Research Article

Impact of Hemolysis on UCH-L1 Quantification in Serum and Plasma

Paula García Martín[1*], Silvia Álvarez Rodríguez[1], Covadonga Solar Macarro[1], Rafael Venta Obaya[1] Eduardo Martínez-Morillo[1]

[1]*Department of Biochemistry San Agustin University Hospital, Camino de Heros, Avilés, Asturias, Spain*

Article Info

[*]***Corresponding Author:***
Paula García Martín
Department of Biochemistry, San Agustin University Hospital,
Camino de Heros, 6, 33401, Avilés, Asturias, Spain
E-mail: paula.garciammamar@sespa.es

Keywords

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Abstract

Introduction: Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is a neuron-enriched deubiquitinase widely used for the evaluation of mild traumatic brain injury (TBI). Although minimal hemolysis-related interference has been reported for the Abbott TBI assay, routine laboratory observations indicate that hemolysis may substantially influence UCH-L1 measurements. This study aimed to evaluate the effect of hemolysis on UCH-L1 concentrations in plasma and serum samples and its potential clinical implications.

Methods: Hemolysis interference was evaluated in accordance with CLSI C56-A and EP07 guidelines using pooled plasma samples and paired individual patient samples. Controlled hemolysis was induced by adding graded amounts of erythrocyte hemolysate to non-hemolyzed samples. UCH-L1 concentrations were measured using the Abbott Alinity i system. In addition, a retrospective analysis of routine TBI biomarker requests was conducted to estimate the potential rate of hemolysis-induced false-positive results.

Results: In pooled plasma, UCH-L1 increased linearly (R^2 = 0.992) with the hemolysis index (HI), with a 10% analytical bias observed at HI values of 100 or lower. Erythrocyte-derived samples showed UCH-L1-related signals more than 20-fold higher than plasma. In individual patient samples, hemolysis induced a dose-dependent but highly variable positive bias. Retrospective analysis suggested that hemolysis could result in false-positive UCH-L1 results in up to 21.5% of TBI-negative patients with concentrations near the clinical cut-off.

Conclusions: Hemolysis induces a clinically relevant positive bias in UCH-L1 measurements, even at moderate HI levels. These findings underscore the importance of strict preanalytical control and cautious interpretation of UCH-L1 results obtained from hemolyzed samples.

Abbreviations: CT: Computed Tomography; CV: Coefficient of variation; GFAP: Glial Fibrillary Acidic Protein; HI: Hemolysis Index; I: Interfered; Int (%): Percentage of interference; NI: Non-interfered; TBI: Traumatic Brain Injury; UCH-L1: Ubiquitin Carboxy-Terminal Hydrolase L1; UCH-L3: Ubiquitin Carboxy-Terminal Hydrolase L3.

Introduction

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is a member of the ubiquitin C-terminal hydrolase family of deubiquitinases, which also includes UCH-L3, UCH-L5, and BRCA1 Associated Protein 1. Its primary function is to hydrolyze ubiquitin from small C-terminal extensions, and it is highly expressed in neurons, with lower expression in gonadal tissue [1,2]. Due to its neuronal enrichment, UCH-L1 was identified in the early 2000s as a promising biomarker for traumatic brain injury (TBI). In combination with glial fibrillary acidic protein (GFAP), UCH-L1 is currently used in clinical practice to aid in the evaluation of patients with suspected mild TBI (Glasgow Coma Scale score of 13–15). Large validation studies have demonstrated that this biomarker panel can reduce the number of unnecessary cranial computed tomography (CT) scans by approximately 40%, thereby limiting radiation exposure and long-term risks associated with imaging [3].
Abbott's TBI assay is an immunoassay panel for in vitro diagnostics used to measure GFAP and UCH-L1 in human plasma and serum, with established cut-offs of 35 and 400 pg/mL for GFAP and UCH-L1, respectively. This assay aids in the evaluation of patients aged over 18 years old with suspected mild TBI within 12 hours of injury. The results help determine whether a cranial CT scan is required. A result exceeding either threshold is considered positive and supports the need for CT evaluation [4].
According to the Abbott package insert (H22846R01, B4Z233, created in 2021) for TBI assay, hemolysis does not produce a significant interference (≤10% bias) in UCH-L1 measurement at hemoglobin concentrations up to 1,000 mg/dL, which are generally considered equivalent to a hemolysis index (HI) of approximately 1,000 arbitrary units [4]. Despite this manufacturer's claim, our laboratory observations suggest that hemolysis may influence measured UCH-L1 concentrations. To further investigate this potential source of analytical variability, the present study was designed to assess the effect of hemolysis on plasma and serum UCH-L1 concentrations using both pooled and individual samples.

