

Long Title:

Wilson's Disease in Children: A position paper by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee

Short Title:

Wilson's Disease in Children:

A position paper by the ESPGHAN Hepatology Committee

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ABSTRACT

Background. Clinical presentations of Wilson's disease (WD) in childhood ranges from asymptomatic liver disease to cirrhosis or acute liver failure, while neurological and psychiatric symptoms are rare. The basic diagnostic approach includes serum ceruloplasmin and 24h-urinary copper excretion. Final diagnosis of WD can be established using a diagnostic scoring system based on symptoms, biochemical tests assessing copper metabolism and molecular analysis of mutations in the *ATP7B* gene. Pharmacological treatment is life-long and aims at removal of copper excess by chelating agents as D-penicillamine, trientine or inhibition of intestinal copper absorption with zinc salts. Acute liver failure often requires liver transplantation. This publication aims to provide recommendations for diagnosis, treatment and follow-up of WD in children.

Methods. Questions addressing the diagnosis, treatment and follow-up of WD in children were formulated by a core group of ESPGHAN members. A systematic literature search on WD using MEDLINE, EMBASE, Cochrane Database from 1990 to 2016 was performed focusing on prospective and retrospective studies in children. Quality of evidence was assessed according to the GRADE system. Expert opinion supported recommendations where the evidence was regarded as weak. The ESPGHAN core group and ESPGHAN Hepatology Committee members voted on each recommendation, using the nominal voting technique.

Key words: Wilson's disease, hepatitis, liver, diagnosis, treatment, children.

What is known:

- Guidelines on diagnosis and treatment of Wilson's disease concerning mainly adults

What is new:

- The most updated systematic review of literature related mainly to management of Wilson's disease in childhood
- Specific criteria for diagnosis of Wilson's disease in children, including diagnosis in early childhood and screening
- Recommendations on choice of therapy depending on age and severity of liver damage in children with Wilson's disease

INTRODUCTION

Wilson's disease (WD) is an autosomal recessive genetic disorder of copper metabolism with an estimated prevalence of about 1:30,000 (1). It is caused by mutations in the *ATP7B* gene encoding a copper transporting P-type ATPase required for copper excretion into the bile (2). This defect results in progressive toxic accumulation of copper in the liver that begins in infancy when copper-containing solids are introduced in the diet. With increasing copper overload over time, deposition of copper in other organs, such as the nervous system, corneas, kidneys and heart, occurs usually during the second decade or later. If WD is not recognized and adequately treated, the progression of liver disease to cirrhosis and liver failure can be very rapid or irreversible brain damage can occur. Diagnosis of WD is difficult in children because they are often asymptomatic and conventional criteria established for adults may not be appropriate (3, 4).

The aim of this position paper was to recommend appropriate steps for diagnosis, treatment and follow up of children with WD.

METHODS

A core group of ESPGHAN members (PS, WJ, AD, LD'A, ST, RI, PV, RH) formulated questions relevant for the diagnosis and treatment of WD in children, which were agreed by the ESPGHAN Hepatology Committee (BF, AD, NH, LH, JJ, VM, VN, FS, HV, UB, DD). To approach these questions, systematic reviews, prospective and retrospective cohort or controlled studies from 1986 to 2016 in children <18 years and adults if

evidence in children was lacking were searched in EMBASE, MEDLINE, Cochrane Database of Systematic Reviews and Cochrane Central Register of Controlled Clinical Trials. The following key words, Wilson's disease, symptoms, diagnosis, liver, ceruloplasmin, copper, treatment, penicillamine, zinc, trientine, children, were used to identify relevant papers. Articles in languages other than English and French, animal studies and abstracts presented only during conference proceedings were excluded.

Using the GRADE system (1 for strong recommendation; 2 for weak recommendation), the quality of evidence of each recommendation was graded as follows (5):

1. **High (A):** Further research is unlikely to change our confidence in the estimate of effect.
2. **Moderate (B):** Further research is likely to have impact on our confidence in the estimate of effect and may change the estimate.
3. **Low (C)** Any estimate of effect is very uncertain.

Consensus Meeting and Voting

A first draft of the position paper was discussed with the ESPGHAN Hepatology Committee members. The core group and Hepatology Committee members voted on each recommendation, using the nominal voting technique. Expert opinion supported recommendations where the evidence was regarded as weak.

Recommendations were accepted if they received >75% positive votes and are presented in table 1.

WHEN SHOULD WD BE SUSPECTED IN CHILDREN ?

The accumulation of copper in diverse organs accounts for the wide range of clinical manifestations shown in table 1. Most children present with liver disease (6) ranging from incidental finding of increased serum transaminases in otherwise asymptomatic children > 1 year of age (7), acute hepatitis, hepatomegaly, hyperechogenic liver on ultrasound to acute liver failure (ALF) or cirrhosis (8-12). WD may present at any age between 3 to 74 years (average 13.2 years), but WD is rarely symptomatic before 5 years of age (13, 14). In a paediatric series of 100 children from Bangladesh, chronic liver disease (76%), most often limited to increased serum transaminases, was the most common presenting feature (15). The finding of another possible cause of liver dysfunction, such as acute viral hepatitis A, non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH) (16), and autoimmune hepatitis (AIH) should not exclude WD. Low-titre autoantibodies (mainly antinuclear antibodies) are commonly found in WD patients (17), but cases of WD and concomitant AIH have been reported (17, 18).

