value (minimal false negative and false positive results) was 94.23 UL-1 (95% sensitivity, 93% specificity, positive predictive value: 95, negative predictive value: 93). M30 serum levels were elevated in patients with severe steatosis [median: 162.35 UL-1 (120.65–794.58)] compared with patient with moderate steatosis [median: 123.65 UL-1 (98.96–243.31)], minimal or no steatosis [median: 113.70 UL-1 (86.68–279.47)] (<math>P = 0.004</math>).</p> <p>Aminotransferase values showed no statistically significant differences either among HAI groups (AST <math>P = 0.22</math> and ALT <math>P = 0.22</math>) as well as among fibrosis stages (AST <math>P = 0.60</math> and ALT <math>P = 0.25</math>).</p> <p>It is well known that approximately 25–30% of HCV patients have normal serum aminotransferase levels, even though most of these patients show histological evidence of chronic liver damage. Consistent with literature, 30% of the studied patients had normal aminotransferase levels at time of biopsy.</p>
<b>Conclusions</b>	The present study demonstrates that M30 was markedly increased in serum samples of HCV-infected paediatric patients and confirms its low presence under normal conditions. These findings point out the usefulness of serum M30 as a marker of liver damage in paediatric patients

## Kronenberger, 2005

<b>Title of the Publication</b>	<i>Apoptotic cytokeratin 18 neoepitopes in serum of patients with chronic hepatitis C</i>
<b>First and last Author</b>	Kronenberger B.; Zeuzem S.
<b>Journal</b>	J Viral Hepat. 12:307-14 (2005)
<b>Disease</b>	HCV
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	cHCV patients with elevated ALT levels (n=72), and persistently normal ALT levels (n=27) and healthy controls (n=19).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters):</b> M30 (ccK18) <b>Histopathology:</b> cHCV histologic activity according to Knodell and Ishak
<b>Principal Findings</b>	ccK18 serum levels were strongly correlated with ALT $r=0.659$ ; ( $p<0.0001$ ) and the histology activity index $r=0.374$ ; ( $p<0.001$ ). Patients with chronic hepatitis C and persistently normal ALT levels had higher apoptotic ccK18 levels than healthy controls ( $p=0.03$ ) but lower levels than patients with chronic hepatitis C and elevated ALT levels ( $p<0.001$ ). Highest serum ccK18 levels were observed in patients with cirrhosis ( $p=0.002$ )
<b>Conclusions</b>	ccK18 (M30) serum levels in patients with chronic hepatitis C are associated with ALT level and histological liver damage and are elevated both in patients with chronic hepatitis C with elevated ALT levels as well as in patients with normal ALT levels indicating that also patients with chronic hepatitis C and normal ALT have an increased hepatocyte loss by apoptosis.

**Bantel, 2004**

<b>Title of the Publication</b>	<i>Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury</i>
<b>First and last Author</b>	Bantel H.; Manns M. P.
<b>Journal</b>	Hepatology. 40:11078-87 (2004)
<b>Disease</b>	HCV
<b>Type of Study</b>	Pilot study, single centre, observational, prospective
<b>Patients</b>	cHCV patients with elevated ALT levels (n=59), with elevated ALT levels (n=43), and persistently normal ALT levels (n=16 plus 9) and healthy controls (n=7).
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18) <b>Histopathology</b> cHCV histologic activity according to Batts and Ludwig.
<b>Principal Findings</b>	cHCV patients with different grades of disease activity revealed considerably elevated ccK18 levels mean: 498U/L. As shown by regression analysis the serum concentrations of ccK18 correlated with AST ( $r=0.76$ ) and ALT ( $r=0.73$ ). The mean serum levels of ccK18 in healthy controls, patients with normal or with elevated aminotransferase levels were 173U/L, 235U/L, and 596U/L, respectively; ( $p<0.00005$ ).
<b>Conclusions</b>	In HCV patients with normal Liver Function Tests (LFT) (n=25) detection of elevated ccK18 serum levels was significantly ( $p<0.05$ ) associated with higher stages of fibrosis. Whereas patients negative for ccK18 only showed fibrosis stage 0 (53.3%) and 1 (46.6%), patients with elevated ccK18 were of stage 0 (20%) and 1 (50%), but also stage 2 (20%) and 4 (10%).
<b>Recommendations</b>	At a cut-off value of ccK18 (M30) 221.5U/L the AUROC value was 0.765 for detecting fibrosis stages of 2 or higher. The measurement of apoptotic ccK18 in serum might represent a sensitive non-invasive tool for detecting HCV-infected patients with normal aminotransferase values but histologically active hepatitis and progressive fibrosis.

