

Wilson disease in children – diagnostic criteria and scores

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ABSTRACT

Wilson's disease is a genetic autosomal recessive transmitted pathology, that causes disorders of copper metabolism, leading to its accumulation in target tissues. It is a multisystemic affection and has a polymorphic clinical picture. Establishing a positive diagnosis can be difficult, with no single test that confirms or definitely excludes the disease. Diagnostic criteria for the Wilson disease in adult patients were established, then re-evaluated for the child. Establishing early diagnosis allows early chelation therapy, which provides a better prognosis, delaying the progression of the disease. This is the reason why the importance of family screening must be emphasised.

Keywords: children, Wilson's disease, diagnostic criteria

INTRODUCTION

Wilson's disease (WD) is a severe disorder of copper metabolism, which is clinically expressed through a wide range of symptoms involving the liver, central nervous system, the eyes, the kidney, psychiatric function, haematological, and rare endocrinological systems, bones, skin etc. WD is determined by mutations at the ATP7B gene encoding copper transport ATPase, resulting in copper accumulation in the target organs (1),(2). The incidence of affection has been increasing in recent years, estimated at 1: 30,000 (3). It is a chronic, invalidating condition that requires chelation therapy throughout life. Establishing early diagnosis and initiating chelation therapy slow down disease progression and provide good prognosis. For these reasons, family screening is highly recommended as a diagnostic method since the asymptomatic or pre-symptomatic period (4) and it is also recommended to screen the previous generation (5).

The positive diagnosis of WD is difficult to establish because the clinical picture is variable, patients may have different clinical phenotypes. None of the available laboratory tests is specific to WD

and does not clearly establish the diagnosis (6). If this condition is not recognized in order to start treatment, the progression of hepatic disease can be rapid to cirrhosis and hepatic failure. Also, with delayed therapy, irreversible damage to the central nervous system may occur (3).

The patient with WD suspicion requires a complex, multidisciplinary assessment: liver function assessment, hepatic imaging, histological studies, neurological and neuroimaging evaluation, psychiatric, ophthalmic and genetic testing. At the same time, special attention should be paid to the family by screening in order to identify asymptomatic patients (7).

Below we will present the types of manifestations that can signal WD in the child, and we will review the main biochemical, imagistic and genetic tests, which in conjunction with the clinical evaluation can establish the diagnosis of WD.

Clinical manifestations in Wilson's disease in children

According to recent studies, it has been established that BW can occur at any age between 3

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years and 74 years (13.2 years on average) but rarely symptomatic before the age of five (8). WD is a multisystemic affection with a large range of clinical manifestations. In the first decades of life, liver manifestations prevail. After the age of 20, 75% of cases present with neurological manifestations and 25% with both hepatic and neurological manifestations (9,10).

Hepatic damage in WD is due to the toxic accumulation of copper in the hepatocyte, causing irreversible injury and apoptosis. Most children initially develop liver disease, generally after the age of five (11). However, cases of severe hepatic WD have been reported in younger children between the age of 2 and 3 years (12). The severity of liver disease varies from accidental discovery of hepatic cytolysis to acute hepatitis, hepatomegaly, hepatic impairment and cirrhosis (13). Of the patients who have an indications of emergency liver transplantation, due to acute liver disease, 6-12% have WD (14). In a study of a 156 polish WD patients 94.23% presented with liver manifestations, of which 16.23% had liver failure (15). Hepatic cirrhosis may be present in some cases from diagnosis (16,17).

The new recommendations from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) are to suspect WD in any child over 1 year of age who has any sign of hepatic disease from asymptomatic cytolysis to cirrhosis of the liver with hepatomegaly and ascites or acute liver failure. (Grade 1A recommendation, 86% agreement level) (3).

Neurological manifestations of the disease are due to predominant copper deposits in the basal ganglia (caudate nucleus, putamen, globus pallidus), but also at the level of the thalamus and occasionally in the cerebral trunk (18). Although neurological / psychiatric symptoms usually occur in the second or the third decade of life, these manifestations may occasionally occur before the age of 10 years and have been reported in 4%-6% of cases of children with hepatic onset (11).

