Plasma-Signature-Model for End-Stage Liver Disease Score to Predict Survival in Severe Alcoholic Hepatitis

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BACKGROUND & AIMS: Severe alcoholic hepatitis (AH) is a highly lethal condition and it is still a challenge to predict the outcome. We previously identified and validated a composite score of hepatic 123-gene prognostic signature and the model for end-stage liver disease (MELD) score: gene signature–MELD. However, the need for liver biopsy limits its clinical application. Therefore, we aimed to identify a plasma protein–based surrogate of the gene signature and independently validate its prognostic capability.

METHODS: All patients were diagnosed with severe AH at Cliniques universitaires de Bruxelles Hôpital Erasme (Brussels, Belgium), and the plasma samples were collected at admission before any treatment. The primary outcome was death or liver transplantation within 90 days. Using our computational pipeline, named translation of tissue expression to secretome (TexSEC), a hepatic-transcriptome–based prognostic signature was converted to a plasma-based secretome signature, which was optimized in 50 patients by comparing their hepatic molecular dysregulation status and combining it with the MELD score. The composite score was validated independently in 57 patients.

RESULTS: The TexSEC and optimization process identified a 6-plasma-protein panel as a surrogate for the 123-gene signature. A composite score with the MELD score, the plasma-signature (ps)-MELD score, was created by using the coefficients of the gene signature–MELD equation. In the validation cohort, the high-risk ps-MELD (n = 23; 40%) was associated significantly with death or liver transplantation within 90 days (adjusted hazard ratio, 4.57; 95% CI, 2.15–9.30; P < .001). The ps-MELD score showed a stable, high prognostic association (time-dependent area under receiver operating characteristics curve, >0.80) and was well calibrated over time; it consistently outperformed existing clinical scores as indicated by various model performance indices.

CONCLUSIONS: The high-risk ps-MELD score was associated with short-term survival in patients with severe AH.

Keywords: Severe Alcoholic Hepatitis; Biomarker; Death; Liver Transplantation.

Severe alcoholic hepatitis (AH) is a highly lethal condition with a 3-month mortality rate of up to 50%. Corticosteroids have been the only available medical therapy that mitigates short-term mortality in a subset of patients who respond to the therapy despite its limited applicability owing to increased risks of infection and gastrointestinal bleeding. Early liver transplantation may be an effective therapy in patients with a poor prognosis, although this procedure is resource-demanding and restricted to highly selected patients. Thus, prognostic prediction before initiating any treatment will improve therapeutic decision making significantly by identifying patients at a high mortality risk to guide indications of existing and future experimental therapies.

Several clinical/histologic prognostic scores have been proposed, including the model for end-stage liver disease (MELD) and Lille score, but their prognostic capability is not satisfactory or requires initiation of corticosteroids to observe a 7-day response for survival prediction. To enable accurate pretreatment prognostic prediction, we reported a composite score of a hepatic

Abbreviations used in this paper: AH, alcoholic hepatitis; aHR, adjusted hazard ratio; gs, gene-signature; MELD, model for end-stage liver disease; ps, plasma-signature.

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transcriptomic signature and MELD score, the gene-signature (gs)-MELD score, which predicts both short- and long-term survival in treatment-naïve severe AH and outperforms existing clinical scores.\(^4,5\) However, the requirement of a liver biopsy specimen diminishes its clinical applicability. Here, we have implemented a blood-based surrogate of the prognostic hepatic transcriptomic signature and developed the plasma-signature (ps)-MELD score.

