

Practical and Multidisciplinary Review on Wilson Disease: The Portuguese Perspective

Filipe Calinas^{a,b} Hélder Cardoso^{c,d} Sofia Carvalhana^e José Ferreira^f
Cristina Gonçalves^g Marina Magalhães^h Helena Pessegueiro Mirandaⁱ
José Presa^j Carla Rolanda^{k,l} Arsénio Santos^{m,n} Rui M. Santosⁿ

^aCentro de Responsabilidade Integrado de Gastrenterologia, Unidade Local de Saúde de São José, Lisbon, Portugal; ^bCentro Clínico Académico de Lisboa, Lisbon, Portugal; ^cServiço de Gastrenterologia, Unidade Local de Saúde de São João, Porto, Portugal; ^dFaculdade de Medicina da Universidade do Porto, Porto, Portugal; ^eServiço de Gastrenterologia, Hospital de Santa Maria, Unidade Local de Saúde de Santa Maria, Lisbon, Portugal; ^fServiço de Gastrenterologia, Unidade Local de Saúde de Santo António, Porto, Portugal; ^gUnidade de Gastrenterologia e Hepatologia Pediátricas, Hospital Dona Estefânia, Unidade Local de Saúde de São José, Lisbon, Portugal; ^hServiço de Neurologia, Unidade Local de Saúde de Santo António, Porto, Portugal; ⁱUnidade de Transplantação Hepática e Pancreática, Centro Hospitalar e Universitário do Porto, Porto, Portugal; ^jUnidade de Hepatologia, Unidade Local de Saúde Trás-os-Montes e Alto Douro (ULSTMAD), Vila Real, Portugal; ^kServiço de Gastrenterologia, Hospital de Braga, Unidade Local de Saúde de Braga, Braga, Portugal; ^lEscola de Medicina e Instituto de Investigação em Ciências da Vida e da Saúde (ICVS) – Universidade do Minho, Braga, Portugal; ^mServiço de Medicina Interna, Hospitais da Universidade de Coimbra, Unidade Local de Saúde de Coimbra, Coimbra, Portugal; ⁿFaculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal

Keywords

Wilson disease · Diagnosis · Treatment · Monitoring · Adherence

Abstract

Wilson disease (WD) is a genetic disorder of copper metabolism caused by mutations in the ATP7B gene resulting in toxic copper accumulation in several organs. WD can manifest as liver disease, a progressive neurological disorder, a psychiatric illness, or a combination of these. Other clinical manifestations can also occur. Diagnosis is challenging and typically requires a range of biochemical tests, imaging, genetic testing for ATP7B, and/or liver biopsy. WD is treatable with

chelating agents, such as D-penicillamine and trientine, and/or zinc salts alongside with dietary copper restriction. Liver transplantation may be indicated in WD patients with severe hepatic disease, and cautiously considered in patients with neurological WD. Treatment success highly depends on patient adherence and treatment persistence. Therefore, effective interventions for improving patient adherence and close monitoring are key for preventing WD progression. In Portugal, there are no reference centers for WD, and patients are dispersed across numerous medical specialists. This review aimed to summarize the most recent and relevant information for the

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diagnosis, treatment, and monitoring of WD in Portugal, as well as possible interventions for stimulating adherence to treatment.

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Revisão Prática e Multidisciplinar sobre a Doença de Wilson – Perspectiva portuguesa

Palavras Chave

Doença de Wilson · Diagnóstico · Tratamento · Monitorização · Adesão

Resumo

A doença de Wilson é uma doença genética do metabolismo do cobre, causada por mutações no gene *ATP7B*, que levam à acumulação tóxica de cobre em diversos órgãos. A doença de Wilson pode manifestar-se como doença hepática, perturbação neurológica progressiva, doença psiquiátrica ou como uma combinação destas patologias. Outras manifestações clínicas também podem ocorrer. O diagnóstico é complexo e normalmente requer a combinação de análises bioquímicas, imagiologia, testes genéticos para o gene *ATP7B* e/ou biópsia hepática. A doença de Wilson é tratável com agentes quelantes, como a D-penicilamina e a trientina, e/ou sais de zinco, em conjunto com uma dieta com baixo teor de cobre. O transplante de fígado pode ser indicado em doentes com doença hepática grave, e deve ser cuidadosamente considerado em doentes com manifestações predominantemente neurológicas. O sucesso do tratamento é altamente dependente da adesão do doente e da persistência no tratamento. Portanto, intervenções eficazes para melhorar a adesão do doente ao tratamento, bem como a monitorização rigorosa, são cruciais para prevenir a progressão da doença de Wilson. Em Portugal não existem centros de referência para a doença de Wilson e os doentes encontram-se dispersos por numerosos especialistas médicos. Este artigo de revisão pretende reunir informação recente e relevante para o diagnóstico, tratamento e monitorização da doença de Wilson em Portugal, assim como possíveis intervenções para estimular a adesão ao tratamento.

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Introduction

Wilson disease (WD) is an autosomal recessive disorder of copper metabolism, caused by mutations in the *ATP7B* gene, which encodes a transmembrane copper transporting

ATPase, leading to copper overload in the liver, brain, and other organs [1–4]. WD can present with a variety of symptoms, making it important for healthcare professionals to maintain a high level of suspicion for early diagnosis [4–7].

Symptoms of WD can manifest at different ages, more commonly observed between the ages of 3 and 55 years old. Liver-related symptoms are more prevalent during childhood and adolescence, while neurological symptoms tend to develop later, usually about a decade after the liver involvement [5, 7, 8].

The global prevalence of WD ranges from 1:29,000 to 1:40,000 individuals, with some variation based on ethnicity [9]. In Spain (mainland), estimated frequencies of affected individuals with WD are reportedly higher, ranging from 1:6,278 to 1:16,540 [10]. Moreover, recent genetic research suggests that the frequency of *ATP7B* mutations (associated with WD) is higher than the reported clinical prevalence, which could be due to variable clinical presentations, incomplete penetrance, or the influence of modifier genes [11–13].

