

**BLOOD SCIENCES
DEPARTMENT OF CLINICAL BIOCHEMISTRY**

Title of Document: Protocol for the use of copper and caeruloplasmin analysis in the investigation of Wilson's disease.

Q Pulse Reference N^o: BS/CB/DCB/PROTOCOLS/33

Version no: 4

Authoriser: Peter Beresford

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

1) Introduction

1.1 Scope and purpose

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

1.2 Responsibility for document

This document is to be reviewed on an annual basis by the author of the protocol or nominated clinical biochemist.

1.3 Location of Copies

- 1) Duty Biochemist files
- 2) Severn Pathology Website

1.4 References

EASL Clinical Practice Guidelines: Wilson's disease European Association for the Study of the Liver, Journal of Hepatology (2012) 56; 3: 671 - 685
Liver Disease and Laboratory Medicine – ACB Venture Publications (2000), pp68-71
Clinical Chemistry and the Sick Child. Ed. Clayton. Blackwell Scientific Publications (1994), p468
SAS Trace Element Handbook 4th Edition (2006)
PRU Handbook of Clinical Immunochemistry-9th Edition (2007), pp37-42.

1.5 Definitions

Wilson's disease

Wilson's disease is an inborn error (autosomal recessive) of copper metabolism. 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.

1.6 Related documents

No related documents

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Introduction

Wilson's disease 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.

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. Presentation can be acute or chronic and may be with either hepatic or neurological symptoms or both.

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, low haemoglobin, 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).

Kayser-Fleischer rings attributable to copper deposition in the eyes (detectable by slit-lamp microscopic examination) are detectable 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.

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

2.2 Laboratory Findings

About 90% of copper in the circulation is incorporated into caeruloplasmin, with the remaining 10% loosely bound to albumin and histidine (non-caeruloplasmin-bound

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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.

Caeruloplasmin concentrations are age- and gender-dependent, are increased during the acute phase response, by oestrogen medication and in pregnancy, with some anticonvulsant therapy, and may be reduced in copper deficiency and in conditions giving rise to hypoproteinaemia.

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. 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. Therefore additional tests are often necessary before Wilson's disease can be reliably excluded.

3.0 Investigation

3.1 Serum Copper and Caeruloplasmin

The first line investigation of Wilson's disease is to measure serum copper and caeruloplasmin (this should be done on the same sample).

Reference Values

<u>Copper $\mu\text{mol/L}$</u>		<u>Caeruloplasmin g/L</u>	
<u>Age</u>		<u>Males</u>	<u>Females</u>
< 4 months	1.4 – 7.2	< 4 months	0.15 – 0.56
4-6 months	3.9 - 17.3	4 - 6 months	0.24 – 0.83
7-12 months	7.9 - 20.5	6 – 18 months	0.27- 0.91
Children <1 year and adults	13 - 23	18 mths – 3 yrs	0.28 – 0.90
		3 – 9 yrs	0.24 – 0.46
		9 – 12 yrs	0.24 - 0.45
		12 - 19 yrs	0.23 - 0.45
		12 - 19 yrs	0.15 – 0.37
		> 19 years	0.21 – 0.50
			0.21 – 0.40
			0.23 – 0.60

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Interpretation

Caeruloplasmin < 0.1 g/L + KF rings establishes diagnosis

Caeruloplasmin <0.1 g/L strongly suggestive

Borderline requires Ix, normal does not exclude (EASL Clinical Practice Guidelines, 2012)

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 and slit-lamp eye examination may be appropriate. Patients with unexplained liver disease or neurologic symptoms should be referred to secondary care.

Common causes for low copper/caeruloplasmin include:

- Malabsorption e.g. Crohn's or Coeliac disease, post gastric bypass surgery
- Protein loss
- Chronic liver disease (of any cause)
- Excess zinc supplementation
- TPN

If these have been excluded then the next investigation is 24 hour urine copper.

Increased copper/caeruloplasmin are rarely of pathological significance. Levels may be increased due to:

- Pregnancy
- Oestrogen therapy including oral contraceptive pill
- Infection/inflammation or tissue damage
- Anticonvulsants e.g. valproate, carbamazepine and phenobarbital

3.2 Urine testing

The second line investigation to distinguish copper deficiency from possible Wilson's disease is a 24 hour urine copper excretion ($\mu\text{mol}/24\text{ hour}$).

Normal copper excretion is < 0.7 $\mu\text{mol}/24\text{h}$; levels > 1.0 $\mu\text{mol}/24\text{h}$ may indicate Wilson's disease.

3.4 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:

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Hepatic copper content

Penicillamine challenge

DNA analysis

Uncontrolled Copy

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Appendix 1: Table_ 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

@cu2: Low copper/caeruloplasmin. Note that high zinc intake (supplementation, denture fixative) may contribute to copper deficiency. Suggest repeat to confirm in 2-3 months (after stopping supplementation if applicable).

@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 years of age. Suggest repeat to confirm in the first instance. Consider 24h urine copper if caeruloplasmin is persistently low with no obvious cause or there is a family history of liver disease or neurological problems.

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

@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.

@cu4: Low copper/caeruloplasmin. Suggest review for possible protein loss or malabsorption/malnutrition. Undiagnosed Wilson's disease is unlikely in patients > 40 yrs old. Suggest rpt to confirm with serum copper. Consider 24h urine copper if caeruloplasmin is persistently low and there is no obvious cause especially if there is any family history of liver and/or neurological disease. Please telephone if you wish to discuss.