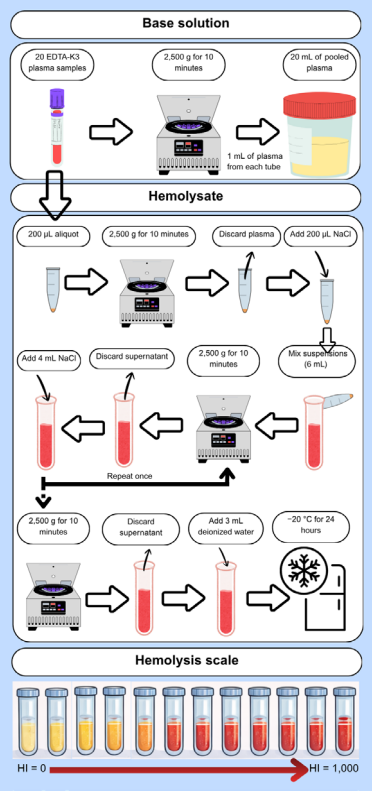
Material and Methods

The procedure for the evaluation of endogenous interferences was adapted from the CLSI C56-A and EP07 guidelines [5,6], with minor modifications.

Pooled sample preparation

A total of 20 EDTA-K3 plasma samples (3 mL purple-top tubes, VACUETTE®) from primary care patients undergoing routine complete blood count testing (presumably healthy individuals) were collected within 24 h of venipuncture, prior to disposal. Only samples visually assessed as non-hemolyzed and with volumes close to the maximum tube capacity (3 mL) were selected. As samples were anonymized immediately after collection by removing all identifying labels prior to specimen handling, written informed consent was not required. This study was approved by the Research Ethics Committee of the Principality of Asturias (approval number: 2025.386).
The UCH-L1 assay is manufacturer-validated for serum and plasma (EDTA-K2/K3 and lithium heparin) [4]. EDTA-K3 plasma was selected for the pooled experimental phase for practical reasons: only samples no longer required for clinical care were deliberately chosen for research use, and hemolysate preparation requires whole blood prior to centrifugation. Unlike serum and lithium heparin samples, which are processed immediately after collection with plasma already separated from the cellular fraction, EDTA tubes are the only collection format routinely stored as whole blood.
Each of the 20 tubes was inverted 10 times to ensure homogenization. Subsequently, to obtain the base solution, the samples were centrifuged at 2,500 g for 10 minutes. From each tube, 1 mL of plasma was collected and pooled into a sterile container, resulting in a total volume of 20 mL of pooled plasma, which served as the base solution for all subsequent experiments (Figure 1).

Figure 1: Schematic representation of pooled plasma preparation and the development of the hemolysis scale.



To obtain the hemolysate, a 200 µL aliquot of whole blood was collected from each EDTA-K3 tube and transferred to a 1.5 mL Eppendorf tube. Following centrifugation (2,500 g, 10 min), the plasma (100 µL, approximately) was discarded, and 200 µL of isotonic saline (NaCl 154 mmol/L) was added to each cellular pellet. The twenty resulting suspensions (300 µL, approximately) were gently mixed and subsequently pooled into a 15 mL Falcon® conical tube, yielding a final volume of approximately 6 mL. This procedure minimized the risk of hemolysis due to ABO incompatibility. Then, the pooled cell suspension was centrifuged (2,500 g, 10 min). The supernatant was discarded, and the pellet was washed twice with 4 mL of isotonic saline solution. After each wash, the suspension was centrifuged again (2,500 g, 10 min) and the supernatant was completely removed. Subsequently, 3 mL of deionized water was added to the washed pellet to induce cell lysis, and to achieve a HI of 10,000 arbitrary units. The resulting lysate was homogenized and stored at −20 °C for 24 hours. After thawing, the hemolysate was thoroughly mixed and centrifuged at 2,500 g for 30 minutes to remove cellular debris (Figure 1).