In Portugal, there are no reference centers for WD, and patients are dispersed across numerous medical specialists. There is also no WD patient association. Data on the distribution, prevalence, genetic variability, and current management of WD in Portugal are not available but are being gathered in a national registry of hepatic diseases. The registry is coordinated by APEF – Portuguese Association for Study of the Liver and is hosted at www.liver.pt.

This review was elaborated by a multidisciplinary team of national medical experts (gastroenterology, internal medicine, neurology, and pediatrics) with large experience in WD. The aim was to summarize important information for the diagnosis, treatment, and monitoring of WD patients in Portugal. It also includes possible interventions for stimulating adherence to treatment. The ultimate goal of this practical review is to promote the adoption of appropriate actions for managing WD patients and to bring awareness on the disease.

Diagnosis

Clinical Manifestations

WD comprehends a wide spectrum of clinical manifestations, including liver disease, progressive neurological disorders, psychiatric symptoms, or a combination of these. Other manifestations involving the eyes, blood, kidneys, and joints can also occur [2, 4–8].

Hepatic symptoms typically manifest during pediatric ages. Liver damage can present in various forms, spanning from asymptomatic abnormalities in liver

biochemistry to severe and life-threatening liver failure [8, 14]. Patients with clinically asymptomatic forms (3–40%) often exhibit hepatomegaly, discretely elevated transaminases, or are identified through family screening [15–17].

Acute hepatitis may develop in up to 25% of patients, presenting with similar symptoms to acute viral hepatitis, such as jaundice and abdominal pain. Up to 20% of patients may present acute liver failure, usually associated with Coombs-negative intravascular hemolysis, coagulopathy, progressive encephalopathy, and renal dysfunction [7, 8, 14].

Chronic presentations of liver impairment encompass chronic hepatitis (10–30%) and cirrhosis (35–60%). Patients may develop cirrhosis-related complications, including ascites, encephalopathy, renal failure, or portal hypertension [14]. Hepatic steatosis can also be observed in up to 80% of biopsies of WD patients [18].

Neurological symptoms typically appear at an older age than hepatic impairment and comprehend a wide spectrum of movement disorders. The most common symptom is dysarthria (46–97%), particularly in the early stages of the disease. Other symptoms include tremor (55%), parkinsonism (12–58%), gait abnormalities/ataxia (28–75%), dysphagia (50%), and chorea (6–30%). Dystonia affecting facial expression and causing involuntary smiling (*risus sardonicus*) is characteristic of WD. In most cases, these symptoms overlap, fluctuate, and can be aggravated by several factors (e.g., stress, emotions, general health conditions, concomitant disorders) [7, 19, 20].

Although most WD patients present with psychiatric symptoms at some stage of the disease, they are present in 10–25% of the patients at diagnosis [7, 21]. In children, it may manifest as a decline in school performance, inappropriate behavior or impulsiveness. In adults, the most common conditions include mood disorders (depressive or bipolar spectrum), behavioral or personality changes (irritability, aggression, and disinhibition), psychotic disorders, sleep disturbances, and subtle cognitive dysfunction [21].

In WD, the most typical ophthalmologic sign is the Kayser-Fleischer ring. This is due to the deposition of excess copper on the inner surface of the cornea in the Descemet membrane. Kayser-Fleischer rings can be detected by optical coherence tomography (preferential method) or slit-lamp examination, which requires an experienced ophthalmologist. These rings are present in almost all patients with neurological symptoms, in 50% of patients with liver manifestations and in 10–30% of

asymptomatic patients. In children presenting with liver disease, Kayser-Fleischer rings are usually absent. Additionally, sunflower cataract is a rare but also characteristic sign. Notably, Kayser-Fleischer rings and sunflower cataract are not associated with impaired vision, and they gradually disappear with effective treatment [7, 22].

Other clinical symptoms are less common and result from the multi-organ damage due to copper accumulation. Hematological manifestations may include hemolysis, thrombocytopenia, and leukopenia. Renal abnormalities comprise tubular dysfunction (e.g., renal tubular acidosis, aminoaciduria, Fanconi syndrome) and nephrolithiasis. Cardiac problems include cardiomyopathy, arrhythmias, and autonomic dysfunction. Bone demineralization and endocrinological symptoms, such as infertility or repeated miscarriages, and hypoparathyroidism may be also observed [23].

Diagnostic Testing

Standard Biology

Laboratory testing should begin with biochemical liver tests, blood counts, and coagulation parameters to assess for liver disease and hemolysis. Abnormal hepatic biochemistry is common in WD, but it is not a specific feature. Of note, a normal hepatic workup does not rule out the possibility of liver involvement [6, 7, 24].

Copper Metabolism Tests

Tests of copper metabolism may include serum ceruloplasmin, serum copper, 24-h urinary copper excretion and liver biopsy for histology, histochemistry, and/or copper quantification (Table 1). Low serum ceruloplasmin, usually <10 mg/dL, is indicative of WD. However, intermediate concentrations of ceruloplasmin, ranging from 10 to 20 mg/dL, are often observed in patients [6, 7, 14, 25, 26]. This test should be carefully interpreted, as false positive or negative results can occur due to other physiological and/or pathological conditions [24, 27–30].

Over 90% of serum copper is bound to ceruloplasmin; thus, in WD, serum copper is usually low in proportion to the decrease of this protein in circulation. Only the determination of total serum copper (ceruloplasmin-bound plus non-ceruloplasmin-bound copper [NCC]) is available, which is generally <635 µg/L (<10 µmol/L) in WD [24, 34]. However, this parameter is no longer useful in WD diagnosis.

