Keratin 18
in Alcohol-associated Liver Disease

The dead cells still count!

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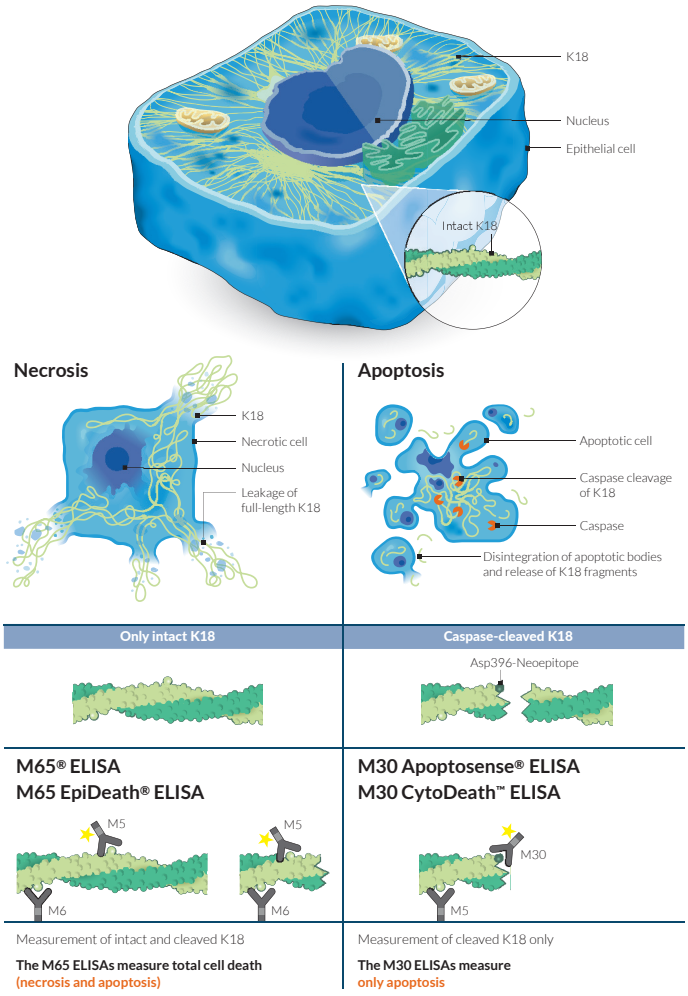
Introduction to Alcohol-associated Liver Disease

Alcohol-associated Liver Disease (ALD) encapsulates a continuum of liver conditions instigated by excessive alcohol intake. This spectrum includes alcohol-associated steatosis, alcoholic hepatitis (AH), fibrosis, and cirrhosis, each escalating in severity and potential fatality. ALD manifests as a leading cause of liver-related morbidity and mortality worldwide, with its progression often covert until critical stages. Traditional diagnosis often relies on liver biopsies, which are invasive and carry risks. Non-invasive biomarkers like M65® and M30® offer a promising alternative for early as well as late-stage diagnosis and management of ALD.

ALD's impact on public health is profound, with escalating incidence rates mirroring rising alcohol consumption trends globally. The silent nature of ALD's progression underscores the necessity for enhanced diagnostic methodologies to identify and intervene in the disease's early phases, potentially reversing its course or mitigating severe outcomes.

Understanding the VLVbio Keratin 18 biomarker assays

Keratin 18 (K18) serves as a biomarker for epithelial cell turnover and liver damage, important in evaluating ALD's severity. K18 leaks from hepatocytes in two main forms to the bloodstream: the full-length form indicative of overall cell death, measured by the two M65® ELISA assays, and the caspase-cleaved fragment (ccK18), a specific marker of apoptosis, assessed by the M30 Apoptosense® ELISA assay. The differential detection of these K18 forms offers insights into the underlying liver pathology, distinguishing necrosis and apoptosis. The hepatocellular release of K18 and ccK18 fragments into the circulation upon cell death makes these markers valuable in diagnosing and assessing liver conditions such as ALD.



