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# Markers of cell death predict therapy response in patients with cirrhosis and hepatorenal syndrome

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## Abstract

**Background and aims:** Hepatorenal syndrome is a major complication in patients with cirrhosis and associated with high mortality. Predictive biomarkers for therapy response are largely missing. Cytokeratin18-based cell death markers are significantly elevated in patients with complications of chronic liver disease, but the role of these markers in patients with HRS treated with vasoconstrictors and albumin is unknown.

**Methods:** We prospectively analyzed a total of 138 patients with HRS, liver cirrhosis without HRS and acute kidney injury treated at the University Medical Center Mainz between April 2013 and July 2018. Serum levels of M30 and M65 were analyzed by ELISA and clinical data were collected. Predictive ability was assessed by Kaplan-Meier curves, logistic regression and c-statistic. Primary endpoint was response to therapy.

**Results:** M30 and M65 were significantly increased in patients with HRS compared to non-HRS controls (M30: $p < 0.0001$; M65: $p < 0.0001$). Both serum markers showed predictive ability for dialysis- and LTX-free survival but not overall survival. Logistic regression confirmed M30 and M65 as independent prognostic factors for response to therapy. A novel predictive score comprising bilirubin and M65 showed highest predictive ability to predict therapy response.

**Conclusions:** Serum levels of M30 and M65 can robustly discriminate patients into responders and non-responders to terlipressin therapy with a good predictive ability for dialysis- and LTX-free survival in cirrhotic patients. Cell death parameters might possess clinical relevance in patients with liver cirrhosis and HRS.

KEYWORDS

cytokeratin-18, hepatorenal syndrome, terlipressin, therapy response

## INTRODUCTION

Chronic liver diseases and cirrhosis are increasing health care problems accounting for 1%–2% of all deaths in Europe.[1,2] Among the most frequent complications in cirrhotic patients is the development of an acute kidney injury (AKI), observed in around 20% of admitted patients with cirrhosis and ascites.[3] Between 18% and 43% of these patients are diagnosed with hepato-renal syndrome (HRS).[4,5]

Traditionally, two subtypes of HRS are distinguished. Type 1 is characterized by acute renal impairment with a sharp increase of serum creatinine >2.5 mg/dl (226 μmol/L), whereas Type 2 is characterized by recurrent or refractory ascites and slow increase of serum creatinine up to 2.5 mg/dl (133–226 μmol/L). Newer classifications emphasize the role of pro-inflammatory cytokines and chemokines and microvascular dysfunction in HRS and have classified HRS type 1 as HRS-AKI, HRS type 2 as renal impairment which fulfills criteria of HRS but not of AKI (non–AKI-HRS; NAKI) and HRS-CKD.[6,7] According to traditional stratification, median OS of Type 2 HRS is 6 months in contrast to only a few weeks for HRS Type 1.[4]

Current treatment guidelines recommend continuous vasopressor therapy (terlipressin) in combination with albumin.[3,8] While response to therapy has significant impact on patient prognosis, response rates are limited and do not exceed 30%–60%.[9] Importantly, treatment-related adverse events as well as death are observed in 20% and 3%, respectively.[9–11] Other therapeutic approaches involve renal replacement, trans-jugular intrahepatic portosystemic shunts (TIPS) or artificial liver-support systems but provide only minimal survival benefit.[3,12] Thus, the only causal therapy option remains liver transplantation.

Given the severe side effects of terlipressin therapy and limited response rate, it seems necessary to stratify patients early and evaluate alternative therapeutic options.

While serum creatinine levels as well as serum bilirubin, Model of end stage liver disease (MELD) score, Child-Pugh Score and international normalized ratio (INR) levels harbor predictive capacity in some studies, no reliable biomarker is currently established.[13]

Decompensated liver cirrhosis is associated with a persistent systemic inflammation,[14,15] which is partially reflected in elevated CRP and leukocyte levels regardless of the presence of an infection. Patient with an advanced cirrhosis show elevated levels of inflammatory cytokines such as IL-6, IL-8, TNFα and markers of oxidative stress.[14,15] The underlying pathomechanism is the translocation of bacteria from the gut to mesenteric lymph nodes, which drives a proinflammatory reaction.[16,17]

Hepatocyte inflammation and cell death triggered by direct damage or via immune response are crucial factors that contribute to progression or decompensation of liver cirrhosis as well as HRS.[18] Cytokeratin-18 (CK-18) filaments are a major component of the cytoskeleton in hepatocytes and cells of the biliary tract and are major substrates for caspase during apoptosis.[19]

During the process of apoptosis, caspases fragmentate CK-18, which exposes a new antigen (M30), that can be detected be specific anitbodies.[19,20] M65 is also an epitope of CK-18 but is not necessarily associated with apoptosis and can be used as a marker of overall cell death. By secondary necrosis, these proteins are released into the blood circulation and are quantified in patients' serum using an enzyme-linked immunosorbent assay.[13,19]

Prognostic ability of cell death markers has been shown in patients with acute (on chronic) liver failure, NAFLD/NASH, hepatocellular carcinoma (HCC) and other tumor entities.[21–23] They are also significantly elevated in patients with decompensated liver cirrhosis.[13] Importantly, M30 and M65 are not influenced by treatment with diuretics, which implies potential superiority over other serum markers including creatinine.[13] However, there is no study prospectively evaluating the predictive ability of cell death markers for therapy response in HRS patients.