# Hepatocellular Carcinoma

*HCC*



**Morris, 2014**

<b>Title of the Publication</b>	<i>Circulating biomarkers in hepatocellular carcinoma</i>
<b>First and last Author</b>	Morris K. L; Dive C.
<b>Journal</b>	Cancer Chemother Pharmacol. 74:323-332 (2014)
<b>Disease</b>	HCC
<b>Type of Study</b>	Multi-centre, observational,
<b>Patients</b>	Fifty-four patients with a median age of 67 years (range 29–80), were recruited between June 2009 and October 2011.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), M65 (K18), Circulating tumour cells.
<b>Principal Findings</b>	The levels of circulating K18 detected by M30 and M65 ELISAs were determined in serum from 48 patients with Hepatocellular Carcinoma. Significant associations ( $p \leq 0.05$ ) between the concentration in serum of caspase-cleaved and full-length K18 concentration (M65) and four of the clinical parameters were observed, specifically tumour node metastases (TNM; stages 1 vs. 2, 1 vs. 3, 1 vs. 4 and 2 vs. 3), BCLC stage (A, B, C vs. D), serum AFP levels (normal vs. elevated) and PS (0 vs. 3, 1 vs. 3). Similarly, there were significant associations observed between the concentration of circulating caspase cleaved K18 (by M30 ELISA) and the same four clinical parameters, except that here the association with TNM stage was only able to significantly discriminate stages 1 versus 3 and 1 versus 4.
<b>Conclusions</b>	Here we report statistically significant associations of HCC clinical parameters with both serum M30 and M65 data. Figure 3a, b and Table 3 suggest that serum M65 data are the most significant in terms of clinical parameter associations. The significant association of circulating K18 with OS in HCC further exemplifies the utility of circulating biomarkers in cancer.

**Waidmann, 2013**

<b>Title of the Publication</b>	<i>Diagnostic and prognostic significance of cell death and macrophage activation markers in patients with hepatocellular carcinoma</i>
<b>First and last Author</b>	Waidmann O.; Piiper A.
<b>Journal</b>	J Hepatol. 59:769-79 (2013)
<b>Disease</b>	HCC
<b>Type of Study</b>	Test and validation study, multi-centre, observational, prospective
<b>Patients</b>	Cohort 1 (n=142) and Cohort 2 (n=125) HCC test cohort (n=115) and validation cohort (n=52) and cirrhotic liver patients without HCC served as control cohort (n=85).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b></p> <p>M30 (ccK18), M65 (total K18) macrophage activation marker: soluble CD163 (sCD163), Alpha-fetoprotein (AFP).</p>
<b>Principal Findings</b>	In the test cohort the area under the curve (AUC) for M30 and M65 were 0.786; (p<0.001) and 0.794; (p<0.001), respectively. In the validation cohort M30 and M65 could also discriminate HCC patients from subjects with cirrhosis without HCC. The AUC for M30 and M65 were 0.826; (p<0.001) and 0.813; (p<0.001), respectively. Whereas cell death parameters showed high sensitivity and specificity for HCC diagnosis, sCD163 was not suitable for diagnostic use with an AUC of 0.526; (p=0.568) and 0.560; (p=0.187) in the two cohorts, respectively. For AFP the AUC for diagnosis of HCC was 0.788; (p<0.001) in the test cohort and 0.842; (p<0.001) in the validation cohort.
<b>Conclusions</b>	59 of the 72 HCC patients (81.9%), in whom HCC nodules were not visible in ultrasound examinations, had elevated M65 serum levels which were diagnostic for HCC. In 51 of the 69 BCLC A patients (73.9%) HCC suspicious nodules were found. In the remaining 18 patients in whom ultrasound was negative eleven HCC patients could be identified by assessment of serum M65.
<b>Recommendations</b>	<p>Total K18 serum levels (M65) could supplement ultrasound screening for HCC diagnosis in all and early stages. K18 levels could differentiate HCC patients from age-, gender- and MELD-matched cirrhotic patients independently from AFP, and K18 could enhance the rate of HCC detection when it was combined with ultrasound screening examinations. Using an algorithm and cut-off values with very high specificity for HCC diagnosis for AFP and K18, more than 70% of HCC patients could be identified with blood based biomarkers. Even in patients with early HCC (within Milan criteria), K18 and AFP could identify more than 50% of HCC patients, proposing K18 as an additional parameter for early identification of HCC patients.</p> <p>In contrast, ccK18 (M30) provided no significant benefit to the K18 (M65) levels. K18 serum levels could also improve HCC detection in combination with abdominal ultrasound imaging in more than 90% of HCC patients and in more than 80% of BCLC stage A patients. Therefore, its diagnostic value could be for the identification of early HCC in cirrhotic patients undergoing HCC surveillance programs.</p>