Clinical Uses of the M30® and M65® biomarker assays in ALD

Diagnosing Early ALD:

The ability of M65® to differentiate between various stages of ALD non-invasively is of significant clinical relevance. It can potentially reduce the reliance on liver biopsies, offer earlier detection of at-risk patients, and facilitate timely therapeutic interventions. The studies suggest that integrating M65® with other non-invasive tests could enhance the diagnostic accuracy for ALD, particularly in asymptomatic patients or those in early stages of disease progression.

Chalin *et al.* (2023):

- Study aimed at evaluating the accuracy of the M65 EpiDeath® ELISA for diagnosing AH in patients undergoing alcohol withdrawal.
- Found that the M65 EpiDeath® ELISA alone had a diagnostic accuracy with an area under the curve (AUC) of 0.82 (test cohort) and 0.90 (validation cohort), which increased to 0.93 and 0.94 respectively when combined with other clinical markers such as transient elastography, alpha-2-macroglobulin, and body mass index. Using two cut-off decision points, M65® was able to classify 46.9% (test cohort) and 34.5% (validation cohort) of patients with 95% sensitivity or specificity.
- Established a scoring system that incorporated M65® and other markers to improve diagnostic precision for AH.

Maccioni *et al.* (2023):

- Investigated the role of the M65® ELISA in identifying early forms of ALD in a cohort of patients with alcohol use disorder (AUD).
- Demonstrated that M65® ELISA levels could distinguish minimal liver disease from early ALD with high accuracy (AUC = 0.8704), proposing an optimal cut-off value of 265.9 U/L for detecting early ALD, shown in **Figure 1**.
- This study highlighted the biomarker's ability to correlate with histological signs of hepatocellular injury and inflammation, further supporting its use in clinical settings.

Combining the findings from these studies presents an argument for the use of M65® as a non-invasive biomarker in the early diagnosis of ALD. The M65® shows high sensitivity and specificity, making it a candidate for early screening of at-risk patients. This approach could minimize the need for invasive liver biopsies, facilitate earlier and more effective intervention, and ultimately improve prognosis for patients with alcohol-related liver damage.

Early ALD vs Minimal liver disease

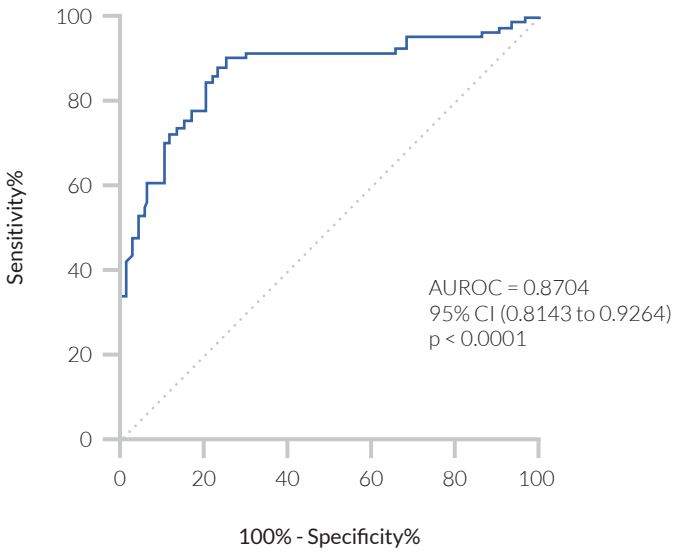


Fig. 1 ROC curves for M65® in detecting early ALD vs minimal liver disease in a training cohort and further increased to AUROC 0.9414 (95% CI 0.8870 to 0.9959. p < 0.0001) in the validation cohort. Adapted from Maccioni *et al.* Alcohol, clinical & experimental research, 2023.

Detecting Severe AH:

Severe AH is a critical condition that necessitates immediate and accurate diagnosis for effective management and treatment. The M30® and M65® ELISAs serve as important biomarkers for diagnosing severe AH. Several studies have investigated their utility, revealing that K18 correlates highly with the degree of liver damage and inflammation, making the biomarkers effective for both diagnosing and gauging the severity of AH.

