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Comparison of transcranial sonography-magnetic resonance fusion imaging in Wilson's and early-onset Parkinson's diseases

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ABSTRACT

Introduction: Wilson's disease (WD) is a hereditary disorder caused by *ATP7B* mutations resulting in systemic copper accumulation. WD may manifest as early-adulthood parkinsonism; and atypical cases may be difficult to distinguish from early-onset Parkinson's disease (EO-PD), a neurodegenerative disorder with onset ≤40 years of age. The aim of our study was to compare transcranial sonography (TCS) −magnetic resonance fusion imaging in WD and EO-PD and examine whether TCS can provide clinically useful information.

Methods: We examined 22 WD, 16 EO-PD, and 24 healthy control subjects. We measured echogenicity and determined presence of MRI signal changes in T2-weighted images in the substantia nigra (SN) and lentiform nucleus (NL). TCS with the capability of magnetic resonance fusion and Virtual Navigator was used. The echogenicity indices of SN and NL were processed using digital image analysis to eliminate subjective evaluation errors.

Results: Mean SN echogenicity index in EO-PD (39.8 ± 5.9 [SD]) was higher compared to WD (28.0 ± 4.6, $p < 0.0001$) and control subjects (28.8 ± 4.9, $p < 0.0001$). Mean NL echogenicity index was higher in WD (117.5 ± 37.0) compared to EO-PD (61.6 ± 5.4, $p < 0.0001$) and control subjects (54.9 ± 11.2, $p < 0.0001$). The SN hyperechogenicity had sensitivity 93.8%, and specificity 90.9%, while the NL hyperechogenicity had sensitivity 95.5% and specificity 93.8% for differentiation of WD and EO-PD. NL hyperechogenicity was more pronounced in WD subjects with putaminal MRI T2 hyperintensity ($p < 0.05$) but was also present in subjects without MRI abnormality.

Conclusions: There are distinct TCS findings in WD and EO-PD complementary to MRI that can be utilized as highly sensitive and specific biomarkers of these disorders.

1. Introduction

Wilson's disease (WD) is a hereditary disorder caused by *ATP7B* mutations resulting in copper accumulation in liver and brain. Timely diagnosis is essential since patients have a good prognosis if the treatment is provided in the early stage [1]. Diagnosis of

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neurological WD is straightforward in the majority of cases when typical biochemical abnormalities are present [2]. However, the diagnosis may be difficult in atypical cases that do not fulfill all the clinical and laboratory criteria [3]. Moreover, genetic confirmation may be difficult since *ATP7B* gene is large and more than 500 mutations were described [1], some of them with questionable pathogenicity [4].

According to guidelines, every patient with young adulthood-onset movement disorder should undergo screening for WD. WD may manifest with parkinsonism, either isolated or in combination with other symptoms such as dystonia, kinetic tremor or ataxia. In early-onset Parkinson's disease (EO-PD), parkinsonism is frequently accompanied by psychiatric symptoms and dystonia [5]. Therefore, the importance of the differential diagnosis between EO-PD and WD may arise, particularly in cases with atypical symptoms and/or inconclusive results of biochemical examinations.

Transcranial sonography (TCS) is a validated tool in the diagnosis of an early stage of PD [6] as well as in the differential diagnosis of PD and atypical parkinsonian syndromes [6,7] and dopa-responsive dystonia [8]. Typical findings in PD consist particularly of the hyperechogenicity and enlarged echogenic size of substantia nigra (SN) [6,9,10]. In the neurological form of WD, the echogenicity of SN is variable, while the hyperechogenicity of the lentiform nucleus (NL) has been consistent across studies [11–15]. It is unclear how echogenicity changes in WD interrelate to MRI abnormalities that typically consist of hyperintensity of basal ganglia, brainstem, and thalamus in T2-weighted (T2w) images [16].