# Liver Transplantation

**Brenner, 2012**

<b>Title of the Publication</b>	<i>Cell Death Biomarkers as Early Predictors for Hepatic Dysfunction in Patients After Orthotopic Liver Transplantation</i>
<b>First and last Author</b>	Brenner T.; Hofer S.
<b>Journal</b>	Transplantation 94:185 (2012)
<b>Disease</b>	Liver Transplantation
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	Liver transplantation (LTPL) (n=100) of which were first-time LTPL (n=77) with the following primary liver diseases: ethyl-toxic cirrhosis 19.5%, viral hepatitis 7.8%, hepatocellular carcinoma 28.6%, and others 44.1% and retransplantation (n=23). Median score in the laboratory model for endstage liver disease (MELD) for all recipients was shown to be 24.0.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), M65 (total K18), sICAM-1, IL-6, TNFalpha plasma levels were measured at baseline (baseline level before transplantation) (Pre), immediately after the end of the surgical procedure (T0), as well as day 1 (T1) and day 3 (T3), day 5 (T5), and day 7 (T7) days after orthotopic liver transplantation.
<b>Principal Findings</b>	In patients with a perfusion disorder (n=30), plasma levels of total K18 were significantly elevated at T0, T3, T5, (p<0.05); T1 and T7 (p<0.01) in comparison to the control group. In patients with an infectious complication (n=27; with sepsis: n=9, without sepsis: n=18) plasma levels of total K18 as well as ccK18 were significantly elevated at T0, T3 (p<0.05); and T1 (p<0.01) compared to the control group (n=37). Cell death biomarkers did not differ significantly between the groups with septic and non-septic diseases at T0, T1, and T3.
<b>Conclusions</b>	Total K18 measurements LTPL cohort of showed plasmatic peak concentrations immediately after the end of the surgical procedure (T0: 7,717U/L), whereas ccK18 levels indicative of apoptotic cell death were characterized by a delay of 24 hr until the peak concentration was reached (T1: 3,789U/L). Plasma levels of both parameters returned to baseline levels within 7 days after transplantation. At T1 the following cut-off values for the development of a complicated course could be calculated for total K18: any complication: AUC=0.692, cut-off=7,592U/L; complication by infections: AUC=0.673, cut-off=6,696U/L, and perfusion disorders: AUC=0.654, cut-off=8,332U/L.
<b>Recommendations</b>	Total K18 measurements are superior to routine laboratory parameters with regards to their prognostic value for a complicated course. In contrast to other previously published diagnostic tools for the detection of patients who underwent LTPL at risk for a complicated course, total K18 measurements were shown to be favorable because they evoked their prognostic capability early (24h versus 7 days after LTPL). Therefore, daily measurements of total K18 in parallel with routine markers of liver impairment (ASAT, ALAT, and lactate dehydrogenase) is recommended.

# Sepsis

**Lorente, 2014**

<b>Title of the Publication</b>	<i>Serum levels of caspase-cleaved cytokeratin-18 and mortality are associated in severe septic patients: pilot study.</i>
<b>First and last Author</b>	Lorente L.; Borrequero-León J.
<b>Journal</b>	PLoS One. Oct 7;9(10):e109618 (2014)
<b>Disease</b>	Sepsis
<b>Type of Study</b>	Prospective, multi center, observational study
<b>Patients</b>	224 severe septic patients
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> Blood samples were collected at the time that severe sepsis was diagnosed to determine serum levels of M30 (ccK18), tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 and IL-10. The end point was 30-day mortality.
<b>Principal Findings</b>	Multiple logistic regression analysis showed that serum ccK-18 levels >391 U/L were associated with 30-day survival (Odds ratio=2.687; 95% confidence interval=1.449-4.983; P=0.002), controlling for SOFA score, serum lactic acid levels and age. Kaplan-Meier survival analysis showed that the risk of death in septic patients with serum ccK-18 levels >391 U/L was higher than in patients with lower values (Hazard Ratio=3.1; 95% CI=1.96-4.84; P<0.001). Serum ccK-18 levels were positively associated with serum levels of IL-6 and lactic acid, and with SOFA and APACHE scores.
<b>Conclusions</b>	Non-surviving patients (n=80) showed higher serum ccK-18 levels (P<0.001) than survivors (n=144).
<b>Recommendations</b>	The largest cohort of septic patients providing data on circulating ccK-18 levels shows that serum ccK-18 levels are associated with mortality in severe septic patients.

