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Performance of Relative Exchangeable Copper for the Diagnosis of Wilson Disease in Acute Liver Failure

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ABSTRACT

Acute liver failure (ALF) can be one of the manifestations of Wilson disease (WD), and due to its severity, prompt diagnosis is essential. A ratio > 15% of the exchangeable copper to total serum copper, known as relative exchangeable copper (REC), has been shown to have a 100% sensitivity and specificity for the diagnosis of WD but this has not yet been studied in an ALF setting. Patients diagnosed with ALF from 1 November 2011 to 31 December 2023, with available REC determination during the acute event, were included. Thirty-three patients were included (11 with WD and 22 without WD). The median age [IQR] at ALF was 12.9 [8.9–20.2] years, range: 0.6–71.0 years. Serum ceruloplasmin (Cp) < 0.20 g/L and 24 h urinary copper excretion > 1.6 μmol/L had both a sensitivity (Se) and specificity (Sp) for the diagnosis of WD of 100% and 72.7%, respectively. A ROC analysis of REC determined that the best cut-off point was 14.4% (AUC 1, $p < 0.01$). All the WD patients had REC values > 14.4%, yielding a sensitivity and specificity of 100. Relative exchangeable copper has 100% sensitivity and specificity for diagnosing Wilson disease in acute liver failure. Relative exchangeable copper has excellent performance in diagnosing Wilson disease in acute liver failure.

1 | Introduction

Wilson disease (WD) [1] is an autosomal recessive genetic disorder caused by biallelic mutations in the *ATP7B* gene. It is characterised by copper accumulation, mainly in the liver and the brain, caused by impaired biliary copper elimination. The diagnosis is based on a pattern of clinical signs, laboratory results, and genetic analysis [2, 3].

The clinical presentation of this disease can be heterogeneous but mainly includes acute or chronic liver disease and neuropsychiatric manifestations. Acute liver failure (ALF) can be the first

Abbreviations: 24 h UCE, 24 h urinary copper excretion; ALF, acute liver failure; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cp, ceruloplasmin.; CuEXC, exchangeable copper; GGT, gamma-glutamyl transferase; INR, international normalised ratio; NCC, non-ceruloplasmin-bound copper; NPV, negative predictive value; PPV, positive predictive value; PT, prothrombin time; REC, relative exchangeable copper; Se, sensitivity; Sp, specificity; TB, total bilirubin; WD, Wilson disease.

manifestation of WD in 10% to 36.5% of cases, according to the series [4], and represents about 3%–6% of paediatric and adult cases of ALF [5, 6]. This presentation is often fatal without liver transplantation, but timely diagnosis of WD and treatment could prevent this outcome. The prognosis of WD-ALF cases can be assessed using validated scores [7, 8] that have been developed to identify patients who will probably not respond to treatment and require liver transplantation, but even in these cases, a timely and appropriate treatment might avoid liver transplantation [9, 10]. Diagnosis of WD is also essential for family screening.

There are a few laboratory findings that are suggestive of WD in an ALF setting, such as Coombs-negative intravascular haemolysis, comparatively low serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and significantly low serum alkaline phosphatase (ALP), but these are not systematically present, particularly in children [5]. The utilisation of the Leipzig score [11] that is standardly recommended to confirm a WD diagnosis is limited in this particular setting given that some parameters necessary to calculate it can be either difficult to obtain (e.g., hepatic copper quantification) or are only available with a significant delay (e.g., genetic studies).

Therefore, several scores have been developed to improve the diagnosis of WD in an ALF context, but their performance is variable according to the studies and less valid for paediatric cases of WD-ALF [4, 5, 12].

The calculated non-ceruloplasmin-bound copper (NCC) has been historically used to monitor the evolution of WD, but it has never been validated for diagnosis. Several limitations have been raised against the utilisation of this method [13], particularly its dependence on the method employed for the ceruloplasmin determination, which can sometimes lead to negative and hard-to-interpret values.

A different assay to directly determine the NCC is known as the exchangeable copper (CuEXC) and represents the labile portion of copper bound to albumin and other peptides but not to ceruloplasmin.

The ratio of the CuEXC to total serum copper is known as Relative exchangeable copper (REC). The REC has demonstrated its utility in the diagnosis of WD [14]. In a previous study, we reported that both the sensitivity and specificity of the REC for the diagnosis of WD were 100%, comparing cohorts of adults and children with WD with other hepatic diseases in a chronic and stable setting, thus not including any case of ALF [1]. The present study aims to evaluate the performance of the REC in diagnosing WD in ALF.

