AASLD
AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES

Highlights of AASLD

King's college hospital charity

James Liu Yin
Aftab Ala
Institute of Liver Studies
Kings' College Hospital

King's

Thanks to King’s College Hospital Charity fund for supporting attendance at AASLD

Wilson's disease at EASL

Symposium: Wilsons disease: update on diagnosis and treatment

General hepatology

Presentations

	Title
1	**Genetics and gene modifiers** Hartmut Schmidt, Germany
2	**Acute liver failure** Anil Dhawan, United Kingdom
3	**Treatment options** Peter Ferenci, Austria
4	**Compliance and monitoring of therapy** Peter Ott, Denmark
5	**Panel Discussion** Panel: Hartmut Schmidt (Panellist), Anil Dhawan (Panellist), Peter Ferenci (Panellist), Uta Merle (Chair), Piotr Socha (Chair), Peter Ott (Panellist)

Wilson's disease at AASLD

Pediatric Liver Disorders SIG Program: The Many Faces of Wilson Disease and Emerging Therapies

Program Chairs: Drs. Jaime Chu and Nanda Kerkar

10:00 AM	Welcoming remarks	Dr. Nanda Kerkar
10:03 AM	Primer on copper metabolism, ATP7B, and epigenetics	Dr. Valentina Medici
10:21 AM	Could this be Wilson disease? Diagnosis and management in children	Dr. Binita Kamath
10:39 AM	Diagnosis and management in adults (including pregnancy)	Dr. Frederick Askari
10:57 AM	Emerging treatments and current trials	Dr. Michael Schilsky
11:15 AM	Panel discussion and concluding remarks	All speakers

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Parallel 5: New Developments in Genetic Diseases of the Liver

EFFICACY AND SAFETY OF ALXN1840 VERSUS STANDARD OF CARE IN WILSON DISEASE: PRIMARY RESULTS FROM AN ONGOING PHASE 3, RANDOMIZED, CONTROLLED, RATER-BLINDED TRIAL

PRIMARY HEPATOCYTE AND iPSC-DERIVED HEPATOCYTE-LIKE CELL TRANSPLANTATION TO TREAT ALPHA-1 ANTITRYPSIN DEFICIENCY ASSOCIATED LIVER DISEASE

CHARACTERIZING LIVER DISEASE (LD) PROGRESSION IN ALPHA-1 ANTITRYPSIN DEFICIENCY (AATD) WITH VERSUS (VS) WITHOUT LUNG DISEASE

COORDINATE REGULATION OF LIVER FERROPORTIN DEGRADATION AND SYNTHESIS DETERMINES SERUM IRON LEVELS IN MICE

ARBM-101 AS A POTENTIAL THERAPEUTIC FOR WILSON DISEASE

ARBM-101

As a potential therapeutic for WILSON DISEASE

[1]Josef Lichtmannegger, [2]Eok Park, [3]Ditte Emilie Munk, [2]Byongkeol Min, [3]Banu Akdogan, [1]Claudia Einer, [4]Judith Nagel, [2]Dasol Kim; [2]TaeWon Kim; [2]Chunwon Jung, [3]Thomas Damgaard Sandahl, [5]Simon Hohenester, [6]Alan DiSpirito, [7]Jeremy Semrau, [8]Andree Zibert, [6]Hartmut H. Schmidt, [1]Bernhard Michalke, [2]Weonbin Im; [2]So-Young Eun, [1,4]Hans Zischka, *[9]Valentina Medici.

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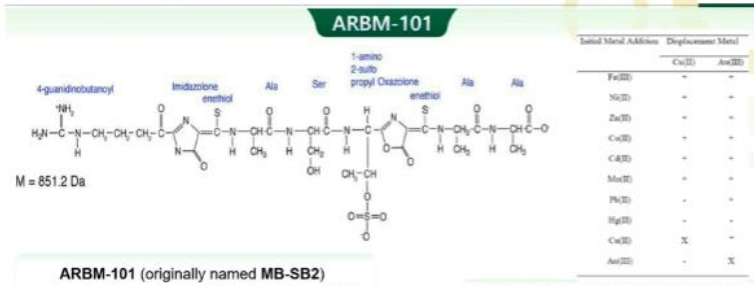
ARBM-101 (MB-SB2): A high-affinity Cu-chelator



Ionic Metal Additives	Displacement Metal	
	Cu(II)	Au(III)
Fe(III)	-	-
Ni(II)	-	-
Zn(II)	-	-
Cu(II)	+	-
Cd(II)	-	-
Mn(II)	-	-
Pb(II)	-	-
Hg(II)	-	-
Cu(II)	X	-
Au(III)	-	X

ARBM-101 (originally named **MB-SB2**) produced and secreted by *Methylocystis sp. SB2*.

ARBM-101 is a selective Cu chelator.

Krentz, B.D., et al., 2010; *Biochem.*;
Bandow, N., et al., 2012; *J. Inorg. Biochem.*;
Bandow N. and DiSpirito AA., 2014; Dissertation

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Nine-day Repeat Dose Study

Scheme of the Study

Once a day for 9 days

A — Main cohort (N=6) sacrificed at d=10 for liver copper analysis.

B — Satellite cohort (N=4) followed up for 60 days or 20% BW loss for survival analysis.

14-15wk (~100D) old LPP rats
: grouped based on ALT levels (>160 U/L).

G1: +/+ WT vehicle Ctrl., IV
G2: -/- KO vehicle Ctrl., IV
G3: -/- D-PA (100 mg/kg), PO
G4: -/- ARBM-101 (100 mg/kg), IV
G5: -/- ARBM-101 (200 mg/kg), IV
G6: -/- ARBM-101 (25 mg/kg), IV
G7: -/- ARBM-101 (50 mg/kg), IV

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Durable Liver Function Improvement

Durable Liver Function Improvement based on ALT/AST and T-BIL Levels

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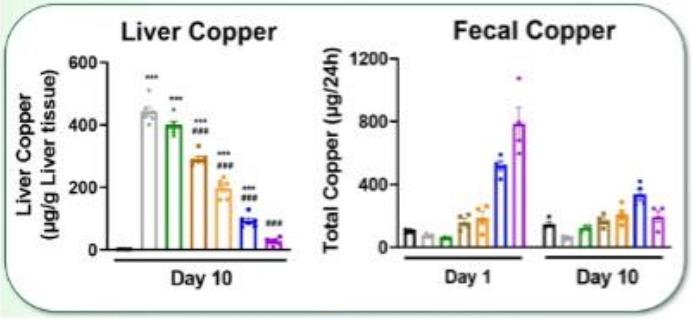
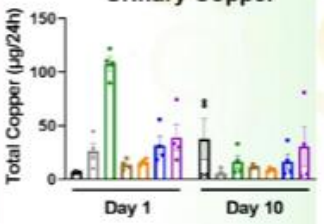
Copper excretion through bile/fecal axis

Dose-dependent copper excretion and reduction of liver copper





*: p<0.05, **: p<0.01, ***: p<0.001. vs G1. Statistics by ANOVA and Dunnett's Multiple Comparison Test.
#: p<0.05, ###: p<0.001. vs G2. Statistics by ANOVA and Dunnett's Multiple Comparison Test.

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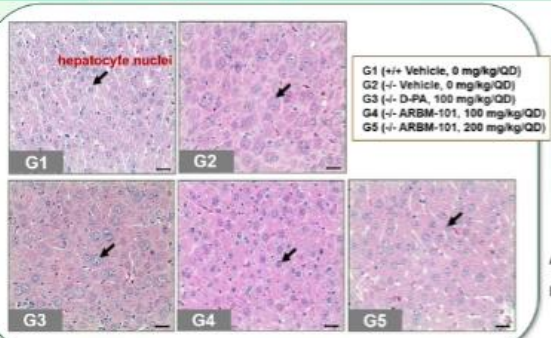
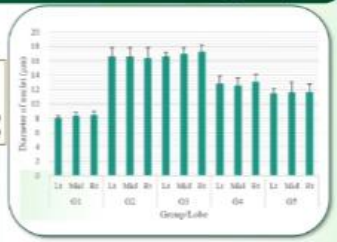
Normalization of hepatocyte nuclei

Histopathology of a representative left lobe from the livers of each group



G1 (+/+ Vehicle, 0 mg/kg/QD)
G2 (-/- Vehicle, 0 mg/kg/QD)
G3 (-/- D-PA, 100 mg/kg/QD)
G4 (-/- ARBM-101, 100 mg/kg/QD)
G5 (-/- ARBM-101, 200 mg/kg/QD)



ARBM-101 higher dose groups showed reduction in size of hepatocyte nuclei.

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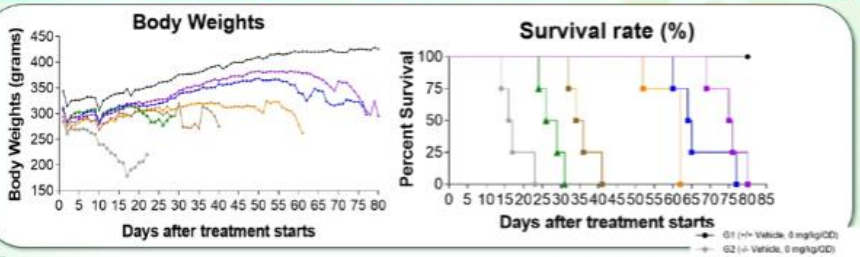
Prolonged Survival

Survival was prolonged in all ARBM-101 dose groups.