Two sample types were prepared using the base solution and the hemolysate:

Non-interfered (NI) sample, consisting of 9 mL of base solution mixed with 1 mL of deionized water.

Interfered (I) sample, consisting of 9 mL of base solution mixed with 1 mL of the hemolysate (HI = 10,000).

Twelve samples were prepared by mixing defined volumes of the NI and I base solutions. Serial combinations of these two matrices yielded samples with estimated HI values ranging from 0 (no hemolysis) to approximately 1,000.

To assess assay precision in the presence of hemolysis, a pooled sample was prepared from 7 EDTA-K3 plasma specimens using the standardized hemolysis protocol, targeting HI of approximately 0, 250, 500, and 1000. HI and UCH-L1 concentrations were measured in quintuplicate for each sample, with imprecision expressed as the coefficient of variation (CV). UCH-L1 concentrations were determined using a chemiluminescent microparticle immunoassay on the Abbott Alinity i module, whereas HI were measured on the Alinity c module using spectrophotometric detection of free hemoglobin, with absorbance readings taken at four wavelength pairs (500/524, 572/604, 628/660, and 524/804 nm) [7].

The degree of interference (Int (%)) was expressed as the percentage difference between the I and NI samples, calculated as Int (%) = 100 * (Ci - C0) / C0, where Ci and C0 represent the measured concentrations in each I and NI samples, respectively. A maximum allowable error of 10% was used as the criterion to define the acceptable limit of interference.

Selection and processing of individual hemolyzed samples

According to our standard operating procedure for sample rejection, a new blood sample is requested whenever the HI measured in plasma or serum exceeds 500. For this study, 20 samples were randomly selected–15 lithium heparin plasma

samples and 5 serum samples–with HI values above 500, for which a second, non-hemolyzed sample was obtained within one hour of the initial sample collection.

Unlike the pooled experimental phase, lithium heparin plasma and serum were used in this phase, as these are the most processed matrices in our laboratory. This allowed hemolysis interference to be assessed under real-world conditions using clinically rejected, hemolyzed samples, each paired with a non-hemolyzed sample from the same patient.

After routine analyses were completed, 700 µL of each sample (600 µL for mixture preparation and 100 µL to account for dead volume) was transferred into 6 mL polystyrene tubes. Only samples from patients without requests for brain trauma markers were included, to avoid potential physiological increases in these markers during the interval between sample collections. Additionally, samples were excluded if less than 500 µL of non-hemolyzed serum or plasma remained available for potential further analyses. All samples were fully anonymized prior to analysis.

Paired non-hemolyzed and hemolyzed samples from each patient were combined to produce four graded levels of hemolysis. The volume of non-hemolyzed serum or plasma was progressively decreased from 300 µL to 0 µL, while the volume of the corresponding hemolyzed sample was increased from 0 µL to 300 µL, resulting in mixtures with hemolyzed fractions of approximately 0%, 33%, 67%, and 100%. UCH-L1 concentrations and HI were measured in each mixture, and the Int (%) was calculated.

To verify the manufacturer's claim of assay suitability across serum, EDTA plasma and lithium heparin plasma, a matrix comparison study was performed. Blood samples were collected from three healthy volunteers in all three tube types, and the standardized hemolysis protocol was applied to generate both non-hemolyzed (HI ≈ 0) and hemolyzed (HI ≈ 1,000) conditions. UCH-L1 concentrations and HI values were measured in triplicate, and differences among matrices were evaluated by repeated-measures analysis of variance (ANOVA) using MedCalc software.

