BASL
British Association for the Study of the Liver

University Hospital Southampton NHS
NHS Foundation Trust

BASL Wilson's Disease Special Interest Group Meeting
14 June 2018

BIOCHEMICAL MONITORING

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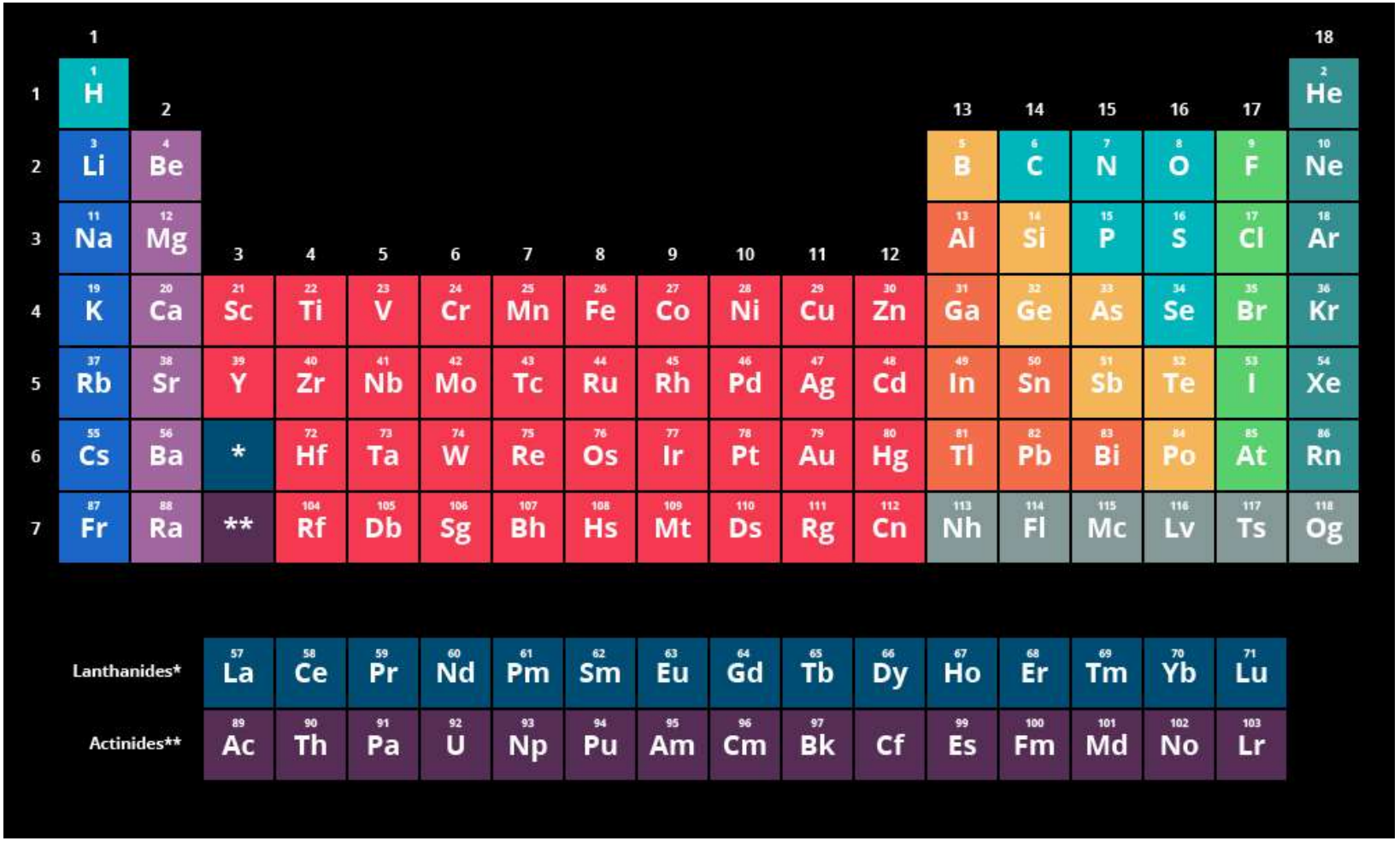
Copper