Urinary copper excretion is negligible in healthy individuals, while in symptomatic WD patients, it is

Table 1. Tests for diagnosis of WD [24, 25, 31–33]

Tests	Normal	Typical findings	False negatives	False positives
Current tests				
Ceruloplasmin	20–40 mg/mL	<10 mg/mL	Increased in: • pregnancy • use of contraceptives • inflammation Normal value in • 50% of WD patients with highly inflammatory liver involvement • 15–36% of children with WD	Decreased: • until 6–10 months of age • healthy heterozygotes for WD • acute, iatrogenic viral hepatitis • Menkes disease • acquired copper deficiency • malnutrition, cachexia • nephrotic syndrome • aceruloplasminemia
24-h urinary copper	<40 µg/24 h <0.6 µmol/24 h	>100 µg/24 h >1.6 µmol/24 h	Normal: • incomplete or inadequate sample collection • in 16–23% of WD patients (children, asymptomatic)	Increased in: • cholestasis • acute liver failure from any cause • healthy heterozygotes for WD (intermediate rates)
Liver copper	<50 µg/g dry tissue 0.2–0.9 µmol/g dry tissue	>250 µg/g dry tissue >3.3 µmol/g dry tissue	Normal or intermediate value due to heterogeneous copper distribution in patients with WD and: • active liver disease • regeneration nodules	High value in: • cholestasis
Genetic analysis	Sequencing of the entire ATP7B gene Whole-genome sequencing permits assessing all liver disease genes, not just WD			
New tests				
CuEXC	39–73 µg/L 0.62–1.15 µmol/L	>132 µg/L ^a >2.08 µmol/L ^a		
REC	3–8.1%	>18.5%		
CuEXC, serum exchangeable copper; REC, relative exchangeable copper; WD, Wilson disease. ^a In the extrahepatic forms.				

typically >100 µg (>1.6 µmol)/24 h. A lower threshold of 40 µg (0.6 µmol)/24 h may indicate WD in asymptomatic individuals or children [6, 7, 25, 31]. When performing this test, written instructions for conducting 24-h urine collections should be provided to the patients together with copper-free containers [6].

The penicillamine challenge test may be helpful in the diagnosis of WD [35]. This test has only been validated in a pediatric population, in which 500 mg of D-penicillamine was administered orally at the beginning and after 12 h during the 24-h urine collection period. A clear differentiation from other liver disorders was found when >1,600 µg copper/24 h (>25 µmol/24 h) was excreted [7, 25, 35].

Serum exchangeable copper (CuEXC) corresponds to the labile fraction of copper in serum. The ratio of CuEXC to total serum copper, called the relative exchangeable copper, is an excellent diagnostic biomarker with a sensitivity and specificity close to 100% for the diagnosis of WD, when its value is >18.5%. This biomarker distinguishes patients with WD from normal individuals, simple heterozygotes and patients with other hepatopathies [32, 36].

CuEXC is shown to be statistically higher in patients with extrahepatic involvement than in patients with hepatic disease; thus, it may be a marker of extrahepatic involvement and severity. A value >132 µg/L (>2.08 µmol/L) was described by some authors and may indicate corneal and brain involvement [37, 38]. CuEXC exhibits promising diagnostic performance and could be determined if available; however, it is not yet accessible in Portugal.

Radioactive copper incorporation is a highly sensitive and specific test for WD; although not commonly available. A ⁶⁴Cu ratio 24 h/2 h <0.3 and a ⁶⁴Cu ratio 48 h/2 h <0.395 are diagnostic of WD [39, 40]. New methods for the determination of the main Cu-species in human serum applying mass spectrometry technology are also under development [41].

Liver biopsy is no longer systematically used for establishing a diagnosis of WD; it should be considered on a case-by-case basis [24]. Liver biopsy permits the weighted determination of intrahepatocyte copper and the assessment of the grade of liver injury [6, 31].

Biopsy specimens with adequate size (at least 5 mm in length) for copper estimation by atomic absorption spectroscopy should be sent in a dry condition in a copper-free container [7, 42]. In Portugal, the Toxicology Laboratory of the Faculty of Pharmacy of the University of Porto has proven experience in performing this analysis.

The copper content in the liver of healthy individuals is typically <50 µg/g of dry weight. In WD patients, the copper levels can exceed 250 µg/g of dry liver tissue [43]. Interpreting the results may be challenging due to heterogenous copper deposition in the liver, sampling error, or the presence of other liver disorders, particularly cholestatic conditions and cirrhosis [31, 26].

Histopathological changes in the liver are usually not specific to WD and can vary depending on the disease stage. Early and common changes often involve mild steatosis, observed in up to 80% of biopsies [18]. Histological features classically associated with chronic hepatitis may be present. In later stages, progressive parenchymal damage may evolve into fibrosis and cirrhosis [44]. Histochemical stains (such as rhodamine) for copper typically have poor sensitivity and a negative stain does not exclude the diagnosis of WD [7, 25].

In clinical practice, converting copper units may be useful; for this, find the unit converter at www.convertunits.com/from/grams+Copper/to/molecule.

Imaging

Brain magnetic resonance imaging (MRI) is used for the differential diagnosis of neurological WD and should be performed in all patients, including asymptomatic or with only hepatic manifestations. Additionally, brain MRI serves as a valuable tool for monitoring progression, detecting structural abnormalities or changes in the brain, and predicting outcomes [19]. The recommended brain MRI sequences include 3D (preferable) or 2D FLAIR, axial T1, axial T2, axial DWI, axial T2*, and coronal T2 (optional). Important anatomical areas to focus on include putamen, caudate, globus pallidus, thalamus, mesencephalon, pons, cerebellum, cortex, and subcortical white matter [45–47].

Neuroimaging abnormalities are present in more than 90% of patients with neurological WD, in 40–70% of patients with hepatic WD, and in 20% of presymptomatic patients [47–49]. The most frequent findings include signal changes in the basal ganglia, thalami, pons, and white matter, as well as atrophy. The increased T2 signal in the midbrain, commonly referred to as the “face of the giant panda sign” is considered pathognomonic for WD, although it is present in only 12% of cases [19, 45, 46]. In patients with abnormal neurological findings, sequential imaging examinations may correlate with progression or recovery [7, 50].