Although neurological/psychiatric symptoms usually develop in the second or third decade of life, they may occasionally be seen before 10 years of age (19-22) (Table 1) and have been reported in 4% to 6% of paediatric cases with hepatic onset (9, 10, 15). However, mild cognitive impairment such as working memory and language difficulties seems quite frequent (23). Kayser–Fleischer (K-F) rings, caused by copper deposition on Descemet’s membrane, are usually not seen on slit lamp examination in children with asymptomatic or mild liver disease, but are almost always present in children with neurological involvement (11).

Acute haemolysis has been described as the initial presentation of WD, sometimes apparently precipitated by infection or drugs, and is prominent in fulminant WD. The prevalence of acute haemolysis was 6.9% in a recent retrospective study of 321 patients with WD with an average onset of 12.6 years (ranging from 7 to 20 years) (24). But, an earlier onset at 3 years of age has been reported (14). Several other extrahepatic manifestations have also been described in children, usually as case reports (Table 2).

Recommendations of the ESPGHAN Hepatology Committee are shown in table 1.

DIAGNOSTIC TESTS FOR WILSON'S DISEASE IN CHILDREN

The diagnosis of WD is usually straightforward in children with advanced liver disease, as the classical biochemical features of disturbed copper metabolism (Table 3) are usually present. However, establishing a diagnosis of WD in young asymptomatic children with mild liver disease is often challenging given that ceruloplasmin levels and urinary copper excretion may be normal, and K-F rings absent.

Liver function tests. In acute presentation of WD with liver failure, typical findings are total high bilirubin levels (>300 micromol/l, >17.5 mg/dL) combined with relatively low serum transaminases levels (100–500 IU/l), low serum alkaline phosphatase level thought to result from zinc deficiency, and a low alkaline phosphatase (IU/l) - to - total bilirubin (mg/dl) ratio < 1 (37, 38). However, these findings are not pathognomonic of WD.

Ceruloplasmin is a copper-carrying protein that is bound to 90% of the circulating copper in normal individuals. Serum ceruloplasmin concentration is low in neonates, then gradually rises with age and peaks in mid childhood before declining slightly during puberty (39). The youngest age suitable for testing serum ceruloplasmin for the diagnosis of WD is 1 year. The concentration of ceruloplasmin is reduced below 20 mg/dl in most

patients with WD because of its impaired biosynthesis and the short half-life of the copper-free molecule apoceruloplasmin. However, decreased levels of serum ceruloplasmin are also found in approximately 20% of heterozygous carriers, patients with liver failure, malabsorption, glycosylation disorders, Menkes disease, protein caloric malnutrition, nephrotic syndrome, protein-losing enteropathy, acquired copper deficiency, and hereditary aceruloplasminemia (40-44). Two main studies aimed at evaluating the diagnostic accuracy of serum ceruloplasmin for the diagnosis of WD (40, 44). The best WD diagnostic threshold of serum ceruloplasmin was below 14 mg/dl (sensitivity 93% and specificity 100%) in a series of 57 WD adults and children with liver dysfunction and/or neurological deficits (40), and below 20 mg/dl (sensitivity 95% and specificity 84.5%) in a series of 40 clinically asymptomatic children with elevated serum transaminases (44).

On the other hand, up to 20% of children and adults with WD may have normal serum ceruloplasmin levels, as reported in patients carrying bi-allelic missense mutations of the *ATPB7* gene (45, 46). Ceruloplasmin levels may increase in WD patients with histologically active chronic hepatitis, in pregnant women or women on estrogens. In addition, misleadingly elevated serum levels may be seen when using the immunological-nephelometric assay which measures both ceruloplasmin and the biologically inactive apo-form (45, 46), the reason why the enzymatic assay measuring oxidase activity should be the preferred method (46).

Total serum copper (which includes non-ceruloplasmin bound copper or « free copper » and copper incorporated in ceruloplasmin) is usually decreased in proportion to the decreased serum ceruloplasmin. However, in WD patients with severe liver injury, serum

copper may be within the normal range or markedly elevated in the setting of ALF due to the release of copper from liver tissue stores and the increase in free copper in the blood. The serum non-ceruloplasmin bound copper concentration can be estimated from the serum copper and serum ceruloplasmin levels, but is dependent on the adequacy of the methods for measuring both serum copper and ceruloplasmin (3, 4). Total serum copper has a poor diagnostic value but could be more valuable for monitoring of pharmacotherapy. Very low values may signal systemic copper depletion that can occur in some patients with prolonged treatment.

Urinary copper excretion. In asymptomatic children or children with mild liver disease, urinary copper values are often normal. The reported optimal basal urinary copper diagnostic cut-off value is 40 μg /24 hours (0.65 μmol)/ 24 hrs) with a sensitivity of 78.9% and a specificity of 87.9% (44). The penicillamine challenge test (i.e 0.5 g D-penicillamine given at the beginning of the 24-h urine collection and 12 h later) is unreliable to rule out the diagnosis in asymptomatic children with a sensitivity of 12% and 46% at the established cut-off diagnostic value of 1575 μg /24 hrs (25 μmol /24 hrs) (44, 47). Lowering the cut-off to 5 times the upper normal limit of basal-urinary copper excretion (200 μg /24 hrs; 3.2 μmol /24 hrs) increased the sensitivity to 88% respectively at the cost of considerable loss in specificity (24.1%) (44). Importantly, plastic or acid washed glass containers should be used for urine collection to avoid contamination with copper.