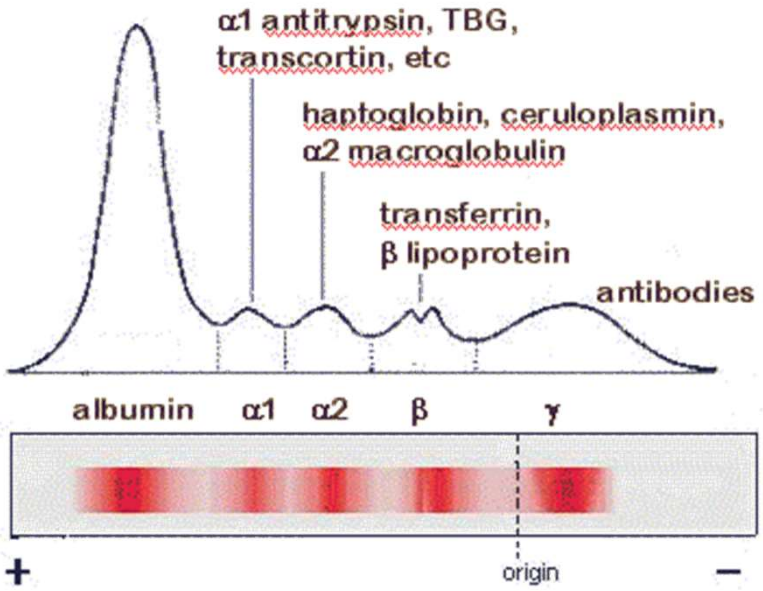

1 18
1 H 2 He
2 Li Be B C N O F Ne
3 Na Mg Al Si P S Cl Ar
4 K Ca Sc Ti V Cr Mn Fe Co Ni Cu Zn Ga Ge As Se Br Kr
5 Rb Sr Y Zr Nb Mo Tc Ru Rh Pd Ag Cd In Sn Sb Te I Xe
6 Cs Ba * Hf Ta W Re Os Ir Pt Au Hg Tl Pb Bi Po At Rn
7 Fr Ra ** Rf Db Sg Bh Hs Mt Ds Rg Cn Nh Fl Mc Lv Ts Og
13 14 15 16 17
3 4 5 6 7 8 9 10 11 12
Lanthanides* La Ce Pr Nd Pm Sm Eu Gd Tb Dy Ho Er Tm Yb Lu
Actinides** Ac Th Pa U Np Pu Am Cm Bk Cf Es Fm Md No Lr

Copper – physiological functions

- Essential cofactor for a number of critical enzymes
  e.g.
  superoxide dismutase (free radical scavenging)
  lysyl oxidase (collagen cross-linking)
  cytochrome c oxidase (oxidative phosphorylation)
  tyrosinase (melanin formation)
  dopamine β-hydroxylase (normetanephrine synthesis)
  caeruloplasmin
- Given its potential toxicity it is bound to copper chaperones within cells and other proteins outside of the cell

Caeruloplasmin

- Caeruloplasmin is an abundant α2-glycoprotein that contains >95% of the copper found in the plasma



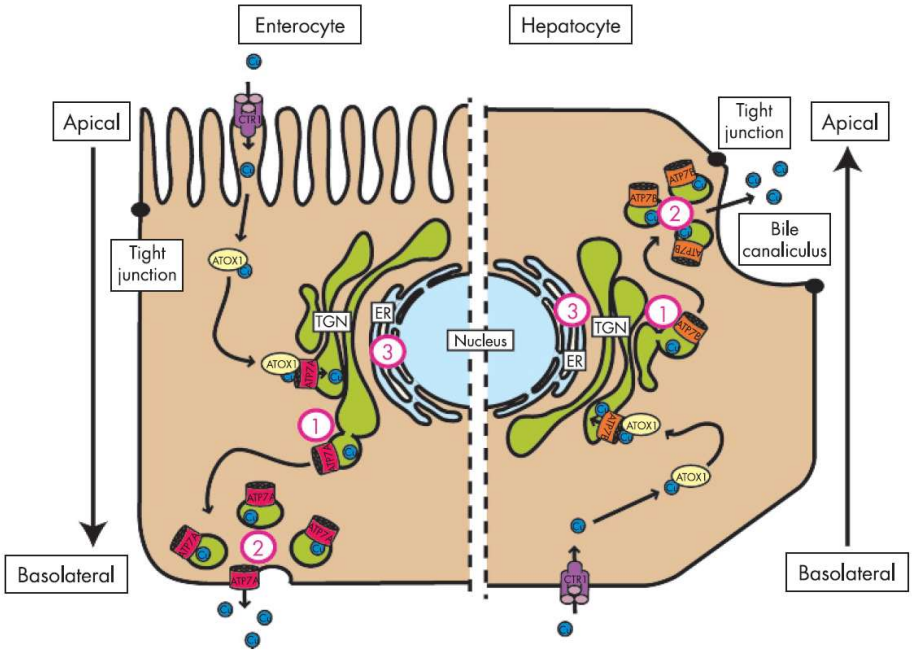
Caeruloplasmin

- Copper is incorporated into caeruloplasmin only during caeruloplasmin synthesis in the liver (apocaeruloplasmin to holocaeruloplasmin)
- Copper not part of the exchangeable pool
- Caeruloplasmin acts as a ferroxidase
- Mobilises and oxidises iron from tissue stores with subsequent incorporation of ferric (Fe^{3+}) iron into transferrin (carrier of iron in blood)
- In the absence of adequate copper the apoprotein is devoid of oxidase activity and is rapidly degraded

Caeruloplasmin

- Most non-hepatic cells also synthesise caeruloplasmin
- Remains bound to the cell (GPI-anchored)
- The role of GPI-anchored caeruloplasmin is to enable iron efflux from cells

Dietary copper release



ATP7A

- When released copper is bound to albumin, transcuprein and amino acids in portal circulation and elsewhere
- This is the exchangeable pool

Wilson's disease

- Primarily a disease of copper excretion into bile, but impairs copper incorporation into apocaeruloplasmin
- Hepatic copper accumulation eventually exceeds storage capacity
- Copper spills into circulation
- Increased urinary copper cannot compensate for the impaired biliary excretion with secondary rise in serum free copper