Considering these different findings in EO-PD and WD patients, TCS could be a time-saving, low-cost, screening examination of these disorders. Potential drawbacks of TCS are limited anatomical clarity and dependence on examiner's subjective evaluation of hyperechogenicity. These obstacles are partially overcome by using Virtual Navigator for fusion of TCS and MRI which helps in precise identification of brain structures [17] and recently validated method of digital image analysis which reduces the sonographer's bias in the assessment of hyperechogenicity [18].

The aim of the study was to compare the echogenicity changes in SN and NL in EO-PD and WD in relation to MRI results and assess the utility of TCS as a screening examination in early-onset parkinsonism.

2. Methods

2.1. Patients

In WD patients, inclusion criterion was neurological WD with diagnosis established according to Leipzig criteria [19]. In EO-PD patients, inclusion criteria were diagnosis in accordance with the UK Parkinson's Disease Society Brain Bank criteria [20], and age at onset $\leq$ 40 years. For both groups, exclusion criteria were presence of deep brain stimulation (DBS) electrodes and insufficient temporal bone window for TCS examination. Out of 37 neurological WD patients from our database, 15 were not reachable or disagreed to participate. Out of 59 EO-PD patients from our database 21 were excluded because of DBS, 21 disagreed to participate and one was excluded due to insufficient temporal bone window. Twenty-two WD and 16 EO-PD patients participated in the study. The control group consisted of 24 healthy subjects with no neuropsychiatric disorder. All subjects signed informed consent and the study was approved by the Ethical Committee of General Teaching Hospital in Prague.

Information about initial clinical symptoms, abnormal serum ceruloplasmin concentration, urine copper excretion, liver copper concentration, and the presence of K-F ring at the time of diagnosis was retrieved from medical records.

Twenty WD patients were on a stable medication while two were *de novo* treatment naïve patients at the time of examination (Table 1). Fifteen PD patients were on a stable antiparkinsonian medication while one was treatment naïve. All PD patients were tested for *PARK2* mutations; heterozygous pathogenic mutation was found in one. Neurological impairment was assessed by the Unified Wilson's Disease Rating Scale (UWDRS) [21] in WD and by the Unified Parkinson's Disease Rating Scale, Part III (UPDRS-III) in EO-PD group respectively. Patients were examined on their usual symptomatic therapy. “Joint parkinsonism subscore” was calculated from items present jointly in both scales in order to compare the severity of parkinsonism in WD and EO-PD groups. This subscore consists of UPDRS-III except items 24, 30 and 31.

2.2. Magnetic resonance imaging

MRI was performed using 1.5 T whole body Philips Achieva system in EO-PD and WD patients. Standard spin echo T1w (resolution = $1.2 \times 1.2 \times 3$ mm^3, TE = 15 ms, TR = 500 ms) and T2w (resolution = $0.5 \times 0.5 \times 2$ mm^3, TE = 233 ms, TR = 2250 ms) sequences covering the whole brain were employed to quantify brain damage and generate anatomical images for TCS fusion. An experienced neuroradiologist blinded to the diagnosis quantified the MRI pathology. Degree of atrophy was graded as absent (0 points) mild (1 point) or severe (2 points) in three locations: 1) cerebellum and brainstem, 2) basal ganglia and subcortical region and 3) cortex. At that, presence of T2 hyperintensities in the caudate nucleus, putamen, globus pallidus, thalamus, mesencephalon, pons and T2 hypointensities in the NL, SN, and dentate nucleus were scored by 1 point each. The total sum of atrophy and signal changes build the composite MRI severity score (maximum 15 points) [22].

2.3. Transcranial sonography

The ultrasound system MyLab Twice (Esaote S.p.A., Genova, Italy) was equipped with the Virtual Navigation (MedCom GmbH, Darmstadt, Germany), which allows real-time image fusion of TCS and MRI images. The ultrasound scanner with a Reusable Tracking Bracket (CIVCO, Kalona, IA, USA) and sensor mount were used. The Virtual Navigator technology was implemented using an electromagnetic tracking system, composed of a transmitter and a small receiver, mounted on the ultrasound probe. SN was imaged in the axial mesencephalic plane (Fig. 1A) while NL and caudate nucleus (CN) were imaged in the axial thalamic plane (Fig. 1B) using the Fusion Imaging technique.

