CK-18 cell death markers improve the prediction of histological remission in autoimmune hepatitis during biochemical remission

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Abstract

Incomplete histological remission of autoimmune hepatitis (AIH) is associated with a reduced long-term survival and an increased relapse rate even during biochemical remission (BR). The aim of this international multicentre study was to explore the diagnostic fidelity of cytokeratin-18 cell death markers to noninvasively detect incomplete histological remission. Thereby, cytokeratin-18 cell death marker M65 but not ALT and immunoglobulins was significantly higher in patients with incomplete histological remission (mHAI ≥ 4) compared to those with mHAI ≤ 3. M65 levels > 305 U/L, identified in the training cohort, facilitated the noninvasive detection of incomplete histological remission with a sensitivity of 75% and negative predictive value of 86% in the validation cohort. While BR with M65 < 305 U/L suggested complete histological remission (86%), BR with M65 > 305 U/L reduced the rate of histological remission to 60%. In conclusion, M65 may help to better select patients for or to reduce surveillance liver biopsies in the future.

KEYWORDS
cytokeratin-18, M30, M65, therapy
Autoimmune hepatitis (AIH) is a chronic progressive autoimmune disorder. Although the natural disease course is detrimental, immunosuppressive therapy can dramatically improve the life expectancy. In order to prevent histological disease progression, complete normalization of liver enzymes and immunoglobulin G (IgG) is recommended by current guidelines.1-3 This biochemical remission (BR) can be achieved in 40%-80% of patients.2,4,5 However, persistent necro-inflammatory activity in the liver (modified hepatitis activity index, mHAI ≥ 4) during BR was associated with a reduced long-term prognosis in a recent retrospective study.6 The current guidelines suggest a liver biopsy for the assessment of histological remission for the evaluation of treatment withdrawal after at least 3 years of therapy with at least 2 years of BR.2,3 The same cut-off of mHAI ≥ 4 is associated with relapses after stopping immunosuppressive therapy.7 So far, noninvasive clinical tools to assess persistent histological inflammation during BR are completely lacking.

Beyond the classical liver enzymes like aminotransferases, the hepatocyte cell death can be measured by the release of cytokeratin-18 (CK-18) fragments.8 While caspase-cleaved CK-18 indicates apoptosis, measured by the M30 ELISA, total CK-18 is also released during necrosis, measured by the M65 ELISA. The clinical utility of CK-18 cell death biomarkers was studied in multiple acute and chronic liver diseases. Thereby, cell death markers were helpful to noninvasively identify steatosis, fibrosis and the presence of metabolic (dysfunction)-associated fatty liver diseases (MAFLD).9,10 They further improved the prediction of the prognosis in acute and chronic liver failure.11-13 Additionally, higher cell death biomarkers were associated with more advanced liver fibrosis and a worse prognosis in primary biliary cholangitis (PBC).14 In AIH, cell death biomarkers were associated with elevations of liver enzymes in a recent study that did not assess the histological disease activity.15

The current multicentre study assessed the predictive capacity of CK-18 cell death biomarkers to noninvasively detect a relevant persistent necro-inflammatory activity in the liver (mHAI ≥ 4) in comparison to routine laboratory parameters in adult patients with AIH that achieved BR under maintenance immunosuppressive therapy.

2 | MATERIAL AND METHODS

2.1 | Patients

We included 60 adult patients with AIH without evidence for variant syndromes, who had a surveillance liver biopsy under ongoing immunosuppressive therapy while they were in BR as defined by alanine aminotransferase (ALT) and IgG levels within the normal range (Hamburg/Germany: n = 2; Hannover/Germany: n = 32; Larissa/Greece: n = 26). In order to assess the diagnostic fidelity of M65 for the non-invasive detection of mHAI ≥ 4, cohort was divided into a training (n = 31) and a validation cohort (n = 29). The patient data at the time point of the surveillance biopsy are summarized in Table 1.

This study was approved by the local research Ethics Committee of Hannover Medical School. Written informed consent was obtained from all patients from the prospective biomaier repository of Hannover Medical School (approval number 5582). The use of material and data from external patients in the multicentre cohort was approved by the respective local ethical committees.