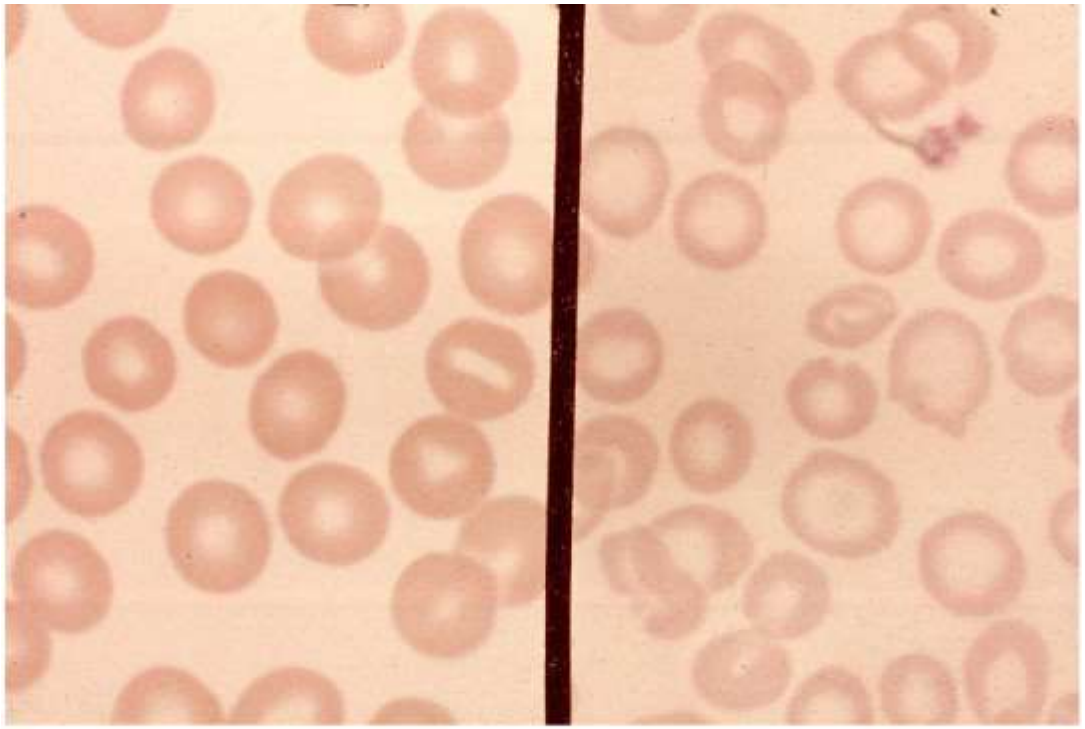
Wilson's disease

- Low total copper
- Low caeruloplasmin
- Free (non-caeruloplasmin-bound copper) serum copper is raised
- Increased urinary copper

Why monitor biochemically?

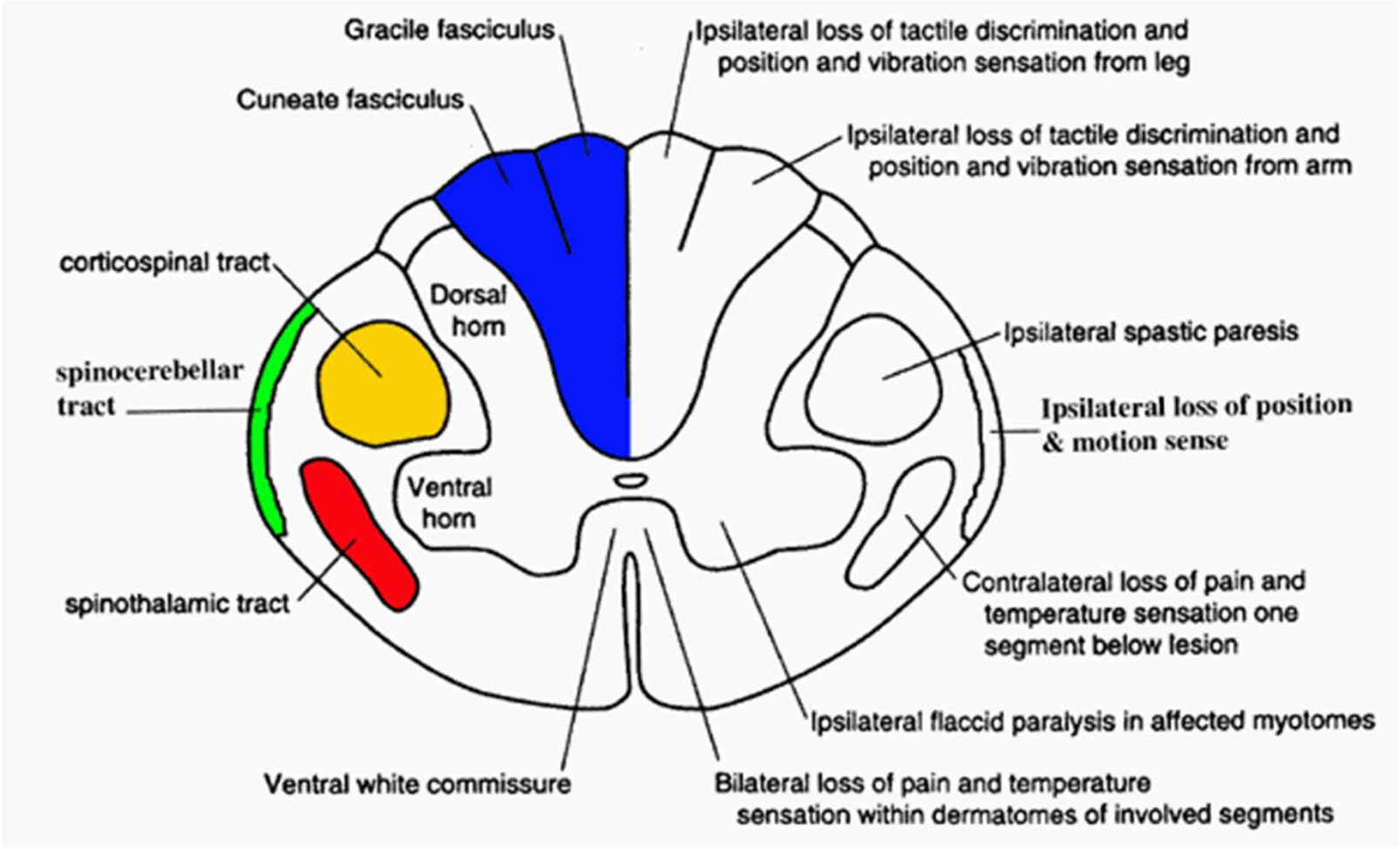
- Prevent over treatment
- Prevent under treatment
- Check compliance
- (Check for complications of therapy)
- (Check for disease progression)

