HVM BIOFLUX
Human & Veterinary Medicine
International Journal of the Bioflux Society

Diagnostic particularities in Wilson's disease as related to age, sex and clinical presentation

[1]Valentin Militaru, [2]Nicolae Miu, [2]Tudor L. Pop, [2]Lucia Burac, [2]Ana Ştefănescu, [1]Sorin Crişan, [1]Ştefan Vesa, [1]Liliana Rădulescu, and [1]Caius R. Duncea

[1] 5th Medical Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Cluj, România; [2] 2nd Pediatric Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Cluj, România. Corresponding author: V. Militaru, Valentin.Militaru@umfcluj.ro

Abstract. Objective: Wilson's disease (WD) is a genetic, autosomal recessive disorder, which affects the liver, brain and cornea. The condition is rare and it has various presentations, hence its diagnosis is difficult. We aimed to study different clinical presentations, their relation with age and sex and the influence of several factors on the diagnostic score (Leipzig score). Material and Methods: We analyzed retrospectively the medical documents of 24 WD patients examined in 2nd Pediatric Clinic and 5th Medical Clinic of Cluj-Napoca, and we collected data concerning the diagnosis. The patients were classified phenotypically (Leipzig classification) and we calculated the Leipzig score. We studied the relation between diagnostic score, clinical phenotype, age at diagnosis and sex. Results: Our group consisted of 7 adults and 17 pediatric patients, F/M = 1/1.4. They were distributed, according to the phenotype, as follows: acute hepatic (H1) – 3 (pediatric, all deceased); chronic hepatic (H2) – 12 (2 adults, 10 pediatric); neurological and hepatic (N1) – 6 (3 adults, 3 pediatric), neurological (N2) – 3 (2 adults, 1 pediatric). The hepatic involvement was inversely correlated with age (p=0.02), which in turn was directly correlated with neurological manifestations (p=0.05). There were no significant differences concerning either distribution on phenotypes in relation with sex and age or between Leipzig score and age, sex, onset modality, presence and severity of hepatic involvement. Conclusion: The presence of hepatic manifestations at the moment of diagnosis of WD decreases with age, while neurological one increases. The distribution on phenotypes was not influenced by age or sex. There is no relation between diagnostic score and studied demographical and clinical factors.
Key Words: Wilson's disease, Wilson's disease clinical phenotypes, Leipzig score.

Rezumat. Obiectiv: Boala Wilson (BW) este o afecţiune genetică, autosomal recesivă, care afectează ficatul, creierul şi corneea. Fiind foarte rară şi variabilă ca prezentare clinică, diagnosticul ei este dificil. Ne-am propus să studiem diferitele modalităţi de prezentare clinică, relaţia lor cu vârsta şi sexul pacienţilor şi influenţa mai multor factori asupra scorului diagnostic (scorul Leipzig). Material si metodă: Am analizat retrospectiv documentele medicale ale 24 pacienţi cu BW consultaţi în Clinicile Pediatrie II şi, respectiv, Medicală V, din Cluj-Napoca, culegând o serie de date privind diagnosticul. Pacienţii au fost clasificaţi fenotipic (clasificarea Leipzig) şi am calculat scorul Leipzig. Am studiat relaţia între scorul diagnostic, fenotipurile clinice, vârsta la diagnostic şi sexul pacienţilor. Rezultate: Lotul a cuprins 7 pacienţi adulţi şi 17 pediatrici, F/M = 10/14. Distribuţia pacienţilor pe fenotipuri a fost următoarea: hepatică acută (H1) – 3 (pediatrici, decedaţi toţi); hepatică cronică (H2) – 12 (2 adulţi, 10 pediatrici); neurologică şi hepatică (N1) – 6 (3 adulţi, 3 pediatrici), neurologică (N2) – 3 (2 adulţi, 1 pediatric). Prezenţa afectării hepatice s-a corelat invers cu vârsta (p=0,02), pe când manifestările neurologice au fost în relaţie directă cu vârsta (p=0,05). Nu au existat diferenţe semnificative privind distribuţia pe fenotipuri în raport cu vârsta sau sexul pacienţilor, nici între scorul Leipzig şi vârstă, sex, modalitatea de debut, prezenţa şi severitatea afectării hepatice. Concluzii: Prezenţa manifestărilor hepatice la momentul diagnosticului BW descreşte cu vârsta, pe când cea a manifestărilor neurologice creşte. Distribuţia pe fenotipuri nu este influenţată de vârstă sau sex. Nu exista o relaţie între scorul diagnostic şi factorii demografici şi clinici studiaţi.
Cuvinte cheie: Boala Wilson, fenotipurile clinice de boala Wilson, scorul Leipzig.