Following parameters were used: penetration depth of 16 cm, penetration high, dynamic range 7 (50 dB), frequency 1–4 MHz, enhancement 3, density 2, view 9, persistence 7, dynamic compression 0, gain 36%, grey map 0, S-view off, 2 focuses in 5 and 10 cm, mechanical index 0.9, tissue indices TIs 1.0, TIB 1.0 and TIC 2.1.

The butterfly-shaped structure of the mesencephalic brainstem and the region of SN in mesencephalic section and CN and LN in the thalamic section were depicted and images were saved in 8-bit grayscale DICOM format for offline analysis. Predefined elliptical ROIs were manually placed in the region of contralateral CN and LN and ipsilateral SN (Supplementary Figure). The echogenicity indices were calculated separately for SN, CN and LN from both right and left temporal bone windows using the B-Mode Assist software for digital image analysis [18,23]; the higher value of both measurements was used for analysis.

Standard visual assessment of SN and LN echogenicity [24] was also performed and results were compared with those obtained by digital image analysis. The mean SN echogenic area from both sides

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Table 1
Demography, clinical and TCS findings.

	WD	EO-PD	Control
Number of patients (females)	22 (11)	16 (9)	24 (16)
Age; mean ± SD (years)[a]	43.9 ± 8.7	42.2 ± 5.2	37.6 ± 10.3
Duration of disease; mean ± SD (years)[b]	17.1 ± 9.9	7.7 ± 4.7	–
Duration of therapy; mean ± SD (years)[b]	13.5 ± 7.2	3.8 ± 1.9	–
UPDRS-III subscore; mean; median (interquartile range)	–	21.3; 16.0 (12.0–32.0)	–
UWDRS neurologic subscore; mean; median (interquartile range)	19.6; 13.5 (7.0–30.5)	–	–
UWDRS/UPDRS-III joint parkinsonism subscore; mean; median (interquartile range)[c]	9.1; 4.5 (2.0–14.3)	14.9, 13.0 (9.0–21.0)	–
L-DOPA equivalent; mean ± SD (mg)	–	623.4 ± 453.2	–
Number of patients on d-PEN/Zinc/combination treatment	7/8/4	–	–
Composite MRI severity score; mean; median (interquartile range)[b]	5.9, 6.0 (2.0–8.5)	2.3, 2.0 (1.0–3.5)	–
TCS results			
SN echogenicity index; mean ± SD[d]	28.0 ± 4.6	39.8 ± 5.9	28.8 ± 4.9
NL echogenicity index; mean ± SD[e]	117.5 ± 37.0	61.6 ± 5.4	54.9 ± 11.2
CN echogenicity index; mean ± SD[f]	47.5 ± 16.5	39.6 ± 9.2	34.4 ± 7.4
SN echogenic area (cm^2); mean ± SD[d]	0.20 ± 0.03	0.26 ± 0.04	0.19 ± 0.03
Subject with abnormal SN echogenicity; n (%)[g]	4/22 (18%)	12/16 (75%)	3/24 (13%)
Subjects with abnormal NL echogenicity; n (%)[g]	15/22 (68%)	3/16 (19%)	3/24 (13%)
Established WD diagnostic methods			
Subjects with abnormal serum ceruloplasmin (<0.2 g/l); n (%)[g]	17/22 (77%)	2/15 (13%)	N.A.
Subjects with abnormal 24-h urine copper excretion (>1 μmol/24 h); n (%)[g]	21/22 (95%)	2/12 (15%)	N.A.
Subjects with positive K-F ring; n (%)[g]	15/22 (68%)	0/12 (0%)	N.A.
Subjects with abnormal liver copper concentration (>250 μg/g dry tissue); n (%)	20/20 (100%)	N.A.	N.A.
Subjects with confirmed pathogenic mutation of *ATP7B*; n (%)[h]	16/22 (73%)	N.A.	N.A.