**Methods**

**Patients**

All patients included in this study were recruited from Cliniques universitaires de Bruxelles Hôpital Erasme (Brussels, Belgium) and had the same following criteria. All patients were chronic alcohol consumers defined as consuming more than 60 g/d for males and more than 40 g/d for females, had onset of jaundice within 90 days of enrollment, a serum bilirubin level greater than 5 mg/dL, and fewer than 60 days of abstinence before the onset of jaundice. Histologic confirmation of AH was performed in all patients by a transjugular liver biopsy. Histologic criteria included the presence of steatosis, ballooned hepatocytes, Mallory bodies, with infiltration of polymorphonuclear neutrophils,\(^6,7\) and all had a Maddrey’s discriminant of 32 or greater.\(^8\) In the optimization cohort (n = 50), patients were recruited between July 2006 and December 2013.\(^4\) The validation set included 57 patients who were recruited between January 2014 and December 2019. Written informed consent was obtained from all individuals, and the Ethical Committee at CUB Hôpital Erasme approved the study.

**Studied Clinical Variables and End Points**

The following values were collected or calculated at blood collection (day 0) unless otherwise specified: age, sex, international normalized ratio, bilirubin level (on day 0 and day 7), albumin level, serum creatinine level, Maddrey’s discriminant function, and MELD scores. The Lille score was calculated on day 7.\(^9\)

The primary outcome was death or liver transplantation, and follow-up time was defined as the period from the date of blood sample collection to 90 days.\(^10\)

**Plasma Samples**

Plasma samples were collected at admission before corticosteroid initiation, aliquoted, and stored immediately after blood collection at -80°C until use. Analysis of archived deidentified samples and clinical information was approved as an exempt study (category 4) by the institutional review board (approval: STU 062018-058).

**Derivation of Alcoholic Hepatitis–Associated Prognostic Liver Proteome Candidates**

We considered proteins in molecular pathways associated with the tissue transcriptome signature in addition to proteins encoded by the 123-gene signature member genes. To systematically identify the relevant pathways in an unbiased manner, we surveyed 2261 gene sets of well-defined molecular pathways from the Molecular Signature Database (MSigDB, v7.2)\(^11\) using gene set enrichment analysis in the data sets,\(^12\) including 4 independent cohorts of 154 patients with AH extracted from in-house and publicly available data sets\(^4,13,14\)

Enrichment of each pathway gene set in each cohort was assessed on rank-ordered genes by correlation with that of the prognostic gene signature in each patient. The enrichment of each pathway gene set across the cohorts was synthesized using the Fisher inverse chi-square statistic\(^15\) (random permutation, test-based, false-discovery rate, <0.10). For each of the associated pathways, proteins encoded by shared leading-edge genes contributing to the enrichment as well as their putative upstream signals were added to the list of candidate proteins.

**Conversion of a Tissue-Based Gene Expression Signature to a Blood-Based Secretome Signature**

To convert the 123-gene prognostic signature member genes and their relevant proteins to a blood-based secretome signature, we used our integrated bio-informatical pipeline, translation of tissue expression to secretome.\(^16\)

**Multiplex Plasma Protein Assay**

Among the computationally identified plasma proteins, validated proteins available for a multiplex assay were implemented in a Food and Drug Administration–approved multiplex clinical diagnostic technology, xMAP platform (Luminex, Austin, TX), and run on the Bio-Plex 200 systems (Bio-Rad, Hercules, CA) at the University of Texas Southwestern BioCenter according to the manufacturer’s protocol. The abundance of each protein was measured as the median fluorescent intensity corrected for background signals from negative control probes and normalized to built-in dilution series of positive control probes as the standards in each 96-well assay plate.

**Definition of Semiquantitative Score and Plasma Signature Model for End-Stage Liver Disease Score**

To minimize the influence of the potential variation in the measurements and ensure the robust prognostic
performance of the assay in a clinic, we converted the continuous protein abundance values (i.e., normalized median fluorescent intensity) into high or low abundance by top quintile cut-off value in the optimization set, and calculated a semiquantitative score as follows:

$$\sum_{i=1}^{6} \begin{cases} 1 & \text{for high abundance of} \\ -1 & \text{for high abundance of} \\ 0 & \text{otherwise} \\ \end{cases}$$

