PROTOCOL FOR THE USE OF COPPER AND CAERULOPLASMIN ANALYSIS IN THE INVESTIGATION OF WILSON'S DISEASE

1.1 Scope and purpose

The purpose of this protocol is to describe the investigation of patients with suspected Wilson's disease.

1.2 References

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1.3 Related documents

BS/CB/DCB/GEN/13 DCB Handbook (Part 3 test information)

2. Introduction

Wilson's disease (WD) is an inborn error (autosomal recessive) of copper metabolism. It has an estimated world-wide prevalence of between 1:100,000 and 1:300,000 with a heterozygote frequency of 1:100. It leads to the accumulation of copper in organs and tissues, initially in the liver and then progressively in the kidneys, eyes, brain and other tissues. It is caused by a defect in the gene ATP7B located on chromosome 13 (13q14.3) which leads to defective ATPase dependant copper excretion by the liver. Presentation can be acute or chronic and may be with either hepatic or neurological symptoms or both.

2.1 Clinical Presentation

Onset of symptoms can occur at any time, but the peak is in adolescence. Cases presenting at <3 or > 60 years are very rare with the majority of patients presenting between the ages of 5 and 35 years old. However, a diagnosis of WD cannot be excluded on age alone and older patients with neurological or psychiatric symptoms and concurrent biochemical or histological findings consistent with WD warrant further investigation.

Liver disease is the most frequent presentation in the 8-16 year old age group and may precede neurologic symptoms by up to 10 years. Patients may present with acute liver failure with rapid deterioration (predominantly young females) or chronic hepatitis and cirrhosis which is often indistinguishable from other causes. Some patients may present with isolated hepatosplenomegaly. There should be a high suspicion in patients with deep jaundice, Coombs negative haemolytic anaemia, moderately raised transaminases and low ALP.

Neurologic signs including tremor, ataxia and dystonia may appear before, concurrently with, or many years after liver disease and may be mild, intermittent or rapidly developing to severe disability. Mean age of onset in 2nd or 3rd decade but can be much later (usually symptomatic by age 50). Psychiatric symptoms may precede neurologic and hepatic signs (~1/3 patients). In adults, the most common psychiatric conditions associated with WD include mood disorders (depressive or bipolar spectrum), psychotic disorders, sleep disturbances, and subtle cognitive dysfunction. Children and adolescents may exhibit non-specific behavioural issues or decline in academic performance.

Kayser-Fleischer rings attributable to copper deposition in the eyes are detectable by slit-lamp microscopic examination (requires specialist referral) in up to 95% of patients presenting with neurologic symptoms and ~50% of those with liver disease only. However, they are very often absent in young children presenting with acute liver disease. Rarely KF rings may be found in chronic cholestatic diseases and neonatal cholestasis therefore are not completely specific. The combination of Kayser-Fleischer rings and a caeruloplasmin of <0.1 g/L is considered diagnostic for Wilson's disease (EASL guidelines).

Coombs negative haemolytic anaemia may be the only presenting symptom (~10%) but is more usually associated with severe liver disease.

Involvement of other organs has been described and may be present at the time of diagnosis or develop later. These include renal abnormalities characterized as a Fanconi

syndrome which may in turn lead to muscle weakness due to hypokalaemia, hypouricaemia, nephrolithiasis and decreased urinary acidification.

Skeletal abnormalities have been noted particularly in the Indo-Pakistani subcontinent and resemble rickets.

Cardiac problems may include cardiomyopathy and arrhythmias and can present in childhood and independently of treatment.

Endocrine abnormalities include hypoparathyroidism, infertility or repeated miscarriages. Pancreatitis has been observed on presentation, attributed to copper deposition in the pancreas.

2.2 Laboratory Findings

The 'classic' laboratory findings in Wilson's Disease are those of low serum/plasma caeruloplasmin and thus low serum/plasma copper levels. Nevertheless, copper is increased relative to the caeruloplasmin due to an increase in non-caeruloplasmin-bound copper. This necessitates measurement of both copper and caeruloplasmin.

About 90% of copper in the circulation is incorporated into caeruloplasmin, with the remaining 10% loosely bound to albumin and histidine (non-caeruloplasmin-bound copper). Caeruloplasmin concentration is thus the primary determinant of copper concentration. Copper is incorporated into caeruloplasmin at a late stage during hepatic synthesis. Normally caeruloplasmin without incorporated copper (apocaeruloplasmin) is not released into the circulation or rapidly metabolised if it is released. In Wilson's disease there may be more circulating apocaeruloplasmin than usual. The shorter circulating half-life of apocaeruloplasmin compared to caeruloplasmin results in lower concentrations in the blood.