2 | Methods

For this retrospective study, the medical records of adult and paediatric patients admitted to either the Lyon or Besançon university hospitals (France) with the diagnosis of ALF between November 2011 and December 2023 were reviewed; patients with available determination of REC within a week of ALF diagnosis were included.

ALF diagnosis was established in the study centres in line with current guidelines according to age. For patients < 18 years of age, the Pediatric Acute Liver Failure Study Group criteria were used: acute onset of symptoms, biochemical evidence of acute liver injury, liver-derived coagulopathy defined as prothrombin time (PT) ≥ 15 s or international normalised ratio (INR) ≥ 1.5 uncorrectable by parenteral administration of vitamin K in the presence of hepatic encephalopathy; or PT ≥ 20 s or INR ≥ 2.0 regardless of the presence or absence of clinical encephalopathy. For patients ≥ 18 years of age, ALF was defined as new onset liver disease characterised by elevated INR > 1.5 and hepatic encephalopathy in absence of any known liver disease [15].

Demographic, clinical, and laboratory data were collected from medical records. The Leipzig score was applied retrospectively once all biochemical and molecular parameters were available to correctly classify the patients in the WD group (score ≥ 4 and genetic confirmation) or the non-WD group (score < 4). All WD patients had a genetic confirmation of the diagnosis. Patients with an indeterminate ALF aetiology also underwent a genetic panel test, including the most common variants of WD.

CuEXC was measured according to the method described by El Balkhi et al. [14]. Briefly, blood samples were collected in BD Vacutainer Serum tubes (Becton Dickinson, Ref 368 815) and centrifuged. Serum was then mixed with a solution of EDTA (8.9 mM, 1:1), vortexed, incubated for an hour, and then ultrafiltered using Amicon Ultra-4R centrifugal filters with a 30-kD cut-off to retain ceruloplasmin. CuEXC was then determined using inductively coupled plasma mass spectrometry (ICP-MS NexION 350; PerkinElmer, Waltham, MA, USA). Total serum copper was measured using ICP-MS, and the REC (%) was calculated as the ratio of CuEXC to serum total copper. Quantitative determination of serum ceruloplasmin concentration was performed using an immunoturbidimetric assay (Architect c8000; Abbott, Abbott Park, IL, USA).

2.1 | Statistical Analysis

Qualitative variables are presented as relative frequency (percentage), as well as mean and standard deviation (SD) for normally distributed quantitative variables and median (interquartile range, IQR) for non-normally distributed variables.

Comparisons were made using the Mann–Whitney non-parametric test using SPSS software version 23.0 (IBM Corp. Armonk, NY, USA). The sensitivity, specificity and positive and negative predictive values were calculated for all the relevant variables. The receiver operating characteristic (ROC) analysis was performed when necessary to determine the best cut-off. $p < 0.05$ was considered significant.

2.2 | Ethics

The local research ethics committee approved this study, approval number 23-5295.

Written informed consent was obtained from all participants or their legal guardians.

3 | Results

A total of 33 patients were included in the study; 11 patients had a confirmed diagnosis of WD and 22 had other hepatic diseases. Among the total population, 45.5% were female; among the WD group, 63.6% were female. In the total population, the median [IQR] age at ALF was 12.9 [8.9–20.2] years, range 0.6–71.0, and there was no significant difference according to whether the aetiology was WD or not (Table 1). There were 6/11 (54.5%) patients < 18 years old at ALF in the WD group and 18/22 (81.8%) in the non-WD group.

Aetiologies of ALF in the non-WD group were autoimmune hepatitis ($n = 8$), viral hepatitis ($n = 2$), acetaminophen hepatotoxicity ($n = 2$), Budd-Chiari syndrome ($n = 1$), sickle cell disease ($n = 1$), homocystinuria ($n = 1$), metastatic hepatoblastoma ($n = 1$), and indeterminate aetiology in the remaining six patients.

Regarding laboratory parameters, between patients included in the WD and non-WD, there were significant differences in the median levels of AST 130 [100–149] U/L versus 1892 [662–3335] U/L, $p < 0.01$; ALT 72 [50–108] U/L versus 1257 [482–4038] U/L, $p < 0.01$; GGT 169 [78–218] U/L versus 60 [37–100] U/L, $p < 0.05$; and ALP 121 [90–296] U/L versus 323 [175–427] U/L, $p < 0.05$; Table 1.