Abbreviations: WD – Wilson disease, EO-PD, early-onset Parkinson's disease, UWDRS – Unified Wilson's Disease Rating Scale, UPDRS-III – Unified Parkinson's Disease Rating Scale, Part III; d-PEN – d-penicillamine, SN – substantia nigra, NL – lentiform nucleus, CN – caudate nucleus, N.A. – not available.

[a] $p < 0.05$ WD compared to controls (ANOVA and Tukey's post-hoc test).
[b] $p < 0.01$ WD compared to EO-PD (Mann-Whitney *U* test).
[c] $p < 0.05$ EO-PD compared to WD (Mann-Whitney *U* test).
[d] $p < 0.0001$ EO-PD compared to WD and controls (ANOVA and Tukey's post-hoc test).
[e] $p < 0.0001$ WD compared to EO-PD and controls (ANOVA and Tukey's post-hoc test).
[f] $p < 0.01$ WD compared to controls (ANOVA and Tukey's post-hoc test).
[g] $p < 0.001$ (Fischer's exact test).
[h] Sequencing of coding regions of the *ATP7B* gene revealed homozygous p.[His1069Gln] mutation in 10 and compound heterozygous mutation p.[His1069Gln] and: [Gly1176Arg] in 1; [Arg778Gly] in 1; [Val1262Phe] in 1; [Gly710Ser] in 2; and [Ala1135fs] in 1 subject. Homozygous p.[Lys1248Thrfs*83] mutation was detected in 1 subject.

was compared in WD, EO-PD, and control group. In the calculation of sensitivity/specificity for differentiation of WD and EO-PD, the larger echogenic area of the two sides was employed while SN hyperechogenicity was defined as echogenic area >0.24 cm^2. Echogenic signals from NL in comparison to surrounding tissue were assessed visually and rated as “normal” or “hyperechogenic”. The echogenicity of NL was regarded as abnormal when hyperechogenic signal was detected at least on one side. A single sonographer who was blinded to the patients' diagnoses but not to movement disorders symptoms performed examinations and image analyses.

2.4. Statistics

The Shapiro-Wilk test was used for normality testing. Data with a normal distribution are reported as mean ± standard deviation. Variables not fitting the normal distribution are presented as a mean, median and interquartile range. Comparisons between two groups were performed using the Mann-Whitney *U* test. Group differences among WD, EO-PD, and control groups were tested using one-way ANOVA with post-hoc Tukey's test. Receiver operating characteristic (ROC) curves were plotted and sensitivity/specificity was calculated for determined cut-off points of SN, NL and CN echogenicity. Sensitivities/specificities were calculated from contingency tables for serum ceruloplasmin, 24-h urine copper excretion, presence of K-F ring, and visually assessed SN and NL hyperechogenicity; frequencies were compared by Fischer's exact test. Dependence of variables was assessed using Pearson correlation coefficient. All reported p-values are two-tailed. Statistical evaluations were performed using the GraphPad Prism version 6 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Clinical assessment

Demographic and medical data are shown in Table 1. The age-range was wider and mean age slightly lower in control subjects but age and echogenicity indices in examined structures were not significantly correlated (data not shown), in accordance with a population study examining SN echogenic area [9]. Twenty-one WD subjects fulfilled the clinical criteria for hypokinetic-rigid syndrome (i.e. presence of akinesia and one of the following: rigidity, tremor or postural instability). Parkinsonian symptoms were more severe in the EO-PD group as documented by higher joint parkinsonism score ($p < 0.05$). Four WD cases initially manifested with isolated parkinsonism and one EO-PD case manifested with leg dystonia. Sensitivities/specificities for WD and EO-PD differentiation were calculated for established screening methods reaching 77/88% for serum ceruloplasmin concentration, 95/83% for 24-h urinary copper excretion and 68/100% for K-F ring.