Mutation analysis. Over 500 mutations within the *ATP7B* gene (locus 13q14.3.) have been identified and most affected individuals are compound heterozygotes (48). Predominant mutations have been reported in specific populations, such as in Eastern

Europe (H1069Q), Spain (Met645Arg), Sardinia (c-441 427del15), Japan (229insC, Arg778Leu), Costa Rica (Asp1279Ser) and China, Korea, Taiwan (Arg778Leu) facilitating molecular diagnosis (49-53). Next-Generation Sequencing (NGS) can identify both mutant alleles in 95% of affected subjects, but several limitations should be considered (54). First, molecular defects outside the coding regions and in adjacent intron/exon junctions of the gene and deletions can still be missed using these techniques. Second, their high yield comes with the risk of identifying Variants of Unknown Significance (VUS) which pose diagnostic difficulties.

Liver biopsy and liver copper content- In equivocal cases, measurement of liver copper content is recommended as the next step for diagnosis of WD. A copper content greater than 250 µg/g dry weight (normal <50 µg/g dry weight) in non-cholestatic patients is considered diagnostic for WD in adult series. Lower concentrations are reported in up to 20% of WD patients possibly related to sampling error because the distribution of copper in the liver is not homogeneous (3, 4, 11, 55-58). The accuracy of liver copper measurement is improved with an adequately sized specimen (preferably >1 cm long, min. 0.5 cm) that should be placed on a small piece of paper for drying, and in a dry plastic copper-free container for atomic absorption analysis on fresh tissue. It has also been proposed that two liver biopsy passes be performed and that an entire core of the sample be used for copper determination (57, 58). The most comprehensive study analyzed liver samples from 691 patients with various liver diseases, including 178 with WD (58). Mean liver copper content was significantly higher in WD patients with liver dysfunction than in asymptomatic patients or patients with neurological dysfunction without signs of liver disease ($p=0.001$). All WD patients with liver dysfunction had liver

copper levels over 250 µg/g dry weight, but a high proportion (47.8%) of patients with primary biliary cirrhosis or primary sclerosing cholangitis also had liver copper values ≥ 250 µg/g dry weight.

There have been only a few studies evaluating the diagnostic accuracy of liver copper content in WD children (44). Liver copper content is increased physiologically in early infancy up to 14 months of age (59), in healthy heterozygotes and in cases of chronic cholestatic diseases such as biliary atresia (60, 61). Nicastro et al reported an increase in liver copper > 250 µg/g dry weight in 28 of 30 WD children with mild liver disease (mean 813 µg/g dry weight) (44). Among WD patients, 2 children (7%) had liver copper level < 75 µg/g dry weight while 4 (6%) of 24 controls had liver copper levels > 50 µg/g dry weight. Liver copper exceeded 250 µg/g of dry weight in 2 children with congenital disorders of glycosylation mimicking WD.

Liver histology alone cannot be used to establish the diagnosis of WD. The main features are non-specific and include microvesicular and macrovesicular fatty deposition, mallory hyaline glycogen-containing vacuoles in the nuclei, portal fibrosis and inflammation resembling AIH with interportal fibrous bridging or cirrhosis (62). Copper deposition may be demonstrable by rhodanine, orcein or rubeanic acid staining but has limited diagnostic value: negative staining does not exclude increased liver copper content (62), while positive staining is seen in many liver diseases associated with impaired bile secretion (63).

Scoring system. In 2001, an international consensus of experts proposed a scoring system to facilitate the diagnosis of WD (referred to as the Ferenci score), using the previously discussed biochemical parameters and molecular diagnostics. It was subsequently

adopted for the Eurowilson database (Table 4) (64). The identification of only one disease-causing mutation appears adequate to confirm the diagnosis of WD only in the presence of definite clinical symptoms and biochemical signs of impaired copper metabolism. Otherwise in asymptomatic children, identification of 2 disease-causing mutations becomes necessary to confirm the diagnosis of WD with certainty (64, 65). In children the Ferenci score provided a relatively good combination of sensitivity and specificity for the diagnosis of WD in children - 98.14% and 96.59% respectively in one study (66), and 90% and 91.6% respectively in the other (44). In this latest study, considering 40 µg/24 hours instead of 100 µg/24 hours as the urinary copper excretion cut-off increased the sensitivity of the scoring system to 93% with no change in the specificity (44).

Other tests are being used in some centers to improve diagnosis. These include the measurement of the incorporation of radiolabelled copper into ceruloplasmin which is impaired in WD (67), and the measurement of serum exchangeable copper (68, 69). Exchangeable copper (CuEXC) corresponds to the labile fraction of copper in the serum complexed to albumin and other peptides. Recently, relative exchangeable copper (namely exchangeable copper-to-total copper ratio) has been reported to provide 100% sensitivity and 100% specificity for the diagnosis of WD in adults with a cut off value of 15% and also showed promising results for family screening of asymptomatic patients (68, 70). Further studies are needed to evaluate its diagnostic accuracy in children with liver disease.

Recommendations of the ESPGHAN Hepatology Committee are shown in table 1.

The diagnostic approach is illustrated on figure 1.

THE IMPORTANCE OF FAMILY SCREENING FOR WD

Genetic counseling is essential for families of WD patients, and screening first degree relatives is recommended by both European and American guidelines (3, 4).

It is essential to screen siblings of any patient newly diagnosed with WD because the chance of being a homozygote and developing clinical disease is 25%. Assessment should include physical examination; serum ceruloplasmin, liver function tests and molecular testing for ATP7B mutations or haplotype studies if not available. Newborn screening is not warranted and screening may be delayed until 1 to 2 years of age.