Copper deficiency



Normal red blood cells Microcytic anemia

Copper deficiency



Wilson's disease

- Low total copper
- Low caeruloplasmin
- **Free (non-caeruloplasmin-bound copper) serum copper is raised**
- **Increased urinary copper**

Copper chelators (pencillamine and trientene)

- *Adequacy of treatment*

  24 hour urinary copper 12-32 umol initially then

  24 hour urinary copper 3.2-8 umol

- *Non-compliance*

  24 hour urinary copper < 3.2 umol/l and serum free copper >2.36 umol/l

- *Over treatment*

  Serum free copper < 0.79 umol/l (over treatment)

  24 hour urinary copper < 0.79 umol or 0.56 umol

Copper chelators (pencillamine and trientene) – off treatment

- *Adequacy of treatment*

  24 hour urinary copper < 1.6 umol/l

  Serum free copper < 2.4 umol/l or 3.9 umol/l

- *Non-compliance*

  24 hour urinary copper > 1.6 umol/l and serum free copper >2.36 umol/l or 3.9 umol/l

Copper chelators (ammonium tetrathiomolybdate)

- *Adequacy of treatment*

  Serum free copper 0.79 – 2.36 umol/l

Inhibition of copper uptake (zinc)

- *Adequacy of treatment*

  Serum free copper 0.79 – 2.36 umol/l

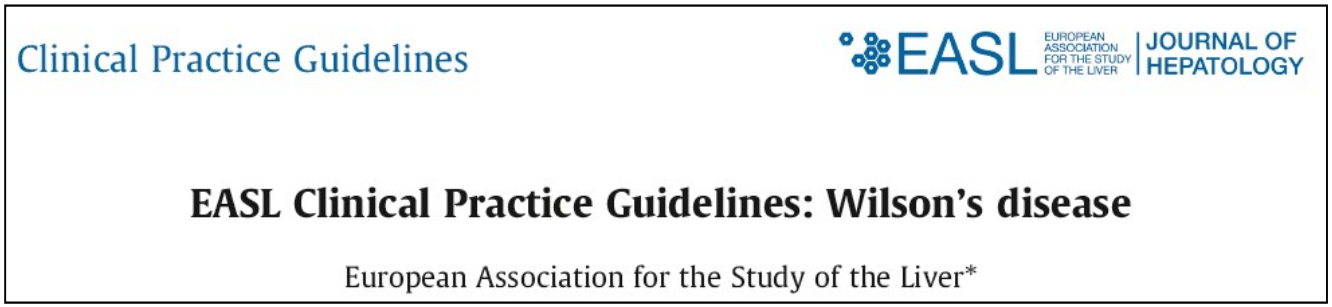
  24 hour urinary copper < 1.2 umol

  24 hour urinary zinc > 30.6 umol
- *Over treatment*

  24 hour urinary copper < 0.57 umol

Non-caeruloplasmin-bound copper (NCBC)

- Non-caeruloplasmin-bound copper = free copper = toxic copper
- Calculated NCBC (umol/l) = total serum copper (umol/l) – (47 x serum caeruloplasmin (g/l))
- Introduced in 1950's and its use is recommended in guidelines



- Despite giving rise to physiologically impossible negative results in 20%

Children < 1
Treated Wilson's
Ultrafiltered + Cu
Ultrafiltered + Cu
Adults

Table 1. Reported copper and caeruloplasmin concentrations and calculated non-caeruloplasmin-bound copper.

Specimen	Number of results	mean concentration	Range	Standard deviation	Coefficient of variation, %
Caeruloplasmin (g/L)					
237X	48	0.057	0.00 to 0.18	0.064	112
A	29	0.187	0.13 to 0.24	0.029	16
B	27	0.079	0.05 to 0.12	0.014	18
C	5	0.009	0.0 to 0.02	0.008	96
D	6	0.01	0.0 to 0.02	0.007	76
E	29	0.303	0.26 to 0.37	0.033	11
Copper (μmol/L)					
237X	34	2.02	<0.1 to 7.4	0.047	23
A	29	11.4	9.8 to 14.9	0.96	8
B	29	3.9	2.76 to 8.0	1.07	7
C	29	6.9	3.3 to 10.9	1.17	17
D	29	2.3	1.01 to 5.0	0.64	18
E	29	17.4	14.3 to 19.5	1.17	7
Calculated non-caeruloplasmin-bound copper (μmol/L)					
237X	9	0.42	−0.46 to 1.74	0.84	199
A	29	2.7	−0.09 to 8.8	1.89	70
B	27	0.3	−1.84 to 5.18	1.54	600
C	5	6.7	5.35 to 7.9	0.97	14
D	6	1.7	0.07 to 2.6	0.87	50
E	29	3.1	−0.39 to 6.14	1.64	53
Calculated percentage non-caeruloplasmin-bound copper (μmol/L)					
237X	9	4.6%	−78 to 65%	43.6	940
A	29	22.4%	−0.8 to 60%	13.4	60
B	27	1.7%	−1.9 to 5.2%	12.9	739
C	5	64.6%	5.4 to 7.9%	10.9	17
D	6	15.3%	0.1 to 2.6%	8.3	50
E	29	26.3%	−0.4 to 6.1%	12.4	47

