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Research Article

Clinical Utility of Serum Ceruloplasmin in the Early Identification of Wilson's Disease in Pediatric Patients with Unexplained Liver Disorders

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Abstract

Early, accurate identification of Wilson's disease in children presenting with unexplained liver dysfunction remains challenging because clinical features are heterogeneous and many routine biomarkers lack specificity. This study evaluated the clinical utility of serum ceruloplasmin for early identification of Wilson's disease among pediatric patients with indeterminate hepatobiliary presentations. A prospective diagnostic-accuracy design was implemented in a tertiary referral setting, enrolling consecutive children with unexplained liver disorders and applying standardized sampling, uniform assay methodology, and blinded reference classification. The prespecified objective was to define an age-appropriate, analytically validated ceruloplasmin threshold and quantify its incremental diagnostic value over standard liver tests. The analysis plan included ROC modeling, net reclassification improvement, and decision-curve analysis. The study demonstrated that a lower, pediatrically optimized ceruloplasmin cut-off meaningfully improved discrimination, reduced false-positive assignments in inflammatory liver disease, and retained high sensitivity in ATP7B-confirmed cases. Results and discussion emphasize statistically robust estimates with tight confidence intervals for sensitivity, specificity, and likelihood ratios, and show consistent performance across subgroups defined by acute versus chronic presentation and hemolysis status, supporting immediate clinical applicability. The conclusions argue that early integration of rigorously measured ceruloplasmin into pediatric diagnostic pathways can accelerate confirmatory testing and targeted therapy, potentially averting hepatic decompensation while minimizing unnecessary investigations. Keywords: Wilson's disease; ceruloplasmin; pediatric hepatology.

Introduction

Wilson's disease is a genetic disorder of copper metabolism with autosomal recessive inheritance that leads to toxic accumulation of copper in the liver, brain, and other tissues. In pediatrics, the initial clinical signal is frequently hepatic, yet the spectrum ranges from asymptomatic elevations of aminotransferases to acute liver failure. This variability, compounded by the overlap with common pediatric liver conditions, makes early identification difficult and invites both under- and over-diagnosis. Against this background, there is sustained interest in readily available biomarkers that can triage children with unexplained liver disease toward or away from specialized confirmatory testing without delaying therapy.1-5

Serum ceruloplasmin has long been embedded in diagnostic algorithms because dysfunctional ATP7B trafficking impairs copper incorporation into apoceruloplasmin and shortens its half-life, leading to lower circulating concentrations. Historically, a threshold near 0.20 g/L (≈20 mg/dL) has been used as a screening cut-off. However, several realities complicate its interpretation in children. First, ceruloplasmin is a positive acute-phase reactant and may normalize transiently in inflammatory states, obscuring underlying copper dysregulation. Second, concentrations are age-dependent and lower in younger children, especially under five years, which widens the overlap between affected and unaffected patients if adult cut-offs are applied. Third, immunologic assays quantify total immunoreactive protein rather than oxidase-active holo-ceruloplasmin, and analytical variation across platforms can shift measured values by clinically meaningful margins. Fourth, hepatic synthetic failure, nephrotic syndrome, malnutrition, and heterozygous ATP7B carriage can reduce ceruloplasmin independently of Wilson's disease, generating false positives if clinical context is ignored.6-10

Recent work has sharpened understanding of these constraints and has proposed refined approaches. Pediatric studies applying ROC analysis suggest lower thresholds than the adult convention can enhance specificity while preserving sensitivity, especially when pretest probability is high. Investigations of ceruloplasmin oxidase activity indicate that functional assessment may outperform immunologic quantification in some settings, although standardization remains incomplete. Decision-analytic perspectives underscore that the value of any biomarker depends on pretest probability, downstream testing availability, and the harms of misclassification. In practical terms, an informative test for pediatric Wilson's disease should

accomplish three things: concentrate true positives early enough to influence management, reduce distracting false positives in children with inflammatory or cholestatic liver disease, and integrate seamlessly with confirmatory tests such as ATP7B sequencing, 24-hour urinary copper excretion, slit-lamp examination for Kayser–Fleischer rings, and quantitative hepatic copper.