The occurrence of WD in 2 consecutive generations has been reported in apparently non-consanguineous families suggesting a benefit for screening WD in offspring of an affected parent (71-74). Moreover, the risk of occurrence of WD in offspring is increased in consanguineous families and in specific populations with a high carrier frequency.

Finally, considering the possibility of late onset of WD, parents of a child newly diagnosed with WD should also be screened by performing liver tests, explorations of copper metabolism, and suitable genetic testing, as illustrated in a recently reported family (75).

TREATMENT OF CHILDREN WITH WILSON'S DISEASE

Treatment is based on the removal of copper excess by chelating agents such as D-penicillamine, trientine or by blocking the intestinal copper absorption with zinc salts. Dietary copper restriction does not prevent accumulation in WD, and there is a lack of evidence that it improves the outcome once chelators are initiated. However, avoiding copper-rich food (shellfish, nuts, chocolate, mushrooms, and organ meats) is advised

until remission of symptoms and biochemical abnormalities (3). Treatment should be initiated upon diagnosis in pre-symptomatic children identified by family screening as soon as 2 to 3 years of age, promptly in symptomatic children to prevent progression of liver and/or neurological disease. High-quality evidence is lacking to estimate the optimal first line treatment choice in WD. Treatment is life-long as well as the monitoring of compliance and early detection of complications. Prognosis is excellent provided compliance to therapy is adequate.

Treatment options

D-penicillamine was introduced in 1956, and remains the standard treatment for WD. It chelates copper and favors its urinary excretion. Experimentally, D-penicillamine also has a copper “detoxifying” effect by inducing the endogenous hepatic metallothionein, a cytosolic metal-binding protein which sequesters copper and thereby limiting damage to the liver.

D-penicillamine has been shown to efficiently prevent the progression of disease in asymptomatic children. This drug improved liver symptoms in over 80% of symptomatic children within a mean time of 16 months (28), including those presenting with liver failure but no hepatic encephalopathy (76). However, worsening of neurologic symptoms has been reported (77, 78).

Significant adverse effects are reported with the use of D-penicillamine resulting in drug withdrawal in up to 30% of cases in children or adults (3, 12, 28, 79, 80). Early adverse effects include sensitivity reactions characterized by fever and cutaneous eruptions, neutropenia or thrombocytopenia, lymphadenopathy, and proteinuria. Other adverse effects that may occur at any time in the medium and long term include a lupus-like

syndrome characterized by hematuria, proteinuria, arthralgia, bone marrow toxicity with severe thrombocytopenia or aplasia, and skin changes related to D-penicillamine's anti-collagen effects such as *elastosis perforans serpiginosa*, *cutis laxa*, pemphigus, *lichen planus*, and aphthous stomatitis (81). Elevations in serum antinuclear antibodies are frequent, but there is no clear correlation with the development of immune-mediated diseases (82).

In children, the dose of D-penicillamine is usually increased progressively to 20 mg/kg/day given in 2 or 3 doses, with close follow-up for the occurrence of adverse events such as hypersensitivity and proteinuria, hematologic toxicity warranting immediate discontinuation and switching to trientine or zinc salts (83) (Table 5). As food inhibits the absorption of D-penicillamine, this drug should be administered one hour before or two hours after meals. There is lack of recent evidence as to pyridoxine deficiency in patients receiving penicillamine hence there has been variable personal practice in its supplementation. Moreover, pyridoxine intake is relatively high as many food products are supplemented with water soluble vitamins.

Trientine, triethylene tetramine hydrochloride, was initially introduced in 1969 as a second line chelating agent in WD patients who developed adverse events related to D-penicillamine (84). There have been only rare reports of allergic reactions, arthralgias, muscle cramps, and sideroblastic anemia induced by trientine (85-87). Given its safety profile it is being increasingly used as first line chelation therapy, although there is a lack of robust clinical studies evaluating the efficiency of trientine in treating WD compared to D-penicillamine. Similar efficacy of trientine compared to D-penicillamine was shown

in one large study in WD adults with liver symptoms (88). However, first line treatment with trientine was associated with a higher risk of neurologic worsening of symptomatic neurologic patients compared to D-penicillamine. The only available paediatric study analysed the efficacy of trientine as second-line therapy in 16 children with D-penicillamine intolerance or adverse events. Liver function normalized in the majority of children, but trientine did not improve accompanying neurological or psychiatric symptoms (86).

The recommended dose of trientine in children is 20 mg/kg/day in 2-3 divided doses. Recently, a small prospective pilot study in adults with WD has suggested that a once daily trientine regimen at a dose of 15 mg/kg provided good efficacy and safety but it should be further evaluated as maintenance therapy to improve compliance (89). Trientine also chelates iron, and therefore if iron supplementation is necessary, it should be administered at a different time of the day. The drug is best given 1 hour before or 2-3 hours after food for optimal absorption (Table 5). Trientine tablets must be kept refrigerated, which could be a problem for patients residing in or travelling to hot countries.