Non-caeruloplasmin-bound copper

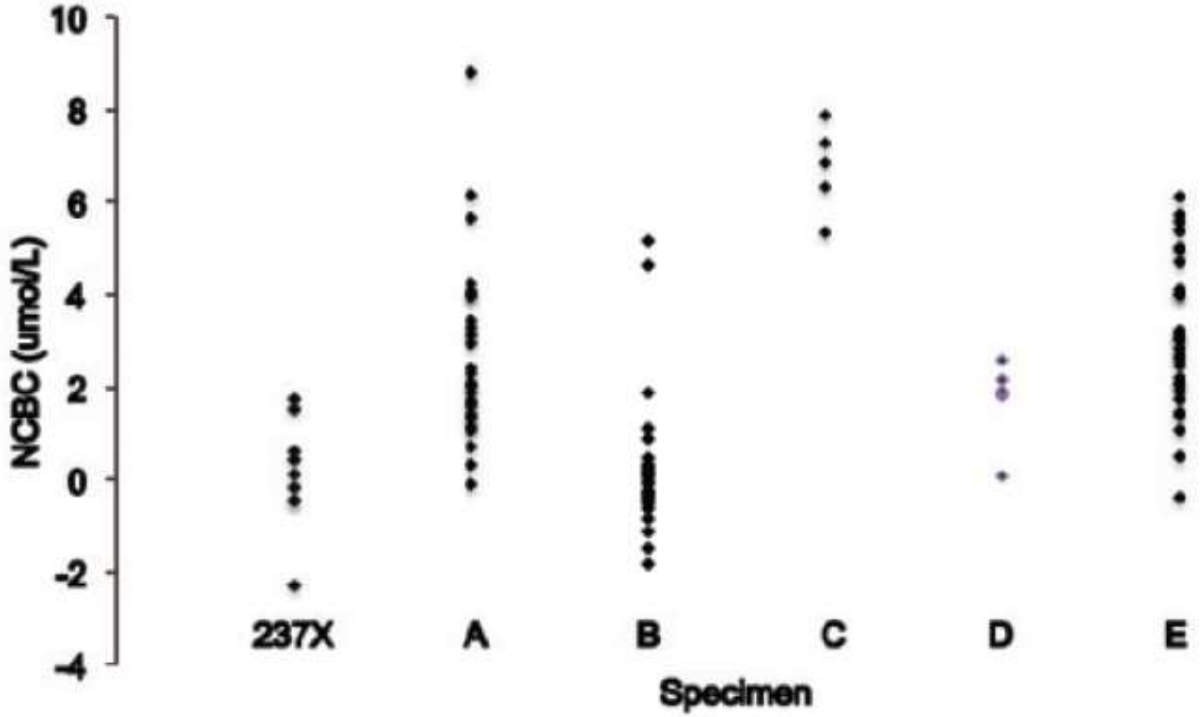


Figure 1. Calculated NCBC results in six specimens distributed for analysis.

Non-caeruloplasmin-bound copper (NCBC)

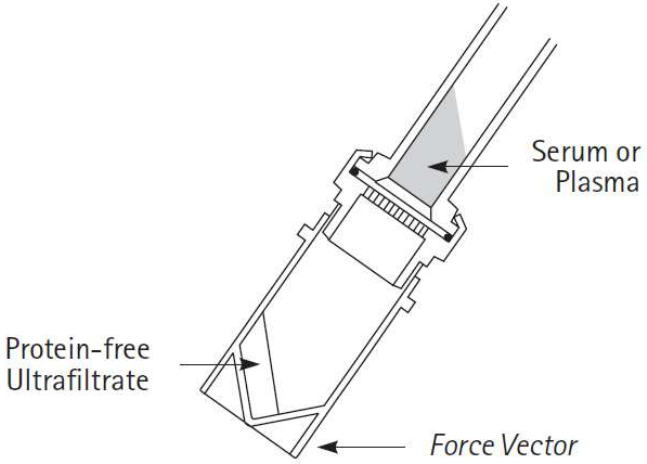
- Normal range (calculated) 1.5-2.4 umol/l