3.2. TCS findings

The EO-PD group exhibited SN hyperechogenicity and the WD group NL hyperechogenicity in comparison with the control group (Fig. 2). Group analysis of digital image analysis results was significant for both brain areas, SN ($F[2, 59] = 30.0$, $p < 0.0001$) and NL ($F[2, 59] = 47.2$, $p < 0.0001$). Post-hoc Tukey's test showed that SN echogenicity in the EO-PD group was significantly higher than in the WD ($p < 0.0001$) and control ($p < 0.0001$) groups and that NL echogenicity was significantly higher in the WD group compared to the EO-PD ($p < 0.0001$) and controls ($p < 0.0001$). Similar pattern as

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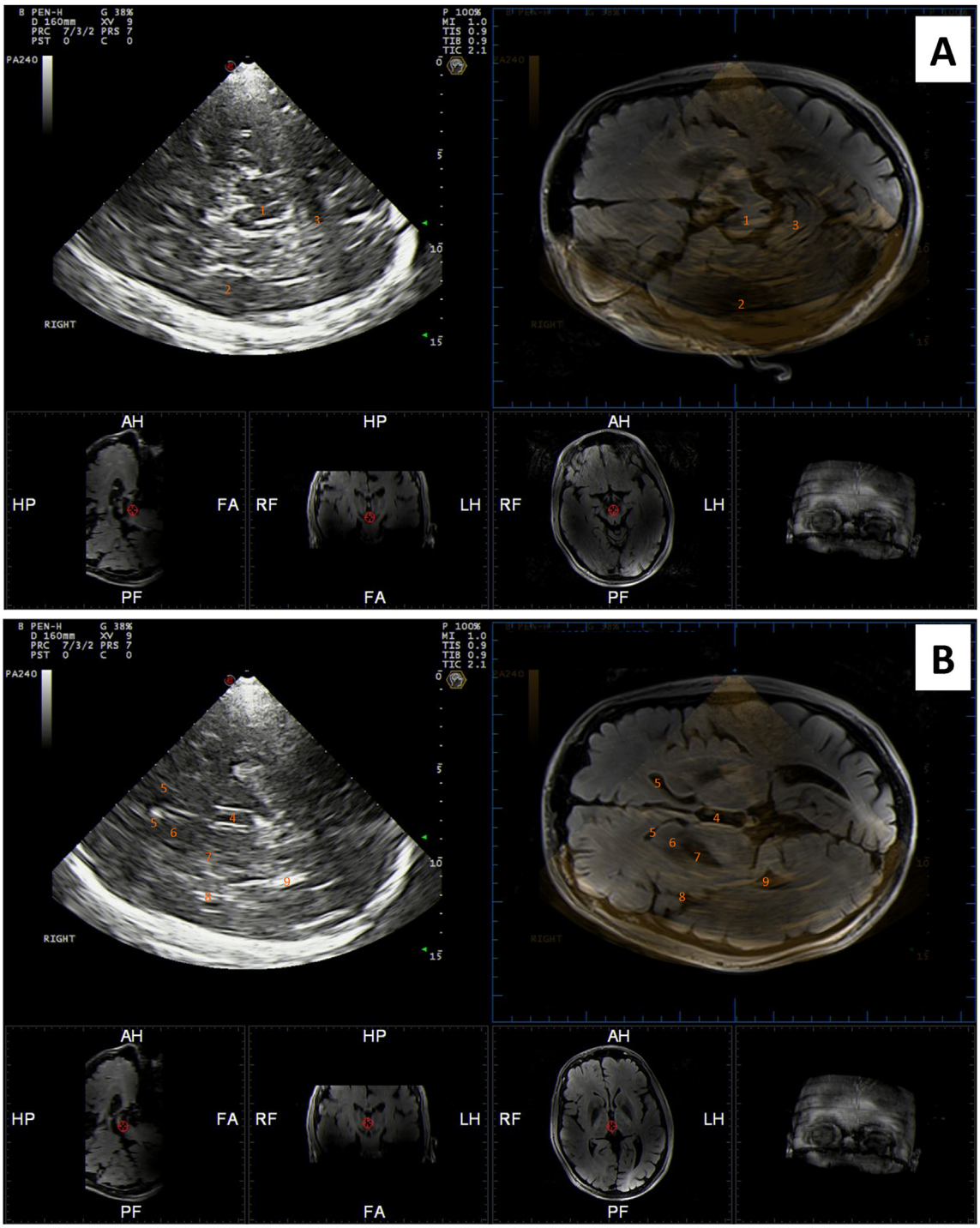