Liver ultrasound should be performed in all patients with suspected WD, irrespective of their clinical presentation. Hepatic steatosis is the most common finding,

Table 2. Leipzig score for diagnosis of WD [58]

Typical clinical symptoms and signs	
Kayser-Fleischer rings	
Present	2
Absent	0
Neuropsychiatric symptoms ^a	
Severe	2
Mild	1
Absent	0
Serum ceruloplasmin	
Normal (>20 mg/dL)	0
10–20 mg/dL	1
<10 mg/dL	2
Coombs-negative hemolytic anemia	
Present	1
Absent	0
Other tests	
Liver copper (in the absence of cholestasis)	
>250 µg (>4 µmol/g dry weight)	2
50–249 µg (0.8–4 µmol/g dry weight)	1
Normal: <50 µg (<0.8 µmol/g dry weight)	–1
Rhodanine-positive granules ^b	1
Urinary copper (in the absence of acute hepatitis)	
Normal	0
1–2 × ULN	1
>2 × ULN	2
Normal, but >5 × ULN ^c after D-penicillamine ^d	2
Mutation analysis	
On both chromosomes detected	4
On one chromosome detected	1
No mutations detected	0
Assessment of the WD score	
Total score	Evaluation
4 or more	Diagnosis established
3	Diagnosis possible, more tests needed
2 or less	Diagnosis very unlikely

MRI, magnetic resonance imaging; ULN, upper limit of normal.
^aOr typical abnormalities at brain MRI. ^bIf no quantitative liver copper available. ^cThe cutoff of >1,600 µg copper/24 h is more reliable. ^dPenicillamine challenge test is only validated in children.

reported in 35–88% of patients. Signs suggesting cirrhosis could also be present. Computed tomography or MRI can also show evidence of liver cirrhosis and portal hypertension [6, 51, 52]. Liver ultrasound is the gold standard for screening hepatocellular carcinoma, although the occurrence of hepatobiliary malignancies is rare in WD [53].

Liver stiffness measurement by transient elastography should be performed in all adults at diagnosis. A study by

Paternostro et al. [54] suggested that a liver stiffness measurement value ≥9.9 kPa accurately identified cirrhosis in patients with recently diagnosed WD (positive predictive value: 74%, negative predictive value: 100%). Another study reported that a liver stiffness measurement of 8.4 kPa could differentiate advanced fibrosis stages from milder stages [55].

Genetic Testing

The number of known variants in the *ATP7B* gene, which exceeds 900, without a clear clinical relationship, limits the usefulness of genetic tests in routine diagnostics. However, sequencing of the entire *ATP7B* gene is very important to make the diagnosis in asymptomatic patients or in the early phases of disease. Genetic testing is recommended for all patients suspected of having WD and, importantly, for family screening. In Portugal, it may take some time to obtain results, so genetic confirmation should not delay the initiation of treatment [6, 56, 57]. Clinicians should be aware that a genetic diagnosis of WD should always be corroborated with clinical and biochemical findings, and the absence of two pathogenic mutations does not exclude a diagnosis of WD [6].

Diagnostic Strategies

No diagnostic test is per se specific for WD; therefore, a range of tests has to be applied [25]. In symptomatic patients, the diagnosis could be established when Kayser-Fleischer rings are present, serum ceruloplasmin is below the lower limit of normal, and urinary copper excretion is above 100 µg/24 h (>1.6 µmol/24 h) [58]. Otherwise, several additional investigations might be required.

Diagnostic scoring systems and algorithms provide a structured approach to diagnosis. The Leipzig scoring system for general diagnosis combines key laboratory and clinical findings and it is validated in adult and pediatric patients (Table 2) [6, 7, 25, 58–60]. Additionally, several indices based on standard biochemistries can be used to establish the diagnosis and prognosis of acute liver failure due to WD [61, 62].

Algorithms can facilitate diagnosis, but their interpretation should be cautiously performed considering the limitations. Algorithms for diagnosing WD in patients with liver disease and/or neuropsychiatric manifestations, adjusted to the Portuguese medical settings, are presented in Figures 1 and 2, respectively [7].

Family Screening

Within families, the risk of WD among siblings of an index case is 25%; while among the progeny, the risk is 0.5% [63]. Therefore, first-degree relatives should be screened for WD. Family screening should involve a