Bissonnette *et al.* 2017:

- This research assessed the diagnostic performance of K18 in a cohort undergoing evaluation for alcoholic hepatitis, which often progresses to severe AH if untreated. This study confirmed high levels of M30 Apoptosense® and M65 EpiDeath® values in patients with severe disease. It highlighted that plasma levels of these biomarkers were significantly elevated in patients with severe AH compared to those without, supporting their use in clinical settings.
- The study also emphasized the biomarkers' capacity to predict disease severity, which is critical for determining the urgency and type of treatment required. M65 EpiDeath® and M30 Apoptosense® both had AUROCs of 0.84 to estimate the presence of AH.
- For the M65 EpiDeath®, a cutoff of 2000 U/L had a positive predictive value of 91%, while a cutoff of 641 U/L had a negative predictive value of 88%, shown in **Figure 2**.

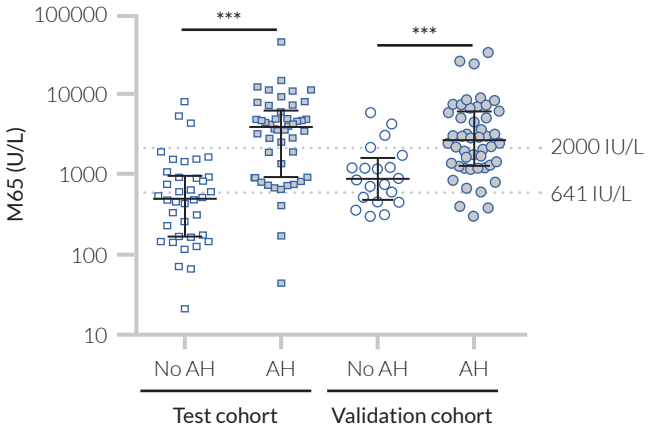


Fig. 2 Plasma levels of M65® were higher in biopsy-confirmed AH compared to non-AH samples. Similar results were shown for M30® levels. Adapted from Bissonnette *et al.* Hepatology, 2017.

Incorporating the M30® and M65® biomarker assays into the diagnostic workflow for patients suspected of severe AH can significantly enhance diagnostic accuracy and patient care. These biomarkers offer a non-invasive, reliable method for detecting severe disease, guiding treatment decisions, and potentially improving outcomes by facilitating earlier and more targeted therapeutic interventions.

Prognosis for Survival in Severe AH Patients:

Predicting patient outcomes in AH, particularly survival prospects, is critical for determining treatment urgency and scope. The M65® and M30® levels provide significant prognostic value.

Vatsalya *et al.* 2020:

- This study confirmed the utility of K18 in predicting mortality in patients with acute AH, a condition that often progresses to severe AH if untreated. Both M30® and M65® showed high prognostic accuracy for short-term mortality (60-day and 90-day), with their levels being substantially elevated in patients who did not survive.
- M30® and M65® serum levels identified patients who died within 90 days with greater accuracy than common prognostic scores such as MELD, ABIC, GAHS, or Maddrey's DF.

Woolbright *et al.* 2017:

- Focused on the use of K18 as a prognostic marker in severe AH, Woolbright *et al.*'s study demonstrated that the ratios of M30®/M65® were particularly indicative of prognosis. Elevated ratios were associated with increased mortality within 30 days, as shown in **Figure 3**.
- M65 EpiDeath® values (cutoff of 8,403 U/L) and the M30®/M65® ratio (cutoff of 0.3884) are capable of predicting early-stage mortality. M30®/ M65® ratio is a more specific and sensitive prognostic marker than ABIC or MELD (90% spec & 86% sens).