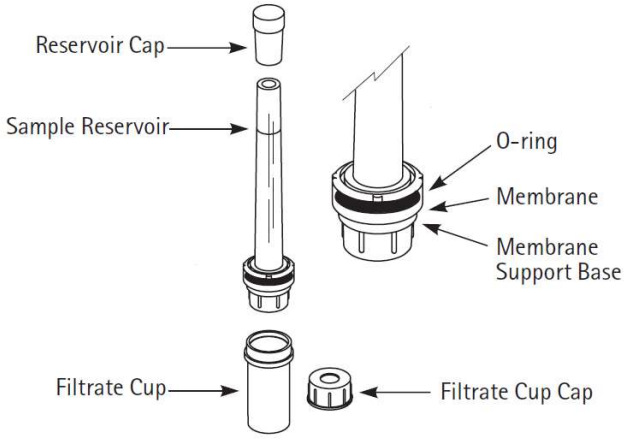
Non-caeruloplasmin-bound copper (NCBC)

Table 1 Reference values of ultrafiltrable copper (CuUF) and exchangeable copper (CuEXC) in plasma in 44 presumably healthy subjects (29 females and 15 males)

	Total Cu (µmol/L)	CuUF (µmol/L))	CuUF (% of total Cu)	CuEXC (µmol/L)	CuEXC (% of total Cu)	NCC µmol/L (n=25)
Mean	16.8	0.102	0.64	0.90	5.64	−0.66
Median	15.0	0.101	0.60	0.91	5.87	−0.75
5–95 percentiles	11.7–28.8	0.071–0.153	0.31–1.07	0.64–1.12	3.44–8.02	
Range	11.5–30.8	0.071–0.156	0.29–1.07	0.57–1.12	3.24–8.58	−3.52–2.2

Free copper





- **Separate blood within 30 minutes**
- **Add 1 ml serum to 1 ml EDTA in NaCl (1:1)**
- **Ultrafilter**
- **Measure ultafiltrate copper (ICP-MS)**

Relative Exchangeable Copper

Sample	Filter 1 Ex Cu	Filter 2 Ex Cu	Mean Ex Cu	Total Cu 1	Total Cu 2	Mean Total Cu	RATIO
1	0.63	0.64	0.64	16.8	16.5	16.7	3.81
2	0.77	0.82	0.80	22.8	22.3	22.6	3.53
3	0.91	0.96	0.94	24.8	24.4	24.6	3.80
4	0.82	0.52	0.67	16.3	15.9	16.1	4.16
5	0.50	0.51	0.51	18.9	18.1	18.5	2.73
6	0.48	0.56	0.52	18.5	18.1	18.3	2.84
7	0.47	0.51	0.49	21.9	22.2	22.1	2.22
8	0.59	0.55	0.57	17.4	18.7	18.1	3.16
9	0.65	0.62	0.64	23.3	23.3	23.3	2.73
10	0.50	0.50	0.50	18.7	18.1	18.4	2.72
11	0.78	0.77	0.78	39.1	38.4	38.8	2.00
12	1.09	1.08	1.09	40.5	40.5	40.5	2.68
13	0.60	0.58	0.59	15.0	14.8	14.9	3.97
14	0.87	0.84	0.86	16.0	15.6	15.8	5.41
15	1.00	1.07	1.04	21.8	19.7	20.8	4.99
16	1.20	1.30	1.25	27.2	27.0	27.1	4.61
17	0.61	0.60	0.61	13.0	12.9	13.0	4.68
18	0.63	0.59	0.61	18.6	18.9	18.8	3.25
19	0.85	0.92	0.89	17.4	17.3	17.4	5.10
20	1.44	1.43	1.44	22.9	22.2	22.6	6.36
21	1.79	1.72	1.76	34.6	33.9	34.3	5.12
22	0.98	0.68	0.83	17.7	17.8	17.8	4.68
23	0.60	0.60	0.60	19.1	19.2	19.2	3.13
24	0.82	0.95	0.89	17.1	17.4	17.3	5.14
25	0.96	0.89	0.92	18.2	18.3	18.3	5.05
26	0.78	0.73	0.76	16.3	16.6	16.5	4.59
27	0.73	0.94	0.83	38.7	38.8	38.8	2.15
28	0.50	0.53	0.52	8.6	8.3	8.5	6.09
29	0.74	0.79	0.77	20.9	21	21.0	3.65
30	0.89	0.70	0.80	17.8	17.7	17.8	4.48
31	0.94	0.90	0.92	19.6	18.7	19.2	4.80
32	1.23	1.18	1.21	19.2	19.2	19.2	6.28

Relative Exchangeable Copper

Table 1 Reference values of ultrafiltrable copper (CuUF) and exchangeable copper (CuEXC) in plasma in 44 presumably healthy subjects (29 females and 15 males)