We show here for the first time that M30 and M65 serum levels might have a diagnostic and predictive impact in patients with HRS treated with vasopressor and albumin therapy. We can demonstrate that M30 as well as M65 are independent predictors for therapy response and can robustly stratify patients prior to therapy initiation.

**Key Summary**

**Summarize the established knowledge on this project**

- Hepatorenal Syndrom (HRS) is a severe complication of liver cirrhosis associated with a high mortality
- Only 30%–60% respond to terlipressin therapy and therapy response is crucial for overall survival (OS)
- So far, there are no reliable predictive markers for therapy response

**What are the significant and/or new findings of this study?**

- Cytokeratine-18 based cell death markers—M30 and M65—are increased in patients with HRS
- M30 and M65 can robustly predict response to terlipressin/albumin treatment prior to therapy start and might be a useful tool for early patient stratification

## METHODS

### Patient cohort and clinical data

Eighty one HRS patients treated at University Medical Center Mainz between 2013 and 2018 were prospectively included in this study. Inclusion criteria for the HRS group were age over 18 years, diagnosed cirrhosis, first episode of HRS Type 1 or 2 according to guidelines and receiving terlipressin treatment ($N$ = 81).[24] Forty three cirrhotic patients without kidney injury and 10 patients with diagnosed AKI, that was not classified as HRS, were included in this study and served as a control to conclusively rule out increase in markers due to kidney damage. Inclusion criteria were age over 18, confirmed liver cirrhosis without acute decompensation, but regular

serum creatinine levels or AKI (e.g., pre-renal kidney failure) that did not meet criteria for HRS.

Patients undergoing a volume-shortage shock, recent therapy with nephrotoxic substances or parenchymatous kidney diseases (proteinuria higher than 500 mg/day, positive urine sediment, microhematuria and an abnormal sonographic exam) were excluded. Patients with HCC or trans jugular intrahepatic portosystemic shunt (TIPS) implantation during the last 6 months have been excluded as well.

Clinico-pathological data as well as disease-specific serum parameters have been assessed for every patient. Complete response was defined as decrease in serum creatinine levels <1.5 mg/dl without dialysis, partial response as a reduction of creatinine levels >50% from the initial value. Follow-up was done until death or last contact. The prospective cohort study was approved by the local ethics committee of the University Medical Center Mainz and was performed in accordance with all relevant data protection criteria and the Declaration of Helsinki. All patients agreed to this study by signing informed consent.

## Blood sample collection

Serum samples were collected between day 0 (terlipressin start) and day 14 (or day of discharge), Samples for M30 and M65 assessment were collected at terlipressin start, centrifuged at 4°C at 3000 rpm and stored within 30 min at −80°.

## ELISA

Quantitative analysis of serum levels of M30 and M65 were performed using the ELISA-kit M30 Apoptosense and ELISA-kit M65 from Peviva, VLVbio, Sweden according to the manufactures protocol. All reagents were used at room temperature. All samples were analyzed in duplicates.

## Statistical analyses

Statistical analysis was performed with Graphpad Prism Software (Version 8.3.0, Graphpad, USA) and R Studio (version 1.2.5019, R Foundation for Statistical Computing, Austria). For comparison of both groups, normal distribution was verified by D'Agostino and Pearson test and variance homogeneity by *F*-test. Statistical significance between groups was then analyzed using *t*-Test, Welch-Test or Mann-Whitney *U* Test, respectively. For more than two groups Kruskal-Wallis test or ANOVA multiple comparison were used. Correlation analysis was performed using Pearson correlation. Overall survival was calculated from date of therapy initiation till death. Overall survival between stages was compared using Gehan-Breslow-Wilcoxon for short time evaluation or log-rank test for long term effects. *p*-values <0.05 were considered statistically significant. Predictive ability was assessed using the c-index. Specifically, we predicted HRS with logistic regression models which all contained age, sex in addition to combinations of Bilirubin levels, MELD score and log transformed M65 levels eventually including interactions. Robustness of the c-index was assessed by bootstrapping with 10.000 repetitions. We also computed bootstrapped confidence intervals where applicable, specifically the bias corrected and accelerated variant.[25] C-indices were computed with R (Version 4.02) and the DescTools package (Version 0.99).

# RESULTS

## Patient baseline characteristics

A total of 134 patients were prospectively enrolled and analyzed at the University Medical Center of Mainz (Figure 1). Patients' baseline characteristics were collected from the clinical patient data system and are shown in Table 1. 61.5% ($n = 88$) of the 134 patients were male and 38.5% ($n = 55$) female. The median age of HRS patients was 58.2 years. Mean creatinine levels at terlipressin start in the HRS group or at time of admission for the two control groups were 2.7 mg/dl (Table 1). 50% of the patients were diagnosed as HRS Type 1% and 50% as HRS Type 2 (Table 1). The median duration of therapy was 6 days in the HRS group. In accordance with our previous studies, HRS patient cohort showed response rates of 49,4% to combined terlipressin and albumin therapy.[8,26]

M30 and M65 serum levels are significantly elevated in HRS patients compared to non-HRS patients.

We aimed to investigate a possible association of HRS and cell death. Therefore, we analyzed serum levels of M30 and M65. In the HRS group we detected a median M30 serum level of 563.7 U/l and median M65 of 935.4 U/l, respectively. Both, M30 and M65 serum levels, were significantly lower in patients without HRS as compared to the HRS group (M30 - cirrhosis group: median 171.4 U/l; M30-AKI group: mean 259.6 U/l, $p < 0.001^{***}$, D'Agostino and Pearson test: $p < 0.001^{***}$, Kruskal Wallis test: $p = 0.0001^{***}$; M65 - cirrhosis group: median 243.5 U/l; M65-AKI group: median 423.6 U/l, respectively. D'Agostino and Pearson test: $p < 0.001^{***}$, Kruskal Wallis test: $p = 0.0001^{***}$, Figure 2).