The high-risk of plasma signature–based prediction was defined by a cut-off value to maximize the concordance with that of gene expression–based prediction. By using regression coefficients in the original gs-MELD score, ps-MELD was defined as follows: ps-MELD score = $1.102 \times (1 \text{ if high-risk prediction by plasma signature prediction or } 0 \text{ if otherwise}) + 0.102 \times$ MELD score, with the original cut-off value of greater than 2.66 to designate high-risk prediction.4

### Statistical Analysis

For time-to-event analyses, prognostic associations of the clinical variables and ps-MELD were assessed using Kaplan–Meier curves and multivariable Cox regression modeling. The risk-predictive performance of ps-MELD was assessed and compared with that of established scores using the integrated Brier score, Harrell's c-index, Royston's D index,17 and time-dependent areas under the receiver operating characteristics curve. The integrated Brier score is an indicator of overall model performance integrating discrimination and calibration simultaneously and is defined as the area under the prediction error curve, for which lower values indicate better overall model performance.14 Higher values for the D index indicate greater discrimination, in which an increase of 0.1 over other risk scores is a good indicator of improved prognostic separation.15 Calibration of high- and low-risk ps-MELD was assessed by plotting predicted and observed survival probability at 30 and 90 days. The observed and predicted survival rates were calculated by the Kaplan–Meier method and Cox regression model, respectively. All statistical analyses were performed using the R (version 4.0.3) statistical language (www.r-project.org).

### Results

#### Computational Derivation and Implementation of Plasma-Protein–Based Prognostic Assay

We identified 212 pathways (22 poor-prognosis– and 190 good-prognosis–associated pathways) associated with the 123-gene prognostic transcriptomic signature (Supplementary Table 1). Among genes in the pathways and the signature, 846 protein-encoding genes (143 poor-prognosis– and 703 good-prognosis–associated genes) were modulated transcriptionally in association with the transcriptomic signature. These genes were translated into 274 candidate proteins (41 poor-prognosis– and 233-good-prognosis–associated proteins) potentially secreted into circulation by the translation of tissue expression to secretome pipeline.16

#### Optimization and Construction of the Plasma Signature Model for End-Stage Liver Disease

Among the candidate proteins, validated antibodies available for multiplex assay, including 8 proteins, were implemented in the xMAP assay and tested in the optimization set (Figure 1). We observed significant correlations of 6 candidate plasma proteins (75%) with the tissue 123-gene signature status, including 3 poor-prognosis–associated proteins (interleukin-1–receptor-like 1, lymphocyte cytotoxic protein 2, and anti-leukoproteinase) and 3 good-prognosis–associated proteins (collagen $\alpha$-1[I] chain, protein S, and thrombospondin-2) (Figure 2A). Interleukin-1–receptor-like 1, a decoy receptor of interleukin 33, was reported as a prognostic biomarker in severe AH,13 supporting the validity of our unbiased approach. Reduction of the collagen $\alpha$-1[I] chain (a good-prognosis–related protein) in association with poor prognosis likely reflects acute-on-chronic liver failure, in which the physiological wound-healing fibrogenic response is impaired together with other homeostatic functions of the liver owing to severe AH. The high risk of the 6-protein semiquantitative score was defined as greater than 0 based on the concordance with that of gene expression-based prediction. The final 6-protein panel yielded a concordant prognostic prediction with the hepatic tissue transcriptomic signature from our previous study with statistical significance (Fisher exact test, $P = .014$) (Figure 2B). Using the optimized plasma protein signature assay, we constructed the ps-MELD score as follows:

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**Figure 1.** Study design. TexSEC, translation of tissue expression to secretome.
ps-MELD score = 1.102 × (1 if poor prognosis prediction by 6-protein signature prediction or 0 if otherwise) + 0.102 × MELD score. A cut-off value of greater than 2.66 was used to distinguish patients with a poorer prognosis compared with the rest, as we previously reported.4

In the optimization set, 19 deaths and 2 liver transplantations were observed during the follow-up evaluation. A high ps-MELD score (n = 21; 42%) was associated significantly with short-term prognosis (adjusted hazard ratio [aHR], 5.14; 95% CI, 2.02–13.1) in the optimization set (Figure 2C). Of note, prognostic performance of ps-MELD score was comparable with that of the gs-MELD score and consistently outperformed the MELD score over time (Figure 2D), indicating successful optimization of the ps-MELD score.