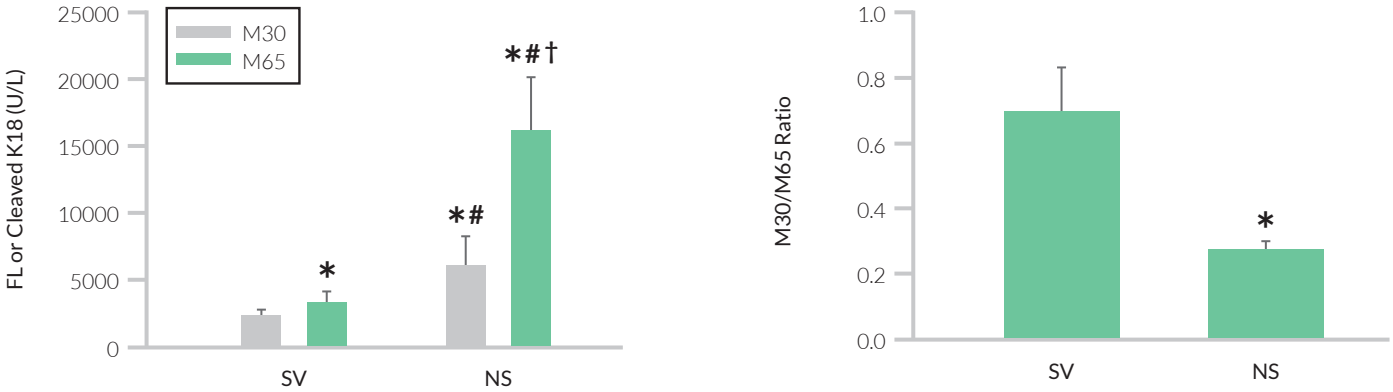


Fig. 3 M65 EpiDeath® ELISA and M30®:M65® ratio in 90-day surviving (SV) and non-surviving (NS) patients. Adapted from Woolbright *et al.* Gene Expression, 2017.

M30® and M65® serve as robust prognostic biomarkers in severe ASH, correlating strongly with 60-day and 90-day mortality. These biomarkers enhance the accuracy of prognosis and their inclusion in clinical protocols can significantly impact patient management, leading to more effective care strategies.

Predicting Treatment Response to Corticosteroids:

For severe AH patients, the only available pharmaceutical treatment is through corticosteroids, a treatment that comes with high risks of infectious side effects. The effectiveness of corticosteroids in treating severe AH varies significantly among patients. Identifying which patients are likely to respond favourably to corticosteroid treatment is a critical clinical decision, one that can be aided by utilizing the K18 biomarkers.

Atkinson *et al.* 2020:

- Atkinson *et al.* explored the correlation between serum ccK18 levels and the response to prednisolone treatment in severe AH, shown in **Figure 4**. They found that patients with higher serum M30® levels (>5k U/L) exhibited a statistically significant therapeutic benefit from prednisolone, with an odds ratio indicating reduced mortality (OR: 0.433) compared to those with lower K18 levels, where prednisolone treatment was potentially harmful.
- This study demonstrated that M30® and M65® could be used to stratify patients based on their likelihood of benefiting from corticosteroid therapy. Application of this cutoff in practice could restrict prednisolone usage to around 13% of patients—maximizing benefit while dramatically reducing the risk of steroid-related complications.