In accordance with previous reports, a significant increase of M30 as well as M65 could be shown in more advanced liver cirrhosis (D'Agostino and Pearson test: $p < 0.001^{***}$, Kruskal Wallis test: $p = 0.0001^{***}$ for M30 and M65, respectively, supp. Figure 1a). To exclude a possible epiphonema due to a higher Child Pugh score in HRS patients, we compared the M30/M65 levels of our HRS cohort only with those patients from the control group, who had a matching Child Pugh score (B and C). Again, this demonstrated a significant difference in serum levels. To provide a more robust dataset, we have added another cohort of 43 patients without HRS that included only stage B and C cirrhotic patients which was collected and retrospectively analyzed at the Hannover Medical School (Cohort B, Hannover, patient baseline characteristics are shown in supp. Table 2). This analysis confirmed the finding of increased M30 and M65 in HRS patients compared to non-HRS patients of cohort A, considering only

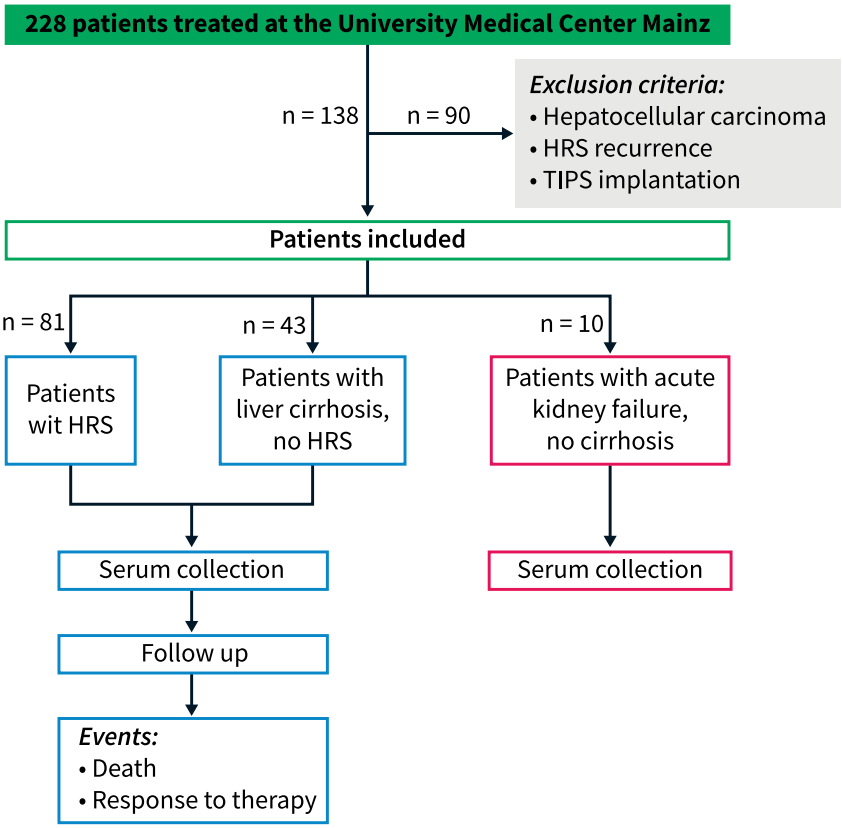


**FIGURE 1** Flow chart study design and exclusion criteria.

Child Pugh B and C patients, as well as the second cohort of only Child Pugh B and C patients from Hanover (Kruskal-Wallis multiple comparison test $p < 0.0001$ and $p < 0.0001$ for M30 and M65, respectively, supp. Figure 1b). We also assessed the correlation between serum parameters reflecting liver, renal function as well as markers of infection with M30 or M65 serum levels. As expected, M30 and M65 showed a strong correlation ($r = 0.96$, $p < 0.0001$, Table 2), whereas there was only a moderate positive correlation with leucocytes ($r = 0.39$, $p = 0.0002$ and $r = 0.36$, $p = 0.0008$, respectively, Table 2) as well as bilirubin ($r = 0.35$, $p = 0.0012$ and $r = 0.38$, $p = 0.0004$, respectively). No correlation with CRP, INR or creatinine could be revealed.

## M30 and M65 levels are associated with LTX and dialysis-free survival

Given the elevated M30 and M65 serum levels in HRS patients, we evaluated if both serum parameters possess predictive capacity in therapy response. Since liver transplantation, dialysis and TIPS are established treatment options upon progression of HRS, we considered these factors in the analyses of clinical outcomes. M30 as well as serum M65 levels alone or in combination could significantly discriminate patients according to LTX- and dialysis-free survival (Gehan Breslow Wilcoxon test $p = 0.0056$, $p = 0.0049$ and $p = 0.0031$, respectively, Figure 3). High and low were defined as the upper or lower 50%. Same data resulted using the first and fourth percentile (data not shown). M30 and M65 serum levels alone or in combination are significantly associated with LTX-, dialysis- and TIPS-free survival in HRS patients (Gehan Breslow Wilcoxon test $p = 0.0006$ and $p = 0.0027$, respectively, Figure 3, supp. Figure 2). As expected, levels of the cell death markers did not discriminate OS (Log rank test: $p = 0.784$, Figure 3, supp. Figure 2), confirming the dismal outcome of these patients in the absence of effective treatment.