Analysis of Results from Patients with a Routine Request for TBI Markers

Data from patients for whom the UCH-L1 test was requested at our hospital between August 2024, and July 2025 were retrospectively retrieved from the laboratory information system. The dataset was analyzed to assess the potential impact of hemolysis on the occurrence of false-positive UCH-L1 results and its possible implications for clinical decision-making in TBI.

Only samples corresponding to requests for TBI biomarkers were included. To minimize the influence of severe preanalytical interference, analyses were restricted to samples with a HI below 100. No clinical records were reviewed, and no personal or identifiable patient data were accessed or collected.

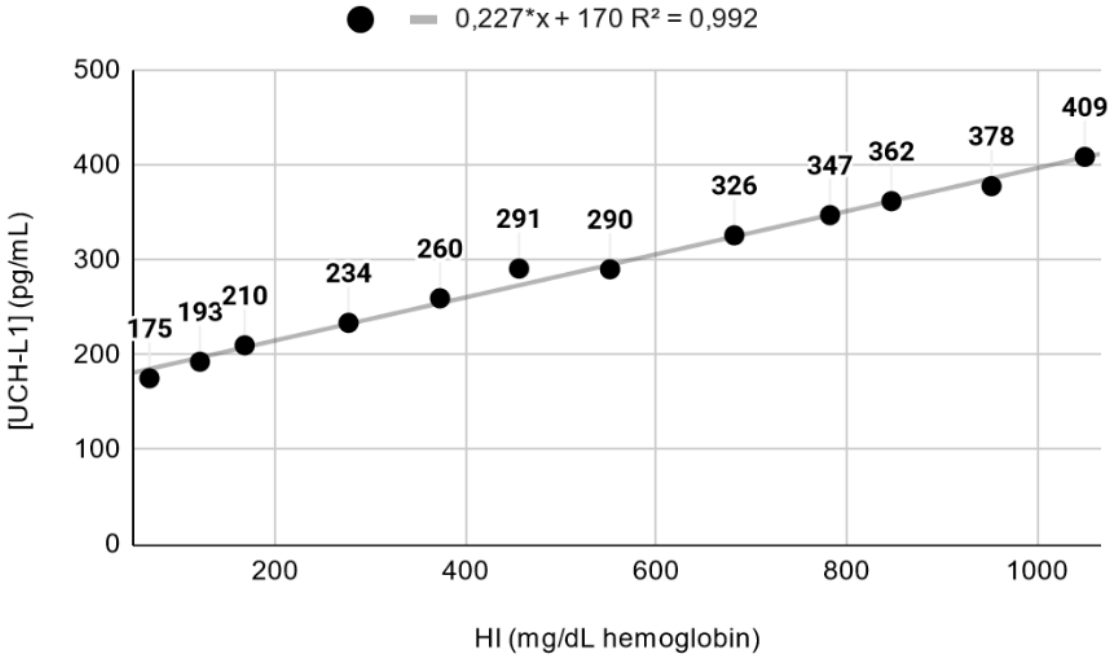
Results

Pooled samples

In the pooled plasma experiment evaluating imprecision, the mean HI values across quintuplicates were 27, 240, 447, and 867, with corresponding mean UCH-L1 concentrations of 196, 305, 399, and 556 ng/L. CVs for UCH-L1 were 6.1%, 8.9%, 5.1%, and 8.6%, respectively, showing no apparent association with the degree of hemolysis, while CVs for HI remained below 3% in all cases. Fitting a quadratic model to the mean values, a 10% increase in UCH-L1 concentration was estimated at HI = 63, and an absolute concentration of 400 ng/L, corresponding to the manufacturer's recommended decision threshold for ruling out brain injury, was estimated at HI = 525. Nearly identical estimates were obtained when the model was fitted using only the first individual measurement from each quintuplicate (HI = 63 and 523, respectively).

In the 20 pooled plasma experiment, UCH-L1 concentrations increased linearly with increasing HI (Figure 2). The regression equation was UCH-L1 = 0.227 (95% confidence interval: 0.212-0.242) x HI + 170 (95% confidence interval: 161-179); with an R^2 value of 0.992. The baseline UCH-L1 concentration at HI = 68 was 175.4 pg/mL. According to this model, a 10% deviation from baseline (corresponding to an increase of approximately 17 pg/mL) would be reached at an HI of 101.