Introduction. Wilson's disease (WD) is a genetic, autosomal recessive condition, which consists in copper overload of certain tissues, mainly hepatic, cerebral and corneal endothelium (Roberts & Schilsky 2008; Tanner 1999). It is caused by mutations in the

ATP7B gene, located on the 13th chromosome, resulting in an impaired biliary elimination of the excess of copper (de Bie et al 2007; Bugbee & Cox 2011). As a consequence, copper is stored in hepatocytes, thus damaging the liver. Its release into blood flow leads to secondary deposition in basal nuclei of the brain, cornea and other tissues (renal tubules). There are 3 main types of clinical manifestations: neurological and/or psychiatric, hepatic and ocular. These manifestations may be present in different combinations, leading to a great clinical variability and to numerous confusions and errors (Prashanth et al 2004). Because of clinical variability and rarity of the disease (30 patients/1 million persons), its diagnosis is difficult and often delayed (Walshe & Yealland 1992). The diagnosis delay, which can be sometimes unbelievably long (Walshe & Yealland 1992, Merle et al 2007, Militaru et al 2011), can have severe consequences (Mak et al 2006). Following a meeting of experts in Wilson's and Menkes' diseases in Leipzig, Germany in 2001, a scoring system was proposed in order to ease and accelerate the diagnosis of WD. Based on clinical, imaging, pathological and laboratory parameters, the score indicates the probability that a patient has WD: higher the score, higher the likeliness that diagnosis of WD is correct. The same authors defined several clinical phenotypes according to the major organ involvement: acute hepatic (H1), chronic hepatic (H2), neurological associated with liver disease (N1), neurological not associated with liver disease (N2), neurological not investigated for the liver (Nx) and others (O). The authors stated in their paper that "Before accepting this scoring system as gold standard for diagnosis of Wilson disease it has to be evaluated prospectively" (Ferenci et al 2003). We did not find any paper in medical literature, so far, that evaluated if there was a relation between the patients' sex or age at diagnosis and clinical presentation (clinical phenotype) or if Leipzig score was influenced by variables not used for its calculation: demographical (sex, age) or clinical (presence and severity of hepatic involvement, onset modality). We stated that the numeric value of the score could be regarded as an indicator of how easy the diagnosis can be set in a particular patient. We hypothesized that Leipzig score (i.e. the easiness of reaching the correct diagnosis) is not influenced by the above-mentioned parameters and that no sub-group of patients (sub-division according to age or sex) is favored in this regard.

Objective. We aimed to study the relative frequency of clinical phenotypes and their relation with sex and age. We also aimed to assess if some factors not comprised in the formula of the score (age, sex, presence and severity of hepatic involvement, onset modality) could influence the numeric value of Leipzig score, rendering diagnosis easier in certain sub-groups.

Material and Methods. Our group of study was made of 24 patients suffering from WD, consulted or admitted in 2nd Pediatric Clinic and 5th Medical Clinic in Cluj-Napoca, Romania, from January 1st, 2003 until October 31st, 2010. The neurological examination was realized in Neurological Clinic (adults, i.e. over 18 years of age), respectively in Children Neurological Clinic (pediatric patients, i.e. under 18 years of age). The patients underwent slit-lamp ophthalmologic examination, looking for Kayser-Fleischer (KF) ring, either in Ophthalmologic Clinic (affiliated to "Iuliu Haţieganu" University of Medicine and Pharmacy), or in an outpatient clinic in Cluj-Napoca. The biochemical and hematological analyses, the abdominal ultrasonography and the digestive endoscopy, if needed for diagnosis, were performed in laboratories of the above-mentioned clinics. The patients were tested for mutations known to produce WD by Prof. Ferenci and his co-workers of Innere Medizin III – Vienna Medical University, Austria.

The patients were assigned to a clinical phenotype of WD according to the classification of Leipzig (Ferenci et al 2003) adapted to the needs of our study. Table 1 shows the definition we used for clinical phenotypes.

The Leipzig score was calculated, using the definition of same authors (Ferenci et al 2003).