Within this landscape, a prospective evaluation of ceruloplasmin in consecutive pediatric patients with unexplained liver abnormalities addresses a tangible clinical gap. Consecutive enrollment mitigates spectrum bias, and a blinded adjudication of Wilson's disease status based on standardized criteria curbs review bias. Harmonizing preanalytical handling and analytical methods for ceruloplasmin is critical; precise timing relative to febrile illness, hemolysis, and corticosteroid exposure should be recorded, and the assay's coefficient of variation documented. Establishing an a priori pediatric threshold using ROC optimization guards against data dredging, while complementary metrics—likelihood ratios, diagnostic odds ratio, calibration plots, and decision curves—translate statistical separation into bedside relevance.

Subgroup analyses deserve particular emphasis in pediatrics. Children who present with acute hemolytic anemia or fulminant hepatic failure may display paradoxical patterns, including transient elevations of non–ceruloplasmin-bound copper with variable ceruloplasmin levels. Younger age strata, especially under eight years, may demand age-specific references. Coexisting conditions such as autoimmune hepatitis, nonalcoholic fatty liver disease, and metabolic disorders can distort ceruloplasmin dynamics and must be accounted for. Finally, any pediatric diagnostic pathway must recognize resource variability; robust single-test screening strategies hold special value where genetic testing turnaround is prolonged.

The present study was designed to quantify the diagnostic utility of serum ceruloplasmin for early identification of Wilson's disease in children with unexplained liver disorders using contemporary analytical standards and clinically relevant decision metrics. The central hypothesis was that a pediatrically optimized ceruloplasmin threshold, measured under strict preanalytical control, would deliver superior specificity at preserved sensitivity compared with the conventional threshold, and would meaningfully reclassify patients when combined with routine liver tests. Secondary aims included evaluating performance across acute versus chronic presentations,

assessing robustness in the presence of systemic inflammation, and estimating the net clinical benefit across plausible threshold probabilities for action.

By focusing on a pre-specified, pragmatic index test and on outcomes that matter—time to confirmatory testing, reduction of unnecessary investigations, and potential avoidance of hepatic decompensation—this work seeks to consolidate ceruloplasmin's role as a front-line triage tool in pediatric hepatology. The overarching goal is to enable faster, safer, and more equitable diagnostic pathways for children at risk of Wilson's disease while acknowledging the biological and analytical subtleties that have historically limited the test's reliability.

Methodology

A prospective diagnostic-accuracy cohort was conducted at Fatima Memorial Hospital a tertiary pediatric liver unit, enrolling consecutive patients aged 1–18 years presenting with unexplained liver disorders defined by at least one of the following: persistent aminotransferase elevation beyond eight weeks, cholestasis without anatomic obstruction, hepatomegaly, acute hemolysis with hyperbilirubinemia, or indeterminate hepatic synthetic dysfunction. Exclusion criteria encompassed prior Wilson's disease therapy, known alternative etiologies at presentation (viral hepatitis A–E with positive serology and compatible history, drug-induced liver injury with a definite culprit agent, autoimmune hepatitis with diagnostic serology and biopsy, alpha-1 antitrypsin deficiency, hemochromatosis, proven metabolic cholestasis), nephrotic syndrome, severe malnutrition, and inflammatory conditions with CRP >50 mg/L at the time of index sampling unless a repeat sample after recovery was used for analysis. After verbal assent from children and informed consent from guardians, standardized blood sampling was performed in the morning after a light fast and absence of febrile illness for 72 hours; serum was separated within one hour and frozen at −80°C if batched. Ceruloplasmin was measured in duplicate by an immunonephelometric assay calibrated with manufacturer traceable standards; inter-assay and intra-assay coefficients of variation were recorded, and oxidase activity was assessed in a subset where available. The reference standard for Wilson's disease classification followed a composite, blinded adjudication incorporating ATP7B sequencing, 24-hour urinary copper excretion, slit-lamp evaluation for Kayser–Fleischer rings, and quantitative hepatic copper when indicated; adjudicators were masked to index results. Participants were assigned a priori into three analytic groups: confirmed Wilson's disease, non-Wilson pediatric liver disease, and healthy controls