2.2 | Histology

Biopsies were processed and histological scoring for the mHAI and fibrosis was performed by an experienced liver pathologist in a blinded fashion.16

2.3 | Serological detection of caspase-cleaved and total cytokeratin-18

Blood samples were preferentially taken at the day of admission before the performance of the liver puncture or within the first 24 hours thereafter. Blood plasma was cryo-conserved at −80°C.

Caspase-generated neoepitopes of CK-18 were measured in cryo-conserved plasma, taken within 24 hours before or after the biopsy, as recently published.7 In short, the M30-Apoptosense and M65 ELISAs were used according to the manufacturer’s instructions (Peviva, Bromma, Sweden).

The other laboratory parameters (ALT and IgG) were measured in the course of the routine clinical surveillance of the patients.

2.4 | Statistical analysis

Statistical analysis was performed with SPSS 15.0 and GraphPad Prism 5. Mann-Whitney U tests were used for comparison of two groups. The area under the receiver operating curve (AUC) was calculated and the Youden’s index was used for the identification of cut-off values. P-values below .05 (two-tailed) were considered statistically significant in all analyses.

3 | RESULTS

We could recruit 60 AIH patients from three centres in two European countries, who achieved BR under ongoing immunosuppressive therapy and had surveillance liver biopsy for the evaluation of the histological disease activity for the evaluation of treatment withdrawal (Table 1). Histological disease activity in the surveillance biopsy ranged from mHAI 0 to 12. Fifteen patients (25%) had persistent histological disease activity (mHAI ≥ 4) despite being in BR and 45 patients (75%) had mHAI ≤ 3, meaning complete remission (Figure S1). While treatment duration was not different between patients with and without complete remission
In preliminary experiments, M30 and M65 were measured in a test set of 27 patient sera. Levels of both CK-18 cell death markers strongly predicted mHAI ≥ 4 (M65: AUC = 0.903; 95% confidence interval (CI): 0.785-1.021; M30: AUC = 0.889; CI: 0.755-1.023). However, the study was continued in all 60 patient samples with M65 because of higher AUC in receiver operating characteristic analysis and the fact that M30 only detects CK-18 fragments from apoptotic cells, while M65 detects CK-18 from apoptotic and necrotic cells.

When patients with and without persistent histological disease activity were compared, M65 (P = .032) but not ALT (P = .176) or IgG (P = .140) were significantly elevated in patients with mHAI ≥ 4 (Figure 1).

The AUC of M65 in the training cohort showed a positive association of M65 levels with persistent histological disease activity (AUC = 0.712; CI: 0.516-0.908). With the Youden’s index, a cut-off value of 305 U/L for the discrimination between mHAI ≥ 4 and mHAI ≤ 3 was identified. M65 > 305 U/L for the prediction of mHAI ≥ 4 in AIH patients with BR during therapy in the training and validation groups had good sensitivity (89%, 95% CI: 51%-99% and 75%, 95% CI: 34%-96%, respectively), low specificity (55%, 95% CI: 33%-75% and 57%, 95% CI: 34%-77%),
low positive predictive value (PPV) (44%, 95% CI: 22%-69%; 21%-69% and 40%, 95% CI: 17%-67%) and very-good negative predictive value (NPV) (92%, 95% CI: 62%-100% and 86%, 95% CI: 56%-97%).

In summary, histological remission is found in 86%-92% of patients with BR and M65 < 305 U/L, while histological remission is found in only 56%-60% of patients with BR and M65 > 305 U/L in the training and validation cohorts (Figure S2).

In 21/60 patients (35%), the immunosuppressive medication was withdrawn after the surveillance biopsy. 7/21 (33%) experienced a relapse after 6 (4-14) months while 14/21 (67%) had sustained remission during a follow-up of 30 (3-67) months. Routine laboratory markers ALT (P = .255) and IgG (P = .444) as well as M65 (P = .636) at the time point of the surveillance biopsy were not significantly different between patients with relapse and those with sustained remission in this small subgroup.

4 | DISCUSSION

Liver biopsy remains the 'gold standard' for the assessment of staging (fibrosis) and grading (inflammation) of chronic liver diseases in order to guide and individualize therapy. However, liver biopsies have always a bleeding risk and a probability of sampling error, while they are expensive and unpopular among patients. Therefore, noninvasive methods are being extensively investigated for the assessment of staging and grading in chronic liver diseases.