Caeruloplasmin concentrations are increased during the acute phase response. Oestrogen medication and pregnancy as well as some anticonvulsant therapy also result in an increased plasma caeruloplasmin concentration. Levels may be reduced in be reduced in copper deficiency and in conditions giving rise to hypoproteinaemia e.g. renal protein loss, protein losing enteropathy, severe chronic liver disease. In addition absolute copper deficiency or zinc excess will also result in a decreased caeruloplasmin.

Accumulation of copper in the liver and other tissues usually results in marked increases in urinary copper excretion, although this might not be the case with very young children. Patients presenting with liver disease may demonstrate misleadingly normal serum caeruloplasmin and copper particularly if they present with acute hepatitis. This has been attributed to an increase in apocaeruloplasmin that is also detected in the immunological assay for caeruloplasmin resulting in overestimation. Therefore, additional tests are often necessary before Wilson's disease can be reliably excluded.

Liver transaminases will be increased in the majority of cases except in infancy, although the degree of elevation may be relatively mild. AST elevation may be greater than ALT rises in those with cirrhosis. Transient hyperbilirubinaemia may be associated with episodes of

haemolysis as may low or low-normal levels of ALP. Serum uric acid may also be decreased at presentation because of associated renal tubular dysfunction.

Investigation

3.1 Serum Copper and Caeruloplasmin

The first line investigation of Wilson's disease is to measure serum caeruloplasmin. If found to be a low then the patient should be evaluated for common causes of low caeruloplasmin (table 1) and a repeat sample ordered along with serum copper.

If common causes have been excluded and caeruloplasmin/copper are persistently low then the next investigation is 24 hour urine copper.

Note high oestrogen states e.g. pregnancy and OCP can increase copper up to 40 µmol/L.

Reference Values

Age	Copper µmol/L	Caeruloplasmin g/L
< 4 months	1.4 – 7.2	0.20 – 0.32
4-6 months	3.9 - 17.3	
>6 months and adults	11.0 – 25.0	

Interpretation

Caeruloplasmin 0.20 – 0.32 g/L – Wilson's disease is highly unlikely
Caeruloplasmin 0.10 – 0.19 g/L – Wilson's disease is not excluded
Caeruloplasmin <0.1 g/L – Highly suggestive of Wilson's disease
Caeruloplasmin < 0.1 g/L + KF rings establishes diagnosis of Wilson's disease

Low caeruloplasmin levels have also been reported in up to 20% of heterozygotes for mutations in the ATP7B gene.

Increased copper/caeruloplasmin are rarely of pathological significance.

Normal levels of copper and/or caeruloplasmin do not exclude Wilson's disease. If there is a high clinical suspicion, then further investigations including 24 hour urine copper and slit-lamp eye examination may be appropriate. Patients with unexplained liver disease or neurologic symptoms should be referred to secondary care.

If common causes of reduced caeruloplasmin/copper have been excluded then the next investigation is 24 hour urine copper.

Estimation of non-caeruloplasmin bound copper (NCC) is no longer recommended due to inherent inaccuracies in the immunologic measurement of caeruloplasmin resulting in zero or negative values for NCC.

Table 1: Other causes of abnormal caeruloplasmin results

Causes of Low Caeruloplasmin	Causes of High Caeruloplasmin
Protein loss (renal, GI, malabsorption)	Oestrogen containing medication (including OCP)
Cirrhosis (loss of synthetic function)	Pregnancy
Absolute copper deficiency (zinc excess, malabsorption/malnutrition, TPN)	Inflammation/infection
Menkes disease	Tissue damage
Aceruloplasminaemia	Anticonvulsant therapy (valproate, carbamazepine, phenobarbital)
MEDNIK syndrome (AP1S1 disorder)	
AP1B1 disorder	
Niemann-Pick type C	
Congenital glycosylation disorder (PGM1-CDG, CCDC115-CGD, TMEM199-CDG)	

3.2 Urine testing

The second line investigation to distinguish copper deficiency from possible Wilson's disease is a 24 hour urine copper excretion (μmol/24 hour).

Normal copper excretion is < 0.7 μmol/24h; Levels > 1.6 μmol/24h are indicative of Wilson's disease.