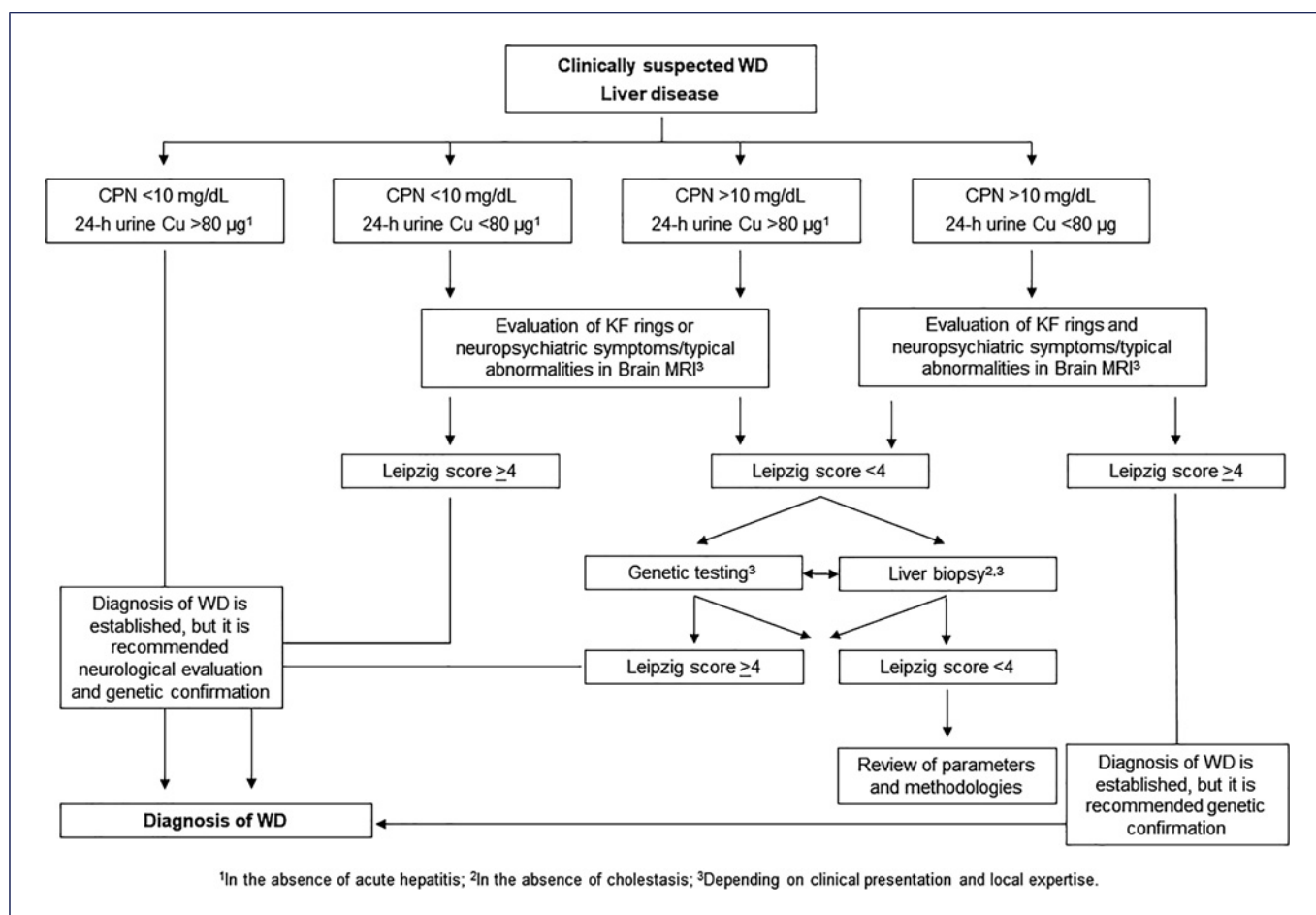


Fig. 1. Algorithm for diagnosing WD in patients with liver disease [7, 58]. CPN, ceruloplasmin; Cu, copper; KF, Kayser-Fleischer; WD, Wilson disease.

clinical examination, routine investigations including standard biology and copper metabolism tests, and genetic testing for familial *ATP7B* mutations. For children of the index case, screening should be performed after they complete 3 years [6, 7, 24].

Treatment

Adults and children aged >3 years old with WD, including asymptomatic patients, should initiate treatment immediately after diagnosis. In children <3 years old, timing to treatment should be individualized according to the degree of organ damage [7, 14].

Currently, lifelong oral pharmacotherapy and dietary copper restriction are recommended to treat WD. Liver transplantation, which corrects the underlying hepatic defect, is also a therapeutic option for WD [7, 64].

Treatment depends on the disease severity and should be driven by the drug safety and efficacy in the individual patient. Initial treatment should include chelating agents and/or zinc salts. Time to observe a clinical response is variable, but liver function tests and neurological symptoms usually begin to improve within 6 months. After a period of sustained clinical and biochemical response, typically at least 2 years, the drugs should be reduced to the lowest effective dose [7, 14].

Pharmacological Treatment

Chelating agents, such as D-penicillamine and trientine, non-specifically bind to copper in the body, facilitating its urinary excretion [65–67]. Trientine also chelates copper in the intestinal tract, thereby preventing its absorption [66–68]. Zinc salts reduce the absorption of copper from the gastrointestinal tract (Table 3) [69, 70, 71].