01 Most upon high doses of 101 **survived until completion of the study (> 60 days).**

02 ARBM-101 high dose groups **gained BWs for ~50 days** after discontinuation of treatment.

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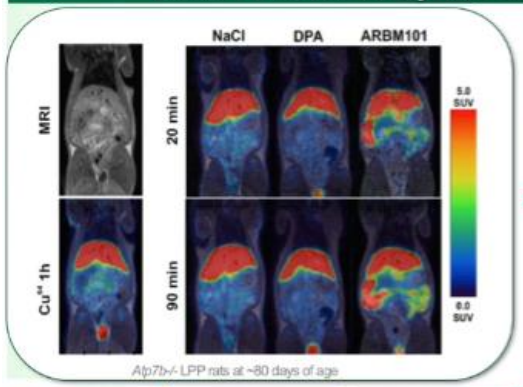
Liver copper mobilization into the gut

In vivo PET-SCAN Analysis: Prompt copper mobilization



01 Radioactive isotope ^{64}Cu injected i.v. in the range of 2.5 to 11.9 MBq.

02 Subsequently ARBM-101 or DPA injected i.p. (2 hrs 40 min. post ^{64}Cu inj.)

03 Cu mobilization monitored for 24 hrs.

Within 30 minutes, ARBM-101 started to mobilize copper into the gut, while DPA did not.

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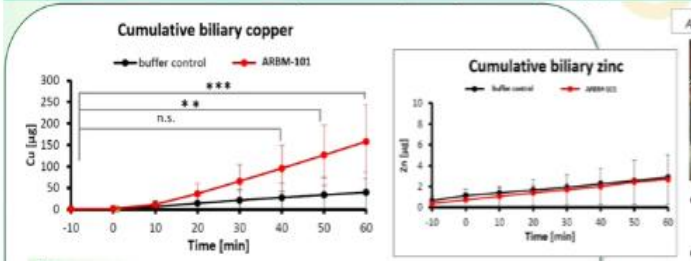
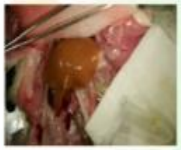
Liver copper mobilization by ARBM-101 into bile

Liver Perfusion Experiment: prompt biliary copper mobilization



Atp7b-/- LPP at about 80 days of age.



- Liver perfusion via cannulation of portal vein
- at infusion rate of 25 ml/min with 30 mg for 1 hr.
- Collection of bile via cannulation of bile duct every 10 minutes.

01 **ARBM-101 immediately mobilized hepatic copper,** showing significantly more copper detected in the bile, compared to the vehicle group.

02 Zinc was not affected showing no excretion more than basal levels.

N=2 for buffer control, N=5 for ARBM-101. *ps0.05, **ps0.005, ***ps0.001 One-way ANOVA.

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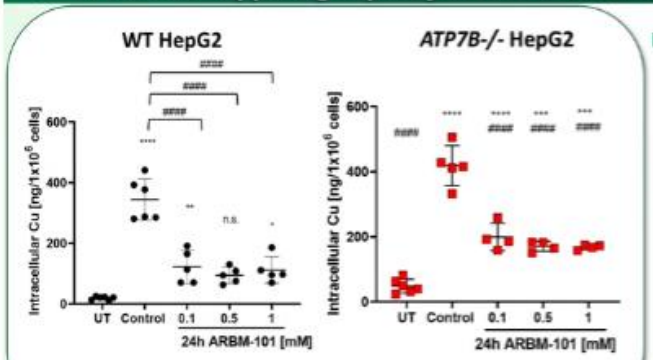
De-coppering Capacity of ARBM-101

In vitro de-coppering capacity of ARBM-101 from the hepatocytic cells



- Statistics done by One-way ANOVA.
- Significance refers to either Control #### ps0.0001 or to UT *ps0.05 **ps0.005, ****ps0.0001.

ARBM-101 was able to **reduce copper levels** from the copper-burdened HepG2 cells with or without functional ATP7B.

UT: Untreated (48h incubated)
Control: HepG2 cells treated 24h with 0.5 mM CuHis + 24h culture medium
101: HepG2 cells preloaded 24h with 0.5 mM CuHis + treated 24h with 0.1, 0.5, or 1 mM ARBM-101

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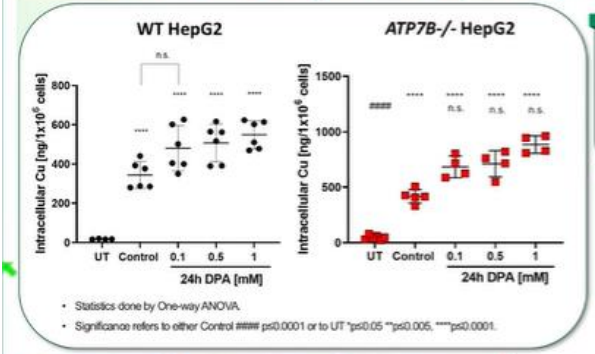
No ameliorating effect of DPA on copper burden

No reduction of copper burden upon DPA in the hepatocytic cells



- Statistics done by One-way ANOVA.
- Significance refers to either Control #### ps0.0001 or to UT *ps0.05 **ps0.005, ****ps0.0001.

Copper burden was not **ameliorated by DPA** in the presence or absence of ATP7B.

UT: Untreated (48h incubated)
Control: HepG2 cells treated 24h with 0.5 mM CuHis + 24h culture medium
D-PA: HepG2 cells preloaded 24h with 0.5 mM CuHis + treated 24h with 0.1, 0.5, or 1 mM D-PA

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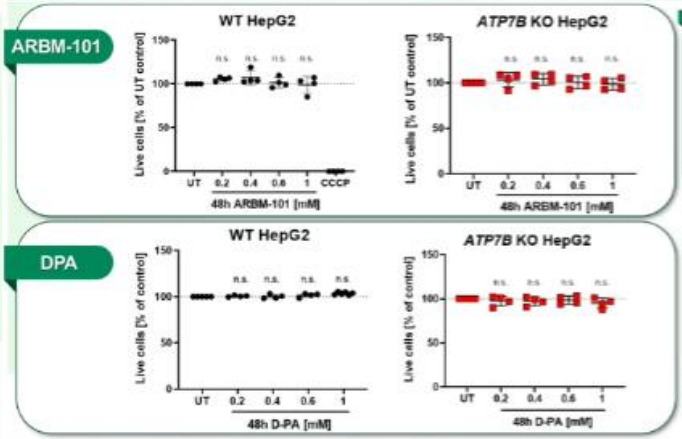
Minimum Cytotoxicity of ARBM-101 in HepG2



No evident cytotoxicity observed up to 1mM of ARBM-101 or DPA by *in vitro* CTG assay .

* CCCP: 0.25 mM treated for positive control of CTG assay that measures ATP production.

- Statistics done by One-way ANOVA.
- Significance refers to either Control #### ps0.0001 or to UT *ps0.05 **ps0.005, ****ps0.0001.

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Conclusion

ARBM-101 as a potential therapeutic for WILSON DISEASE

Efficacy

- **Massive fecal copper excretion** → normalized liver copper contents, improved liver function, with improved hepatocyte histopathology
  : indicated by **improved LFTs, normal BWs, and prolonged survival**.
- Positive effects were durable for +30 days after discontinuation of treatment
- Superior efficacy on overloaded Cu depletion from the WT and *ATP7B-/-* HepG2 cells *in vitro*.

Safety

- No observed toxicity up to 200 mg/kg for 9 consecutive days in *Atp7b-/-* LPP rats.
- No evident toxicity up to 400 mg/kg in normal SD rats for 5 consecutive days (Data not shown).
- No cytotoxicity up to 1 mM either in WT or in *ATP7B-/-* HepG2 cells *in vitro*.

Primer on Copper Metabolism, ATP7B and Epigenetics

Valentina Medici, MD, MAS, FAASLD
University of California Davis

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HDAC: Histone deacetylases

- Histone deacetylases (HDACs) remove acetyl moieties from lysine residues of histone tails, resulting in transcriptional repression
- HDAC levels are greatly influenced by dietary factors, therefore lying at the interface between nutrition and epigenetic regulation of gene expression

HDAC5 plays fundamental roles in many biological events, including cell proliferation, differentiation, apoptosis, inflammation, and lipogenesis.