We tested the relation between clinical phenotypes and patients' sex and age. Age was regarded both as a numeric variable and as a category (pediatric/adult), being

tested in both ways. The relation between patients' age at diagnosis and presence of hepatic and of neurological manifestations, respectively, was also assessed.

The numeric value of WD diagnosis score (Leipzig score) was tested for its correlations with patients' age, sex, presence and severity of hepatic involvement and onset modality. Out of the 24 patients, only one had the liver copper concentration dosed through laparoscopic liver biopsy, which could have artificially increased his diagnostic score and biased the results (for all other patients we used zero value for liver copper when calculating the Leipzig score according to its definition, but it was a statistically false zero, coming from absence of data). This is why we performed all the statistical tests concerning the Leipzig score twice: first with the true diagnostic score of the above-mentioned patient, then with a score ignoring copper dosage in the liver (lower by 2 points).

Table 1

Definition of the clinical phenotypes of Wilson's disease – adapted after the classification of Leipzig conference (Ferenci et al 2003)

Phenotype name		*Phenotype code*	*Short presentation*
Hepatic*	Acute	H1	Icterus in a previously (apparently) healthy patient and/or Coombs negative haemolytic anemia.
	Chronic	H2	Chronic liver disease (with or without symptoms).
Neurological	Associated with symptomatic liver disease	N1	Usually cirrhosis is present at the time of the diagnosis.
	Not associated with symptomatic liver disease	N2	Absence of severe liver disease proven by an imagery and/or histology**.
	Not investigated for liver disease	NX	No investigation conducted for liver disease.
Other		O	

* Assignment of o patient to H1 or H2 required exclusion of neurological symptoms through an examination by a neurologist.
** The authors of the Leipzig Classification state that a liver biopsy is needed in clinical protocols to prove the absence of severe liver disease. The reasons for which we did not perform a systematic biopsy are explained in the discussions section.

For the statistical analysis we used SPSS 17 software. Chi-square, Kruskal-Wallis and Mann-Whitney test were used accordingly. For the definition of an age threshold for the hepatic involvement we used ROC curve analysis.

Table 2

Distribution of the 24 WD patients according to sex and clinical phenotype

		Phenotype				*Total*
		H1	*H2*	*N1*	*N2*	
Sex	M	1	8	3	2	14
	F	2	4	3	1	10
Total		3	12	6	3	24

The initials of the clinical phenotypes are explained in Table 1.

Results. The group we studied consisted of 7 adult patients and 17 pediatric patients, with an average age of 20.33 years and a median age of 14.5 years. Sex ratio was F/M = 1/1.4. There were no patients belonging to NX or O phenotypes. Table 2 shows the distribution of patients on the remaining four clinical phenotypes, separately for males and females. The Chi-square test revealed no significant difference (p=0.71) among clinical phenotypes concerning sex of the patients.

We used Kruskal-Wallis test, as the age of patients at diagnosis did not follow a normal distribution, to study if there was any relation between age and clinical phenotypes. First, we considered age as a numeric variable (expressed in years) and we did not find a statistical signification (p=0.31). Neither was there any significant difference if the age was regarded as a category variable: adult/pediatric (using as cut-off level 18 years, which is the legal age of adulthood in Romania); in this case p=0.14 and the distribution of the patients is depicted in Table 3.

Table 3

Distribution of the 24 WD patients according to age at diagnosis and clinical phenotype

		Phenotype				*Total*
		H1	*H2*	*N1*	*N2*	
Age	Pediatric	3	10	3	1	17
	Adult	0	2	3	2	7
Total		3	12	6	3	24

The initials of the clinical phenotypes are explained in the Table 1.

As the clinical phenotypes are defined by a combination of organs' involvement, we studied if there was any relation between age and presence of the suffering of one of two major organs affected by the WD: liver and brain. We used Mann-Whitney test to analyze the relation between age at diagnosis (expressed in years) and the presence of hepatic involvement. There was an inverse correlation, statistically significant (p=0.02), of a medium power (correlation coefficient: r=-0.46). The correlation is also clinically significant, but these aspects will be detailed in the discussions section. For the neurological affectation, the correlation with age (years) is direct and statistically significant, with a medium power (p=0.05, r=0.41), and we considered it also clinically significant.