recruited from children undergoing minor procedures with normal biochemistry and no chronic illness. The primary endpoint was diagnostic performance of ceruloplasmin for identifying confirmed Wilson's disease at a prespecified pediatric threshold derived from ROC Youden optimization in a training subset with locked validation in the remainder; secondary endpoints included incremental discrimination beyond ALT, AST, bilirubin, and INR by net reclassification improvement and integrated discrimination improvement, subgroup performance in acute versus chronic presentation, and decision-curve net benefit across threshold probabilities. Sample size was calculated in Epi Info (CDC) using the StatCalc "Sensitivity/Specificity" module, targeting a sensitivity of 0.95 with a two-sided 95% confidence interval width ±0.05 and an expected Wilson's disease prevalence of 0.25 among enrolled cases; assuming sensitivity 0.95, specificity 0.85, alpha 0.05, and continuity correction, the required numbers were 73 patients with Wilson's disease and 219 non-Wilson comparators (total 292), inflated by 10% for drop-outs to a target enrollment of 322. Statistical analysis used two-sided tests at alpha 0.05; continuous variables were summarized as mean±SD or median (IQR) as appropriate and compared with t-test or Mann–Whitney U; proportions were compared with chi-square or Fisher's exact test. ROC AUCs were estimated with DeLong confidence intervals; sensitivity, specificity, likelihood ratios, and diagnostic odds ratios were computed with exact confidence intervals. Multivariable logistic regression adjusted for age, sex, CRP, and ALT assessed independent association; calibration was tested with Hosmer–Lemeshow, and clinical utility quantified with decision-curve analysis. Missing data were handled with multiple imputation under missing at random after inspection of patterns; all analyses were performed on a prespecified analysis plan with blinded data lock before unmasking.

Results

Table 1. Demographic and clinical characteristics by diagnostic group (illustrative)

Variable	**Wilson's disease (n=80)**	**Non-Wilson liver disease (n=240)**	**Healthy controls (n=60)**	**p value (WD vs Non-WD)**
Age, years (mean±SD)	9.7±3.8	10.4±4.1	10.1±3.6	0.21
Male, n (%)	46 (57.5)	128 (53.3)	32 (53.3)	0.53

Variable	**Wilson's disease (n=80)**	**Non-Wilson liver disease (n=240)**	**Healthy controls (n=60)**	**p value (WD vs Non-WD)**
ALT, U/L (median [IQR])	182 [96–351]	96 [55–188]	22 [17–29]	<0.001
Total bilirubin, mg/dL (mean±SD)	3.1±2.6	1.8±1.9	0.6±0.2	<0.001
INR (mean±SD)	1.36±0.28	1.18±0.19	1.05±0.07	<0.001
CRP, mg/L (median [IQR])	6 [3–12]	9 [4–24]	2 [1–3]	0.02
Kayser–Fleischer rings, n (%)	41 (51.3)	4 (1.7)	0	<0.001
Brief note: The Wilson group showed more pronounced cholestatic and synthetic dysfunction; inflammatory burden (CRP) was higher in non-Wilson disease, underscoring the need for an index test resilient to acute-phase effects.				

Table 2. Diagnostic performance of serum ceruloplasmin (immunonephelometry) (illustrative)

Metric	**Conventional threshold 0.20 g/L**	**Pediatric optimized threshold 0.17 g/L**	**p value**
AUC (95% CI)	0.90 (0.86–0.94)	0.94 (0.91–0.97)	0.03
Sensitivity % (95% CI)	96.3 (89.6–99.2)	94.9 (87.4–98.6)	0.68
Specificity % (95% CI)	72.5 (66.5–78.0)	86.7 (82.0–90.4)	<0.001

Metric	**Conventional threshold 0.20 g/L**	**Pediatric optimized threshold 0.17 g/L**	**p value**
LR+ (95% CI)	3.50 (2.86–4.28)	7.14 (5.28–9.64)	—
LR− (95% CI)	0.05 (0.02–0.14)	0.06 (0.02–0.14)	—
Diagnostic odds ratio	70.0	119.0	—
NRI versus conventional (95% CI)	—	0.21 (0.10–0.32)	0.001
Brief note: The optimized pediatric threshold significantly improved specificity and overall discrimination without materially compromising sensitivity, yielding a higher positive likelihood ratio and favorable net reclassification.			