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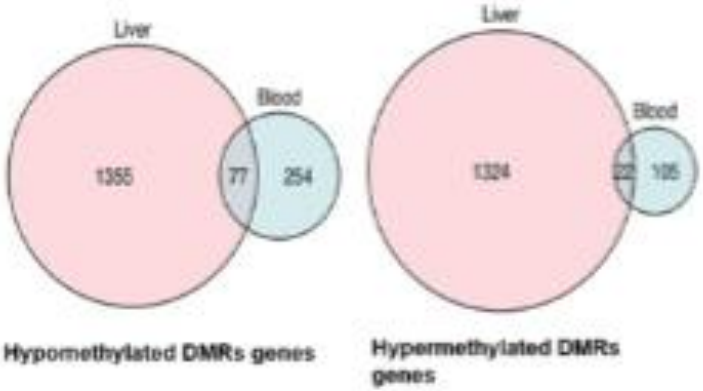
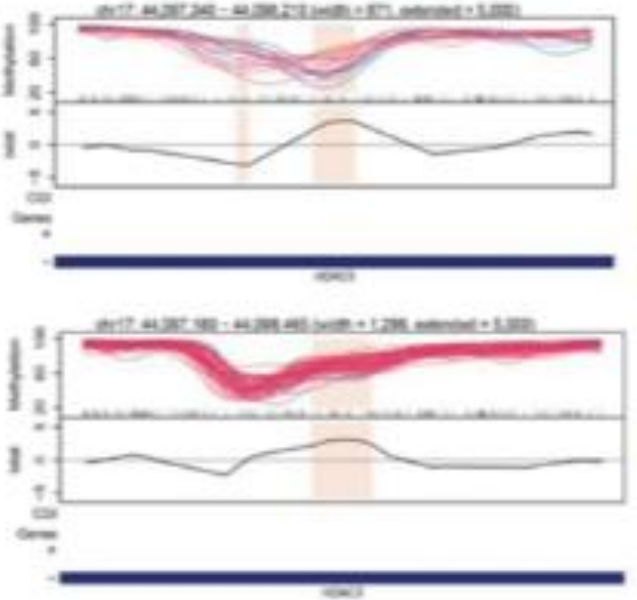
Shared DMRs associated genes in blood and liver

WD-specific DMRs in liver and blood overlap by gene





- About 1/3 of the genes near differentially methylated regions (DMRs) in the blood significantly overlapped with those identified in the liver
- **Histone deacetylase 5 (*HDAC5*)** was significantly hypermethylated with similar direction and gene body locations (FDR<0.05) both in the liver and blood

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Epigenomic signatures in liver and blood of Wilson disease patients include hypermethylation of liver-specific enhancers

Charles E. Mordaunt, Janine M. LaSalle & Valentina Medici, Epigenetics & Chromatin 2019

HDAC5

- Reduced levels in multiple models of WD
- Reduced with liver fibrosis progression
- Levels changed by copper levels and methyl groups availability

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Key Takeaways

Epigenetic mechanisms at the intersection between DNA methylation and histone acetylation affect gene expression in Wilson disease

- Environment/diet affects phenotype
- Hepatic vs Neurological
- Treatment response modulation
- Response to gene therapy

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TETRAETHYLENTETRAMIN AS FIRST LINE TREATMENT OF WILSON DISEASE – LONG-TERM RESULTS

Peter Ferenci1, Isabelle Mohr2, Albert Friedrich Stättermayer1, Rudolf Stauber3, Marlene Panzer4, Petra Steindl-Munda1, Ute Merle2, Michael Trauner1, Karl-Heinz Weiss5

1 Internal Med. III, Medical University of Vienna, Austria, 2 Internal Med. IV, Heidelberg, Germany, 3 Internal Med. Medical University of Graz, Austria,4 Internal Med., Medical University of Innsbruck, Austria, 5 Internal Med., Salem Hospital, Heidelberg, Germany

Methods

Retrospective chart review

in 31 patients who received initial treatment with TETA (treatment with D-Pen for < 60 days was permitted)

Assessment of efficacy:

Changes in liver disease

based on clinical and laboratory (ALT levels, MELD and/or Child-Turcotte-Pugh score [CTP] in cirrhotic patients) data:

improvement:

- ALT normalization
- full recompensation of previous decompensated liver disease
- decrease in MELD score (by ≥2) or in CTP stage

worsening:

- ALT increase > 2x upper limit of normal
- increase in MELD score (by ≥2) or in CTP stage
- hepatic decompensation
- death or liver transplantation

Changes in neuropsychiatric disease

based on clinical examination (by experienced neurologists) or self reported changes

Statistics:

The Kaplan-Meier method to estimate survival probabilities. Statistical analysis was performed with EasyMedStat (version 3.20.2; www.easymedstat.com).

Outcome

2 patients died:

- 1 due to hepatic adenocarcinoma (pankreo-biliary type) (treated for 7 years)
- 1 end stage liver disease (treated for 25 years)

3 patients underwent orthotopic liver transplantation:

- 1 decompensated in the third trimenon of pregnancy, she was transplantated 2 weeks after spontaneous delivery (treated for 6 years)
- 1 had psychiatric problems, did not attend the liver clinic for >3 years and presented with acute liver failure (treated for 14 years)
- 1 endstage liver disease, listed for LTX after 7 years of treatment (ascites, esophageal varices), further worsening after portal vein thrombosis, followed by complications (after 2 years on the waiting list)

all other 26 patients are alive and doing well. No severe side effects to TETA treatment were observed.

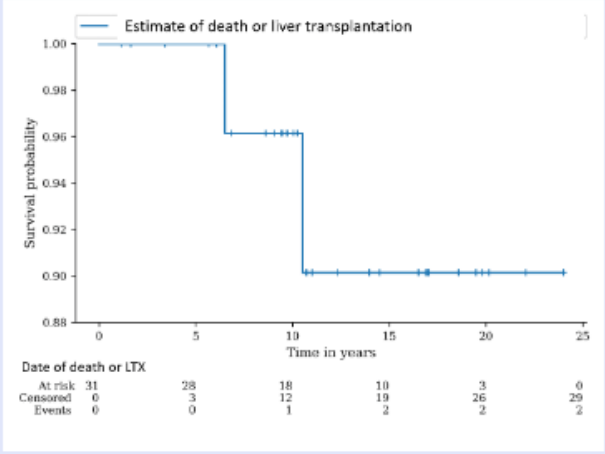
Demographics

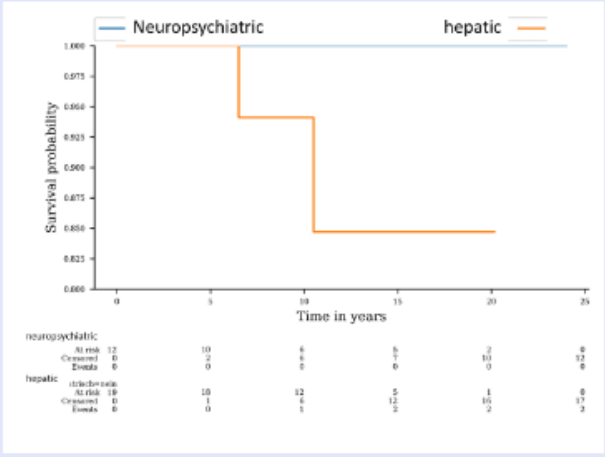
	Baseline	Clinical FU
male, n (%)	13 (41.9)	
age, y*	27.9±12.4	39.9±13.2
treatment, y	12.0±6.0	
cirrhosis/biopsy, n/N	9/19	
hepatic Cu, µg/g	690±452	
neurologic, n (%)	12 (38.7)	
KFR, n (%)	16 (51.6)	
CPL, mg/dL	11.7±5.5	9.5±6.1
Serum-Cu, µg/dL	57.0±32.5	45.1±44.8
24h urine Cu, µg/d	850±1576	144±98.2**
ALT, U/L	97.9±136.1	51.1±54.5
bilirubin, mg/dL	1.7±3.4	3.1±7.1
INR	1.3±0.5	1.2±0.4
Platelets, G/L	179±80	176±84
CTP score, n (%)		
CTP A	25 (80.6)	26 (83.9)
CTP B	4 (12.9)	2 (6.5)
CTP C	2 (6.5)	3 (9.7)
MELD score	9.8±5.4	10.6±8.1

*continuous variables presented as mean±SD

**data available in 24 subjects, drug holiday before sampling in 11/24 subjects

Survival analysis (Kaplan Meier)





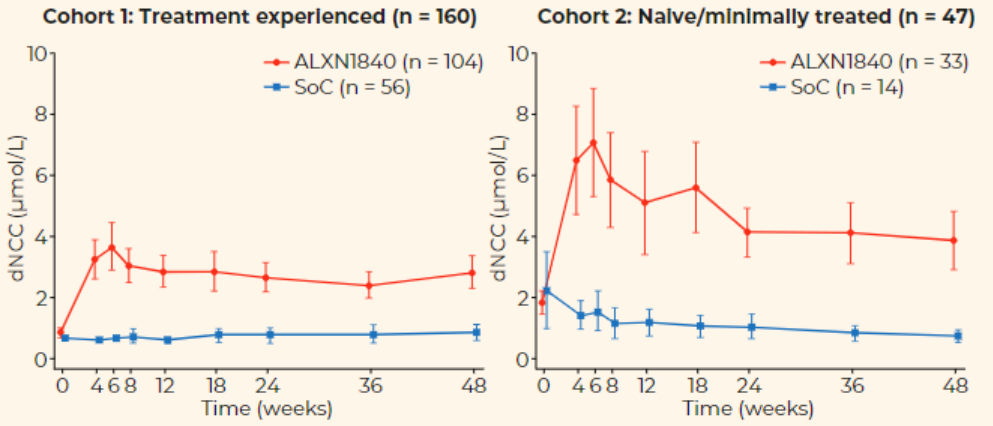
Efficacy and safety of ALXN1840 versus standard of care in Wilson disease: primary results from an ongoing phase 3, randomized, controlled, rater-blinded trial

KARL HEINZ WEISS[1,8], MICHAEL SCHILSKY[2,8], ANNA CZLONKOWSKA[3], FREDERICK ASKARI[4,8], AFTAB ALA[5,6], PETER FERENCI[7], PETER OTT[8], DZHAMAL ABDURAKHMANOV[9], FERENC SZALAY[10], PIOTR SOCHA[11], NORIKAZU SHIMIZU[12], JEFF BRONSTEIN[13], DANNY BEGA[14], SIHOUN HAHN[15], EUGENE SCOTT SWENSON[16], YI CHEN[16], AURELIA POUDOIS[17]*