Zinc salts are being increasingly used as first line therapy for the treatment of pre-symptomatic patients and for maintenance therapy after initial de-coppering with a chelator, but the efficacy of zinc monotherapy in symptomatic patients with liver disease is still under debate (90-96). The postulated mode of action of zinc is the induction of metallothionein in enterocytes (97). Copper absorbed in the small intestine is thereby sequestered in enterocytes which at the end of their life cycle carry copper into the lumen. Zinc also induces hepatocyte metallothionein, and as D-penicillamine may have copper

detoxifying effect. Most studies evaluating the clinical efficacy of zinc when applied as first line monotherapy in the various clinical presentations of WD showed that zinc had a better tolerance profile than penicillamine and could be safely used for treatment of presymptomatic children (90, 92-94). Treatment failure was however reported in symptomatic children presenting with liver disease (79, 91, 95, 96) and patients who relapsed on zinc improved after reintroduction of a chelating agent (91). Initiation of therapy with zinc salts presents also risk of neurological deterioration as is observed with other treatment modalities (91, 98).

Different formulations of zinc salts are available: zinc sulphate, zinc acetate and zinc gluconate. Gastrointestinal problems, such as nausea, vomiting, epigastric pain, gastric/duodenal mucosal ulceration or erosion have been reported mainly with zinc sulphate, and may particularly alter the child's quality of life and lead to poor adherence (95, 99). Gastrointestinal symptoms may resolve when switching formulation from zinc sulphate to zinc acetate. Anemia related to iron deficiency, isolated increase of serum amylase and lipase levels (zinc containing enzymes) without clinical and radiological features of pancreatitis may also be observed.

The recommended dosage is 25 mg twice daily of elemental zinc in children under 5 years of age, 75 mg/day (if body weight < 50 kg) or 150 mg/day (if body weight >50 kg) in three divided doses in children over 5 years of age (3, 92, 94). Zinc should not be taken with food because it interferes with its absorption, and dietary copper restriction is not recommended because zinc blocks copper absorption from the intestinal track (Table 5).

Treatment strategy

Treatment should be individually tailored to the clinical condition of the child defined by the type and severity of organ involvement. A clinically relevant limitation in the long-term use of D-penicillamine is the occurrence of severe adverse events. Lack of adherence and underdosage are the main risk factors for an unfavorable clinical course. To improve adherence to the life-long therapy, the treatment scheme should be as simple as possible.

Paediatric hepatologists vary in the approaches they use in the care for children with WD (100). In asymptomatic children or children with only mild liver symptoms, all available treatments have proven effective (28, 86, 90, 92-94). In symptomatic WD patients, current guidelines favor the use of chelating agents (D-penicillamine, trientine) as first line therapy (3, 4).

A sequential treatment in WD could be proposed based on the hypothesis that after a treatment phase with the more effective chelating agents, smaller dosage or alternative treatment with zinc in de-coppered patients might be sufficient to upkeep copper homeostasis. However, only very limited data are available to decide when and under which conditions a patient can be switched to zinc maintenance therapy and vice versa.

Combination therapy using zinc in conjunction with a chelating agent (administered at widely spaced intervals during the day to avoid interference- and given one hour before or two hours after meals) has a theoretical basis in both blocking copper uptake and eliminating excess copper, and thus might be synergistic effect. Reports have been limited to patients who present with decompensated chronic liver disease, and suggest a favorable outcome for combination therapy with D-penicillamine and zinc (8, 76) or with

trientine and zinc (79, 101). Recommendations of the ESPGHAN Hepatology Committee are shown in table 1. **INDICATIONS FOR LIVER TRANSPLANTATION IN CHILDREN WITH WD**

Indications for LT are rare (<1%), including patients with ALF or those with progression of liver dysfunction to liver failure despite drug therapy (102). Excellent post-LT outcomes are reported (103, 104). Actual patient survival rates were 87% at 5, 10, and 15 years in a French series of 75 adults and 46 children (median age: 14 years, range 7 to 17 yrs) transplanted between 1985 and 2009 for ALF (53%), decompensated cirrhosis (41%), or severe neurological disease (6%) (103). In another study analysing the United Network for Organ Sharing (UNOS) database including 170 WD children who underwent LT between 1987 and 2008, one and five year survival were 90.1% and 89% respectively (104). In both studies, patients transplanted for end stage chronic liver disease had better long term survival than patients transplanted for ALF. Extracorporeal liver support systems as a bridge to LT may improve the outcome (105, 106). Neurological and especially psychiatric involvement may show little improvement with transplantation but LT cannot be considered as a therapy for patients with severe neuropsychiatric involvement (107, 108).

Children presenting with decompensated liver cirrhosis with liver failure but no hepatic encephalopathy can be often rescued with chelation treatment. Response to medical treatment may take time with improvement of PT after a minimum of 1 month and normalization within 3 months to 1 year or more (76). Close follow-up and monitoring of clinical status for hepatic encephalopathy, ascites, sepsis and liver function tests is required in specialized LT units to timely listing the child on the transplant list, a decision

that is extremely challenging. In 1986, Nazer et al devised a scoring system to predict the outcome of patients including adults and children with hepatic decompensation in the setting of WD (109). In 2005, the score was re-examined in the paediatric population by Dhawan et al who proposed a new scoring system (King's Wilson index (WI)) that had a better positive predictive value for mortality without transplantation (Table 6) (8, 102). The WI is reported to be 93% sensitive, 98% specific, with a positive predictive value of 93% (8). However, although useful, the WI may not be entirely accurate and continued investigation of predictors of outcome in WD is necessary.

Recommendations of the ESPGHAN Hepatology Committee are shown in table 1.

MONITORING EFFICACY, SAFETY AND COMPLIANCE TO TREATMENT

Treatment of WD should aim at normal physical examination and normal liver function tests. Patients must avoid alcohol consumption and potential hepatotoxic drug therapy.