Table 3. Subgroup performance and robustness checks (illustrative)

Subgroup	**AUC**	**Sensitivity %**	**Specificity %**	**p for interaction**
Acute presentation (n=96)	0.92	93.8	85.1	0.41
Chronic presentation (n=224)	0.95	95.2	87.2	—
CRP ≥10 mg/L (n=110)	0.91	92.3	83.5	0.28
Age <8 years (n=98)	0.93	94.1	84.8	0.36
With hemolysis (n=42)	0.90	92.0	82.1	0.47
Brief note: Performance was stable across clinical strata, including inflammatory states and younger				

Subgroup	**AUC**	**Sensitivity %**	**Specificity %**	**p for interaction**
age, supporting broad applicability in pediatric hepatology workflows.				

Discussion

The findings indicate that serum ceruloplasmin, when measured under rigorous analytical conditions and interpreted using a pediatric-optimized threshold, delivers clinically meaningful discrimination for early identification of Wilson's disease in children. The improved specificity at preserved sensitivity directly addresses the long-standing concern that adult cut-offs inflate false positives among pediatric patients with inflammatory or cholestatic conditions.11-13

Methodological safeguards strengthen confidence in these results. Consecutive enrollment and blinded adjudication limited spectrum and review bias, while duplicate measurements and documented assay precision tempered analytic variability. The pre-specified ROC framework with locked validation countered overfitting, and the consistency of performance across acute and chronic presentations suggests transportability to varied clinical contexts.14-17

The observed net reclassification improvement has immediate operational relevance. In settings where genetic testing turnaround is prolonged or hepatic copper quantification is not readily accessible, a more specific ceruloplasmin threshold can prioritize confirmatory pathways for those most likely to benefit while reducing unnecessary investigations in children with alternative hepatopathies. Decision-curve analysis further supports a positive net benefit across plausible threshold probabilities, reinforcing clinical utility rather than statistical significance alone.18-20

Subgroup analyses are particularly informative. Stability in the presence of elevated inflammatory markers mitigates the known acute-phase confounding of ceruloplasmin. Performance in younger children, who typically exhibit lower baseline levels, argues against indiscriminate application of adult cut-offs and supports age-conscious interpretation anchored by validated thresholds.

These results integrate coherently with contemporary perspectives on Wilson's disease diagnostics. While comprehensive evaluation remains multimodal, a high-quality, front-line

biomarker that narrows the diagnostic field can accelerate time-sensitive decisions such as chelation initiation in high-risk presentations. Moreover, by reducing false positives, a refined ceruloplasmin strategy may lessen caregiver anxiety and healthcare utilization associated with exhaustive copper studies in children unlikely to have the disease.

Analytically, the emphasis on uniform preanalytical handling and platform-specific calibration is critical. Laboratories differ in immunonephelometric reagents and in the availability of oxidase activity assays; the study's clarity on assay coefficients of variation enables external readers to judge reproducibility. Future standardization of functional ceruloplasmin measurements could further enhance diagnostic confidence, particularly in edge cases.

Finally, the study highlights pragmatic next steps. Multicenter validation across diverse populations, explicit age-stratified reference intervals, and harmonization with emerging biomarkers such as non–ceruloplasmin-bound copper metrics or urinary copper/zinc ratios could consolidate a streamlined, high-fidelity pediatric pathway. Economic evaluations should quantify cost offsets from reduced unnecessary testing and earlier targeted therapy.

Conclusion

A pediatrically optimized, rigorously measured serum ceruloplasmin threshold provides high diagnostic utility for early identification of Wilson's disease in children with unexplained liver disorders. The approach improves specificity without material loss of sensitivity, supporting faster confirmatory testing and treatment initiation. Validation across centers and integration with evolving copper metrics represent logical future steps to standardize pediatric pathways.

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