[1]Krankenhaus Salem der Evang. Stadtmission Heidelberg, Heidelberg, Germany; [2]Yale School of Medicine, New Haven, CT, USA; [3]2nd Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland; [4]University of Michigan Health System, Ann Arbor, MI, USA; [5]Royal Surrey County Hospital Guildford, UK; [6]King's College Hospital NHS Foundation Trust, London, UK; [7]Medical University of Vienna, Vienna, Austria; [8]Aarhus University Hospital, Aarhus, Denmark; [9]Sechenov First Moscow State Medical University, Moscow, Russia; [10]Semmelweis University, Budapest, Hungary; [11]Children's Memorial Health Institute, Warsaw, Poland; [12]Toho University School of Medicine, Tokyo, Japan; [13]Ronald Reagan UCLA Medical Center, Los Angeles, CA, USA; [14]Northwestern University Feinberg School of Medicine, Chicago, IL, USA; [15]Seattle Children's Hospital, Seattle, WA, USA; [16]Alexion, AstraZeneca Rare Disease, Boston, MA, USA; [17]Rothschild Foundation Hospital, Paris, France

*Co-first authors; ¶Presenting author

Figure 3. Primary endpoint: copper mobilization with ALXN1840 was rapid and sustained



Mean daily direct NCC $AUEC_{0-48W}$	Cohort 1: Treatment experienced		Cohort 2: Naive/ minimally treated		Total	
	ALXN1840 n = 104	SoC n = 56	ALXN1840 n = 33	SoC n = 14	ALXN1840 N = 137	SoC N = 70
n[a]	91	51	27	12	118	63
Mean (SD)	2.68 (2.118)	0.72 (0.643)	4.58 (2.526)	1.09 (0.484)	3.12 (2.347)	0.79 (0.629)
LSM (SE)	2.50 (0.150)	0.87 (0.204)	4.76 (0.319)	0.96 (0.487)	3.18 (0.167)	1.00 (0.219)
LSM difference (SE)	1.64 (0.254)		3.79 (0.584)		2.18 (0.244)	
p value	< 0.0001		< 0.0001		< 0.0001	

[a]Patient numbers for calculation of mean; all patients were included in the calculation of LSM, LSM difference and *p* values. $AUEC_{0-48W}$, area under the effect curve from week 0 to week 48; dNCC, directly measured non-ceruloplasmin-bound copper; LSM, least-square mean; NCC, non-ceruloplasmin-bound copper; SD, standard deviation; SE, standard error; SoC, standard of care.

Figure 4. Secondary endpoint: trends toward modest improvements in UWDRS Part II and Part III scores were observed at week 48

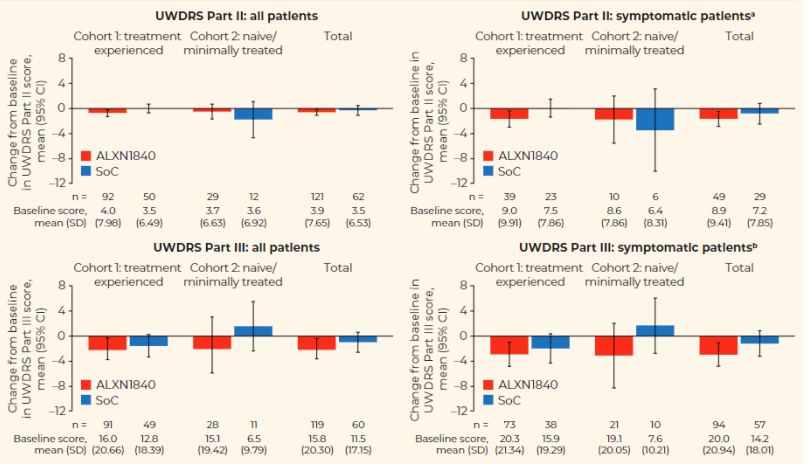


Table 2. Summary of treatment-emergent adverse events over 48 weeks

	ALXN1840 n = 137 PY = 118.9			SoC n = 70 PY = 62.4		
	n (%)	Number of Events	Events per 100 PY	n (%)	Number of Events	Events per 100 PY
Any AE	119 (86.9)	577	100.1	54 (77.1)	192	86.5
Any SAE	18 (13.1)	34	15.1	6 (8.6)	14	9.6
Any non-SAE	117 (85.4)	543	98.4	53 (75.7)	178	84.9
Death	2 (1.5)	2	1.7	0	0	0
AE leading to withdrawal of study drug	8 (5.8)	10	6.7	1 (1.4)	2	1.6
SAE leading to withdrawal of study drug	2 (1.5)	2	1.7	0	0	0
AE by relationship						
Related	56 (40.9)	120	47.1	10 (14.3)	20	16.0
Not related	106 (77.4)	457	89.2	53 (75.7)	170	84.9
AEs reported in ≥ 5% of patients, preferred term						
ALT increased	20 (14.6)	25	16.8	2 (2.9)	2	3.2
Nasopharyngitis	15 (10.9)	18	12.6	12 (17.1)	14	19.2
Fatigue	13 (9.5)	16	10.9	3 (4.3)	3	4.8
Headache	11 (8.0)	12	9.3	6 (8.6)	11	9.6
Tremor	10 (7.3)	12	8.4	2 (2.9)	5	3.2
Upper respiratory tract infection	9 (6.6)	10	7.6	3 (4.3)	3	4.8
Nausea	8 (5.8)	8	6.7	3 (4.3)	3	4.8
Pyrexia	8 (5.8)	8	6.7	0	0	0
Anxiety	7 (5.1)	9	5.9	0	0	0

AE, adverse event; ALT, alanine aminotransferase; PY, patient-years – sum of all patient-years for all patients in the particular treatment group [rate per 100 patient-years [PY] = (#patients / PY) x 100]; SAE, serious adverse event; SoC, standard of care.

Distribution of Laboratory Determined Free Copper and Urinary Copper Excretion in Wilson Disease Patients Regarded Clinically Stable on Maintenance d-Penicillamine: Observations from the CHELATE Trial

Schilsky ML[1], Sandahl T[2], Czlonkowska A[3], Zuin M[4], Cassiman D[5], Poujois A[6], Ala A[7], D'Hollander K[8], Weiss K-H[9], Kamlin COF[10], Ott P[2] on behalf of the CHELATE Trial Investigators

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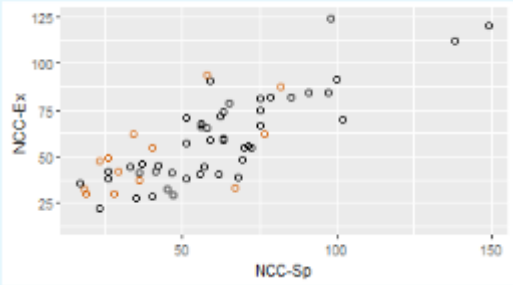
All patients	Mean	Median	Min	Max	SD	IQR
NCC-Sp (mcg/L)	57.1	56.5	17.0	149.0	26.7	36.0, 71.05
UCE (mcg/24hr)	586	494	47	2172	384	339, 751
ALT (U/L)	33.8	26.0	6.0	107.0	22.6	19.0, 43.0
DPA dose (mg/kg)	13.7	13.05	4.4	29.8	4.7	10.5, 16.8
(a) Patients with Serum NCC-Ex < 50 mcg/L						
NCC-Sp (mcg/L)	39.3	36.1	17.0	69.6	15.4	26.0, 49.0
UCE (mcg/24hr)	479	427	323	714	222	323, 714
ALT (U/L)	25.3	22.5	6.0	73	15.2	13.75, 33.0
DPA dose (mg/kg)	14.9	15.4	7.4	29.8	4.7	10.8, 17.45
(b) Patients with Serum NCC-Ex ≥ 50 mcg/L						
NCC-Sp (mcg/L)	74.2	70.7	34.0	149.0	24.6	58.3, 84.3
UCE (mcg/24hr)	636	557	55.9	2172	399	381, 870
ALT (U/L)	39.3	29.5	20.8	56.8	23.8	20.8, 56.8
DPA dose (mg/kg)	12.6	12.3	4.4	24.3	4.8	9.0, 15.8

Figure 1: NCC-Ex vs NCC-Sp



• NCC-Ex was used to screen subjects;