## M30 and M65 serum levels predict therapy response in HRS patients

A positive therapy response to terlipressin/albumin treatment is the key determinant of clinical outcome in HRS patients. Patients were separated into terlipressin responders and non-responders and serum M30 and M65 levels were analyzed. Mean creatinine levels after therapy in responders was 1.25 mg/dl, in non-responders 2.62 mg/dl respectively (supp. Figure 3). Patient baseline characteristics of both groups are shown in supp. Table 1.

49.4% of all HRS patients treated with terlipressin and albumin responded to therapy (Figure 4a). Consistently, the prognostic relevance of therapy response could be confirmed in our cohort (Figure 4b). To evaluate predictive ability of cell death for therapy response, we analyzed if M30 and M65 therapy are associated to response to terlipressin and albumin therapy. Indeed, M30 as well as

**TABLE 1** Patient baseline characteristics cohort A

| | Cirrhosis HRS | Cirrhosis no HRS | Acute kidney failure |
|---|---|---|---|
| Total number | 81 | 43 | 10 |
| Age in years, mean (range) | 58.2 (25–80) | 59 (39–80) | 67.9 (58–85) |
| Male, *n* (%) | 52 (64.2) | 25 (58.1) | 5 (50) |
| Female, *n* (%) | 29 (35.8) | 18 (42.0) | 5 (50) |
| Ethiology, (%) | | | |
| Alcohol abusus | 68.6 | 39.5 | 40 |
| HCV | 10.5 | 32.6 | 0 |
| HBV | 4.7 | 4.7 | 0 |
| PSC/PBC/SSC | 3.5 | 2.3 | 0 |
| NASH | 1.2 | 0 | 20 |
| Cryptogenic | 9.3 | 11.6 | 20 |
| AIH | 0 | 4.7 | 0 |
| Other cause | 2.4 | 4.7 | 30 (no cirrhosis) |
| CHILD Pugh, (%) | | | |
| A | 0 | 67.4 | 30 |
| B | 17.4 | 25.6 | 40 |
| C | 82.6 | 4.7 | 0 |
| No cirrhosis | 0 | 0 | 30 |
| MELD, mean (range) | 28 (14–40) | 9 (6–15) | 19 (0–29) |
| Serum parameters at therapy start or admission (mean + range) | | | |
| Serum Kreatinin (mg/dl) | 2.7 (1.5–6.5) | 0.85 (0.6–1.3) | 2.4 (1.2–4.2) |
| Harnstoff | 49 (20–123) | NA | NA |
| Serum bilirubin (mg/dl) | 5.3 (0.4–47.8) | 1.3 (0.4–2.6) | 0.9 (0.4–5.7) |
| INR | 1.6 (0.8–3.4) | 1.18 (0.9–1.7) | 1.3 (1–2.2) |
| CRP (mg/dl) | 34 (2.2–155) | 7.4 (0.2–80) | 14.5 (1.4–29) |
| Leukocytes (×10^9/L) | 8.9 (1.4–33.5) | 6.3 (2.3–14.6) | 5.9 (2.9–11) |
| M30 (U/l), mean (range) | 552.7 (88–41064) | 260.3 (34–1262) | 260 (80–855) |
| M65 (U/l), mean (range) | 952.4 (219–73484) | 382.6 (59–2268) | 424 (115–860) |
| HRS type I | 50% | | |
| HRS type II | 50% | | |
| Therapy | | | |
| Responders (%) | 49.4 | | |
| Non responders (%) | 50.6 | | |

Abbreviations: INR, international normalized ratio; MELD, Model of end stage liver disease.

M65 serum levels were significantly elevated in non-responders on day 0 (terlipressin start, Mann-Whitney test $p = 0.0011$ and $p = 0.0003$, respectively, Figure 4c). In contrast, there was no difference in creatinine levels or CHILD scores between responders and non-responders. Interestingly, calculated MELD scores were also higher in non-responders (unpaired *t*-test $p = 0.0032$, supp. Figure 4). Since HRS is a type of renal impairment that might affect serum sodium levels, we have assessed serum sodium levels at terlipressin start, during therapy and at end of treatment. Serum sodium levels increased under therapy (supp. Figure 4e). However, sodium levels did not differentiate between responders and non-responders (supp. Figure 4f). Importantly, this predictive ability was independent of HRS Type 1 or Type 2 (Figure 4d). M30 and M65 levels were consistently higher in non-responders, except for M30 in HRS Type I patients. However, there is still a trend towards higher level in non-responders and the missing significance might be due to the low patient number.