Five (45.5%) patients included in the WD group had haemolytic anaemia, as did 1 (4.5%) patient in the non-WD group.

There were significant differences in all the parameters of copper metabolism: serum copper, ceruloplasmin levels, 24 h urinary copper excretion (24 h UCE), exchangeable copper, and REC (Table 1).

The performance for the diagnosis of WD of all the test parameters and scores is shown in Table 2.

Concerning the ceruloplasmin (Cp) levels, no WD patient had a value > 0.20 g/L, and three (27.3%) had values between 0.10 and 0.20 g/L; all the rest (72.7%) have values < 0.10 g/L. In the non-WD group, 6/22 (27.3%) patients had Cp values < 0.20 g/L, 5 (22.7%) between 0.10 and 0.20 g/L, and one (4.5%) < 0.10 g/L. Serum Cp < 0.20 g/L had a sensitivity (Se) and specificity

TABLE 1 | Demographic, clinical and laboratory characteristics.

	Total, $n = 33$	WD, $n = 11$	Non-WD, $n = 22$	
Sex, Female	15 [45.4%]	7 [63.6%]	8 [36.4%]	
Age at ALF (years)	12.9 [8.9–20.2]	15.8 [9.7–32.6]	11.3 [8.5–16.9]	
Hb (g/dL)	11.9 [8.4–13.2]	11.0 [7.7–12.4]	12.3 [8.8–13.3]	
Leucocytes (giga/L)	7.4 [4.4–10.5]	5.5 [3.7–10.9]	7.4 [4.8–11.2]	
Platelets (giga/L)	136 [99–207.5]	126 [91–216]	149.5 [99.5–204.7]	
INR	2.7 [2.2–3.5]	2.4 [1.9–3.1]	2.9 [2.3–3.5]	
Ferritin (μg/L)	167 [33–989.5]	153 [19–1088]	177 [33–1328]	
Creatinine (μmol/L)	48 [40–61.5]	48 [43–78]	44 [37–61.2]	
Albumin (g/L)	31 [24–35]	27 [23–3]	32.5 [26.4–34.2]	
Blood ammonia (μmol/L)	77.5 [52–100.7]	55 [32.7–92.5]	85 [67–103.3]	
AST (U/L)	700 [141–2531]	130 [100–149]	1892 [662–3335]	***
ALT (U/L)	489 [84–1869]	72 [50–108]	1257 [482–4038]	***
GGT (U/L)	78 [46–196]	169 [78–218]	60 [37–100]	
ALP (U/L)	261 [119–385]	121 [90–296]	323 [175–427]	**
Total bilirubin (μmol/L)	89 [34–247]	45 [19–235]	117 [55–260]	
Serum copper (μmol/L)	9.8 [5.7–16.5]	5.4 [3.3–9.3]	13.0 [8.5–17.3]	**
Ceruloplasmin (g/L)	0.19 [0.08–0.29]	0.06 [0.03–0.14]	0.23 [0.16–0.30]	***
24 h UCE (μmol/24 h)[a]	5.2 [1.1–17.4]	16.9 [6.6–34.8]	1.5 [0.6–3.7]	***
CuEXC (μmol/L)	0.6 [0.4–1.4]	2.0 [1.4–3.8]	0.5 [0.4–0.7]	***
REC (%)	5.4 [3.7–26.7]	36 [26.1–61.5]	4.2 [3.3–5.6]	***

Note: Values expressed in median [IQR].
Abbreviations: 24 h UCE: 24 h urinary copper excretion; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CuEXC: exchangeable copper; GGT: gamma-glutamyl transferase; INR: international normalised ratio; REC: relative exchangeable copper.
[a]Available for 23 patients (11 WD/12 non-WD).
**$p < 0.05$.
***$p < 0.01$.

TABLE 2 | Comparison of screening tests for WD in ALF.

	Sensitivity	Specificity	PPV	NPV
Coombs neg– hemolytic anaemia	36.4%	95.5%	80.0%	75.0%
AST/ALT > 2.2	27.3%	77.3%	37.5%	68.0%
ALP/TB < 4	45.5%	31.8%	25.0%	53.8%
ALT/AST or AP/TB combined	45.5%	31.8%	25.0%	53.8%
Ceruloplasmin < 0.2 g/L	100%	72.7%	64.7%	100%
24 h UCE > 1.6 μmol/L	100%	72.7%	64.7%	100%
CuEXC > 1.2 μmol/L	81.8%	100%	100%	91.7%
REC > 15%	100%	100%	100%	100%

Abbreviations: 24 h UCE: 24 h urinary copper excretion; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CuEXC: exchangeable copper; REC: relative exchangeable copper; TB: total bilirubin.