Figure 2: ALL Subjects

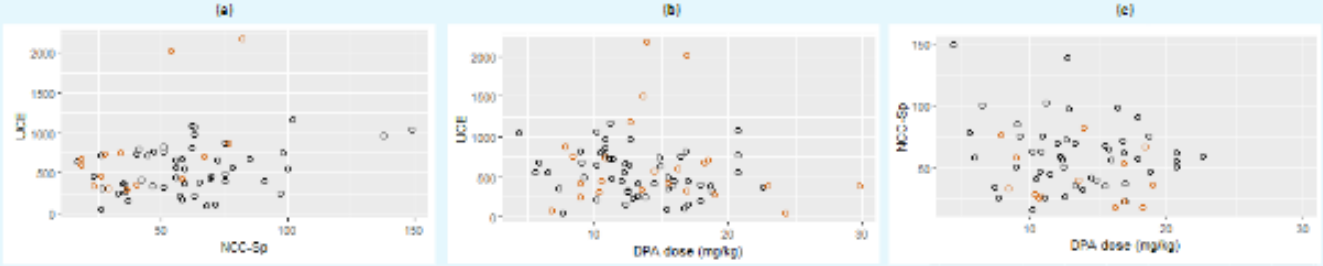


Figure 3: NCC-Ex < 50 mcg/L

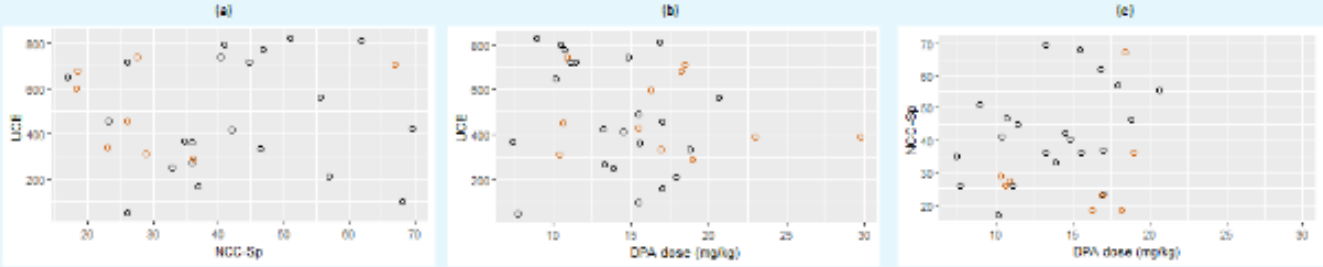
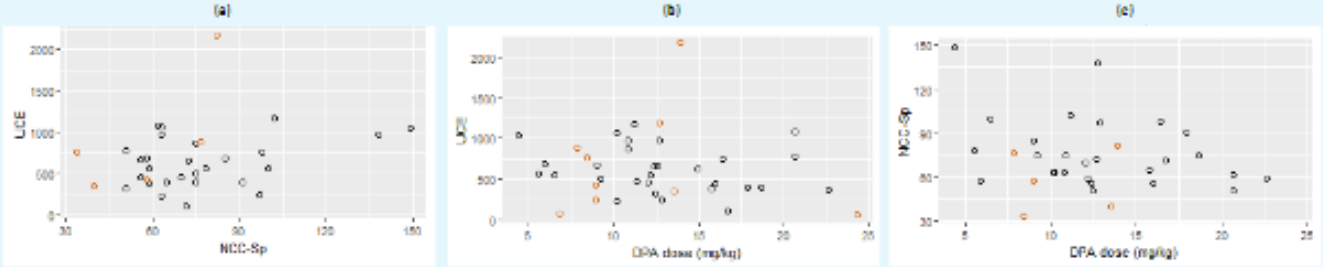


Figure 4: NCC-Ex ≥ 50 mcg/L



Excreation in Patients on Treatment for Wilson Disease?

Bekir Mert Durukan[1], Merve Banu Polat[2], Gül Yalçın Çakmakli[3], Tevhide Şahin[4], Aslı Pınar[2], Bülent Elibol[3], Hatice Yasemin Balaban[4]

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24-hours urinary copper excretion and to evaluate the utility of EC/REC in daily practice.

METHODS AND MATERIALS

- Prospective, cross-sectional, cohort study.
- 38 WD patients with and 43 controls with dyspepsia.
- Interventions:
  - Face-to-face interviews for the history of liver disease, comorbidities, and drug treatments,
  - Review of an electronic system for laboratory results,
  - 10 cc sample of blood for serum copper analysis by inductively coupled plasma mass spectrometry (ICP-MS),
  - REC = EC/total copper
  - Since controls rejected to collect urine, urinary copper was measured only in WD patients by using atomic absorption spectrometry.

RESULTS

- The median age in controls and patients was similar (28 and 31 years; p=0.393).
- 46% of controls and 50 % of patients were male (p=0.928).
- The median age at diagnosis of WD was 10 years.
- There was 55% off only hepatic WD, 42% off both hepatic and neurologic WD, and 3% off only neurologic WD . Psychiatric disease was present in 24% of patients.
- Medical treatment:
  - 62% D-penicillamine and zinc,
  - 16% D-penicillamine alone,
  - 16% trientine and zinc,
  - 3% trientin
  - 3% zinc alone.
- Median 24-hour urinary copper excretion widely ranged (472.5 (32.9-1872) µg/day).
- Serum copper tests:
  - Total serum copper was higher in controls (16.92 vs. 10.55 µmol/l; p<0.001).
  - EC levels were similar in patients and controls (0.53 vs. 0.81 µmol/l; p=0.101).
  - REC was lower in controls than in patients (2.93% vs. 9.25%; p<0.001).
- In comparison with patients with both hepatic and neurological involvement, patients with only hepatic-WD had higher serum total copper (11.2 vs. 5.75 µmol/l; p=0.025), EC (1.02 vs. 0.63 µmol/l; p=0.068) and 24-hour urinary copper excretion (591.4 vs. 406.19 µg/day; p=0.617), although REC was similar (9.25% vs. 9.82%; p=0.37).

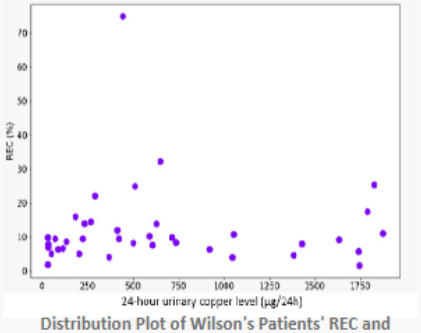
Treatment Period; Year	0,005	0,978	0,118	0,488	0,173	0,298	0,157	0,348
Hemoglobin; g/dl	-0,03	0,797	-0,004	0,972	0,015	0,895	0,398	0,013
White Blood Cell Count; x10³ /ml	0,315	0,004	-0,047	0,682	-0,253	,026	-0,085	0,613
Platelet Count x10³ /ml	0,554	<0,0001	-0,117	0,309	-0,507	<0,0001	-0,205	0,217
AST; IU/L	-0,289	0,01	-0,02	0,859	0,174	0,128	0,189	0,255
ALT; IU/L	-0,237	0,037	0,101	0,377	0,216	0,057	0,092	0,582
GGT; IU/L	-0,382	0,001	0,163	0,154	0,36	0,001	0,212	0,201
ALP; IU/L	-0,381	0,001	0,033	0,774	0,338	0,003	-0,027	0,873
Total Bilirubin; mg/dl	-0,252	0,029	-0,012	0,916	0,256	0,026	0,204	0,22
Direct Bilirubin; mg/dl	-0,248	0,032	0,01	0,929	0,27	0,019	0,004	0,98
Albumin; g/dl	0,199	0,081	-0,206	0,068	-0,283	0,012	-0,202	0,223
Total Serum Protein; g/dl	0,037	0,761	-0,098	0,421	-0,083	0,496	-0,389	0,016
INR	-0,269	0,102	-0,048	0,776	0,248	0,134	-0,039	0,816
Creatinine; mg/dl	-0,013	0,912	-0,012	0,917	-0,034	0,772	0,119	0,477
LDL; mg/dl	-0,07	0,603	-0,082	0,539	-0,038	0,777	0,08	0,71
Triglyceride; mg/dl	-0,248	0,065	0,179	0,179	0,238	0,075	0,193	0,353
AFP; ng/ml	-0,040	0,824	0,103	0,563	0,161	0,365	0,182	0,303
MELD-Na Score	-0,243	0,141	-0,035	0,833	0,231	0,163	-0,17	0,92
FIB-4 Score	-0,291	0,1	0,058	0,614	0,272	0,17	0,308	0,06
Ceruloplasmin; mg/dl	0,56	<0,0001	0,348	0,032	-0,345	0,034	-0,039	0,816

Serum copper tests were associated with several clinical parameters:

- The correlation of platelet count with total copper (r=0.554; p<0.0001) and REC (r=-0.507; p<0.0001) were in opposite direction.
- ALP and GGT were negatively correlated with serum total copper (r=-0.381; p=0.001 and r=-0.382; p=0.001, respectively) and positively correlated with REC (r=0.338; p=0.003 and r=0.36; p=0.001, respectively).
- Ceruloplasmin positively correlated with serum total copper (r=0.56; p<0.0001) and EC (r=0.348; p=0.032).

None of the serum total copper tests did not correlate with 24-hours urinary copper excretion.

REC did not correlate with 24-hours urinary copper excretion.



Distribution Plot of Wilson's Patients' REC and 24-Hour Urine Copper Excreation

urinary copper excretion and EC representing serum-free copper. The inability to find such a positive correlation in our study can be explained by several reasons:

- Urinary loss of copper and other heavy metals is shown to be associated with proteinuria. So, (non)selective proteinuria for any reason will increase copper excretion independent from the level of serum-free copper. So serum EC level will be lower than expected due to urinary loss of copper., meaning that EC (free copper) and urinary copper (free copper and proteinuria-associated copper) may be representing different pools of copper.
- The type of chelator treatment may be confounding since, it is known that penicillamine can cause proteinuria.
- , Unlike EC, urinary copper excretion depends on compliance with chelator treatment and glomerular filtration rate.

CONCLUSIONS

Serum EC/REC measurements are technically difficult, although it is more practical than 24-hour urine collection which is a test of choice in routine clinical practice to evaluate body copper load.

There was no association between serum copper tests and urinary copper excretion in this study.

The possible explanations for this lack of correlation might be the facts that:

- These tests may be showing different copper pools in the body,
- Type and/or compliance to chelators may be changing serum and urine copper distribution,
- Renal function and proteinuria may be affecting urinary copper excretion,
- Methodological differences in serum and urine copper measurements may be misleading.