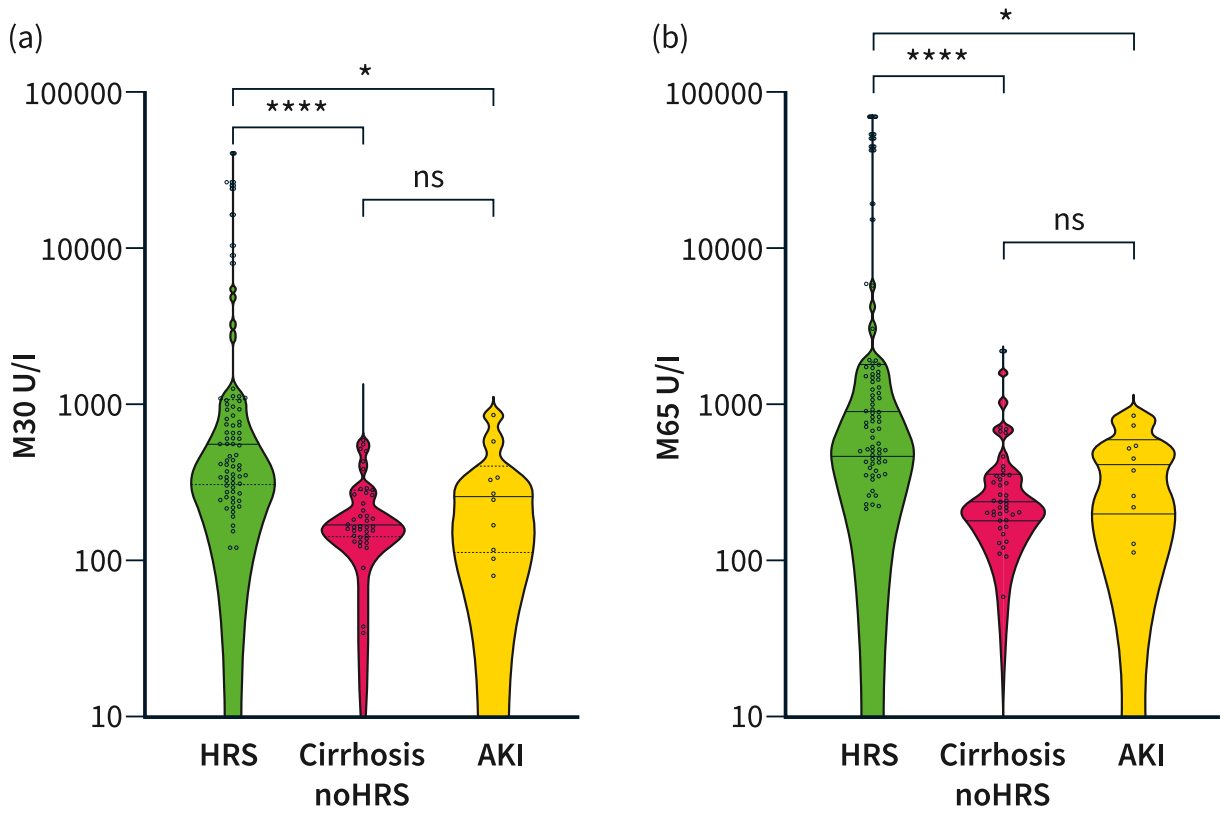


**FIGURE 2** Serum M30 and M65 levels for each group. (a) Serum M30 levels are significantly different between Hepatorenal Syndrom (HRS) patients and patients with cirrhosis without HRS or patients with acute kidney injury (AKI) (Kruskall Wallis test: $p < 0.0001$****). (b) Serum M65 levels are significantly different between HRS patients and patients with cirrhosis without HRS or patients with AKI (Kruskall Wallis test: $p < 0.0001$****).

**TABLE 2** correlation analysis of M30 and M65 with patient serum markers

| | M30 | | M65 | |
|---|---|---|---|---|
| **Parameter** | ***r*** | ***p* value** | ***r*** | ***p* value** |
| CRP | 0.16 | 0.16 | 0.12 | 0.33 |
| Leucocytes | 0.4 | 0.0002*** | 0.36 | 0.0001** |
| INR | 0.24 | 0.04* | 0.23 | 0.04* |
| Bilirubin | 0.34 | 0.002** | 0.38 | 0.001** |
| Creatinin | 0.06 | 0.27 | 0.1 | 0.37 |
| MELD | 0.26 | 0.02* | 0.28 | 0.01* |
| CHILD | 0.16 | 0.17 | 0.18 | 0.11 |
| M30/M65 | 0.96 | 2.20E-16**** | | |

Abbreviations: INR, international normalized ratio; MELD, Model of end stage liver disease.

Next, we analyzed if serum levels of M30 and M65 can predict response to terlipressin therapy. Besides cell death markers, parameters of liver function as well as inflammation and etiology of liver cirrhosis were included in the analyses. Logistic regression revealed that M30 and M65 indeed predict therapy response to terlipressin therapy (logistic regression, $p = 0.0067$ and $p = 0.0037$ respectively, Table 3). In addition, MELD score, and bilirubin levels were also associated with therapy response.

To investigate if cell death markers are independent factors associated with therapy response, bootstrap confidence intervals for C-index were computed (based on 10,000 bootstrap replicates). C-index is equivalent to the area under the curve index. It ranges from 0 to 1 where 0 predicts no concordance, 1 predicts perfect concordance, and 0.5 predicts a random distribution between two variables.

Given the almost optimal correlation of M65 and M30 we only included M65 for further analysis. After testing for collinearity of MELD and bilirubin, both values were included for statistical analysis. Clustering analysis graphically demonstrated the close relationship between all parameters as well as the association with therapy response (supp. Figure 5).