The most common neurological and psychiatric manifestations of WD are listed in Table 1.

According to ESPGHAN recommendations, WD should be excluded in any teenager with cognitive, psychiatric or unexplained motor disorders (Grade 1A Recommendation 96% agreement) (3).

The Kayser-Fleischer pericorneal ring (KFR) and the “sunflower cataract” are the ophthalmic manifestations of WD. Both manifestations are reversible with drug therapy or after hepatic transplantation. KFR is the mark of Wilson’s disease

TABLE 1. Neurological and psychiatric manifestations frequently associated with WD in children.

Neurologic manifestations	Psychiatric manifestations
Motor disorders (tremor, involuntary movements, coreea)	Depression
Hypersalivation, dysarthria	Personality disorders
Rigid dystonia	Psychosis
Pseudobulbar paresis	Neurotic behaviour
Disautonomy	
Migraine headache	
Insomnia	
Seizures	

and occurs after copper deposits occur in the Descemet corneal membrane. Some patients may only have KFR sketches. Its presence is highly suggestive for the diagnosis. It may be missed in younger patients, in those with hepatic pathology. The KFR is present in 95% of cases with neurological symptoms (19).

Coombs-negative haemolytic anemia may be the only initial manifestation of Wilson’s disease. Marked hemolysis is commonly associated with chronic hepatic disease. Hepatic cell damage releases large quantities of copper that aggravates haemolysis (1). Severe haemolytic anemia associated with hepatic impairment may be an indication for WD. Isolated haemolysis is rare in the absence of significant hepatic impairment. The diagnosis is more difficult to establish if haemolytic anemia is the inaugural symptom (20).

Other manifestations or forms of presentation of WD that are less common. These are listed in Table 2.

TABLE 2. Rare manifestations of WD in children (21-25)

Target organ	Clinical manifestation
Kidney	Renal tubular dysfunction (Fanconi syndrome, renal tubular acidosis, aminoaciduria) Nephrolithiasis Nephrocalcinosis
Heart	Cardiomyopathy Arrhythmias
Endocrine system	Hypogonadotropic hypogonadism secondary to chronic liver disease Hypoparathyroidism Disorders of the menstrual cycle Infertility
Osteoarticular system	Osteopenia Osteoporosis Arthritis
Skin	Lipoma Lunae cerulae
Gastrointestinal	Pancreatitis

Paraclinical tests used to diagnose Wilson's disease in children

Evaluation of liver function. In mild hepatic impairment, a moderate hepatic cytolysis syndrome with transaminase values between 100-500 UI can be observed. Severe bilirubin levels ($> 17.5\text{ mg / dl}$) and low alkaline phosphatase (IU) / bilirubin (mg/dl) are typically found in severe liver disease associated with hepatic impairment < 1 . These characteristics are not, however, pathognomonic for WD (3).

Serum ceruloplasmin is the major copper transporter in the blood. It is also an acute phase marker. Concentration of serum ceruloplasmin is decreased in neonates, then gradually increases with age reaching a peak in the middle of the childhood before decreasing slightly during puberty (26). For this reason, it is recommended to determine ceruloplasmin after 1 year of age. Ceruloplasmin concentration is $< 20\text{ mg/dl}$ in most patients with WD (27). Low serum levels are also found in approximately 20% of heterozygous carriers, patients with hepatic impairment, malabsorption, glycosylation disorders, Menkes disease, proteinoreal malnutrition, nephrotic syndrome, protein-deficient enteropathy, acquired copper deficiency, and hereditary aceruloplasminemia (6). Recent studies have shown the best threshold of ceruloplasmin value that gives diagnosis accuracy $< 14\text{ mg/dl}$ (93% sensitivity and 100% specificity) in a study involving 57 WD adults and children with hepatic dysfunction and / or neurological deficits (28). The cut-off of ceruloplasmin $< 20\text{ mg/dl}$ had 95% sensitivity and 84.5% specificity in a study involving 40 children with asymptomatic cytolysis (29).