Validation of the Plasma Signature Model for End-Stage Liver Disease

Demographics of patients in the validation set are summarized in Table 1. The 6-protein panel shows a clear pattern (Figure 3A). In the validation set, 24 deaths and 7 liver transplantations were observed during the follow-up evaluation. The high-risk ps-MELD (n = 23; 40%) was associated significantly with death or liver transplantation within 90 days (aHR, 4.57; 95% CI, 2.15–9.30; P < .001) (Figure 3B). The time-dependent area under the receiver operating characteristics curve (AUROC) of ps-MELD, gs-MELD, and MELD in the optimization set. aHR, adjusted hazard ratio; FDR, false-discovery rate; gs-MELD, gene-signature–model for end-stage liver disease; ps-MELD, plasma-signature–model for end-stage liver disease.
Discussion

We successfully developed and validated a blood-based noninvasive biomarker for precise prediction of 90-day prognosis in severe AH. The ps-MELD score will substantially improve the care of patients by informing a likelihood of high mortality before initiating any therapy, unlike the Lille score, which requires 1 week of corticosteroid treatment. It will enable refined indication of liver transplantation at an early phase of care. In addition, the member proteins of the plasma signature associated with pathogenesis of severe AH may serve as a rational therapeutic target to be monitored by the ps-MELD score. Although the prognostic performance is fairly consistent throughout the process of the initial gMELD/ps-MELD score derivation and validation, the current study still was a single-center evaluation. Further multicenter/regional validation is warranted for its

Table 1. Demographics of the Optimization and Validation Sets

<table>
<thead>
<tr>
<th>Variable</th>
<th>Optimization set (n = 50)</th>
<th>Validation set (n = 57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>53 (47–60)</td>
<td>53 (46–56)</td>
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<tr>
<td>Male sex, n (%)</td>
<td>32 (64)</td>
<td>35 (59)</td>
</tr>
<tr>
<td>Corticosteroid therapy, n (%)</td>
<td>50 (100)</td>
<td>28 (53)</td>
</tr>
<tr>
<td>INR, median (IQR)</td>
<td>1.92 (1.69–2.31)</td>
<td>1.85 (1.61–2.23)</td>
</tr>
<tr>
<td>Bilirubin level, mg/dL, median (IQR)</td>
<td>11.0 (6.7–22.5)</td>
<td>16.0 (9.3–23.0)</td>
</tr>
<tr>
<td>Creatinine level, mg/dL, median (IQR)</td>
<td>0.8 (0.6–1.1)</td>
<td>0.8 (0.7–1.6)</td>
</tr>
<tr>
<td>Albumin level, mg/L, median (IQR)</td>
<td>26.5 (23.0–28.8)</td>
<td>26.0 (24.0–29.0)</td>
</tr>
<tr>
<td>ps-MELD score, median (IQR)</td>
<td>2.50 (2.14–3.54)</td>
<td>2.65 (2.24–4.17)</td>
</tr>
<tr>
<td>MELD score, median (IQR)</td>
<td>24 (21–27)</td>
<td>24 (22–23)</td>
</tr>
<tr>
<td>Maddrey score, median (IQR)</td>
<td>55.9 (47–84)</td>
<td>59.8 (42.3–79.3)</td>
</tr>
<tr>
<td>Lille score, median (IQR)</td>
<td>0.30 (0.15–0.60)</td>
<td>0.17 (0.10–0.59)</td>
</tr>
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</table>

INR, international normalized ratio; IQR, interquartile range; ps-MELD, plasma signature–model for end-stage liver disease.