Interestingly, all parameters showed a relatively high C-Index as single values (Bilirubin 0.76; M65 0.77; MELD score 0.71) respectively, Table 3). To further improve prediction, we calculated a composite score combining the two highest parameters, bilirubin and M65. Indeed, a combination of both parameters resulted in a c-index of 0.81 (Table 3), a superior prediction compared to each individual laboratory parameter alone. Furthermore, we evaluated an optimized cut-off for the respective parameters, which resulted in 6.45 mg/dl for bilirubin and 951.73 U/l for M65 based on bootstrap sampling. The threshold was determined with multivariable models, including

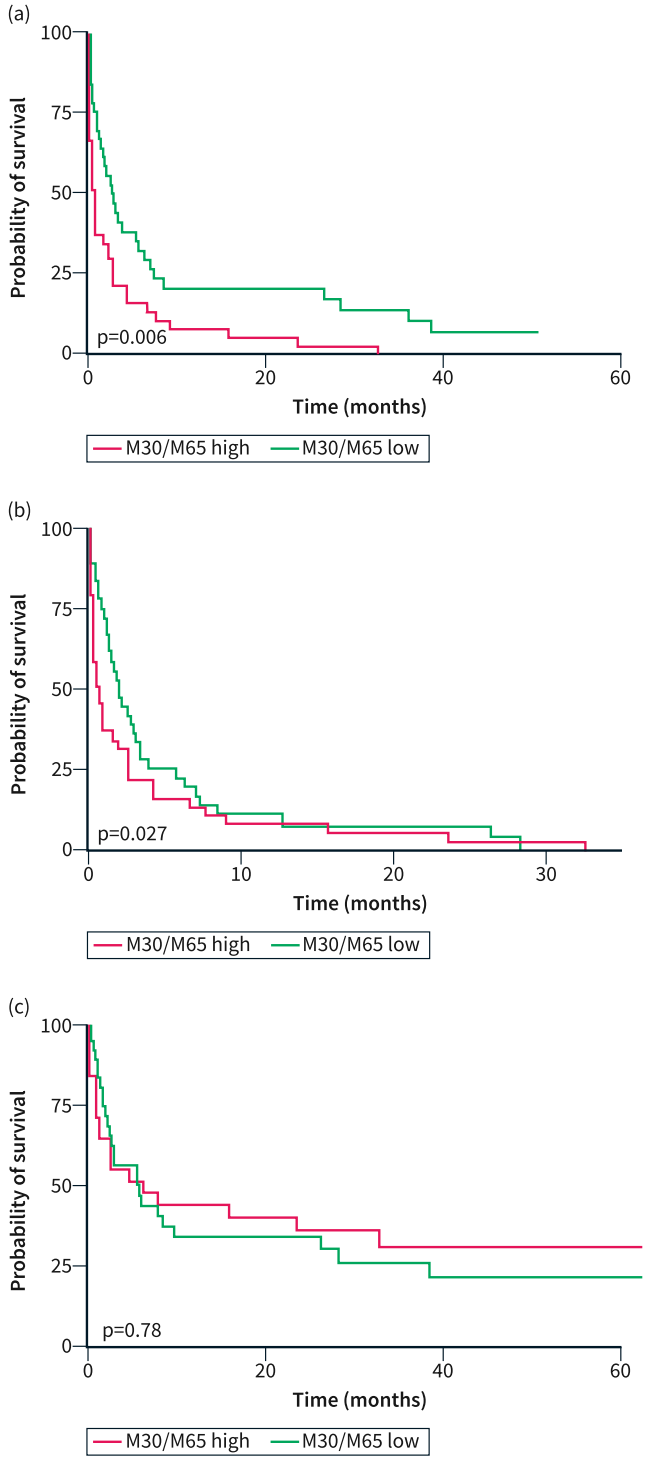


**FIGURE 3** Kaplan Meier analysis according to both, M30 and M65 high or low, serum levels. Survival curves show (a) HD and LTX free survival times ($p = 0.006^{**}$), (b) HD, LTX and trans-jugular intrahepatic portosystemic shunts (TIPS) free survival times ($p = 0.027^{*}$) and (c) overall survival (OS) times ($p = 0.78$). For TIPS free survival two patients had to be excluded. Differences in survival were assessed by Gehan-Breslow-Wilcoxon-test.

age, sex and bilirubin. The sensitivity for a M65 value below the stated threshold indicating therapy response is 0.683 (95% CI: 0.5263,0.8125), the specificity is 0.7 (95% CI: 0.5366, 0.8250). The positive predictive value is 0.7 (95% CI: 0.5402, 0.8261) and the negative predictive value is 0.683 (95% CI: 0.5238, 0.8182; meaning that 70% of the patients with a value of M65 smaller than the defined threshold are therapy responders).

We compared the two models based on c-indices computed from 10.000 subsamples drawn from the original dataset with replacement (bootstrapping). Specifically, we computed the difference of the c-index between models which contained M65 in addition to bilirubin with models, lacking M65 but contained bilirubin. Importantly, for the median of the bootstrap samples addition of M65 to bilirubin considerably increased the c-index of the single values. In 88% of the subsamples, the c-index was higher when including M65, confirming the improved prediction by using the composite score (supp. Figure 5).

## DISCUSSION

Hepatorenal Syndrom is a life-threatening complication of decompensated cirrhosis and is associated with high mortality.[3,9] Importantly, reversal of HRS significantly improves pre-transplant patient survival and reduces post-transplant need for dialysis in affected patients.[9,10,27] Acute hepatic inflammation and hepatocyte death are critical factors influencing liver homeostasis and predispose HRS development. Thus, cell death-based markers are of clinical relevance in HRS. While a prognostic relevance of serum-based cell death markers was demonstrated for several chronic liver diseases as well as acute liver failure, the impact in HRS patients remains unclear.[21,23,28] We here present the first prospective study evaluating clinical relevance as well as predictive ability for therapy response of M30 and M65. Major findings of this study demonstrate that serum levels of M30 and M65 correlate with MELD score, bilirubin and leukocytes, but, interestingly, not with creatinine or CHILD Pugh Score. Notably, this observation contrasts with a recent publication reporting that M65 serum levels correlate with creatinine as well as with CHILD Pugh score in patients with decompensated cirrhosis probably due to the small number of patients with affected kidney function.[13]

We could further demonstrate that M30 and M65 are associated with LTX-, TIPS- and dialysis-free survival. Interestingly, levels of cell death markers were not associated with OS, which might be explained by the dismal outcome of the patients in the absence of either response to vasopressor-albumin therapy or definitive treatment, that is, dialysis or transplantation.