Future studies controlling these factors would reveal the potential of serum tests as an alternative to urine collection for evaluating body copper load in WD patients on treatment.

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ETHNICITY DOES NOT PREDICT LONG-TERM OUTCOME OF LIVER DISEASE IN PAEDIATRIC-ONSET WILSON DISEASE: A NORTH AMERICAN MULTI-ETHNIC COHORT

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PATIENTS & METHODS

A single-centered, retrospective study involving clinically and/or genetically diagnosed WD probands seen at The Hospital for Sick Children between 1962 and 2022. Patients diagnosed by family screening or who had co-existing liver disease were excluded.

Hepatic presentation was classified as compensated cirrhosis in patients with portal hypertension (PHTN), decompensated cirrhosis in patients with PHTN AND ascites requiring diuresis OR variceal bleeding requiring therapeutic endoscopy. All acute liver failure patients met the PALF definition.

Event-free survival (EFS) was defined as the length of time subjects were free of hepatic decompensation (ascites requiring diuresis or variceal bleeding), liver transplantation, or liver-related death.

RESULTS

Out of 56 WD probands (28 male), 52% (n=29) were CAUC, 21% (n=12) were SA, and 27% (n=15) were from other ethnicities from 11 different countries. Most (87%) study subjects presented with hepatic WD, with a median age of 11.5 years (IQR 8 – 14) and were followed for a median of 4.2 years (IQR 1.9 – 6.7).

Compared to 27 non-CAUC, **CAUCs had a later disease onset and were less likely to present with chronic liver disease.**

CONCLUSION

Although SA probands were more likely to be younger at presentation and when experiencing a liver-related event, **ethnicity did not influence long-term outcome of paediatric WD in a resource-rich setting.**

Female preponderance is a well-recognized feature of severe WD.[3] **Our study reveals that this holds true regardless of ethnicity.**

Further multi-centered studies with larger sample sizes are crucial to re-confirm our novel findings.

Table 1. Clinical Presentation and Outcome of Caucasian vs non-Caucasian WD Probands

	Non-CAUC	CAUC	*p*-value
n	*27*	*29*	
Demographics, % (n)			
Female	33% (n=9)	66% (n=19)	**0.01**
Age at presentation, y (IQR)	10 (7-12)	14 (10-15)	**0.002**
Hepatic presentation, %	26	28	
Chronic liver disease	73% (n=19)	32% (n=9)	**0.003**
Compensated cirrhosis	7.7% (n=2)	25% (n=7)	0.14
Decompensated cirrhosis	3.8% (n=1)	14.3% (n=4)	0.35
Acute liver failure	15.4% (n=4)	28.6% (n=8)	0.24
Long-term outcomes			
Hepatic decompensation event, % (n)	33.3% (n=9)	41% (n=12)	0.53
Age at first event, y (IQR)	11.2 (9.1 – 13.4)	15 (13.5 – 16.8)	0.08

In contrast, **SAs were younger than non-SAs (n=44) at presentation and when experiencing a hepatic decompensation event** despite having comparable disease severity at presentation.

EFS showed no significant differences in long-term outcome between CAUCs and non-CAUCs by 18 years (log-rank *p*=0.86), between SAs and non-SAs (log-rank *p*=0.14), and between SAs and CAUCs (log-rank *p*=0.26).

Figure 1. Event-free Survival Among All Ethnicities

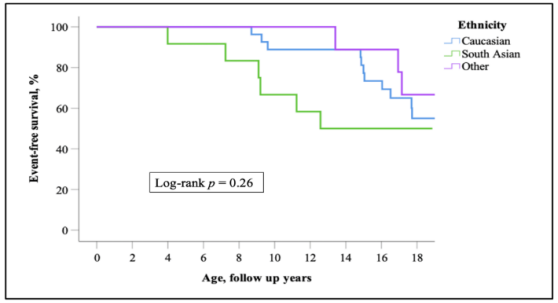
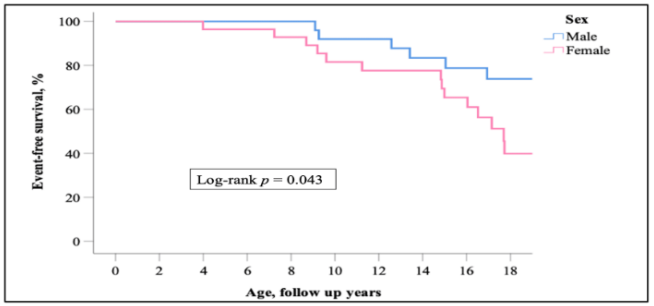


Table 2. Clinical Presentation and Outcome of South Asian vs non-South Asian WD Probands

	SA	Non-SA	*p*-value
n	*12*	*44*	
Demographics, % (n)			
Female	33% (n=4)	55% (n=24)	0.19
Age at presentation, y (IQR)	10.5 (8.5-11)	13 (8-14)	**0.03**
Hepatic presentation, %	11	43	
Chronic liver disease	63.6% (n=7)	49% (n=21)	0.38
Compensated cirrhosis	18.1% (n=2)	16.3% (n=7)	1.0
Decompensated cirrhosis	0%	11.6% (n=5)	0.57
ALF	18.1% (n=2)	23.2% (n=10)	1.0
Long-term outcomes			
Hepatic decompensation event, % (n)	50% (n=6)	34% (n=15)	0.33
Age at first event, y (IQR)	9.2 (7.7 – 10.7)	15 (14.1 – 17)	**0.002**

By 18 years old, **females were found to have significantly higher incidence of hepatic decompensation events** than males, regardless of ethnicity.

Figure 2. Event-free Survival Comparing Females to Males



Exchangeable and Relative Exchangeable Copper Correlate with Multiparametric Liver MRI Parameters in Patients with Wilson Disease

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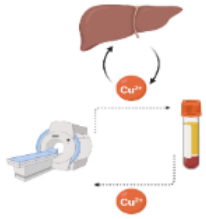
Introduction

Exchangeable copper (EC) and relative exchangeable copper (REC) are serum tests reported to be superior to urinary copper excretion for diagnosis of Wilson's disease (WD), but they have not been validated for usage in routine Daily practice.

MRI is the most sensitive radiologic method for diagnosis and follow-up of liver disease. Multiparametric MRI methods enable simultaneous measurement of several liver parameters: MR elastography (MRE), proton density fat fraction (PDFF), T1, T2, and T2* mapping, R2*.



This study was aimed to investigate the correlation of EC and REC with clinical and multiparametric liver MRI parameters.

Methods and Materials

This prospective, cross-sectional, cohort study included 35 WD patients.
The clinical parameters were collected through face-to-face interviews for the history of WD, and from electronic system records of laboratory results.
A 10 cc sample of blood was taken for serum total serum copper and EC analyses which were done by inductively coupled plasma mass spectrometry (ICP-MS).
REC was calculated as the ratio of them (REC = EC/total serum copper).
Multiparametric liver MRIs were performed by using a 1.5-T MR imaging system (Magnetom Aera, Siemens Healthcare, Erlangen, Germany).
Multiparametric liver MRIs were reported by the same expert radiologists for liver parenchymal stiffness (MRE), liver fat content (PDFF), and iron accumulation (corrected T1, cT1). T1, T2, T2*, and R2* measurements were made, and cT1 was calculated according to the following formula: T1-420+20*(T2*).

Results

The median age was 32 (18-58) years, and 54% was male.
The median age at diagnosis of WD patients was 10 years.
There was %97 had hepatic involvement.
The median liver MRE value was normal (2.45 (1.71-6.2) kPa) with a distribution o:

- 69% no fibrosis,
- 6% Stage 1 fibrosis,
- 11% Stage 2 fibrosis,
- 8% Stage 3 fibrosis,
- 6% Stage 4 fibrosis.

MR-PDFF technique showed mild (5-14%) adiposity in 38% of patients, and moderate (15-20%), or severe (>20%) hepatosteatosis was not detected in any patients.

Multiparametric MRI findings of Wilson Patients

Median Liver Parenchymal Stiffness; (n=35); (Min-max); kPa	2,45(1,71-6,2)
Liver Parenchymal Stiffness; (n=35); n (%)	
• Normal	24 (69%)
• Stage1	2 (6%)
• Stage2	4 (11%)
• Stage3	3 (8%)
• Stage4	2 (6%)
Median Liver Fat Ratio by MRI-PDFF; (n=26); (Min-max); %	3.75 (1.4-11.5)
MR-PDFF ile Karaciğer Yağ Oranının Sınıflaması; (n=26); n (%)	
• Normal (<5%)	16 (62%)
• Mild (5-14%)	10 (38%)
• Moderate (5-19%)	0 (0%)
• Severe (>20%)	0 (0%)
T2*; (n=34); Median (Min-max); ms	28.4 (13.8-38.2)
T2; (n=40); Median (Min-max); ms	48.5 (43.5-55.8)
cT1; (n=39); Median (Min-max); ms	948.5 (550-1305)
T1; (n=35); Median (Min-max); ms	780 (496-1215)
R2*; (n=26); Median (Min-max); 1/ms	29.4 (25.6-57.4)

The liver stiffness was positively correlated with and age (r=0.514; p=0.002), AST (r=0.445; p=0.007), GGT (r=0.549; p=0.001), ALP (r=0.365; p=0.031), INR (r=0.404; p=0.016), creatinine (r=0.344; p=0.043), FIB-4 score (r=0.694; p<0.0001); and negatively correlated with white blood cell (r=-0.49; p=0.003) and platelet (r=-0.607; p<0.0001) counts.
The fat fraction was in positive correlation with serum triglyceride level (r=0.532; p=0.013).
T2* was a negatively correlated with total bilirubin (r=-0.394; p=0.019) and creatinine (r=-0.382; p=0.023).
T2 was negatively correlated with hemoglobin (r=-0.405; p=0.016).
R2* indicating iron accumulation in the liver was negatively correlated only with GGT without statistical significance (r=-0.306; p=0,129) .
There was no correlation between cT1 and any clinical or laboratory parameters.