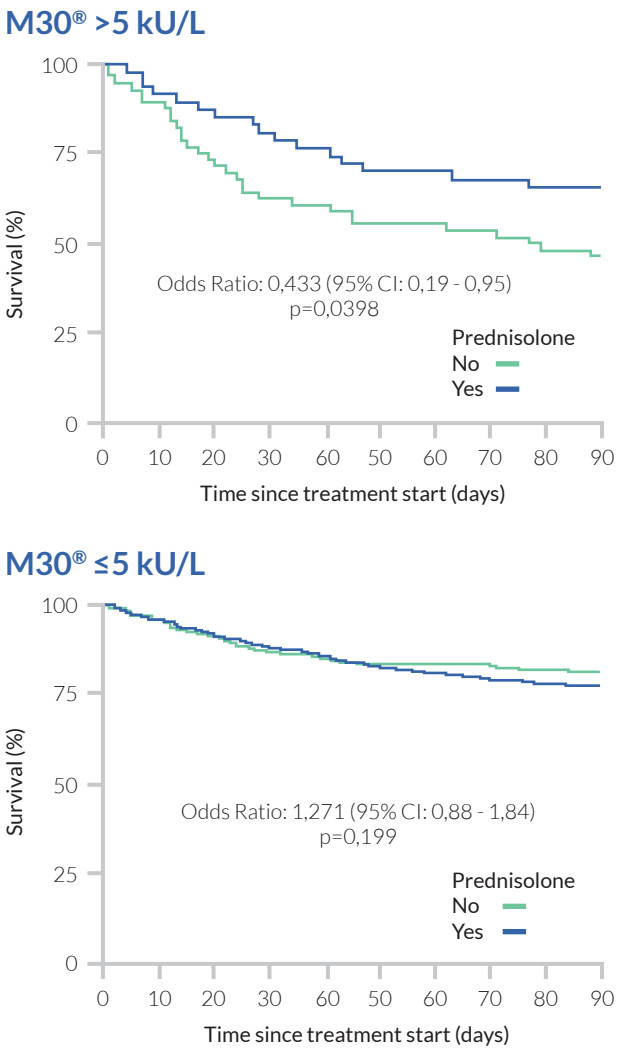


Fig. 4 M30® levels predicting therapeutic benefit from prednisolone treatment. Adapted from Atkinsson *et al.* Am J Gastroenterol, 2020.

Implementing VLVbio Keratin18 Biomarkers in Clinical Practice

The transition from research findings to clinical application involves establishing clear guidelines and standardized protocols for K18 biomarker use. The work by Vatsalya *et al.* (2023) emphasizes the importance of setting reference ranges for M65® and M30® levels that correspond with specific ALD stages and outcomes. Understanding the nuances of what elevated M65® and M30® levels signify in the context of ALD can enhance diagnostic accuracy, facilitate early intervention, and inform ongoing patient management. Below in **Figure 5** is a collection of cut-off values from clinical research papers which help lay an important foundation to provide perspective when utilizing K18 biomarkers in clinical practice.

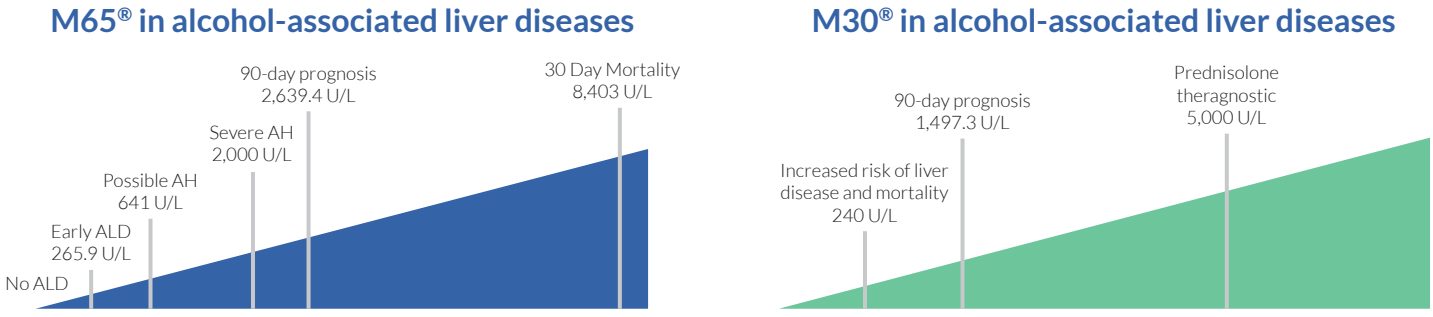


Fig. 5 Compilation of M65® and M30® cut-off values published in different clinical studies. Note that the figure includes cut-off values using both M65® and M65 EpiDeath®, which can differ depending on which assay is used.

VLVbio™ Product Line	
ELISA Products	**Prod. No**
M30 Apoptosense® ELISA	10011
M30 CytoDeath™ ELISA	10900
M65® ELISA	10020
M65 EpiDeath® ELISA	10040
M65 EpiRat™ ELISA	10060

How to order

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For More Information:

For healthcare providers seeking more information on K18 biomarkers and their application in ALD and access the detailed findings from the referenced studies, please contact VLVbio.

The information provided in this brochure aims to support and enhance the clinical management of ALD through the use of K18 biomarkers. It is part of our commitment to advancing healthcare through research, education, and collaborative practice.

The VLVbio K18 assays are CE IVD marked in Europe, not for clinical or diagnostic use in the US.

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