Total serum copper includes the value of free copper, unbound ceruloplasmin and copper bound to ceruloplasmin. In WD, the value of copper may be low, normal or increased. Unbound ceruloplasmin „ free copper” can be calculated using the following formula:

$$[\text{total serum copper } (\mu\text{g / dl}) - (3.15 \times \text{serum ceruloplasmin } (\text{mg / dl}))]$$

Free copper values are reduced in patients receiving treatment, usually between 5 and 15 $\mu\text{g/dl}$. This marker is very useful in monitoring the treatment of patients with BW (30,31).

Urinary copper/24 hours, quantifies copper urinary excretion in 24 hours. In children with asymptomatic forms or in children with mild liver problems, copper values are often normal. The diagnostic threshold was set at 40 mg /24 h (0.65 mmol / 24h) with a sensitivity of 78.9% and a spec-

ificity of 87.9% (29). The penicillamine challenge test is performed by administering of 500 mg of D-penicillamine at the beginning of urine collection for 24 hours and another 500 mg given 12 hours later. It is not a reliable test to exclude diagnosis in asymptomatic cases (sensitivity of 12% and 46%) for the cut-off of 1,575 mg/24 hours (25 mmol/24 hours). Reduction of the cut-off to 200 mg/24 hours (3.2 mmol/24 hours) increased sensitivity to 88%, respectively, but resulted in a considerable decrease in specificity (24.1%) (3,32).

Relative exchangeable copper measurement is a new test proposed and used in some centers for WD diagnosis. This test refers to the determination of the labile serum copper that is bound to albumin and other peptides. A recent study has shown that this test has 100% specificity and sensitivity for WD diagnosis in adults, with a cut-off value of 15% (15). According to the ESPGHAN recommendations, this test requires additional studies in children with hepatic disease, to assess its diagnostic accuracy (3).

Genetic testing has remarkably increased the rate of diagnosis, especially in cases where clinical and paraclinical data are difficult to interpret. There are more than 500 mutations of the ATP7B gene involved in WD. Heterozygous compounds have also been identified (32,33). There is a regional distribution of genetic mutations. Some mutations have been described with greater frequency in different geographic regions: in Eastern Europe, the most common mutation is H1069Q, in Spain - Met645Arg, in Sardinia the c-441 427del15 mutation was identified more frequently in Japan - 229inC, Arg778Leu, Costa Rica - Asp1279Ser and in China, Korea and Taiwan the Arg778Leu mutation is more common. Knowing the frequency of these mutations facilitates molecular diagnosis by performing rapid, specific genetic tests (30,34). In Romania, we have difficulties in this stage of work because of the low availability of genetic tests and associated high costs. For genetic testing, we have some tests that study 4 mutations of the 500 mutations (H1069Q, R778L, A874V and N1270S). Gene sequencing is not available in our country. It can be done in other European countries but costs are very high.

Liver biopsy for BW diagnosis is recommended only in cases where the diagnosis is unclear. Hepatic biopsy can quantify copper hepatic load. A single histological study cannot establish the diagnosis in the absence of the other criteria. Histological features are nonspecific in WD and include: aspects of liver microsatellite and macrovascular

hepatic steatosis, glycogenic deposits in peripheral hepatocyte nuclei and focal hepatocytic necrosis, Mallory cortical structures that are composed of eosinophilic cytoplasmic inclusions formed by cytoskeletal proteins and inflammation resembling that of the autoimmune hepatitis (35). Copper deposition can be highlighted by the stains: rhodanine, orceine or rubic acid staining, but has a limited diagnostic value: the negative staining cannot exclude the increase in the amount of hepatic copper, while the positive staining is observed in many disorders associated with biliary disorders (3).