(Sp) for the diagnosis of WD of 100% and 72.7%, respectively (Table 2).

24 h UCE levels were available for 23 patients (11 WD/12 non-WD). 24 h UCE levels were significantly higher in the WD group, 16.9 [6.6–34.8] μmol/24 h versus 1.5 [0.6–3.7] μmol/24 h ($p < 0.05$). All the patients in the WD group had values > 1.6 μmol/24 h, and 6/12 (50%) patients of the non-WD group had values > 1.6 μmol/24 h, range 2.9–17.5 μmol/24 h. Using a cut-off of 5.29 μmol/24 h, the Se was 90.9% and Sp 84.6% (AUC 0.91 $p < 0.001$; Table 2).

The sensitivity and specificity of the alkaline phosphatase (ALP) to total bilirubin (TB) ratio < 4 for diagnosing ALF in WD were 45.5% and 31.8%, respectively. AST: ALT ratio > 2.2 yielded a sensitivity of 27.3% and a specificity of 77.3%. Combining those two ratios yielded values of 45.5% and 31.8% for sensitivity and specificity, respectively (Table 2).

Using a cut-off of > 25 μg/dL for the calculated NCC, it yielded a sensitivity of 18.2% and a specificity of 63.4% (PPV 20.0%, NPV 60.9%).

The CuEXC was significantly higher in the WD group: 2.0 [1.4–3.8] U/L μmol/l versus 0.5 [0.4–0.7] μmol/l ($p < 0.01$). A cut-off of 1.2 μmol/L had a sensitivity of 81.8% and a specificity of 100% (AUC 0.91, $p < 0.001$; Table 2).

For REC, a ROC analysis found that the best cut-off point was 14.4% (AUC 1, $p < 0.01$). All non-WD patients had REC values < 14.4%, ranging from 2.6% to 9.6%, and conversely, all the WD patients had values ≥ 14.4%, yielding both a sensitivity and specificity of 100% (Table 2 and Figure 1).

We calculated the performance of the score developed by Feng et al. [16] for paediatric ALF using 5 biochemical parameters (ALT, AST, AST/ALT ratio, ALP, and ALP/total bilirubin ratio) and age. Among the patients aged < 18 years included herein patients this score had a sensitivity of 83.3% and specificity of 77.8% (PPV 55.6%, NPV 93.3%). As an exploratory test, we also applied this score to the total population; both sensitivity and specificity were 72.7% (PPV 57.1%, NPV 84.2%).

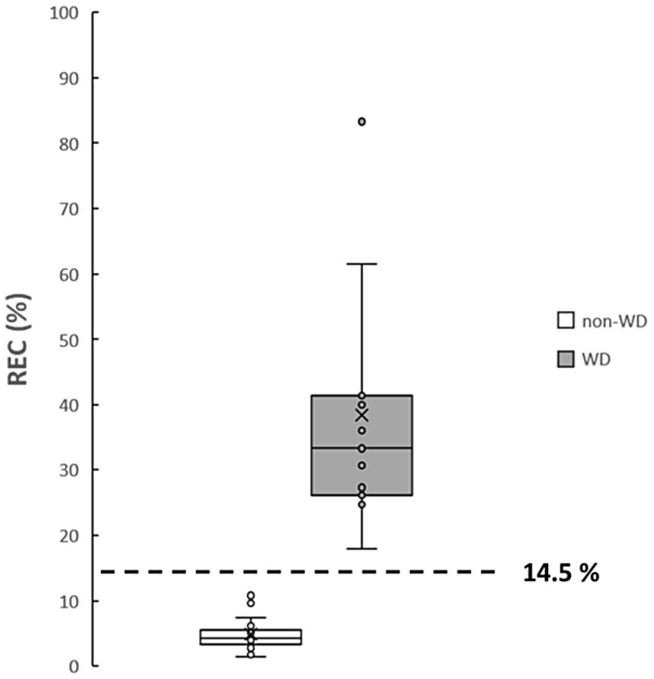

FIGURE 1 | Relative exchangeable copper values comparison between the WD-group and the non-WD group.