Monitoring should be performed once a week at initiation of therapy, especially while increasing penicillamine dosages, every 1 to 3 months until remission and every 3 to 6 months afterwards. Non-adherence to therapy can lead to life-threatening deterioration and monitoring intervals should be shorter, especially in adolescents, in whom compliance is uncertain.

Monitoring includes physical examination (search for new WD symptoms or related to therapeutic adverse events of therapy) and liver function tests which should normalize progressively within 3 to 12 months. Twenty-four hour urinary copper excretion should increase after initiation of therapy with D-penicillamine or trientine, followed by a decrease once liver function tests return to normal indicating a reduction in the body load

of copper. In general pre-symptomatic children excrete less copper than those with symptomatic disease. Urinary copper excretion should lie between 200-500 $\mu\text{g}/24\text{ h}$ during maintenance therapy with D-penicillamine or trientine. Monitoring of patients on zinc therapy must ensure a reduction in urinary copper excretion (initially below 100 $\mu\text{g}/24\text{h}$, and between 30 - 75 $\mu\text{g}/24\text{h}$ on maintenance therapy); low levels below 30 $\mu\text{g}/24\text{h}$ suggest zinc overdosage. Additionally, serum zinc levels and urinary zinc excretion should be maintained above 125 $\mu\text{g}/\text{dL}$ and 1.5-2 g/d respectively; lower levels generally indicate poor compliance to therapy (110).

It is important to also monitor blood cell counts, and screen any therapeutic related adverse events (such as proteinuria). Neutropenia and anemia may be due to failed iron mobilization, with transaminase elevation due to increased hepatic iron accompanied by increased ferritin indicating over treatment. Temporary discontinuation of therapy, with close observation, is warranted followed by reintroduction of therapy at a reduced dose (see Table 6).

Yearly slit-lamp examination should document fading or disappearance in patients with KF rings if they are being adequately "de-coppered". Appearance of KF rings in patients with persisting abnormalities of liver function tests on maintenance therapy indicates non compliance. Search for appearance of changes at magnetic resonance imaging of the brain may be useful in patients non-compliant to therapy (111).

KEY RECOMMENDATIONS

Appropriate steps for diagnosis, treatment and follow up of children with WD were discussed collectively between the core group and ESPGHAN hepatology members. An agreement was obtained on the recommendations shown in table 1.

DISCLAIMER

“ESPGHAN is not responsible for the practices of physicians and provides guidelines and position papers as indicators of best practice only. Diagnosis and treatment is at the discretion of physicians”.

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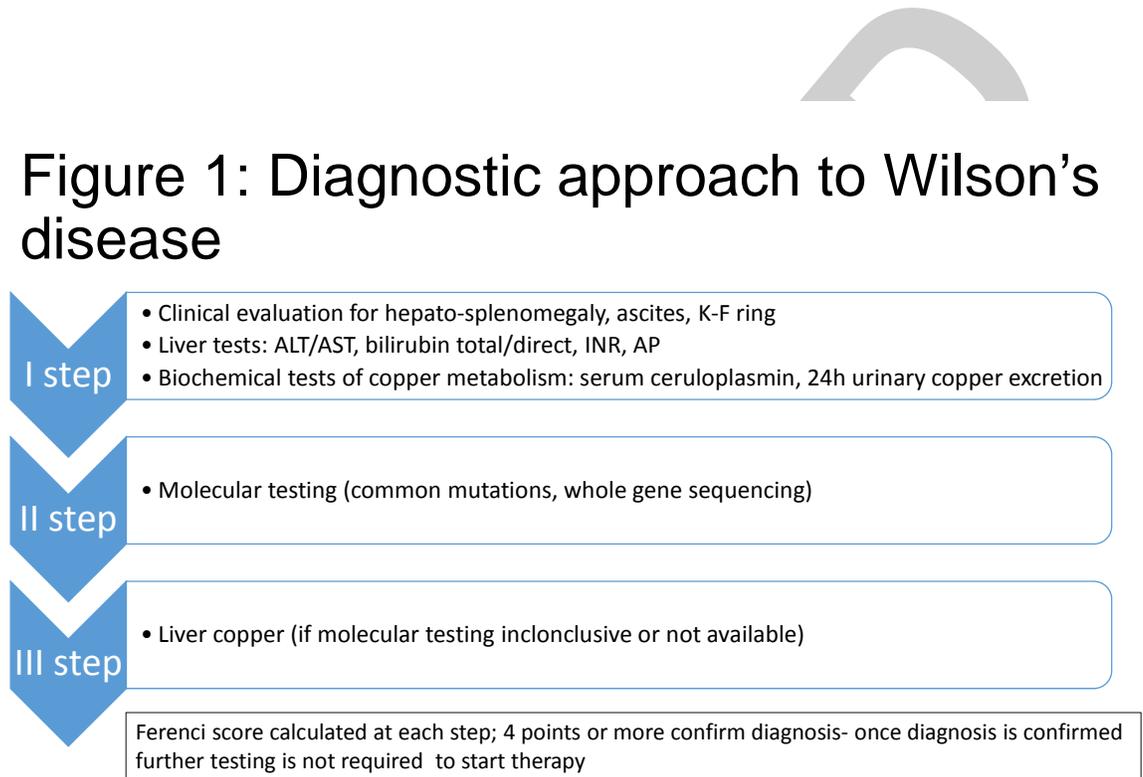
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Figure 1: Diagnostic approach to Wilson's disease