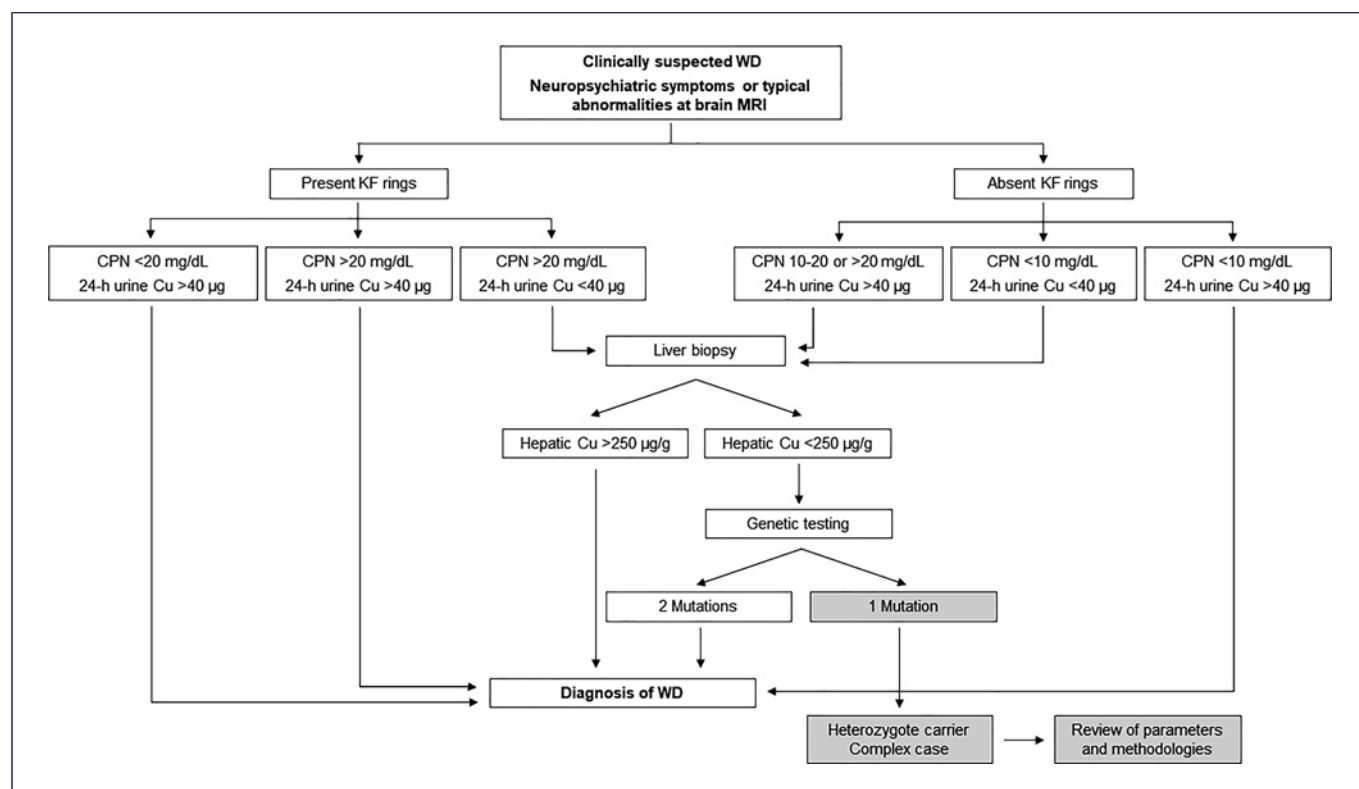


Fig. 2. Algorithm for diagnosing WD in patients with neuropsychiatric manifestations [7, 58]. CPN, ceruloplasmin; Cu, copper; KF, Kayser-Fleischer; WD, Wilson disease.

D-Penicillamine is the most used therapy for WD [6, 14, 25, 72]. Hepatic improvements are observed in patients under D-penicillamine, but its efficacy in neurologic WD is less satisfactory [73, 74].

D-Penicillamine carries numerous adverse effects, which may lead to prompt drug discontinuation in up to 30% of patients [6, 75–77] (Table 3). Paradoxical worsening of neurologic symptoms may occur at the onset of treatment, affecting 10–20% of patients with pre-existing neurological symptoms. This adverse reaction has also been observed with other anti-copper therapies (e.g., trientine, zinc) and can be mitigated by gradually titrating the doses [6, 75–77]. Oral D-penicillamine is rapidly but incompletely absorbed [75].

Trientine is effective for WD with fewer adverse reactions than D-penicillamine (Table 3) [78, 79]. Trientine, in the form of dihydrochloride or tetrahydrochloride salt, is commonly used in patients who are intolerant to D-penicillamine or at increased risk of adverse effects and it is now becoming a preferred treatment for WD [14, 25, 66, 67, 78, 80].

The recently available trientine tetrahydrochloride formulation has demonstrated efficacy comparable to

that of the dihydrochloride salt and has shown good tolerability [81, 82]. Trientine tetrahydrochloride has also shown non-inferior efficacy to D-penicillamine when used as oral maintenance therapy in WD [83]. Trientine tetrahydrochloride is characterized by more favorable pharmacokinetics (increased systemic exposure) compared with the dihydrochloride salt, resulting in a reduced pill burden [84, 85]. The tetrahydrochloride form is a more stable salt of trientine that can be stored at room temperature [82].

Zinc is not recommended as a first-line treatment in symptomatic forms but can be used in presymptomatic or asymptomatic forms and for long-term maintenance therapy after optimal decoppering with chelators [24, 70]. Zinc has demonstrated good efficacy in WD, particularly in patients with neurological manifestations and in asymptomatic siblings. However, its effectiveness may be reduced in case of symptomatic liver disease [86]. If zinc is used, careful monitoring of transaminases is necessary [25].

The most common side effect of zinc is gastric irritation (Table 3). Different zinc salts (sulfate, acetate, gluconate) can be used; however, acetate salts are

Table 3. Available drugs for treatment of WD: dosages and adverse effects [6, 24, 65, 66, 67, 69, 70, 71]

Drug	Initial dose		Maintenance dose (typically after 2 years)	Precautions	Adverse effects	Management of adverse effects
	children	adults without neurological or psychiatric symptoms	adults with neurological or psychiatric symptoms			
D-Penicillamine	Progressively increase dose up to 20 mg/kg/day in 2–4 divided doses	600–2,100 mg/day in 2–4 divided doses	150–300 mg/day, slowly increasing by 150–300 mg/week, to 600–2,100 mg/day in 2–4 divided doses	Gradual titration of the dose. Administration should be done 1 h before or 3 h after meals ^a . Avoid administration with antacids or iron supplements. Pyridoxine supplementation (25–50 mg/day) is advised, particularly in children, pregnant women, patients with malnutrition and intercurrent illness.	<i>Early reactions:</i> Hypersensitivity reactions (fever and rash), proteinuria, bone marrow suppression, altered sense of taste or smell, and paradoxical neurological worsening. <i>Late reactions:</i> lupus-like syndrome, Goodpasture syndrome, elastosis perforans serpiginosa, cutis laxa, and poor wound healing	<i>Hypersensitivity reactions:</i> Prednisolone 10–30 mg/day + drug discontinuation. Reintroduction of the drug at low doses followed by gradual increase. D-Penicillamine can be substituted by trientine. <i>Cytopenia:</i> Dose reduction or drug discontinuation. Reintroduction of the drug at low doses followed by gradual increase.
Trientine dithydrochloride	400–1,000 mg/day in 2–4 divided doses	800–1,600 mg/day in 2–4 divided doses	150–200 mg/day, slowly increasing by 150–200 mg/week to 800–1,600 mg/day in 2–4 divided doses	Gradual titration of the dose. Administration should be done 1 h before or 3 h after meals ^a . Avoid administration with antacids or iron supplements.	Nausea, skin rash, anemia, aplastic anemia, sideroblastic anemia, dystonia, tremor, lupus-like syndrome, colitis, duodenitis, paradoxical neurological worsening during initial phase of treatment	<i>Bone marrow suppression:</i> Immediate drug discontinuation. <i>Neurological worsening:</i> Dose reduction followed by gradual increase. Change to trientine or to zinc salts. <i>Late reactions:</i> Drug discontinuation and substitution by zinc salts. Corticoids and symptomatic treatment. In some cases, chelating agents could be maintained at low doses. Cryotherapy for cutaneous lesions.
Trientine tetrahydrochloride	225–600 mg/day in 2–4 divided doses	450–975 mg/day in 2–4 divided doses	150–200 mg/day, slowly increasing by 150–200 mg/week to 450–975 mg/day in 2–4 divided doses	Gradual titration of the dose. Administration should be done 1 h before or 3 h after meals ^a . Avoid administration with antacids or iron supplements.	Nausea, skin rash, pruritus, erythema, urticaria, sideroblastic anemia, iron deficiency anemia, duodenitis, colitis, paradoxical neurological worsening during initial phase of treatment	