**Hofer, 2009**

<b>Title of the Publication</b>	<i>Cell death serum biomarkers are early predictors for survival in severe septic patients with hepatic dysfunction</i>
<b>First and last Author</b>	Hofer S.; Weigand M.
<b>Journal</b>	Crit Care. 13:R93 (2009)
<b>Disease</b>	Sepsis
<b>Type of Study</b>	Pilot study, single centre, observational
<b>Patients</b>	Sepsis (n=101, with survivors, n=52 and non-survivors, n=49), postoperative patients after major abdominal surgery (n=28) healthy volunteers (n=18).
<b>Methodology</b>	<p><b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b></p> <p>M30 (ccK18), M65 (total K18), sICAM-1, sVCAM, IL-6, TNFalpha plasma levels were measured at baseline at the time of sepsis diagnosis (t0), and 48 hours (t48) and 120 hours (t120) later; samples from healthy volunteers were collected once, and from postoperative patients, once immediately after surgery.</p>
<b>Principal Findings</b>	At the time of diagnosis of sepsis, levels of total K18 and ccK18 were significantly higher in the non-surviving subgroup. The levels of ccK18 remained significantly higher at 48 hours and decreased to comparable values at 120 hours. At t120 levels of ccK18 in surviving patients were still lower (p=0.073). ccK18 was also significantly lower at t0 in survivors 357.7U/L compared to non-survivors 475.4U/L; (p=0.035) and t48 in survivors 355.4U/L compared to non-survivors 603.6U/L; (p=0.007) and total K18 at t0 in survivors 1,581.9U/L compared to non-survivor 2,006.5U/L; (p=0.038).
<b>Conclusions</b>	Postoperative patients showed comparable levels of apoptotic activity, but necrotic cell death was markedly increased, probably due to surgical tissue injury. In contrast, patients with severe sepsis, and especially non-survivors of the septic group showed increased levels of markers for both apoptotic and necrotic cell death. In severe septic patients with liver dysfunction, necrosis is increased relative to severe septic patients with intact hepatic function.
<b>Recommendations</b>	The measurement of ccK18 (M30) and total K18 (M65) appears to be an early predictor for survival in severe septic patients with hepatic dysfunction. Measurement of ccK18 and total K18 appears to be an early predictor for survival in patients with severe sepsis and hepatic dysfunction.