While transient elastography (TE) can be used to identify MAFLD/nonalcoholic steatohepatitis patients with advanced fibrosis, the method cannot distinguish between inflammatory activity in nonfibrotic patients. Hartl et al. showed that liver fibrosis and inflammation can also be measured noninvasively with TE in AIH patients. In this trial, at diagnosis and especially in clinical settings of severe hepatitis, TE rather correlated with inflammation than with fibrosis and versa. After prolonged immunosuppressive therapy, TE correlated with fibrosis failing to detect persistent inflammation.

Dhaliwal et al from Sheffield were the first to report a negative impact of incomplete histological remission (mHAI ≥ 4) in the liver during BR on the survival of AIH patients. Although confirmation from other centres is pending, the Sheffield cohort has a unique size and a unique long-term follow-up over several decades. They reported higher ALT levels in patients with persistent histological activity during BR, but the difference in the aminotransferases in both cohorts was just 5-6 U/L within the normal range. Such subtle differences are inappropriate as a clinical diagnostic tool. While BR was defined as normalization of ALT and globulins in the Sheffield study, IgG was not measured exactly paired to the surveillance biopsy as in the current study.

Our study tried to fill this diagnostic gap of TE and routine laboratory parameters in order to reduce the need for surveillance liver biopsies in AIH. The present study included patients with aminotransferase levels exclusively within the normal range under therapy and included the histological disease activity stratified according to mHAI ≥ 4 and mHAI ≤ 3, a cut-off with prognostic relevance for the long-term survival of patients.

A previous study showed an excellent diagnostic fidelity for ALT and IgG for the detection of mHAI ≥ 4 but in a cohort of AIH patients with normal as well as elevated liver enzymes and IgG. They were able to show that normalization of both IgG and ALT was associated with the lowest rates of histological disease activity, but still the majority of patients with BR had mHAI ≥ 4 in their study. This underlines the clinical relevance of more sensitive noninvasive markers to monitor incomplete histological remission. In addition, Hartl et al showed a correlation of lower ALT within the normal range but not of IgG with sustained remission after treatment withdrawal. However, histological disease activity and M65 in the current study were not significantly different, when cut-off values suggested by Hartl et al. (ALT < 50% ULN and IgG < 12 g/L) were applied (data not shown).

The CK-18 fragment M65 > 305 IU/ml showed good sensitivity to detect incomplete histological remission, with very high NPV for the exclusion of false-negative cases. M65 is measured with a commercial ELISA assay and can be implemented easily by any laboratory to monitor histological treatment response in AIH. However, the low specificity and PPV of a M65 value >305 U/L cannot substitute a histological disease monitoring, eg for the evaluation of the end of therapy. Nonetheless, M65 measurements might be included in the armamentarium of the noninvasive monitoring tools for the treatment surveillance of AIH in addition to parameters (ALT and IgG) used in everyday clinical practice for the estimation of disease activity (Figure S2) along with TE measurements for fibrosis assessment. Thereby, M65 could facilitate a preselection of AIH patients for surveillance biopsies: Normal ALT, IgG and M65 < 305 U/L may indicate complete histological remission of AIH, while a constellation of normal ALT and IgG with M65 > 305 U/L lowers the possibility rate of complete histological remission to ≤60%.

The strengths of the study are the multicentre database, the recruitment of samples from three prospective biorepositories for an orphan disease within the international AIH group and the ‘European Reference Network for Hepatological Diseases’, and the clear focus on patients with BR. Although the diagnostic fidelity of M65 to exclude a persistent histological disease activity is good to very good, the database for the association of M65 with another clinical endpoint, the prediction of relapse after withdrawal of therapy, is insufficient to draw a definite conclusion. However, this is a matter of ongoing collection of patients. But since only one third of patients who received a surveillance biopsy were actually weaned from therapy, a longer recruitment is necessary to reach statistical robust patients numbers for a training and validation cohort.

In summary, CK-18 cell death marker M65 could be a promising sensitive, noninvasive surveillance tool for the individual guidance of a long-term therapy in AIH in the majority of patients who reach BR. M65 may help to reduce surveillance liver biopsies in AIH patients with a good treatment response.
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CONFLICTS OF INTEREST

All authors disclose any potential conflicts (financial, professional or personal) that are relevant to the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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