A total of 4/11 (36.3%) patients from the WD group underwent liver transplantation (LT); 3 F/1 M. Two of the patients had a revised King's Score ≥ 11; the other two recovered from the acute period but progressively developed chronic complications of cirrhosis, ultimately requiring LT. One patient with a King's Score ≥ 11 listed for LT recovered with chelation therapy only. Three patients in the non-WD group underwent LT, and one patient in this group died of multiple organ dysfunction and uncontrolled sepsis before LT.

4 | Discussion

Common findings that are usually associated with the diagnosis of WD, like the presence of the Kayser–Fleischer ring, low ceruloplasmin, high urinary copper excretion, or high copper liver content, can be absent, are not readily available, are not achievable, or are less specific in an ALF setting.

For instance, the Kayser–Fleischer ring's detection rate is dependent on age at presentation, with rates going from < 50% to > 85% [4, 5, 17], and moreover, it can also be found in other hepatic diseases [18].

In the present study, ceruloplasmin < 0.20 g/L had a sensitivity of 100% and a specificity of 72.7%, but in another study, it only yielded a sensitivity of 56% and specificity of 63% [12]. According to our results, a normal or high value of ceruloplasmin could help rule out the diagnosis of WD, and very low values are particularly indicative of WD.

Urinary copper excretion is rarely used to discriminate WD from other aetiologies of ALF since it is difficult to obtain; renal failure is often associated with this feature and the fact that copper excretion can be increased in other aetiologies as a consequence of hepatocyte necrosis potentially overlapping with WD [12, 19]. However, when it can be obtained, we showed that it could be a good marker, raising the cut-off to 5.29 μmol/24 h to increase the diagnostic yield; however, our assessment is limited, considering that 24 h UCE data was lacking in 10/22 patients from the non-WD group. Other paediatric studies proposed a cut-off > 2.6 μmol/24 h or > 3.8 μmol/24 h to obtain a sensitivity of 70% and 91.7%, respectively, and a specificity of 100% for both cut-offs [16, 19].

Being Coombs-negative haemolytic anaemia, a frequent characteristic of ALF-WD, haemoglobin values have been proposed as an element of a scoring system for diagnosing WD using a cut-off point of < 9.15 g/dL [19]. A paediatric study showed a 66.7% sensitivity and 78.3% specificity when using a cut-off point of < 7.7 g/dL but did not consider including it in their proposed scoring system since many factors influence the haemoglobin level, hence limiting its performance [16]. In our study, we did not find a significant difference in the haemoglobin values of both groups, but the presence of coombs-negative hemolytic anaemia was very specific to WD (95.5% specificity).

Many methods to differentiate WD-ALF from other etiologies have been proposed. Korman et al. [12] showed that the AST: ALT ratio > 2.2 had a sensitivity of 94%, a specificity of 86%, and a sensitivity of 94% as well as a specificity of 96% for the alkaline phosphatase to total bilirubin ratio < 4; moreover, a sensitivity and specificity of 100% can be reached when combining these ratios. In our study, these tests performed much less well, yielding a sensitivity and specificity of 45.5% and 31.8%, respectively, when combining the ratios. This could be explained by the important proportion of paediatric patients included in our study, particularly in the non-WD group, where they were significantly more prevalent. A meta-analysis of paediatric cases of ALF showed a sensitivity of 49% using the combined ratios [5]. These tests have the value of being readily accessible and with minimum delay, but they have a more limited value in paediatric cases.

For these reasons, two other scores were developed for paediatric cases of ALF to differentiate WD from other etiologies [16, 19]. Both comprise age and several biochemical parameters that are common: AST, ALT, AST/ALT ratio, alkaline phosphatase, and alkaline phosphatase to total bilirubin ratio. The score proposed by Gungor et al. [19] also includes haemoglobin, platelets, albumin, cholesterol, LDL-cholesterol, GGT, uric acid, and total and direct bilirubin. They seem to perform very well with a sensitivity of 88.9% [19] and 100% [16] and a specificity of 87.9% [19] and 97.8% [16]. Nevertheless, further validation will be necessary since we could not confirm the results of the score published by Feng et al. [16] in our cohort. We could not test the score proposed by Gungor et al. [19] using 14 biochemical parameters and age since some of those parameters were not included in our collected dataset.

The calculated NCC has been historically used to monitor the evolution of WD's patients, but its use for diagnosis has never been validated. A cut-off of > 25 μg/dL has been proposed as most untreated WD patients have these values [2], but this can also be the case in many other circumstances, like liver failure of any aetiology, hence limiting its value for diagnostic purposes. Our results illustrate this problem, given the inadequate performance this method showed in diagnosing WD in an ALF scenario.