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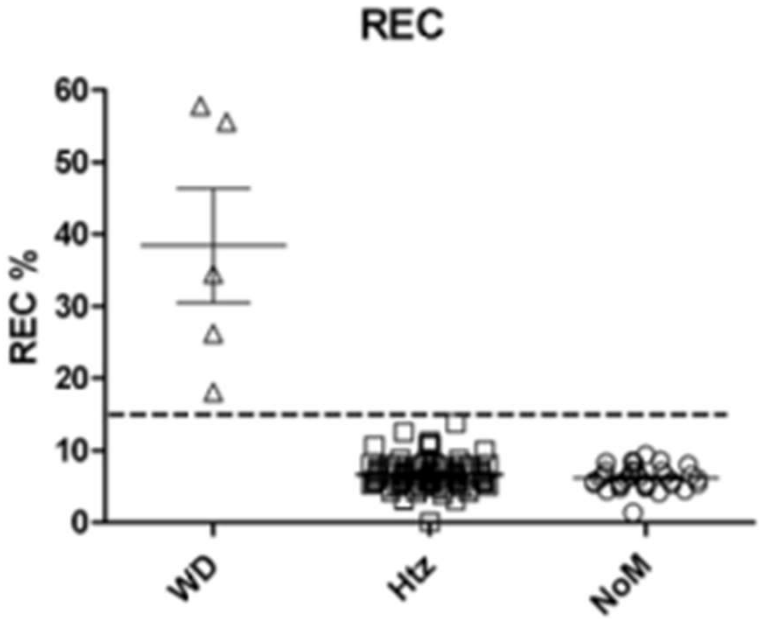
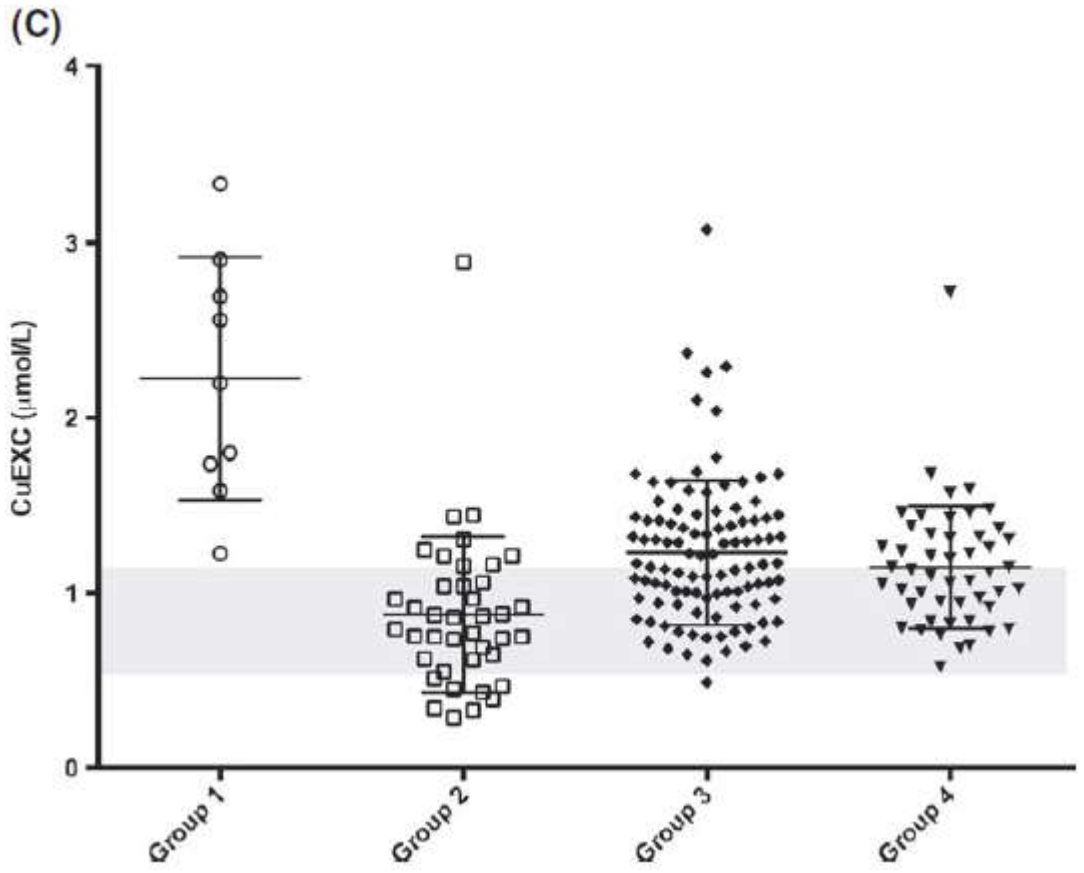
Range	11.5–30.8	0.071–0.156	0.29–1.07	0.57–1.12	3.24–8.58	−3.52–2.2
			UHS	0.50 – 1.25		