ACG

Table 1: Recommendations of the ESPGHAN hepatology committee

- #1 WD should be considered in the differential diagnosis of children above the age of 1 year presenting with any sign of liver disease ranging from asymptotically increased serum transaminases to cirrhosis with hepatosplenomegaly and ascites or ALF. **Grade 1A. [level of agreement: 86%]**
- #2 WD should be ruled out in any teenager with unexplained cognitive, psychiatric or movement disorder. **Grade 1A [96%]**
- #3 Diagnostic testing for WD in suspected patients should include liver function tests (serum transaminases, conjugated and total bilirubin; alkaline phosphatase and prothrombin time/INR), serum ceruloplasmin, and 24-hour urinary copper. **Grade 1A [96%]**
- #4 The Ferenci scoring system should be applied to children for diagnosis of WD. Mutation analysis of the *ATPB7* gene may facilitate the diagnosis. **Grade 1A [91%]**
- #5 Copper estimation in the liver tissue could be helpful in children where the diagnosis is uncertain. **Grade 1C [100%]**
- #6 Once WD diagnosis is confirmed in the proband, WD should be sought in first degree relatives including siblings, offspring and parents by performing liver function tests, explorations of copper metabolism, and targeted molecular analysis. **Grade 1A [100%]**
- #7 Given its safety profile, zinc salts, preferably zinc acetate, could be used in pre-symptomatic children identified through family screening, or as maintenance therapy after de-coppering with chelators as long as serum transaminase levels remain normal. **Grade 2C [96%]**
- #8 Children with signs of significant liver disease, such as cirrhosis or abnormal INR, should be preferably treated with copper chelating agents. **Grade 2B [96%]**

- #9 Dietary restriction of copper rich foods is advised until remission of symptoms and normalization of liver enzymes in children treated with copper chelating agents. **Grade 2C. [82%]**
- #10 Children with ALF or decompensated liver cirrhosis should be transferred to and managed in paediatric liver transplantation centers. **Grade 1A [100%]**
- #11 Children with decompensated liver cirrhosis should be treated with a chelating agent or a combination of zinc salts and a chelating agent that may preclude the need for a liver transplantation. The King's Wilson index should be monitored for prognostic assessment and timely decision for LT. **Grade 2B [96%]**
- #12 Because liver transplantation corrects the enzymatic defect, chelating agents or zinc treatment is no longer required after transplantation. **Grade 1A [96%]**
- #13 All children should be closely followed-up during the first month following initiation of therapy, then every 1 to 3 months until remission, and every 3 to 6 months thereafter. **Grade 1C. [100%]**
- #14 Monitoring includes physical examination, biochemical tests (i.e blood cell count, liver function tests, urea, creatinine, proteinuria), serum copper, and 24 hr-urinary copper in order to assess efficacy, over-dosage or non-adherence to therapy and adverse events. **Grade 1C. [96%]**
- #15 Evidence for non-adherence to zinc can be assessed by measuring serum zinc levels and/or urinary zinc/copper 24h excretion. **Grade 2B. [91%]**
- #16 If increased transaminases remain or relapse despite treatment, poor compliance should be suspected. **Grade 2B. [96%]**
- #17 The occurrence of penicillamine related adverse events should prompt discontinuation and switching to trientine or zinc salts according to the severity of liver disease. **Grade 2B. [100%]**

Legend: Voting results are indicated in brackets for each recommendation .

ACCEPTED

Table 2. Clinical presentations of Wilson’s disease in childhood.

| Clinical symptoms | Age at onset of symptoms | References |
|--|--|---|
| HEPATIC <ul style="list-style-type: none"> • Incidental finding of increased serum transaminases • Acute hepatitis • Hepatomegaly • Fatty liver • Acute liver failure with haemolysis • Portal hypertension: esophageal varices, splenomegaly, low platelet count • Decompensated cirrhosis with ascites | >2 years | (6-15, 17, 18) |
| NEUROLOGICAL AND PSYCHIATRIC <ul style="list-style-type: none"> • Dysarthria • Dysphagia, excessive salivation • Mood/behaviour changes including depression, irritability • Incoordination (e.g. handwriting deterioration) • Declining performance at school • Resting and intention tremors • Gait disturbance, dystonia, rigidity • Mask-like face, risus sardonicus, • Stroke-like symptoms | Usually > 15 years Case reports 7-9 years | (20-22, 25-28) |
| OPHTHALMIC <ul style="list-style-type: none"> • KF rings at slit lamp examination | > 10 years | (10, 28) |
| HAEMATOLOGICAL <ul style="list-style-type: none"> • Acute/chronic haemolytic anaemia | > 7 years | (24) |
| OTHER <p>Renal</p> <ul style="list-style-type: none"> • Renal tubular dysfunction (Fanconi’s syndrome, tubular acidosis, aminoaciduria) • Nephrolithiasis • Nephrocalcinosis <p>Cardiac</p> <ul style="list-style-type: none"> • Cardiomyopathy, subclinical dysfunction • Arrhythmia <p>Endocrine</p> <ul style="list-style-type: none"> • Hypoparathyroidism. <p>Other</p> <ul style="list-style-type: none"> • Pancreatitis • Skin lipomas <p>Skeletal</p> <ul style="list-style-type: none"> • Rickets/osteopenia/osteoporosis • Arthropathy | Case reports, age can not be defined | (29) (30) (31) (32) (33) (34-36) |

Table 3. Explorations of copper metabolism.