The exchangeable copper and its ratio referred to serum copper, known as relative exchangeable copper (REC), have a proven value in multiple situations related to WD. The first studies showed the utility of the REC for the diagnosis of WD, where values > 14% or > 18% had 100% sensitivity and specificity [1, 14]. A more recent study from a different team evidenced that the cut-off of > 14% allowed for 100% specificity and a sensitivity of 93.8% [20]. Our findings indicate that the best REC cut-off is > 14.4%, which is in line with previous studies. We have previously published a study comparing the REC values of two WD cohorts (recently diagnosed or non-stabilised and well-controlled patients) with two groups (adults and children) of other liver diseases [1]. As stated before, this study showed that a REC value > 14% has excellent performance for the diagnosis of WD, but both groups, WD and non-WD, included either chronic patients or patients who were not in a situation of ALF. Our study is the first to evaluate this performance in an ALF setting, and it shows that it is comparable to the results obtained in previous studies analyzing chronic cases of WD.

Even though the REC is a reliable parameter to diagnose WD, the main limitations remain its availability, since it is only performed in specialised laboratories and the significant delay required to obtain it, which can vary from 3 to 7 days depending on the test's availability and the local logistical organisation. This is an extremely important matter when dealing with ALF. In contrast, genetic testing, such as rapid exome sequencing, is becoming increasingly accessible and faster, offering a potentially more efficient diagnostic alternative in many settings. Despite these advancements, CuEXC/REC determination is still of value, since it represents a direct measure of biochemical alterations associated with copper metabolism, providing functional evidence that complements genetic data or serves as a diagnostic tool in resource-limited settings where genetic testing is not yet widely accessible.

Considering the REC's current limitations, we suggest that the simpler scores previously discussed, which use basic and more readily accessible biochemical parameters, should still be used as the first exploratory tests to start treatment promptly when deemed necessary. The REC could be employed as a mid-term

confirmatory test until it becomes more available and readily accessible.

Other than its use for diagnostic purposes, the CuEXC has also demonstrated that it is correlated with extrahepatic involvement [21]; it can help assess treatment compliance [22] and is a potential marker to monitor the disease [23]. However, potential under- and overestimation of this method has been reported [24, 25].

There is a predominance of females (63.6%) in the WD group, consistent with previous adult and paediatric reports [5, 26]. It has been hypothesised that female sex hormones may contribute to the occurrence of ALF. However, a preliminary animal study points towards a more significant influence of sex hormones in the evolution rather than in the onset of acute hepatic injury [27].

The study's main limitations are the retrospective design, which restricts the correct characterisation of ALF cases, and the small number of WD included. However, the conclusions remain in line with previous publications about the relative exchangeable copper's performance in the diagnosis of WD.

5 | Conclusion

A relative exchangeable copper value ≥ 14.4% has 100% sensitivity and specificity in the diagnosis of Wilson disease in an ALF setting.

Author Contributions

Daniela Spirea: collected data, major contribution in the reporting and writing of the manuscript. **Claire Vanlemmens and Teresa Antonini:** data collection and manuscript approval. **Abdelouahed Belmalih:** data collection, regulatory submissions, and manuscript approval. **François Parant:** performed biochemical analysis, wrote, and approved the manuscript. **Muriel Bost:** performed biochemical analysis, and approved the manuscript. **Alain Lachaux, Olivier Guillaud, Jerome Dumortier:** study conception and manuscript approval. **Eduardo Couchonnal:** conceived and planned the project, performed statistical calculations, and made a major contribution in the reporting and writing of the manuscript.

Ethics Statement

The local research ethics committee approved this study, approval number 23-5295. Written informed consent was obtained from all participants or their legal guardians.

Conflicts of Interest

Eduardo Couchonnal received consulting fees, honoraria for presentations, or support to attend meetings from ORPHALAN, UNIVAR, ALEXION, and VIVET THERAPEUTICS. Olivier Guillaud received consulting fees, honoraria for presentations, or support to attend meetings from ORPHALAN, ABBVIE, GILEAD, AMGEN. Daniela Spirea1, Claire Vanlemmens, François Parant, Teresa Antonini5, Muriel Bost, Alain Lachaux, Abdelouahed Belmalih, and Jerome Dumortier declare that they have no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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