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Serum free copper levels: a possible biomarker of neurodegeration in Multiple Sclerosis patients?

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Objectives

To evaluate the serum free copper (f-Cu) levels in a cohort of Multiple Sclerosis (MS) patients and to explore the relationship between f-Cu and disability measures.

Materials and Methods

Fifty-one MS patients [25 relapsing remitting (RRMS) and 26 progressive MS (PMS)] were retrospectively enrolled in the study.
f-Cu levels were assayed by spectrum-fluorimetry (*Test C4D, figure 1)* on serum samples obtained at the time of MS diagnosis. For all patients 6 months clinical follow-up was available.
Thirty-three patients had usable brain MRI scans, obtained by 1.5 Tesla scanner, within one month from diagnosis. Using SIENAX we calculated the following cerebral volumes from the MRI scan: Total Brain Volume [TBV], Grey Matter Volume [GV, total and peripheral] and White Matter Volume [WMV].

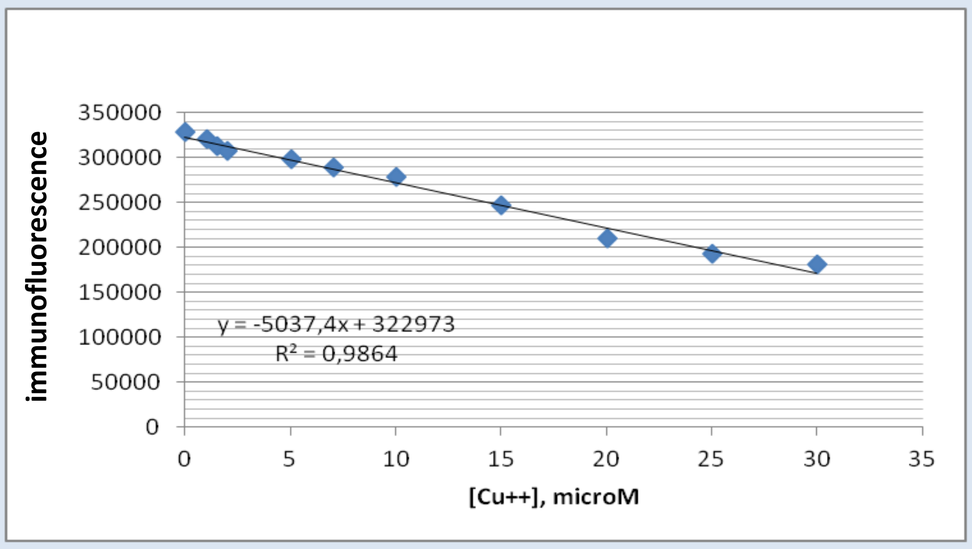

Figure 1. Test CD4
The test CD4 performs a quantitative dosage of f-Cu using a spectrum-fluorimetry tool.
It is compounds by a fluorescent probe to switch off having a scaffold coumarin.
The probe consists of two portions: the former to chelate f-Cu in the sample and the latter to emit a fluorescent signal. When the f-Cu is captured, the probe reduces the intensity of fluorescent emission proportionally to the amount of f-Cu present in the sample.

Results

Table1. Basal features of MS cohort. Values expressed as media (SD) and mediana (range)

	Total	**RRMS**	**PMS**	**p**
N° patients	51	25	26	
F/M	23/28	13/12	10/16	0.2
Age at disease onset, yrs	37.38±12.04	32.66±13.18	41.9±8.9	0.005
Age at diagnosis, yrs	44.1±13	37.11±13.01	50.9±8.25	<0.0001
Disease duration at diagnosis, months	81.8±117.22	54.13±112.06	108.38±118.01	0.1
EDSS at baseline	4.0 (1-6)	2.5(1-4.5)	4.5 (3-6)	<0.0001
Free serum copper levels, µmoli/L	**2.52±1.03**	**2.22±0.65**	**2.82±1.24**	**0.03**
Duration of follow up, yrs	3.38± 2.73	1.60±1.14	5.08±2.74	<0.0001
EDSS at last follow-up	4 (1-8)	2 (1-5)	6 (3-8)	<0.0001

- *The serum f-Cu levels were higher in PMS patients compared to RRMS [2.82±1.24 vs 2.22±0.65 µmol/L, p=0.03].*
- *Serum f-Cu levels correlated with age among male patients (r=0.4;p=0.03)*

- *Patients increasing EDSS score of more than 1.5 point during follow-up showed higher f-Cu levels compared to patients with a lower EDSS changes [3.27±1.26 vs 2.32±0.88 µmoli/L; p=0.006].*
- *A logistic regression analysis (dependent variable: EDSS change at last follow-up ≥1.5 points; covariates: age, sex, baseline EDSS, serum f-Cu levels and disease duration) revealed that higher serum f-Cu levels (OR:1.17, I.C.95%:1.2-8.7; p=0.02), male gender (OR:2.6, I.C.95%:1.35-140.3; p=0.03) and higher EDSS at baseline (OR:1.4, I.C.95%:1.2-13.3; p=0.02) were independent predictors of EDSS progression at follow-up.*

Table2. Serum copper levels in MS patients stratified according to median value of each brain volume.

Brain Volumes (x 10^3 µ/L)		**Serum free Copper (µmoli/L)**	**p**
TGV 701.28 (533.78-843.64)	Low group	2.2 (0.6-4.2)	n.s.
	High group	1.95 (1.10-5.2)	
PGV 544.34 (413.58-688.41)	Low group	2.2 (0.6-4.2)	n.s.
	High group	1.95 (1.10-5.2)	
TBV **1475.12 (1227.63-1695.84)**	**Low group**	**2.3 (1.3-5.2)**	**0.01**
	High group	**1.95 (0.6-2.85)**	
WMV **765.87 (593.39-921.16)**	**Low group**	**2.35 (1.10-5.20)**	**0.04**
	High group	**2.05 (0.60-2.85)**	
CSF Volume 48.51 (19.78-119.42)	Low group	2.20 (1.30-2.85)	n.s.
	High group	2.05 (0.6-5.20)	

- *Stratifying the patients in two groups according to the median value of each cerebral volume ("low" volume and "high" volume groups) we found that patients with "low" TBV and "low" WMV showed higher f-Cu serum levels compared to patients with "high" TBV and WMV (p=0.01 and p=0.04 respectively).*

- *A logistic regression analysis, using TBV and WMV as dependent variables (covariate: age, sex, MS course, disease duration and serum f-Cu levels) demonstrated that the age at diagnosis (OR:0.85, IC 95%:0.73-0.98; p=0.03) and f-Cu levels (OR:0.07, IC 95%: 0.005-0.97; p= 0.04) were the independent predictors of TBV.*

Conclusions

Our preliminary data suggest that serum f-Cu levels are higher in patients with progressive MS course, grater brain atrophy at diagnosis and higher disability accumulation over time. These results suggest a possible role of serum f-Cu as biomarker targeting neurodegeneration in MS.