Figure 3. Validation of the ps-MELD score. (A) Pattern of the 6-protein signature abundance and associated clinical variables. (B) Association of ps-MELD with survival. The hazard ratio was adjusted for sex, age, and use of corticosteroids. (C) Time-dependent area under the receiver operating characteristics curve (AUROC) of ps-MELD and clinical scores. (D) Calibration plot of ps-MELD at 30 and 90 days. The grey dashed line indicates ideal calibration. aHR, adjusted hazard ratio; MDF, Maddrey’s discriminant function; MELD, model for end-stage liver disease; ps-MELD, plasma-signature–model for end-stage liver disease.


Table 2. Prognostic Performance of ps-MELD and other Clinical Prognostic Scores Within 90 Days in the Validation Cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>ps-MELD score</th>
<th>MELD score</th>
<th>Maddrey score</th>
<th>Lille score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroid-treated (n = 28)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Overall (n = 57)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Integrated Brier score (95% CI)</td>
<td>2.88 (1.88–4.39)</td>
<td>1.18 (1.11–1.28)</td>
<td>1.18 (1.01–1.03)</td>
<td>1.40 (1.12–1.75)</td>
</tr>
<tr>
<td>Harrell’s c-index (95% CI)</td>
<td>0.79 (0.70–0.88)</td>
<td>0.73 (0.62–0.83)</td>
<td>1.84 (1.44–2.81)</td>
<td>1.43 (0.65–2.40)</td>
</tr>
<tr>
<td>ps-MELD score (95% CI)</td>
<td>0.136 (0.066–0.217)</td>
<td>0.139 (0.068–0.296)</td>
<td>0.175 (0.166–0.203)</td>
<td></td>
</tr>
<tr>
<td>Model performance&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 (0.70–0.86)</td>
<td>0.77 (0.67–0.86)</td>
<td>0.73 (0.62–0.83)</td>
<td></td>
</tr>
<tr>
<td>Prognostic association&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.11 (0.68–4.78)</td>
<td>2.11 (0.68–4.78)</td>
<td>1.22 (0.65–3.60)</td>
<td>1.27 (0.38–3.16)</td>
</tr>
</tbody>
</table>

- aHR, adjusted hazard ratio; CI, confidence interval; MELD, model for end-stage liver disease; ps-MELD, plasma signature model for end-stage liver disease.
- Integrated Brier score: 0.575 for ps-MELD, 0.856 for MELD, 0.790 for Maddrey, 0.514 for Lille.
- Harrell’s c-index: 0.79 for ps-MELD, 0.77 for MELD, 0.77 for Maddrey, 0.73 for Lille.
- ps-MELD score: 2.88 (1.88–4.39) for corticosteroid-treated, 0.136 (0.066–0.217) for overall.
- Model performance: 0.77 (0.70–0.86) for corticosteroid-treated, 0.77 (0.67–0.86) for overall.
- Prognostic association: 2.11 (0.68–4.78) for corticosteroid-treated, 1.22 (0.65–3.60) for overall.

Clinical deployment and widespread implementation as standard care. The ps-MELD also may serve as a selection biomarker to identify severe AH patients with a high likelihood of poor prognosis to be enrolled in clinical trials of experimental therapies with fewer participants and higher statistical power compared with the traditional all-comer trials.

**Supplementary Material**

Note: To access the supplementary material accompanying this article, visit the online version of Clinical Gastroenterology and Hepatology at [www.cghjournal.org](https://www.cghjournal.org), and at [https://doi.org/10.1016/j.cgh.2021.02.041](https://doi.org/10.1016/j.cgh.2021.02.041).

**References**


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Conflicts of interest
This author discloses the following: Yujin Hoshida serves as an advisory board member for Helio Health and founding share holder for Alentis Therapeutics, and has received research funding from Morphic Therapeutics. The remaining authors disclose no conflicts.

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