Table 3 (continued)

Drug	Initial dose		Maintenance dose (typically after 2 years)	Precautions	Adverse effects	Management of adverse effects
	children	adults without neurological or psychiatric symptoms	adults with neurological or psychiatric symptoms			
Zinc salts	Age <6 years: 25 mg 2x daily Age 6–16 years or <57 kg: 25 mg 3x daily Age >16 years or >57 kg: 50 mg 3x daily	50 mg 3x daily if patient >57 kg	50 mg 3x daily if patient >57 kg	Administration should be done 1 h before or 3 h after meals ^a .	Nausea, abdominal pain, gastritis, zinc accumulation, possible changes in immune function, paradoxical neurological worsening (rare), hepatic function impairment	<i>Gastritis:</i> Symptomatic treatment. Administration of the first dose between breakfast and lunch. Occasional administration with a protein (e.g., gelatine or ham). <i>Neurological worsening:</i> Zinc discontinuation and restart with a chelating agent at low doses. <i>Hepatic function impairment:</i> Zinc discontinuation and restart with a chelating agent at low doses.

Dosing for penicillamine, trientine dihydrochloride, trientine tetrahydrochloride, and zinc salts is based on the respective summary of product characteristics [60–64] and on the references [6, 21]. Higher doses of trientine tetrahydrochloride (up to 1,050 mg/day in adults) could be used, without being associated with increased toxicity. ^aClinicians should assist the patient in planning the drug administration, by elaborating a schedule adapted to the patient routine and lifestyle.

associated with a lower incidence of gastric side effects [7, 14, 25].

Antioxidants, such as vitamin E, vitamin C, N-acetylcysteine, and curcumin, have been proposed as adjunctive treatment. However, no benefit has been definitively proven [7]. When combining treatments, it is important to assess the potential drug interactions. For this, online websites such as <https://www.drugs.com/interaction/list/> can be consulted.

Currently, clinical trials are being conducted to investigate the safety and efficacy of adeno-associated virus (AAV) curative gene therapy for WD [87, 88].

Dietary Copper Restriction

Dietary copper restriction is an essential part of the WD treatment, and a low-copper diet is advised in combination with pharmacological treatment, especially during the initial treatment phase or until liver function tests normalize. Foods high in copper, such as liver and shellfish, should be avoided. Patients should avoid consuming water from copper pipes or vessels and preparing food with copper cookware [6, 7, 89, 90]. After the initial treatment phase, the decision to continue dietary copper restriction may be revised taking into consideration the response to treatment, adherence, and impact on quality of life [6]. Seeking guidance from a dietitian experienced in managing WD can be highly beneficial for obtaining personalized advice on dietary copper restriction, meal planning, and suitable alternatives to copper-rich foods [6, 7].

Liver Transplantation

In WD, orthotopic liver transplantation (OLT) should be considered in adult and pediatric patients with acute liver failure, end-stage liver disease, hepatocellular carcinoma, or disease progression, despite adequate chelating therapy [6, 7, 25, 91]. Acute hepatic WD may be present in up to 20% of WD patients with hepatic presentations and can rapidly progress to hepatic failure, often necessitating emergency liver transplantation. It is predominantly observed in young patients and is typically characterized by moderately elevated aminotransferases and high bilirubin to alkaline phosphatase ratio, along with Coombs-negative hemolytic anemia and encephalopathy. Acute hepatic WD may also occur in patients who were previously treated but stopped their medications [6, 25]. Longitudinal assessment with a prognostic scoring system may facilitate the decision to transplant patients. The New Wilson's Index, based on bilirubin, INR, aspartate aminotransferase, white blood cell count, and serum albumin, accurately predicts survival without OLT, indicating a poor prognosis when the score is ≥ 11 [62, 92].

In patients with neurological WD, OLT might be cautiously considered. Results from a recent systematic review encourage OLT in severe neurological patients not responding or getting worse with anti-copper treatment; however, it is still uncertain which patients with neurological impairment benefit most from OLT and when is the optimal timing for OLT [93]. The Unified WD Rating Scale (UWDRS) score is used in the evaluation and selection of neurological patients for transplantation [94].

Monitoring

Patients should be regularly monitored to confirm treatment efficacy, ensure therapy adherence, and early identify adverse effects [7, 25]. The frequency of follow-up clinical examination is variable, depending on the disease severity. In general, a clinical examination should be performed every 15 days for the first 3 months of treatment; every 3 months for the first year; and every 6 months when therapeutic objectives are met. Patients should be also examined at each dose change and when clinically indicated [7, 25].

Follow-up should include clinical assessment, complete blood count, liver function tests, coagulation profile, renal function, bone profile, serum ceruloplasmin, serum copper and urine tests, including 24-h urine copper and urine dipstick [6, 7, 25]. Monitoring of disease progression and neurologic response to treatment could be facilitated by using rating scales, such as the Unified Wilson's Disease Rating Scale [95] and the Global Assessment Scale for Wilson disease [96, 97].