Fig. 1. Typical TCS findings in WD patient with corresponding MRI images using the fusion imaging technique. A) axial mesencephalic plane showing normal echogenicity of a substantita nigra; B) axial thalamic plane showing hyperechogenic lentiform nucleus. Numbers indicate following anatomical structures: 1 – mesencephalon; 2 – temporal lobe; 3 – cerebellum; 4 – third ventricle; 5 – frontal horn of lateral ventricle; 6 – caudate nucleus; 7 – lentiform nucleus; 8 – insula; 9 – occipital horn of lateral ventricle.

in NL, but with weaker significance, was observed also in CN (Table 1).

The cut-off value for SN and LN echogenicity indices were determined 33.7 and 71.9 respectively. SN hyperechogenicity reached sensitivity 93.8%, specificity 90.9% and area under the ROC curve (AUC) of 0.972 (95% confidence interval = 0.928–1.00, $p < 0.0001$), while NL hyperechogenicity reached sensitivity 95.5%, specificity 93.8% and AUC of 0.992 (95% confidence interval = 0.973–1.00, $p < 0.0001$) for a differentiation of WD and EO-PD (Fig. 3).

The visual assessment of SN echogenicity detected significantly larger mean SN echogenic area in EO-PD subjects compared to WD ($p < 0.0001$) and controls ($p < 0.0001$). The sensitivity/specificity of visually assessed hyperechogenicity was 75/82% in SN and 68/81% in NL for WD and EO-PD differentiation.

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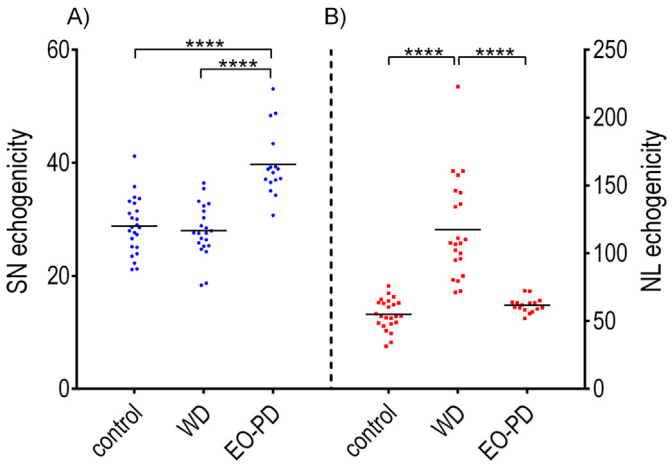

Fig. 2. Echogenicity index in A) substantia nigra (SN) and B) lentiform nucleus (NL) in control subjects, Wilson disease (WD) and early-onset Parkinson's disease (EO-PD) groups. Y-axes are differently adjusted for SN and NL values respectively. Echogenicity index in CN exhibited similar pattern as in NL and is thus not depicted in this graph.