Figure 2: Linear regression of UCH-L1 (pg/mL) concentration versus hemolysis index (HI) in pooled plasma. The line represents the best-fit linear model, illustrating the dose-dependent increase of UCH-L1 with increasing hemolysis.



Assuming a linear increase in UCH-L1 concentration of 0.227 pg/mL per HI unit, we evaluated which HI values would result in UCH-L1 concentrations exceeding 400 pg/mL, depending on the initial baseline concentration. Samples with baseline concentrations below 175 pg/mL did not exceed 400 pg/mL within the HI range tested; samples with baseline concentrations between 175 and 300 pg/mL exceeded 400 pg/mL at intermediate HI values (approximately 400–1,000); and samples with baseline concentrations above 300 pg/mL consistently exceeded 400 pg/mL when HI values were greater than 440 (Table 1).

Table 1: Estimated hemolysis index (HI) required for a sample to exceed the clinical ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) threshold of 400 pg/mL, based on a linear increase in UCH-L1 concentration with hemolysis (0.227 × HI).

Baseline UCH-L1 concentration (pg/mL)	Required increase in UCH-L1 (pg/mL)	Required HI (mg/dL hemoglobin)
150	250	1,101
175	225	991
200	200	881
250	150	661
300	100	441
350	50	220
375	25	110

For each baseline UCH-L1 concentration, the corresponding HI value required to reach 400 pg/mL was calculated. Samples with baseline UCH-L1 concentrations below 175 pg/mL required very high HI values (> 950) to exceed the decision threshold, whereas samples with baseline concentrations of ≥ 300 pg/mL surpassed 400 pg/mL at moderate levels of hemolysis (HI < 450). The calculated increase in UCH-L1 above baseline (shown in the middle column) represents the additional concentration required to reach the pathological cut-off.

The relative abundance of UCH-L1 —or the interfering substance detected by the assay— was also evaluated within erythrocytes compared with plasma. To generate a hemolysate, 2 mL of washed red blood cells were mixed with 3 mL of deionized water, resulting in a 5 mL preparation (I sample) with an approximate hematocrit of 40%, comparable to that of an average individual. The UCH-L1 concentration measured in this erythrocyte-derived matrix was 408.8 pg/mL (Figure 2). In contrast, the mean UCH-L1 concentration in non-hemolyzed patient plasma was approximately 175 pg/mL, slightly overestimated due to a baseline HI of 68 rather than 0. Based on these measurements, the intracellular-to-plasma ratio was approximately 23.5, indicating that the erythrocyte compartment contains more than 20-fold higher UCH-L1–related signal than plasma.

Individual samples

In individual patient samples, UCH-L1 concentrations increased progressively with increasing HI, confirming a positive, dose-dependent interference pattern. This trend was consistent with that observed in the pooled plasma experiment, although considerable variability among patients was evident. To better characterize this relationship, individual quadratic regression models were generated for each patient (Table 2). The quadratic model provided a superior fit compared with a simple linear model, reflecting the non-linear nature of hemolysis interference.

Table 2: Calculated hemolysis index (HI), mg/dL hemoglobin, required to increase ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) concentration by 10% and to reach 400 pg/mL, based on quadratic models.