Correlation Analysis of Liver Multiparametric MRI Results with Clinical and Laboratory Parameters

	Liver Parenchyma Stiffness (kPa)		MR-PDFF Fat Ratio (%)		T1 (ms)		T1* (ms)		R2* (1/ms)		T2 (ms)		cT1 (ms)	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Age; Year	**0,514**	**0,002**	0,048	0,810	0,143	0,236	0,094	0,592	0,112	0,584	0,143	0,454	0,225	0,201
Hemoglobin; g/dl	-0,295	0,588	0,195	0,345	**-0,405**	0,641	-0,254	0,141	-0,190	0,352	**-0,405**	**0,016**	-0,24	0,172
White Blood Cell Count; x10³ /ml	**-0,49**	**0,003**	0,257	0,075	-0,006	0,978	-0,215	0,214	-0,044	0,829	-0,036	0,972	-0,04	0,823
Platelet Count x10³ /ml	**-0,607**	**<0,0001**	-0,104	0,611	-0,094	0,961	0,002	0,641	0,011	0,959	-0,034	0,581	-0,045	0,801
AST; IU/L	**0,445**	**0,007**	0,253	0,232	0,041	0,642	-0,201	0,247	0,008	0,968	0,045	0,795	-0,044	0,805
ALT; IU/L	0,163	0,349	**0,332**	0,12	-0,034	0,897	-0,208	0,169	-0,121	0,556	-0,034	0,847	0,053	0,764
GGT; IU/L	**0,549**	**0,001**	0,331	0,137	0,107	0,678	-0,025	0,888	**-0,306**	0,129	0,107	0,542	0,18	0,307
ALP; IU/L	**0,365**	**0,031**	0,197	0,335	0,054	0,453	-0,135	0,448	0,191	0,358	0,054	0,756	0,134	0,486
Total Bilirubin; mg/dl	0,135	0,374	0,159	0,438	0,154	0,738	**-0,394**	**0,019**	0,18	0,379	0,156	0,371	-0,114	0,519
Total Protein; g/dl	**-0,317**	0,064	0,147	0,475	0,111	0,851	-0,257	0,137	0,158	0,439	0,113	0,509	-0,056	0,752
INR	**0,404**	**0,016**	-0,093	0,65	0,299	0,472	0,131	0,663	-0,144	0,484	0,299	0,081	0,009	0,960
Creatinine; mg/dl	**0,344**	**0,043**	0,178	0,384	-0,116	0,352	**-0,382**	**0,023**	0,169	0,252	-0,116	0,566	-0,033	0,854
Triglyceride; mg/dl	-0,105	0,635	**0,532**	**0,013**	-0,066	0,769	-0,136	0,536	0,126	0,576	-0,066	0,764	-0,061	0,815
MELD-Na Score	0,298	0,082	0,021	0,92	0,314	0,453	-0,112	0,522	0,691	0,688	0,854	0,067	-0,009	0,959
FIB-4 Score	**0,694**	**<0,0001**	0,117	0,569	0,104	0,695	0,004	0,983	-0,179	0,383	0,104	0,554	0,11	0,536
Ceruloplasmin; mg/dl	-0,141	0,405	-0,057	0,781	-0,013	0,669	**0,317**	0,064	-0,003	0,987	-0,013	0,941	0,163	0,274
Serum Total Copper (µmol/l)	-0,112	0,484	0,153	0,454	0,11	0,33	0,214	0,217	-0,145	0,48	0,027	0,876	-0,122	0,52
Exchangeable Copper (µmol/l)	0,261	0,727	**0,134**	**0,005**	**0,371**	**0,028**	-0,158	0,366	0,101	0,623	-0,145	0,484	0,188	0,319
Relative Exchangeable Copper (%)	0,159	0,362	0,305	0,13	0,229	0,186	**-0,422**	**0,012**	0,148	0,237	-0,116	0,588	**0,354**	0,092

Discussion

EC was a positively correlated with MR-PDFF (r=0,534; p=0,005) and T1 (r=0.371; p=0.028), while REC was a negatively correlated with T2 (r=-0.422; and p=0.012).*

The parameters for liver fibrosis at multiparametric MRI are cT1, T1, and T2 relaxation times. The positive correlation between EC and T1 suggests that T1 also increases with copper accumulation in the liver.

T2* and R2* values are parameters used to measure iron accumulation in the liver. Depending on the accumulation of iron in the liver, it is expected that the T2* value will decrease and the R2* value will increase. There is no previous study on the T2* value in the liver in WD. In this study, a negative correlation was found between REC (r=-0.422; p=0.012) and T2*.

Conclusions

Multiparametric liver MRI is sensitive for showing hepatic involvement in WD.
As alternative tests for evaluating body copper load, EC and REC are correlated with the parameters of MRI for steatosis, inflammation, and fibrosis.
Further studies with a larger number of patients are needed to establish the correlation between serum copper tests and multiparametric liver MRI findings.

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Exchangeable and Relative Exchangeable Copper Levels were Similar in Wilson Disease Patients with and without of Cranial MRI Involment

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INTRODUCTION: The cranial MRI is the golden standard radiologic technique for diagnosis and follow-up of neurologic Wilson's disease (WD). Exchangeable copper (EC) and relative exchangeable copper (REC) have diagnostically superior to urinary copper excretion, but their role in follow up have not been evaluated.
This study was aimed to investigate the relationship between serum copper tests and cranial MRI findings among patients treated for WD.

METHODS: This prospective, cross-sectional, cohort study included 36 patients with WD in whom face-to-face interviews for medical history and a review of electronic system records for laboratory results were done.
Analyzes for EC and total serum copper were done from 10 cc of a blood sample by using inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Nexion 2000). For preparation UF; serum was diluted with EDTA (3 mg/L) (1:1) and incubated for precisely 1 hour. Then the mixture was ultrafiltrated on Amicon Ultra-4 (Millipore, Molsheim, France). The REC was calculated as the ratio of EC to total serum copper.
All patients underwent cranial MRI on a 1.5-T MR imaging system (Magnetom Aera, Siemens Healthcare, Erlangen, Germany). The gray matter lesions of WD patients were examined by the same neuroradiologists. A score was assigned to each of the bilateral lenticular nuclei (pallidum and putamen), caudate nuclei, thalamus, pons, mesencephalon, and dentate nuclei involvement. The cranial MRI score ranged between 0 to 6 in WD patients.

RESULTS: The median age was 32 (18-58) years, and 53 % of patients were male. The median age for diagnosing WD was 10 years.
Cranial MRI revealed WD involvement in 45% of patients, and 14% of patients had cranial MRI findings without clinical symptoms of neuro-WD.
The distribution of the patient's subgroups according to cranial MRI scores was as follows;

- 56% no gray matter involvement,
- 33% Score 1-2, 8% Score 3-4, 3% Score 5-6.

Patients with and without gray matter MRI involvement had similar total serum copper (10.67 vs 6,85 µmol/l p=0.168), EC (0.94 vs. 0.65 µmol/l; p=0.158), REC (8.55% vs. 9.47%; p=1) and 24-hour urinary copper excretion (558.56 vs 472.56 µg/day; p=0.582).

	No Gray Matter Involment	Gray Matter Involment	p
Serum Total Copper (µmol/l)	10.67	6.85	0.168
Exchangeable Copper (µmol/l)	0.94	0.65	0.158
Relative Exchangeable Copper (%)	8.55	9.47	1
24 h Urinary Copper (µg/24 h)	558.56	472.56	0.582

DISCUSSION: The literature on neuro-WD has been shown that structural and functional changes can be found in the brain even if the patients do not have neurological/psychiatric symptoms or signs. Also, Poujois et al. have been shown that the serum EC level is higher in newly diagnosed WD patients with neurologic and hepatic involvement than in those with isolated hepatic involvement.
In this cross-sectional study, the reason for similar EC and REC levels in patients with and without cranial involvement is thought to be due to the depletion of cranial copper load under chelator treatment

CONCLUSION: Cranial MRI was more sensitive than clinical symptoms for detecting cranial involvement in WD.
However, EC and REC levels were similar in WD patients with and without of cranial MRI involvement.
Follow-up cohort studies could show a correlation between EC and REC levels with cranial MRI scores or clinical parameters.

Objective Biochemical Endpoints in an Open-Label Randomized Trial of Chelation Therapy in Wilson disease; Can Subject Behavior in a Trial be Identified?

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Background:

- Adherence to treatment is critical for minimizing morbidity and avoiding reduced life expectancy for individuals with Wilson disease (WD)
- Designing placebo-controlled trials evaluating new therapies for WD are not ethical to evaluate long-term exposures to novel therapies; interventions are thus compared against standard of care
- Blinding of subjects and investigators of the intervention is not always feasible.
- An open label trial such as the Chelate trial[1] (NCT03539952) used an objective, quantifiable (non ceruloplasmin copper; NCC-Sp) primary outcome to minimize assessment bias
- Evaluation of clinical and biochemical stability was assessed by an independent adjudication committee (IAC) of 3 WD experts prior to randomization to confirm eligibility and post-randomization to ensure objectivity at primary (24-wk) and extension (48-wk) phase endpoints, as they were masked to subject's allocated therapy
- However, subjects with knowledge of the intervention may be a source of bias, especially adherence.