# Graft-versus-Host Disease after Stem Cell Transplantation

*GvHD after SCT*



**Luft, 2007**

<b>Title of the Publication</b>	<i>Serum cytokeratin-18 fragments as quantitative markers of epithelial apoptosis in liver and intestinal graft-versus-host disease</i>
<b>First and last Author</b>	Luft T.; Dreger F.
<b>Journal</b>	Blood 110:4535 (2007)
<b>Disease</b>	GvHD after SCT
<b>Type of Study</b>	Pilot study, retrospective, single centre, observational
<b>Patients</b>	55 patients with (n=50) and without Graft versus Host Disease (GvHD) (n=5) after allogeneic stem cell transplantation (SCT). GvHD was defined by histology, or, if not available, by accepted clinical criteria (histologically confirmed GvHD of gut (n=13), liver (n=3) and skin (n=3)). Samples taken immediately prior to the escalation of immunosuppressive therapy or, in the case of steroid-resistant (treatment refractory) GvHD, samples taken the day closest to salvage immunosuppressive therapy, were considered as representing maximum GvHD.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> M30 (ccK18), bilirubin, ALAT, AP, GT, creatinin). The serum sampling period comprised pre-SCT and weekly or second weekly sample collections after SCT for a maximum of 1 year.
<b>Principal Findings</b>	Basal pre-SCT ccK18 levels were analyzed in 60 patients using serum samples obtained immediately prior to conditioning. Median ccK18 baseline levels were 152U/L. Longitudinal ccK18 serum kinetics were measured in patients (n=50) with documented episodes of GvHD. For 19 patients, biopsy results were available: 13 intestinal, 3 liver, and 3 skin biopsies. Significantly elevated ccK18 levels were observed during biopsy-proven GvHD of liver (median 1,920U/L) and gut (median 560U/L) compared with pre-SCT levels ( $p<0.001$ ). Episodes of biopsy-proven isolated intestinal GvHD (n=4) showed significant increases in ccK18 levels (median 494U/L) compared with pre-SCT levels ( $p<0.001$ ). ccK18 increased preceding the clinical diagnosis of GvHD (n=9). ccK18 levels rise during the period preceding the initiation of immunosuppressive therapy and correlate with response to immunosuppressive treatment and GvHD severity, but not during toxic hyperbilirubinemia/veno-occlusive disease (n=9), infectious enteritis (n=11), and renal failure (n=2).
<b>Conclusions</b>	In contrast to biomarkers that all reflect the activation status of the immune system or its inflammatory activity, ccK18 mirrors the pathogenetic end point of GvHD, that is, GvHD-induced apoptotic activity in critical epithelial organs (bowel and liver). Although apoptosis is clearly not GvHD-specific, it is the direct cause of GvHD-mediated end organ damage, and therefore should be a much better indicator of clinically relevant GvHD sequelae than the general immune activation status. This study demonstrates that epithelial apoptosis induced by GvHD of gut and liver can indeed be assessed by serum ccK18 measurement. Without exception, ccK18 levels were strongly elevated during uncontrolled clinical hepatointestinal GvHD in comparison to baseline. ccK18 values obtained after clinical response of GvHD to immunosuppressive therapy were always lower than those determined at the start of immunosuppressive treatment. Refractory GvHD was consistently associated with persisting or increasing ccK18 serum levels.
<b>Recommendations</b>	ccK18 monitoring provides a promising tool for sensitive assessment of GvHD-associated apoptotic activity in both intestinal and hepatic GvHD. Although apoptosis is not GvHD-specific, ccK18 may be useful to distinguish active GvHD from GvHD-unrelated conditions with similar symptoms commonly observed after transplantation, and to monitor response of GvHD to immunosuppressive treatment. Prospective studies are on-going to prove its suitability for diagnosis, grading, prognosis, and

treatment guidance of this major complication of SCT.

# Pancreatitis

## Vlachos, 2014

<b>Title of the Publication</b>	Serum profiles of M30, M65 and interleukin-17 compared with C-reactive protein in patients with mild and severe acute pancreatitis.
<b>First and last Author</b>	Vlachos S.; Simopolous C.
<b>Journal</b>	J Hepatobiliary Pancreat Sci. Dec;21(12):911-8 (2014)
<b>Disease</b>	Pancreatitis
<b>Type of Study</b>	Pilot study, multi centre
<b>Patients</b>	150 patients with pancreatitis and 70 controls.
<b>Methodology</b>	<b>Non-invasive Biomarker (in addition to clinical laboratory parameters)</b> The prognostic value of M30, M65 and their ratio M30/M65 is assessed by ELISA. The same method is used for the study of IL-17.
<b>Principal Findings</b>	At 24 h after symptom onset, the concentrations of M30 and M65 as well as their ratio, differed significantly in severe compared to mild disease ( $P = 0.016$ ). C-reactive protein (CRP) was significantly higher ( $P < 0.001$ ) in severe pancreatitis on the same day. The sensitivity of M65 to show severe acute pancreatitis at 24 h was 100% for values above the cut-off point of 428.15 U/l. The sensitivity of CRP was 100% as well. Concerning IL-17, its concentrations were higher in patients than in the control group ( $P < 0.001$ ) in the first 24 h.
<b>Conclusions</b>	In conclusion, this study states that of all the measurements, M65 and M30/M65 can provide future perspectives for early prognosis of severity in acute pancreatitis. Therefore, they could be applied in clinical practice, potentially along with other biomarkers.
<b>Recommendations</b>	Plasma concentrations of M65 and the M30/M65 ratio can be useful in predicting the severity of acute pancreatitis as early as 24 h after the onset of symptoms.

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