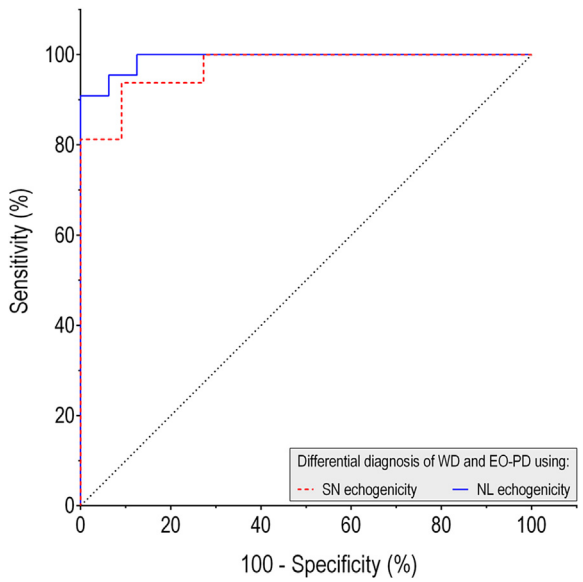

Fig. 3. Receiver operating characteristic (ROC) of substantia nigra (SN, dashed line) and lentiform nucleus (NL, solid line) echogenicity in the differential diagnosis of Wilson's disease and early-onset Parkinson's disease.

3.3. Correlation between TCS, MRI, and clinical parameters

TCS findings of two *de novo* WD patients did not differ from patients on chronic treatment (NL echogenicity index 134.2 and 107.5 vs. 117.1 ± 38.6, p = 0.70). Furthermore, we did not find correlation between the NL echogenicity index and age (r = −0.36, p = 0.10), disease duration (r = −0.32, p = 0.14), treatment duration (r = −0.21, p = 0.36) or UWDRS score (r = 0.36, p = 0.10) in the WD group. In EO-PD group, we did not observe correlation between SN echogenicity index and age (r = 0.06, p = 0.84), disease duration (r = −0.14, p = 0.61) and UPDRS-III score (r = 0.02, p = 0.93).

The composite MRI severity score was higher in the WD compared to the EO-PD group (p < 0.01) (Table 1). MRI signal in the NL in T2w images was normal in six, hypointense in 12, while T2 hyperintense signal in the putamen was present in 13 WD patients. Out of the group of six WD patients with normal MR signal in NL, none had NL echogenicity index within the normal range. NL echogenicity index was higher in WD patients presenting compared to those not presenting with T2 hyperintense signal in the putamen (140.4 ± 13.6 vs 106.9 ± 8.2, p = 0.03) while no difference in NL echogenicity index was observed between patients presenting and not presenting T2 hypointense signal in NL (113.2 ± 9.5 vs 124.5 ± 12.1, p = 0.70). T2 hypointense signal in the SN and/or T2 hyperintense signal in the mesencephalic white matter was present in 13 WD patients. In the EO-PD group, NL was rated as hypointense in four patients while hypointense SN was not found in any patient.

4. Discussion

The results of our study confirmed that the NL and SN echogenicity indices examined by digital image analysis of TCS-MRI fusion images are sensitive and specific markers of WD and EO-PD. The hyperechogenicity of SN in the EO-PD group was detected in 93.8% cases, in accordance with previous TCS studies. In the control group slightly increased NL and SN echogenicities were found in about one tenth of subjects without any clinical correlate. This proportion of healthy subjects with SN hyperechogenicity is consistent across studies and their potential risk of developing parkinsonism is discussed [6].

The NL echogenicity index was abnormal in 95.5% of WD patients. Increased echogenicity index of CN in WD patients was less constant than NL hyperechogenicity. Sensitivity and specificity of NL hyperechogenicity were comparable to routinely used screening methods, i.e. serum ceruloplasmin, urinary copper and K-F ring supporting its potential value as a screening examination of WD. Notably, K-F ring was not detectable in 32% of our WD cases in whom diagnosis was confirmed by genetic examination and/or increased liver copper concentration. This finding, which is in line with several recent studies showing that significant proportion of neurological WD patients may lack the K-F ring [3,25,26], advocates the utility of other screening investigations.

SN echogenicity in WD patients was similar as in control subjects, yielding approximately 10% of subjects with hyperechogenicity. This is in contrast to previous studies showing >50% prevalence of SN hyperechogenicity in WD [12,13].

Our finding of normal SN echogenicity in WD patients is surprising since the majority of the WD patients were affected by parkinsonism. Previous studies suggested a link between parkinsonism in WD and variable combination of pre- and postsynaptic damage of the nigrostriatal pathway [27]. Moreover, 13 WD patients had T2 hypointensity in SN and/or T2 hyperintensity in the mesencephalic white matter tracts. These results suggest that, in the case of SN, its microstructural damage depicted on MRI may not invariably affect its echogenicity.