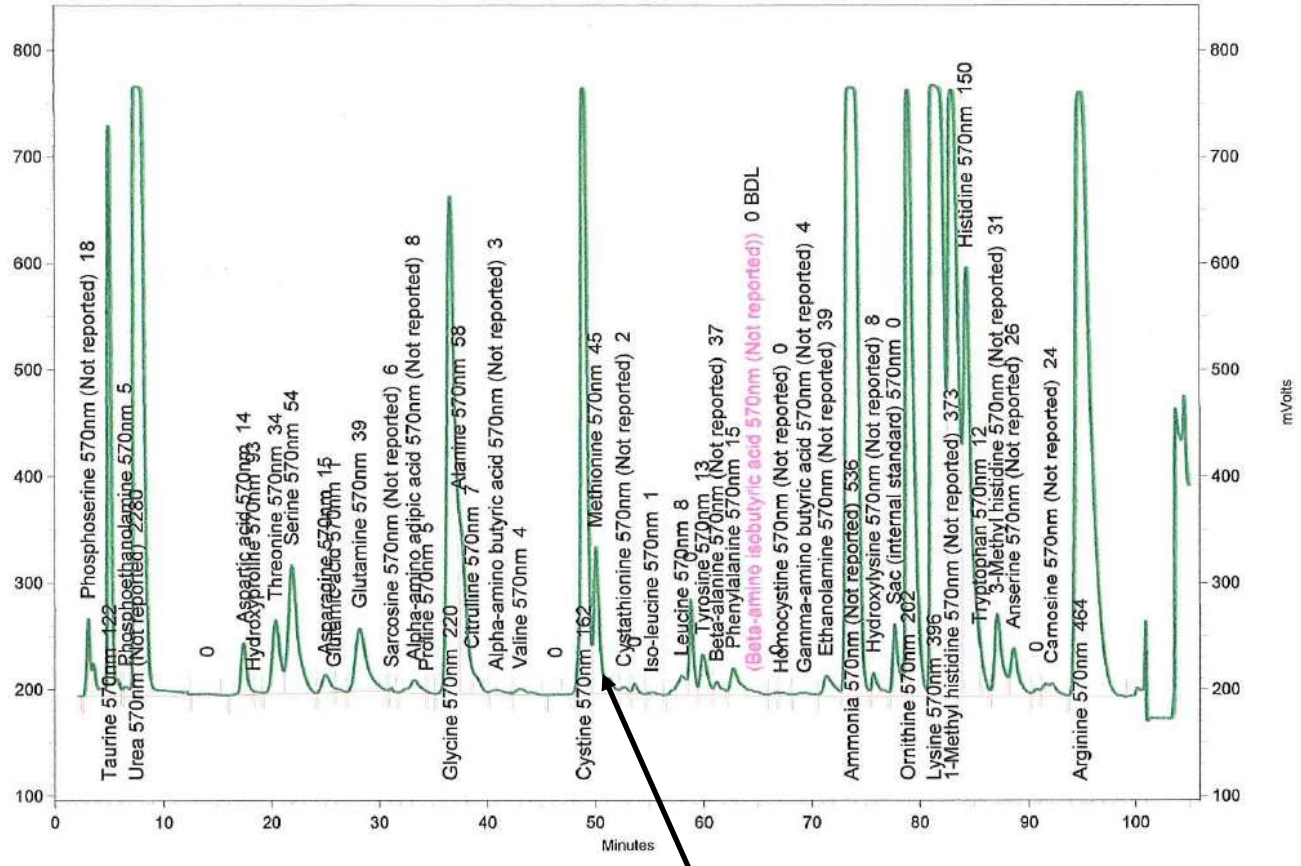
Relative exchangeable copper

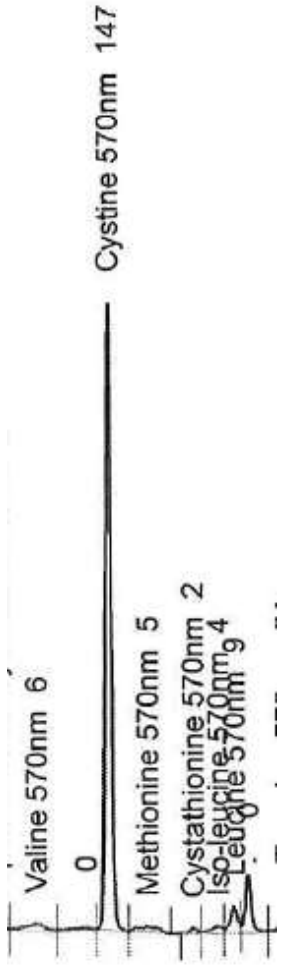


Relative exchangeable copper



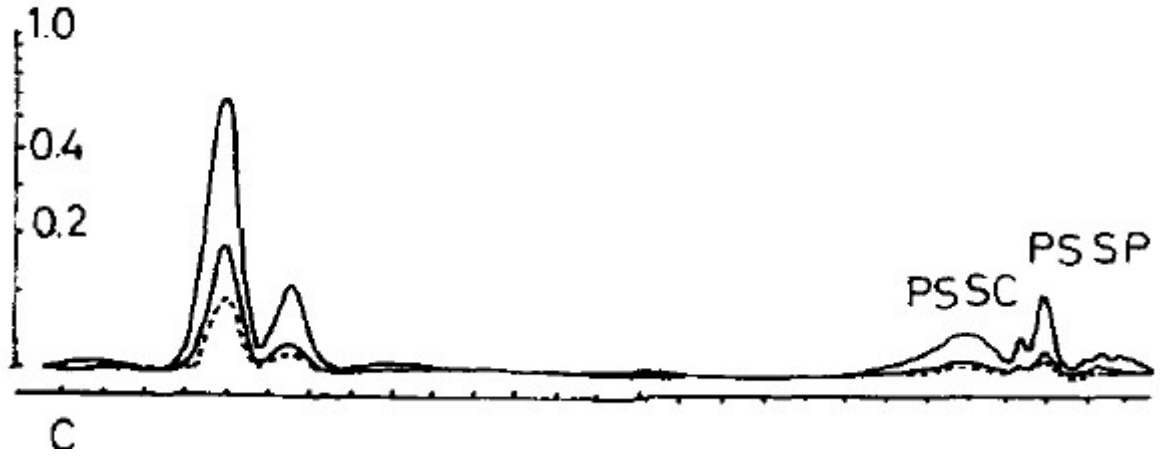
A form of therapeutic (binary) drug monitoring





Cysteinyl-penicillamine

A form of therapeutic drug monitoring



Cysteinyl-penicillamine

Summary

- Monitor – yes
- No evidence – expert opinion
- References all refer back to review articles
- Variation in the literature regarding cut-offs
- Calculated free copper should not be used
- Should chelating agents be stopped prior to 24 hour urine?
- Could laboratory measurement of exchangeable copper be all that is needed?