| | Normal values | High suspicion of WD |
|--------------------------------|--|--|
| Serum Ceruloplasmin | 20 - 40 mg/dl | < 10 mg/dl |
| 24 hr-urinary copper excretion | < 40 μg (< 0.65 μmol) | > 100 μg (1.6 μmol) |
| Liver copper content | < 50 $\mu\text{g/g}$ dry weight | > 250 $\mu\text{g/g}$ dry weight (> 4 $\mu\text{mol/g}$ dry weight) |

Table 4. Diagnostic score in Wilson's Disease, agreed at a consensus meeting (64).

| Score | -1 | 0 | 1 | 2 | 4 |
|---|----------------------|-----------|---------------------------------|---|---|
| Kayser-Fleischer rings | | absent | | present | |
| Neuropsychiatric symptoms suggestive of WD (or typical brain MRI) | | absent | | present | |
| Coombs negative haemolytic anaemia + high serum copper | | absent | present | | |
| Urinary copper (in the absence of acute hepatitis) | | normal | 1-2 x ULN | >2 x ULN, or normal but >5 x ULN 1 day after challenge with 2 x 0.5 g D-penicillamine | |
| Liver copper quantitative | normal | | < 5 x ULN (<250 µg/g) | >5 x ULN (>250 µg/g) | |
| Rhodanine positive hepatocytes (only if quantitative Cu measurement is not available) | | absent | present | | |
| Serum ceruloplasmin (nephelometric assay) | | > 0.2 g/l | 0.1-0.2 g/l | < 0.1 g/l | |
| Disease-causing mutations detected | | none | 1 | | 2 |
| Assessment of the Wilson's Disease diagnostic score | | | | | |
| 0-1: unlikely | 2-3: probable | | 4 or more: highly likely | | |

Abbreviations: ULN, upper limit of normal

Table 5. Dosage and treatment monitoring

| | Zinc salts | D-Penicillamine | Trientine |
|---|--|---|--|
| Dosage in children | <p>Zinc acetate</p> <ul style="list-style-type: none"> • Age > 16 yrs and body weight > 50 kg : 150 mg/day in 3 divided doses. • Age 6 to 16 yrs and body weight < 50 kg: 75 mg/day in 3 divided doses • Under 6 years of age: 50 mg/day in 2 divided doses <p>Zinc Sulphate</p> <ul style="list-style-type: none"> • Age > 16 yrs and body weight > 50 kg : 600 mg/day in 3 divided doses. • Age 6 to 16 yrs and body weight < 50 kg: 300 mg/day in 3 divided doses • Under 6 years of age: 200 mg/day in 2 divided doses | <ul style="list-style-type: none"> • Starting dose: 150-300 mg/day, gradually increasing once a week up to 20mg/kg/day given in 2 or 3 divided doses or 1000 mg (max 1500 mg) in young adults given in 2 or 4 divided doses. • Maintenance dose: 10-20 mg/kg/day up to 750 mg -1000 mg/day in 2 divided doses | <ul style="list-style-type: none"> • Starting dose: 20mg/kg/day or 1000 mg (max 1500 mg) in young adults given in 2 or 3 divided doses. • Maintenance dose: 900 – 1500 mg/day in 2 or 3 divided doses. |
| Administration | 1h before meal or 2h after meal | 1h before meal or 2h after meal | 1h before meal or 3h after meal |
| Adequacy of treatment parameters | <ul style="list-style-type: none"> • Urinary copper excretion: 30 -75 µg (0.5 -1.2 µM) /24hr on maintenance treatment • Serum zinc level > 125 µg/dL • Urinary zinc > 2 mg/24 hr on maintenance treatment | <ul style="list-style-type: none"> • Urinary copper excretion: 200-500 µg (3-8 µM)/24 h on maintenance treatment | <ul style="list-style-type: none"> • Urinary copper excretion: 200-500 µg (3-8 µM)/24 h on maintenance treatment |
| Liver function | Usually 2-6 months, ALT | Usually 2-6 months | Usually 2-6 months |

| | | | |
|-------------------------------------|---|--|--|
| improvement | normalization within 1 year | | |
| Indication for a drug change | <ul style="list-style-type: none"> • Persistent ALT > 3x upper limit of normal and/or INR>1.5 • Poor tolerance e.g. nausea, abdominal pain, gastric ulcerations | Poor tolerance or side effects e.g. hypersensitivity reactions, fever, neutropenia, thrombocytopenia, lymphadenopathy or proteinuria | Poor tolerance or side effects e.g. allergic reactions, arthralgia, sideroblastic anemia |

ACCEPTED

Table 6. Wilson's disease scoring system to predict the outcome of children with hepatic decompensation (King's Wilson index) by Dhawan et al (8).

| Score | Bilirubin ($\mu\text{mol/l}$) | INR | AST | Leukocytes ($10^9/\text{l}$) | Albumin (g/l) |
|-------|------------------------------------|---------|---------|-----------------------------------|------------------|
| 0 | 0-100 | 0-1,29 | 0-100 | 0-6,7 | >45 |
| 1 | 101-150 | 1,3-1,6 | 101-150 | 6,8-8,3 | 34-44 |
| 2 | 151-200 | 1,7-1,9 | 151-200 | 8,4-10,3 | 25-33 |
| 3 | 201-300 | 2,0-2,4 | 201-300 | 10,4-15,3 | 21-24 |
| 4 | >300 | >2,5 | >300 | >15,3 | 0-20 |

* > 11 points - urgent listing for LT