Most importantly, M30 and M65 accurately classified patients according to response to therapy and, thus, might be a clinically meaningful addition for patient stratification. Due to the poor outcome of treatment in non-responders and the potentially severe side-effects of terlipressin treatment, identification of patients most likely to benefit from therapy is crucial.[9] Our results indicate that inflammation and liver cell death are major components driving hepatic decompensation and, thus, leading to HRS. Interestingly, since patients with acute kidney damage independent of HRS show relatively low levels of the markers, our results indicate that hepatic cell

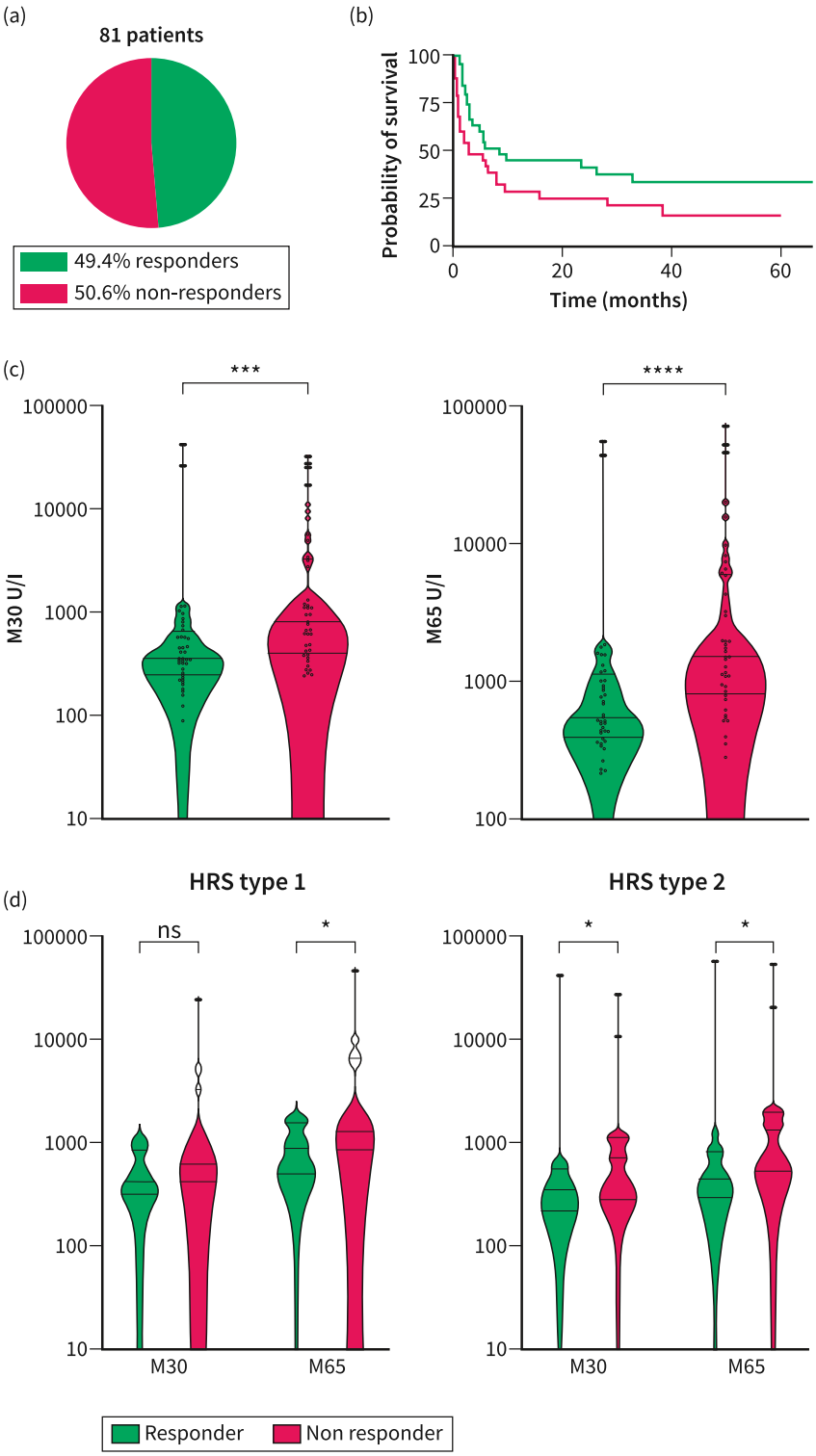


**FIGURE 4** (a) Patient distribution in terlipressin responders and non-responders. (b) Survival analysis of patients according to terlipressin therapy response ($p = 0.0345^{*}$). Difference in survival times was assessed by Gehan-Breslow-Wilcoxon-test. (c) Serum M30 and M65 serum level in terlipressin responders and non-responders. Serum M30 levels are significantly higher in non-responders (Mann-Whitney test: $p = 0.002^{***}$, left). Serum M65 levels are also significantly higher in non-responders (Mann-Whitney test: $p < 0.0001^{****}$, right). (d) Serum M30 and M65 serum level in terlipressin responders and non-responders separated in hepatorenal syndrom (HRS) Type I (left) and Type II (right). M30 and M65 level are significantly higher in non-responders in HRS Type I as well as in Type II.