Aims:

- To describe changes in physician clinical (site PI) and selected laboratory assessments over the course of the CHELATE trial in a post-hoc analysis of data to identify trial effect, independent of treatment allocation

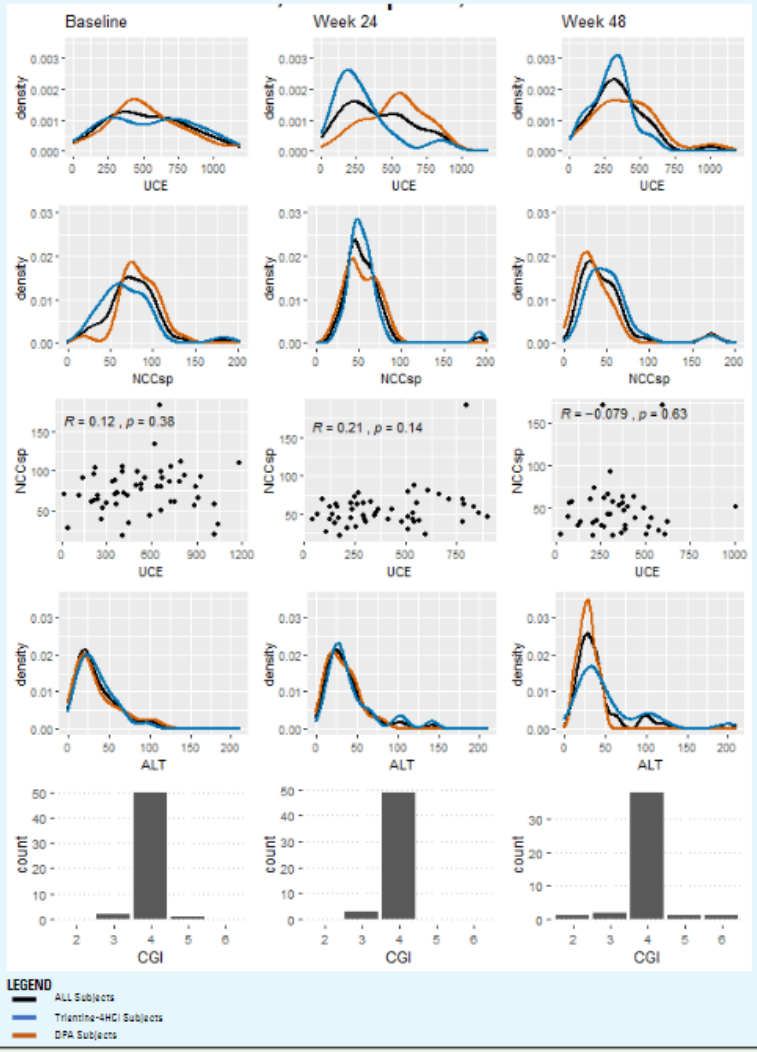
Method:

- Clinically stable patients on D-penicillamine (DPA) ≥1y and on average diagnosed for > 20 years were enrolled into an open label trial
- At the end of a 12-wk block to allow for stabilization of dose, patients who were confirmed by site PI and IAC to be clinically stable, were randomized (baseline) to either DPA or trientine tetrahydrochloride (trientine-4HCl)
- Stability was assessed using clinical global impression of change (CGIC) scores by site PI, and efficacy and safety of the intervention was assessed by evaluation of signs of liver disease, neurological (UWDRS) examination and laboratory investigations including NCC-Sp using novel methodology[2], 24-hour urinary copper excretion (UCE) and liver function tests
- Data were compared at baseline (end of the 12-week run-in period) at 24- and 48-weeks post randomization; the IAC confirmed clinical stability in all subjects post randomization

Results:

- Across all recruiting centers, 77 (38 female) subjects were enrolled with 53/77 (69%) randomized after 12-wk of baseline period (demographics table)
- UCE was widely distributed, breeching guidance targets (200 - 500mcg/L) at baseline
- At week 24, UCE peak with trientine-4HCl was narrower and left shifted; at week 48 although the density for DPA shifted to the left, it showed a smaller peak with a wider distribution
- A similar pattern of increased density and shift to the left was observed with NCC-Sp and alanine aminotransferase (ALT) at week 24 and 48 irrespective of treatment
- No correlation was observed between UCE and NCC-Sp, although at study end this changed from a positive to negative (inverse) relationship

Baseline Characteristics	DPA (N = 27)	Trientine-4HCl (N = 26)
Age (years)	45.2 (13.4)	42.0 (15.6)
Female – no. (%)	16 (59%)	12 (46%)
Weight (kg)	72.7 (16.1)	76.6 (14.4)
Dose of DPA at randomization (mg)	900 (276)	891 (353)
Global neurological (UWDRS) score at baseline*	6.0 (1.0, 15.0)	4.5 (2.0, 8.0)



Conclusions:

- In an open label study, comparing DPA with trientine-4HCl, where subjects remained clinically stable throughout, UCE and NCC showed less variation during course of the study, suggesting a trial effect
- Subjects in the CHELATE trial may have become more compliant with diet and medication, independent of open study design and allocated therapy
- These data demonstrate that WD patients, even those on maintenance therapy, benefit from participating in a randomized trial

Preliminary findings for accurate non-ceruloplasmin copper (ANCC): Potential monitoring biomarker in medically treated Wilson Disease

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BACKGROUND

Treatment monitoring for Wilson disease (WD) involves clinical and biochemical testing. Accurately determined non-ceruloplasmin copper (ANCC) is a new assay (1) proposed to be a useful biomarker for determining overall copper status in WD patients on medical therapy. There are few data on ANCC in WD patients on medical therapy.

AIM

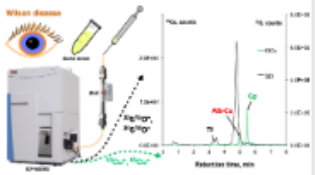
To correlate ANCC with data for copper status in treated WD patients to begin to determine if ANCC is a useful biomarker for treatment monitoring.

MATERIALS AND METHODS

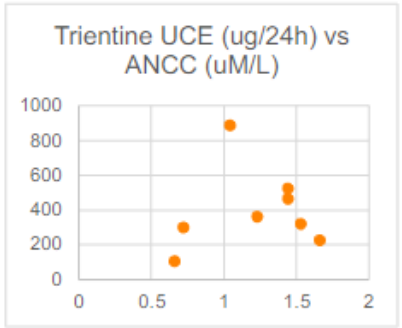
The cohort included patients enrolled in the WD Registry (data coordinating center and biorepository at Yale) where ANCC and 24 h urine copper excretion (UCE) data were available. ANCC was determined from serum from patients on zinc (n=13) or trientine (n=8) therapy using a previously described novel assay based on copper protein speciation and inductively coupled plasma mass spectrometry (1) Prospectively collected clinical and biochemical data and history of WD diagnosis and treatment were reviewed.

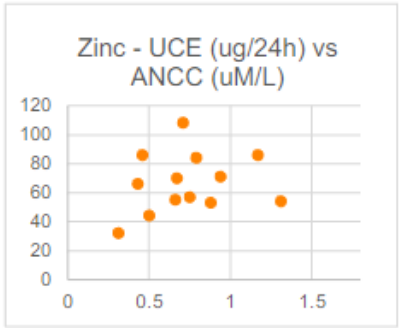
RESULTS

Data was available on ANCC and urine copper excretion in 21 treated WD patients (15 F). The average patient age was 42 y (range 12-75), and treatment for WD with zinc (n=13) averaged 10.4 y while those on trientine (n=8) averaged 7.8 y. ANCC in all ranged from 0.22 to 1.66 uM/L, with the average for zinc treatment being significantly lower at 0.74 ± 0.29 versus 1.21 ± 0.37 for those on trientine (p=0.009). For reference, the ANCC range for a non-WD population was determined to be 1.17 to 3.87 uM/L. As we assumed, UCE showed a poor correlation with ANCC (r=0.21 for zinc, 0.10 for trientine), though average values of UCE were in expected ranges for most long-term treated patients (66.6 ± 20.5 ug for zinc, 398.9 ± 236.2 ug for trientine).



ANCC Determination: Serum proteins were separated by anion exchange chromatography and copper content of protein peaks determined by ICP-MS. Internal standardization is possible using natural sulfur and copper isotopes.





Figures: Shown are the UCE (y axis) and ANCC (X axis) for patients on trientine and zinc therapy for their Wilson disease. Note similarity of ANCC range and wider range for UCE, particularly in patient on chelation therapy

CONCLUSIONS

1) ANCC may be a useful alternative to UCE for patient monitoring as UCE values are highly variable, especially in patients on chelation therapy. This requires further validation in a larger cohort of WD patients on different treatments.

2) Counter to expectations, we observed a wider range of ANCC concentrations between individual therapies for WD, possibly due to the small sample size initially evaluated. Further evaluation is needed to determine if the range should be consistent between therapies.

3) For ANCC to be an accepted biomarker for WD, further evaluation is needed to determine the ranges of values that correlate with clinical and biochemical stability in treated patients.

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