Patient	Baseline UCH-L1 concentration (pg/mL)	Baseline HI	UCH-L1 concentration increased by 10%	HI required for 10% UCH-L1 increase	HI required to reach 400 pg/mL of UCH-L1	Quadratic equation
1	44	3	48	17	2106	43,5 + 0,259x - 4,26E-05x² =y
2*	58	0	64	13	856	58.7 + 0.405x - 7.17E−06x² =y
3*	65	11	71	40	2275	64,6 + 0,161x - 5,97E-06x² =y
4	68	1	74	13	754	66.9 + 0.549x - 1.42E−04x² =y
5	81	2	89	42	1795	78,3 + 0,257x - 7,16E-05x² =y
6*	96	1	106	39	1929	95,5 + 0,272x - 7,05E-05x² =y
7	106	1	116	26	723	105 + 0.441x - 4.59E−05x² =y
8	122	4	134	24	559	122 + 0,502x - 7,68E-06x² =y
9*	134	1	148	194	2103	134 + 0.0668x + 2.84E−05x² =y
10*	136	1	149	73	1484	139 + 0.134x + 2.82E−05x² =y
11	140	3	154	25	369	134 + 0.807x - 2.33E−04x² =y
12	142	10	156	42	775	139 + 0,41x - 9,47E-05x² =y
13	158	5	174	84	1310	156 + 0,216x - 2,27E-05x² =y
14	169	59	186	102	1009	153 + 0,341x - 1,69E-04x²=y
15	178	2	196	33	573	177 + 0.583x - 3.38E−04x²=y
16	232	110	255	171	468	171 + 0,492x - 5,63E-06x²=y
17	247	38	272	195	968	241 + 0.158x + 6.44E−06 x²=y
18	262	73	288	138	269	154 + 1,03x + -4,29E-04x²=y
19	413	54	455	95	N/A	350 + 1,13x + -2,02E-04x²=y
20	1183	164	1301	808	N/A	1206 + -0,232x + 4,33E-04x²=y

The equations were derived from individually fitted quadratic models describing the relationship between the HI and the measured UCH-L1 concentration in samples with progressively increasing hemolysis. The patients' results are shown in order of increasing baseline UCH-L1 concentration.

**Serum samples (*); remaining samples are lithium heparin plasma.*

N/A: not appicable

Baseline UCH-L1 concentrations among patients ranged from 44 to 413 pg/mL, with one patient exhibiting an exceptionally high concentration of 1,183 pg/mL. Calculated UCH-L1 concentrations corresponding to a 10% deviation from baseline varied from 48 to 455 pg/mL, with an outlier sample of 1,301 pg/mL (patient previously mentioned). The HI required to produce a 10% change in UCH-L1 concentration varied between 13 and 195 for most patients, except for one patient, for whom it was 808. Finally, the HI necessary to raise UCH-L1 above the cut-off value of 400 pg/mL (resulting in a false-positive) ranged from 269 to 1,000 in 10 of the 20 patients (50% potential false-positives), while it exceeded 1,000 in the remaining patients, except for patients 19 and 20, whose baseline UCH-L1 concentration was already over 400 pg/ml.

In the matrix comparison study evaluating assay suitability across serum, EDTA plasma, and lithium heparin plasma, the mean HI was 8 in non-hemolyzed samples and 1144 in hemolyzed samples. Under non-hemolyzed conditions, mean UCH-L1 concentrations for the three volunteers were 44.5, 69.8, and 101 ng/L, rising to 308.1, 318.0, and 451.8 ng/L under hemolyzed conditions. No statistically significant differences among matrices were observed under either condition ($p > 0.05$).

Analysis of Results from Patients with a Routine Request for TBI Markers

During the study period, 986 patients presenting with mild TBI underwent assessment, and TBI markers were requested. Among them, 260 patients showed concentrations of both markers (GFAP and UCH-L1) below their respective cut-off values (35 and 400 pg/mL, respectively), with 56 of these "TBI-negative" patients exhibiting UCH-L1 concentrations in the range of 300–400 pg/mL. Based on our regression model, all these patients would have had UCH-L1 concentrations above 400 pg/mL if their sample HI had exceeded 440. This indicates that analyzing hemolyzed samples (HI > 440) from these patients would have resulted in a 21.5% false-positive rate among all patients with a negative TBI result (56 of 260).

Discussion

The linear relationship observed between UCH-L1 concentration and HI demonstrates that increasing hemolysis contributes to higher UCH-L1 values, which can substantially affect the clinical interpretation of this biomarker in plasma and serum. In pooled plasma, the baseline UCH-L1 concentration was 175.4 pg/mL, in close agreement with previous reports indicating that basal levels in patients with a negative TBI are generally below 200 pg/mL [4,8,9] using Alinity ci. This consistency supports the validity of our pooled model. Sample matrix (serum, EDTA plasma or lithium heparin plasma) does not appear to be a determining factor in hemolysis-induced interference, and the use of single measurements rather than replicates does not meaningfully affect the estimates obtained, as these remained consistent with those derived from the quintuplicate determinations.