**TABLE 3** logistic regression analysis

| | Univariate analysis | | | | | |
|---|---|---|---|---|---|---|
| **Parameter** | **Odds ratio** | **P value*** | **95% CI*** | **Parameter** | **C Indices** | **95% CI** |
| Age | 1.03 | 0.28 | 0.98; 1.08 | | | |
| Gender | 0.71 | 0.46 | 0.29; 1.74 | | | |
| HCV | 0.88 | 0.86 | 0.19; 4.01 | | | |
| HBV | 0.86 | 0.91 | 0.1; 7.74 | | | |
| Alcohol | 0.47 | 0.15 | 0.16; 1.29 | | | |
| MELD score | 1.11 | **0.0046**** | 1.04; 1.19 | MELD score | 0.71 | 0.56; 0.78 |
| Albumin | 1.01 | 0.67 | 0.95; 1.08 | | | |
| INR | 1.19 | 0.71 | 0.47; 3.03 | | | |
| Bilirubin | 1.06 | **0.0059**** | 1.02; 1.11 | Bilirubin | 0.76 | 0.61; 0.85 |
| Creatinine | 1.49 | 0.07 | 0.9982.36 | | | |
| CRP | 1.00 | 0.80 | 0.99; 1.01 | | | |
| Leucocytes | 0.98 | 0.62 | 0.92; 1.05 | | | |
| CHILD Pugh Points | 1.16 | 0.26 | 0..90; 1.51 | | | |
| Persistent alcohol abusus | 0.42 | 0.22 | 0.25; 2.01 | | | |
| M30 log | 3.53 | 0.0067** | 1.52; 9.79 | | | |
| M65 log | **4.25** | **0.0037**** | 1.75; 12.6 | M65 log | 0.77 | 0.62; 0.86 |
| | | | | **M65 x Bilirubin** | **0.81** | **0.64; 0.88** |
| | | | | M65 × MELD | 0.77 | 0.61; 0.85 |

*Note*: Univariate and *c* index statistics. Significant values are written in bold.

Abbreviations: INR, international normalized ratio; MELD, Model of end stage liver disease.

death rather than acute kidney damage are the key determinants for effective therapy. Consistently, we could provide evidence that M30 and M65 are independent prognostic factors for therapy response. Logistic regression analysis revealed M30, M65, bilirubin and MELD score as the only predictive parameters for therapy response. A previous study reported that the mean CLIF-SOFA score is lower in responders than in non-responders indicating that a more severe liver failure with consecutive sepsis and inflammation significantly reduces the likelihood of response to treatment.[29] Our study is in line with this suggestion and demonstrates that higher hepatic cell death reflected by higher M30 and M65 serum levels can independently predict response to terlipressin and albumin treatment. Furthermore, diagnostic accuracy can even be improved by combination with bilirubin a routine marker for hepatic function. Consistently, a higher C-index can be achieved by the composite assessment of the exploratory M65 combined with routine bilirubin measurements at a pre-defined cut-off 951.73 U/l and 6.45 mg/dl respectively. Thus, application of the score might help early initiation of dialysis or transplantation and, consequently, prevent overtreatment and potential side-effects of terlipressin.

Given the high predictive ability of the composite score, our data raise the question, if elevated levels of cell death markers as well as bilirubin warrant early initiation of dialysis and, consecutively, offers a higher chance of liver transplantation due to MELD increase and, thus, better clinical outcome. Therefore, future studies should evaluate if an early stratification of patients with high M30 or M65 and bilirubin might lead to improved long-term outcome of this group.

This study is limited by the number of patients treated at a single center. Further, due to the composition of the control group, we cannot proof that M30 and M65 serum levels are increased by more advanced cirrhosis itself. However, high predictive ability of the cell death markers for therapy response favors a functional relevance for HRS over a simple association to liver function. Further, the significant difference between HRS and AKI underscores that M30 and M65 indeed discriminate HRS and not only more advanced cirrhosis.[13] Taken together, the here presented study indicates that the addition of cell death markers to routine serological assessment in HRS might be of significant clinical importance and help to predict response to the commonly used terlipressin and albumin treatment.

## AUTHOR CONTRIBUTIONS

Jens Uwe Marquardt, Marc Nguyen-Tat and Sophia Heinrich have contributed to conception and design of the paper. Sophia Heinrich, Thomas Austgen, Darko Castven, Lena Stockhoff, Bernd Heinrich, Christian Labenz, Carolin Zimpe, Benjamin Maasoumy, Martha Kirstein have contributed collecting the data and performing experiments. Sophia Heinrich, Thomas Austgen, Benjamin Maasoumy, Peter Robert Galle, Harald Binder, Moritz Hess, Hans Heinrich

Wedemeyer, MG and Jens Uwe Marquardt have analyzed and interpreted the data. Sophia Heinrich and Jens Uwe Marquardt have written the manuscript. All authors have reviewed and agreed the final version of the manuscript.

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## CONFLICT OF INTEREST

There are no conflicts of interest for all authors.

## DATA AVAILABILITY STATEMENT

There are no data publicly available.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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