Brain MRI and Kayser-Fleischer rings examination should be done in patients with brain or ocular involvement at baseline, before and during treatment to evaluate response, and in case of any worsening. Fading or disappearance of Kayser-Fleischer rings may be observed in adequately treated patients [7, 22, 25].

Liver stiffness measurement (hepatic elastography [FibroScan[®]]) and non-invasive biological tests for fibrosis (FibroTest, APRI, etc.) could be repeated to monitor the evolution of fibrosis [54, 98]. Hepatocellular carcinoma screening by a 6-monthly ultrasound should be considered in cirrhotic patients [53].

The biochemical response to treatment should be monitored at least annually by 24-h urine copper output and NCC (Table 4), which is estimated using the following formula: $\text{NCC } [\mu\text{g/dL}] = \text{total copper } [\mu\text{g/dL}] - 3.15 \times \text{ceruloplasmin } [\text{mg/dL}]$. Estimated NCC may not accurately reflect real free copper [6, 7, 99, 100]; however, new methods are being developed to directly

Table 4. Biochemical targets for the different treatments of WD [6, 7, 14, 99, 100, 102]

Drug	Treatment initiation	Maintenance treatment				Overtreatment		Treatment failure on chronic therapy		
	24-h urine copper	24-h urine copper, µg/24 h	NCC, µg/dL	AST, ALT	24-h urine copper, µg/24 h	NCC, µg/dL	24-h urine copper µg/24 h ^b	NCC, µg/dL	AST, ALT	
		On treatment	Off treatment ^a							
D-Penicillamine	↑	~200–500	12–40	5–15	Trend to normal					
Trientine	↑	~150–500	12–40	5–15	Trend to normal	<100		>500 (previously in range)	>15	↑
							<5			
Zinc salts	No change, then ↓	~25–75	–	5–15	Trend to normal	<25		>75 (previously in range)	>15	↑

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NCC, non-ceruloplasmin-bound copper. ^aForty-eight hours off treatment. ^bPersistently increase in 24-h urine copper values.

measure NCC, e.g., using anion exchange chromatography coupled to inductively coupled plasma mass spectrometry [101]. CuEXC could be an alternative as soon as it becomes available in Portugal.

Serum aminotransaminase normalization is a good marker of effective treatment, and it positively correlates with the maintenance of 24-h urine copper excretion (Table 4). Treatment safety should be monitored by checking blood cell counts, iron status, proteinuria, and renal function [6, 7, 14, 25].

Measuring copper indices on treatment can be highly informative, although results must be judged in relation to the drug, drug dose, and stage of treatment. Moreover, results from treatment collections can be misleading in patients who do not adequately adhere to treatment. Therefore, a collection after a 48-h washout period (off treatment) should be considered during maintenance treatment [6, 7].

Adherence to Treatment

Long-term effects of treatment are largely dependent on patient adherence to the treatment recommendations and treatment persistence (continuation in drug use) [103]. Low adherence leads to progressive worsening of the clinical condition, and treatment discontinuation can lead to severe organ injury and even death [104, 105]. In fact, a retrospective study demonstrated that clinical improvement or disease stabilization was noted in almost

98% of persistent patients, whereas clinical worsening was observed in 52% of non-persistent patients [106].

Diverse factors influence patient adherence and persistence, including the phenotypic presentation, disease severity, and the delayed manifestation of symptoms after treatment discontinuation [104, 107]. The treatment duration, posology (e.g., single vs. multiple doses, number of pills), drug characteristics (e.g., pill size), and safety profile, as well as the mode of acquiring the medication (in hospital pharmacy) and associated costs are also important factors [104, 107, 108]. Furthermore, family support is fundamental to stimulate patient adherence and treatment persistence [106].

Treatment adherence can be monitored by direct and indirect methods. Direct methods consist of the determination of hepatic biochemistry, 24-h urine copper on treatment and off treatment, 24-h urine zinc and serum copper, both on treatment, and clinical monitoring using rating scales. Indirect methods include patient/caregiver interview and clinician assessments (e.g., using the Morisky scale), monitoring of medication refills, pill counts, and patient self-evaluation on treatment adherence [109].

Non-adherence could be very challenging and should be suspected in patients with abnormalities or great oscillations on the laboratorial measurements and/or failing to achieve treatment targets, and/or when there is a re-appearance of Kayser-Fleischer rings after their previous resolution, an erratic attendance for follow-up appointments and/or irregularity in getting prescription refills [7].

A well-defined treatment plan gradually implemented and adapted to the patient's lifestyle and conditions, with regular clinical and biochemical assessments, and a broadly supportive team-based approach, involving the patient family, are good interventions to improve adherence [7]. The posology of the current treatments still challenges patient adherence; nevertheless, some small studies have reported promising results of a single daily dose for treating WD [110, 111].

Conclusion

WD is a genetic disease of copper metabolism, associated with a multitude of non-specific and highly variable clinical manifestations, which demands a high index of suspicion for prompt diagnosis. A structured approach employing several biochemical tests, imaging, genetic testing for ATP7B and/or liver biopsy is usually considered for the diagnosis of WD. Chelating agents and zinc salts are effective pharmacological options for WD as long as patients adequately adhere and persist on treatment. Liver transplantation is indicated for patients with severe hepatic manifestations and should be cautiously considered in patients with neurological WD. Close monitoring and effective interventions for improving patient adherence to treatment are fundamental for preventing the progression of WD.

In Portugal, there are no reference centers for WD, and patients are followed by various medical specialists. Therefore, the knowledge and adoption of evidence-based actions by clinicians are of utmost importance for the appropriate management of WD patients. Data gathered in the National Registry of Liver Diseases will also shed new light on patients' characterization and current management, potentially serving as a starting point for scientific decisions and collaborations that will ultimately benefit WD patients.

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