Levels between 0.7 – 1.6 μmol/24h are not diagnostic but can be suggestive of Wilson's disease, particularly in asymptomatic children.

3.3 Further Investigations

Where biochemistry tests are indicative of Wilson's disease or there is a high clinical suspicion further investigations may be carried out by a specialist including:

Hepatic copper content
Penicillamine challenge (paediatric cases only, not recommended in adults)
DNA analysis (performed at King's College Hospital)

3.4 Scoring Systems

Scoring systems exist for diagnosis and prognosis of WD. The Leipzig score (also known as the Ferenci score) was developed by expert opinion and available in evidence in 2001. Clinical and biochemical findings associated with WD are each given a graded score with a total score of 4 making a diagnosis of WD highly likely. This system has been validated in children and adults. A modified version recently developed specifically for children is yet to be fully validated.

Prognostic scoring systems to predict treatment failure, ALF and decompensated chronic liver disease and include the Nazer score, New Wilson Index and Alam score.

4. Screening

First-degree relatives of newly diagnosed WD patients should be screened even if asymptomatic. If disease-specific mutations have been identified in the proband then primary screening should be mutation analysis of the *ATP7B* gene.

Clinical assessment should include a thorough clinical history relating to jaundice, liver disease, subtle features of neurological or psychiatric involvement as well as physical examination and slit-lamp eye examination to look for KF rings.

Biochemical testing should include LFTs, conjugated and conjugated bilirubin, AST, caeruloplasmin, serum and 24h urine copper. Biochemical findings suggestive of WD should be confirmed by liver biopsy.

Any family members displaying signs or symptoms of WD should also be fully evaluated.

5. Treatment & Monitoring

Symptomatic patients should be treated initially with a chelating agent, either d-penicillamine or trientine. Asymptomatic patients may be treated with a lower dose of either of these therapies or zinc.

Treatment adequacy is evaluated by clinical and biochemical improvement. Overtreatment may result in hepatic iron overload.

Where there is a good and stable response to treatment maintenance therapy may involve lower doses of the chelating drug or switching to zinc. 24-h urinary copper output while continuing medications (on treatment) should be 3–8 µmol/24 h with chelating agents and 0·5–1·2 µmol/24 h with zinc salts. Patients should be monitored at least twice a year with more frequent monitoring required during the initial phase of treatment.

Hepatocellular carcinoma (HCC) is a rare complication of WD. Screening and surveillance of HCC is recommended in patients with WD and cirrhosis only.

Appendix 1: Results from patients with known Wilson's disease

Patient No	Serum Cu µmol/L	Serum Cp g/L	Urine Cu µmol/24h
1	6.8	0.10	0.8
2	5.4	0.04	-
3	5.1	0.02	-
4	6.6	0.02	5.8
5	4.1	0.02	-
6	4.0	0.02	-
7	7.3	0.03	-
8**	4.8	0.04	-

** This patient was undiagnosed for 10 y, had an equivocal penicillamine test
All of the above patients presented with predominantly neurological symptoms

Appendix 2: Coded Comments

If zinc is high:

@cuz: Low copper. Note that high zinc intake (supplementation, denture fixative) may contribute to copper deficiency. If cause unknown suggest repeat to confirm and request also caeruloplasmin; consider sending a trace element sample (dark blue top) for analysis of both zinc and copper.

1st low caeruloplasmin or copper result:

@cu1: Slightly low copper/caeruloplasmin. Common causes include malabsorption/malnutrition, protein loss and chronic liver disease. Wilson's disease is a rare cause usually presenting before 40 yrs of age. Zinc induced copper deficiency may be seen in chronic high dose supplementation or excessive use of denture fixative. Repeat with serum copper in the first instance. Send a trace element tube for zinc analysis if required.

2nd low caeruloplasmin or copper result:

@cu2: Again, low copper/caeruloplasmin. If no obvious cause consider 24h urine copper to distinguish copper deficiency from copper excess (Wilson's disease).

Caeruloplasmin <0.1 g/L

@cu3: Low copper/caeruloplasmin. Suggest review for possible protein loss or malabsorption/malnutrition. If no obvious cause then Wilson's disease should be excluded. Is there any family history of liver and/or neurological disease? Please send a repeat sample to confirm and check also serum copper and 24h urine copper. Please telephone if you wish to discuss.

High copper:

@cuhi: Increased serum copper commonly seen in response to infection, inflammation or steroid hormones (e.g. pregnancy, OCP, HRT)