With respect to the echogenicity changes of NL and CN, our results are comparable to two previously published studies [12,13]. The first of the studies found NL hyperechogenicity in 100% of neurological WD patients and a significant correlation between NL hyperechogenicity and clinical severity [12]. The second one found NL hyperechogenicity in 81.6% of neurological WD patients and detected a correlation between SN hyperechogenicity and clinical severity [13].

In contrast to these studies, we did not find any correlation between TCS findings, clinical severity and duration of disease or treatment. Taking these contradictory results into consideration, TCS probably cannot be used as a reliable marker for longitudinal follow-up of chelating therapy or disability prognosis.

There are several factors, which may contribute to disparate SN echogenicity results. For more accurate anatomical delineation of examined structures we used real-time MRI fusion imaging

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technology. Although not yet validated in neurological applications, fusion imaging may allow for better identification of brain structures, particularly in oblique imaging planes. Thus, echogenicity indices might be more accurate when using this technique. In addition, we also applied newly developed software for digital analysis of brain structures echogenicity that automatically accounts for the quality of the bone window, different depth, and shades of grey. Its usage eliminates the incorrect manual evaluation of echogenicity and increases sensitivity and comparability [18,23]. Accordingly, sensitivity and specificity for WD and EO-PD differentiation were lower in our cohort when visual analysis of echogenicity was employed.

Although TCS is an easy and cheap examination, it has several limitations. Only one patient (2.6%) from the studied group had to be excluded due to insonability. However, in large population studies insufficient temporal bone window, particularly for NL assessment, is found in more than 10% of subjects [9]. In addition, MRI-TCS fusion with subsequent digital image analysis with duration of 20–30 min is more time consuming compared to standard visual analysis, which may limit its practicability. Further studies should investigate whether similar results can be obtained without MRI-TCS fusion when digital image analysis algorithm would be implemented in the software of routine ultrasound devices. This could decrease the examination duration to 10 min.

Interpretation of TCS findings is hampered by the lack of understanding of neuropathological underpinnings of tissue hyperechogenicity. The widespread hypothesis suggests its connection with the accumulation of iron and copper. Presumed dependence of tissue echogenicity on iron concentration [28] implies that TCS hyperechogenicity should correlate with MRI T2/T2* hypointensity, predominantly caused by paramagnetic iron species [29]. Contrary to this assumption, the degree of NL hyperechogenicity was related to putaminal T2 hyperintensity rather than to T2 hypointensity in NL suggesting that echogenicity is not only determined by iron concentration. In addition, we found NL hyperechogenicity in all WD patients with normal MR signal in this area confirming previous findings that TCS can bring complementary information to MRI [14,15,30]. The echogenicity changes could be also influenced by copper as documented in a post-mortem study on WD brains showing a correlation between echogenicity and copper concentration in NL [11]. Since MRI cannot reliably depict copper [29], we cannot support nor foreclose its effect on tissue echogenicity in our patients. To sum up, the underpinnings of echogenicity contrast in TCS examination are complex and depend not only on metal accumulation but also on associated pathological microstructural tissue changes.

Despite the limitation of the small number of patients, the sensitivity and specificity of the results are obvious and our findings are in good accordance with previously published studies in PD and WD patients. However, in this proof of concept study we did not examine only *de novo* patients, not all the patients with WD have had isolated parkinsonism, and disease duration was different between groups. Therefore, additional studies with untreated *de novo* patients are needed to confirm the utility of TCS in the differential diagnosis of WD and EO-PD.

With regard to the results above and previously performed studies, we can conclude that TCS findings in WD and EO-PD are potentially helpful as screening biomarkers in early-onset parkinsonism. For the future, it could be efficient to include TCS abnormality into supportive neuroimaging criteria for WD.

Conflict of interest

None of the authors report a conflict of interest with respect to financial or personal relationships with organizations that may have an influence on this work.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2016.04.031.

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