Quantification of intrahepatic copper is recommended to be performed in cases with equivocal diagnosis according to the new ESPGHAN recommendations. (3) According to recent publications, the liver density > 250 μ g / g of dry tissue (where normal values are between 15 and 55 μ g/g) accompanied by low serum ceruloplasmin, establishes the diagnosis of WD. It should be stated that increased concentrations of hepatic copper are present in cholestatic disorders (primitive biliary cirrhosis, sclerosing colangitis, biliary atresia, intrahepatic cholestasis) and non-Wilsonian cupric toxics (cirrhosis of the Indian children, endemic infantile cirrhosis, idiopathic copper toxicity) (30). Quantification of hepatic copper cannot be achieved in our country.

Family screening. Genetic counseling is recommended by both European and American guides. (1),(36) It is mandatory to screen the siblings of patients with WD because the chance of being homozygous and making the disease is 25% (3). The work-up should include full physical examination, serum ceruloplasmin evaluation, liver function tests, and molecular tests for ATP7B mutations. Neonatal screening is unjustified and can be postponed until the age of 1-2 (5).

Diagnostic score. To facilitate diagnosis, in 2001, an international consensus of experts in the field proposed a scoring system, making a diagnostic score using clinical aspects, biochemical parameters (serum ceruloplasmin, copper / 24h) and molecular diagnosis. This score, also known as the “Ferenc Score” (Table 3), was subsequently adopted for the Eurowilson database (37).

The diagnostic value of the score was also studied in the pediatric population. For a cut-off value of 4, a 98% sensitivity and 97% specificity was identified for the positive diagnosis. In cases of mild symptomatology, however, the score showed lower positive and negative predictive values. The score limits are related to the fact that the score by the sum of points depends on the correctness of the determinations made. Also, some criteria can not be applied to young children (2). For pediatric cas-

TABLE 3. Diagnostic Score in WD (The Ferenci Score) (6)

Clinical signs and symptoms	Score	Biochemistry	Score
The Kayser-Fleischer ring			
Present	+1	Liver copper quantitative >5 ULN (50-250 μ g/g)	+2
Absent	0	<5 ULN (50-250 μ g/g)	+1
		Normal (< 0.8 μ mol/g)	-1
Neuropsychiatric symptoms suggestive of WD or typical brain MRI		Rhodanine positive hepatocyte (only if quantitative copper is not available)	
Severe	+2		
Moderate	+1	Absente	0
Absente	0	Prezente	+1
Coombs negative haemolytic anemia + high serum copper		Serum ceruloplasmin	
Prezentă	+1	Normal (> 20 mg/dl)	0
Absentă	0	10-20 mg/dl	+1
		< 10 mg/dl	+2
Genetic testing		Urrinary copper, in absence of acute hepatitis	
Two disease-causating mutations detected	+4	Normal	0
One disease-causating mutation detected	+1	1-2 X ULN	+1
None disease-causating mutations detected	0	> 2 ULN	+2
		Normal, dar > 5 ULN after challenge with 2 X 0.5 g D penicillamine	+2
Total score > 4 – highly likely to be WD			
Total score 2-3 – probable			
Total score 0-1 – unlikely			
ULN* – the upper limit of normal			

es several changes were made to the Leipzig score (29). These changes are presented in Table 3.

CONCLUSIONS

WD can embrace many phenotypic aspects. It is important to recognize the main signs and symptoms of the disease to guide the therapeutic work-up needed in order to establish the diagnosis. Sometimes establishing a positive diagnosis can be extremely difficult and laborious. The Ferenci diagnostic criteria aids the clinician by facilitating

diagnosis. Criteria and investigative algorithm must be adapted to the country's socio-economic status. Determination of serum ceruloplasmin and urinary copper/24 hours are available and can guide us to the diagnosis. In uncertain cases, genetic testing or liver biopsy is recommended. Once a case of Wilson's disease has been diagnosed, family screening should be performed. Both the patient's brothers and their parents will be evaluated in order to identify new cases of asymptomatic or pre-symptomatic disease.

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