According to the relationship [UCH-L1] ≈ 0.227 × HI, the degree of hemolysis required for UCH-L1 concentrations to exceed the clinical threshold of 400 pg/mL was evaluated. For baseline UCH-L1 concentrations below 175 pg/mL, only severe hemolysis (HI > 950) could result in falsely elevated values above the threshold; however, samples with such high hemolysis would be rejected. In contrast, when the baseline UCH-L1 concentration exceeded 300 pg/mL, hemolysis levels of approximately 450 were sufficient to raise the calculated concentration above 400 pg/mL (Table 1). These results indicate that the susceptibility to hemolysis-induced false-positives depends strongly on both baseline UCH-L1 concentration and the degree of hemolysis.

Applying the manufacturer's recommended allowable error of 10%, an interference threshold at HI ≈ 100 (corresponding to UCH-L1 = 193 pg/mL) was identified. Interestingly, the manufacturer's insert reports no interference from hemoglobin up to 1,000 mg/dL [4]. In contrast, our findings indicate that beyond HI ≈ 100, hemolysis can meaningfully affect UCH-L1 measurement accuracy. This observation is supported by the FDA memorandum on the Banyan Brain Trauma Indicator, which reported that hemoglobin produced statistically significant interference beyond the prespecified acceptance limit of 500 mg/dL, while showing no interference at concentrations up to 62.5 mg/dL [10].

From a clinical perspective, these findings are relevant because UCH-L1 is increasingly used to guide imaging decisions in TBI. In our laboratory, the clinical decision limit for recommending CT imaging is 400 pg/mL [4,11]. According to the regression model (UCH-L1 = 0.227 x HI + 170), an HI greater than approximately 1,013 would be necessary to raise a baseline UCH-L1 concentration above this pathological threshold, potentially resulting in false-positive interpretations and unnecessary imaging. At such extreme levels of hemolysis, samples should be rejected and recollected; however, lower HI values may be problematic when other published thresholds are applied. For example, the decision cut-offs proposed by Papa et al. (2022), Karamian et al. (2025), and Bazarian et al. (2018, 2021)—189, 225, 327, and 360 pg/mL, respectively [10,12–14]—would correspond to HI values of approximately 84, 242, 692, and 837 according to our model. This suggests that even moderate hemolysis could artifactually elevate UCH-L1 concentrations above these clinical cut-offs, potentially generating false-positive results. It should be noted that these cut-offs were derived using ELISA-based assays in all studies except for Bazarian et al. (2021), who employed the i-STAT Alinity system [14], which has been seen to be highly correlated to Alinity ci [15]. However, these studies differ not only in their proposed clinical cut-offs but also in their analytical methodologies. As shown by the 2024 SEQC-ML external quality assessment for serum indices, different

analysers yield non-identical HI values even when measuring equivalent samples [16]. Such heterogeneity limits the direct comparability of absolute UCH-L1 values and interference thresholds across studies. Nevertheless, regardless of the analytical approach used, our findings highlight that hemolysis can shift UCH-L1 values toward commonly used decision limits, potentially leading to misclassification. This may not only increase healthcare costs but also expose patients to unnecessary radiation and delay appropriate clinical decision-making.

This study also demonstrates that the effect of hemolysis on UCH-L1 measurements is highly variable among patients, and that individual hemolysis–response curves can differ substantially even under identical analytical conditions. Baseline UCH-L1 concentrations ranged from 44 to 413 pg/mL in most individuals, with one outlier reaching approximately 1,183 pg/mL (Table 2). Patients with low baseline UCH-L1 concentrations showed the greatest susceptibility to hemolysis. In these cases, small increases in HI were sufficient to exceed the 10% error threshold, making their results particularly vulnerable to clinical distortion. This supports a proportional relationship in which lower initial UCH-L1 concentrations require smaller increments in HI to generate unacceptable analytical error.

The observed increase in UCH-L1 concentration with rising hemolysis may be explained by several, non-mutually exclusive mechanisms. Immunoassays might be particularly susceptible to hemolysis-related interference, as red blood cell–derived components, including free hemoglobin and intracellular proteases, may degrade target antigens or disrupt antibody–antigen binding, thereby altering signal generation [17]. However, the interference observed may not be directly attributable to free hemoglobin itself, as the manufacturer reports no significant effect at concentrations up to 1,000 mg/dL, but rather to other components released from intraerythrocytic content during hemolysis, which is consistent with the reported impact of non-specific analytical factors such as fibrin, erythrocyte remnants, platelets, or other suspended particles on assay performance [4]. Alternatively, hemolysis may lead to a true release of UCH-L1 or UCH-L1–like proteins from blood cells. Given that UCH-L3 shares approximately 52% sequence homology with UCH-L1, cross-reactivity was considered as a potential contributor [2]; however, the Banyan Brain Trauma Indicator decision memorandum [10], as well as the Abbott package insert [4], reported no detectable cross-reactivity between UCH-L3 and the UCH-L1 assay, making immunological cross-detection an unlikely explanation for the observed bias. Regardless of the underlying mechanism, our data clearly demonstrate that hemolysis is a major pre-analytical variable for UCH-L1 quantification.

Overall, the patient-level analyses indicate that hemolysis interference is neither linear nor uniform and is influenced by both baseline UCH-L1 concentrations and the specific curvature of each HI–UCH-L1 relationship. These findings emphasize that UCH-L1 results from hemolyzed samples must be interpreted with caution, as the magnitude of interference can range from negligible to clinically significant and may bias diagnostic decision-making. The data analysis highlights that even moderate hemolysis can shift UCH-L1 concentrations toward the decision cut-off and generate false-positive results, potentially affecting patient classification in diagnostic algorithms.

To our knowledge, this is the first study to describe hemolysis interference in UCH-L1 measurement at such a low HI. According to Puravet A. et al., UCH-L1 exhibits a positive bias exceeding 10% at HI of 400, identifying this value as analytically relevant [18]. However, their study did not evaluate lower hemolysis ranges due to technical limitations in HI measurement. In contrast, our analysis specifically investigated lower HI values using patient-derived samples and demonstrates that a ≥10% positive bias can already occur at HI ≈ 100, indicating that clinically meaningful interference arises well before the previously reported limit.

Limitations

Limitations include the small number of pooled and individual samples and the focus on a single analytical method. Further studies should evaluate larger, more heterogeneous cohorts, and aim to establish consensus interference thresholds for UCH-L1.

Conclusion

We have demonstrated that hemolysis produces a strong, dose-dependent positive bias in UCH-L1 concentrations, with even moderate hemolysis capable of altering values sufficiently to affect clinical interpretation, particularly at lower thresholds. The linear relationship observed in pooled plasma, together with the high erythrocyte enrichment of UCH-L1 (or the interfering substance) and the potentially substantial proportion of false-positive results in patient samples attributable to hemolysis, underscores the biomarker's marked vulnerability to red blood cell lysis. Consequently, unrecognized hemolysis may lead to false-positive classifications and unnecessary CT imaging in patients with mild TBI. These findings highlight the need for systematic hemolysis detection, assay-specific interference thresholds, and stringent sample quality criteria to ensure the reliable clinical application of UCH-L1.

Research ethics

The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was granted by the Research Ethics Committee of the Principality of Asturias (September 2025, ID: 2025.386)

Informed consent

Not applicable.

Author contributions

All authors have accepted responsibility for the entire content

of this manuscript and approved its submission.

Paula García Martín: Investigation, Validation, Formal analysis, Writing - Original Draft, Visualization.
Silvia Álvarez Rodríguez: Validation, Writing - Review & Editing.
Covadonga Solar Macarro: Validation, Writing - Review & Editing.
Rafael Venta Obaya: Writing - Review & Editing.
Eduardo Martínez Morillo: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing - Review & Editing, Supervision.

Use of Large Language Models, AI and Machine Learning Tools
None declared.

Conflict of interest
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