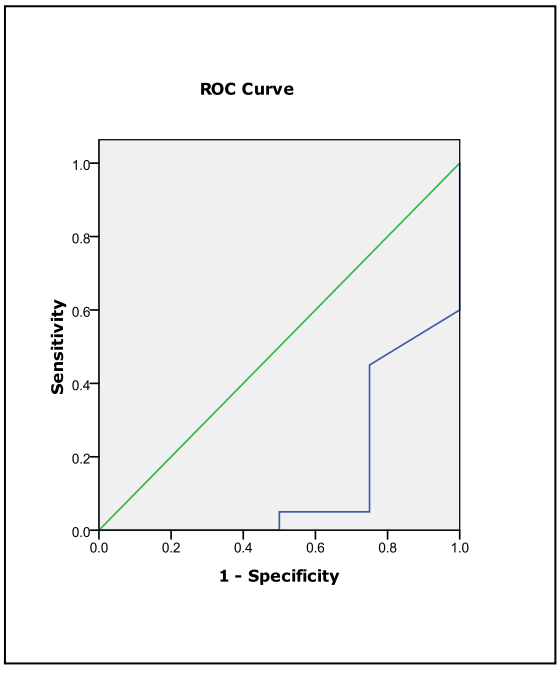

Figure 1. ROC curve for age vs. hepatic involvement.

We tested age as category (adult/pediatric), using the age of 18 years as a cut-off, against presence of hepatic involvement. There was no statistical signification (p=0.108) and we hypothesized that a different age cut-off (without calling the patients "pediatric" or "adults") could offer a statistically significant difference concerning the presence of hepatic involvement. In order to determine this cut-off value we used ROC curve analysis for age (as a numeric variable, expressed in years) as a "diagnostic test" for hepatic involvement and we determined the area under the curve (AUROC). The best sensitivity and specificity were found for the age of 36.5 years (Se=0.05, Sp=0.25); under this age there is significantly more hepatic affectation than over this age. The ROC curve is shown in Figure 1.

The diagnostic score (Leipzig score) was tested for correlations with several parameters not used for its calculation, which could eventually influence it. As we already mentioned, we performed each test twice, using two values of the score of one of the patients: with and without the 2 points he received for the liver copper concentration (he was the only patient of our group to have the liver copper concentration determined). None of the tested factors (demographical and clinical) significantly correlated with the Leipzig score. Table 4 presents the tested variables, the statistical test used and the two results of the test.

Table 4

Results of the assessment of the relation between demographical and clinical factors and the Leipzig score

Tested variable	*Statistical test used*	p_1*	p_2**
Sex	Mann-Whitney	0.86	0.766
Age at diagnosis (years)	Spearman's	0.963	0.988
Age at diagnosis (adult/pediatric)	Mann-Whitney	0.772	0.675
Presence of hepatic involvement	Mann-Whitney	0.055	0.052
Severity of hepatic involvement (cirrhosis/chronic hepatitis)	Mann-Whitney	0.754	0.844
Onset modality (hepatic/neurologic/screening)	Kruskal-Wallis	0.383	0.391

* The statistic for this column was done with true Leipzig score of the patient having liver copper concentration determined.
** The statistic for this column was performed ignoring the determination of liver copper concentration.

Discussion and Conclusions. The clinical phenotypes defined at the Leipzig meeting try to organize the possibilities of WD's symptoms to combine, in order to make it easier for the clinician to recognize WD. The diagnosis score has the same purpose - to facilitate the diagnosis, mainly in atypical cases (Ferenci et al 2003). As the authors state in the paper, the score is subjected to validation by studies (vide supra), which is what we intended to do. In our study we tried to find out if there were any factors "external" to the definitions of clinical phenotypes and Leipzig score which could influence and/or bias them.

When classifying a patient as belonging to the N2 (chronic neurological) phenotype, we did not perform systematically a liver biopsy in order to prove the absence of severe liver disease, though the authors of definition state that it is recommended for clinical studies (Ferenci et al 2003). We considered obvious the absence of severe liver disease from the combination of clinical, laboratory and ultrasound data. Another reason not to perform the biopsy was that our patients presented for neurological conditions and they were rather reluctant to investigate invasively a potential liver disease, without any clinical biological or imaging argument. Exposing them to the potential risk of a liver biopsy, even if the risk is not high, for scientific purposes seemed unethical to us from the medical point of view.

The distribution on clinical phenotypes was not influenced by patients' age or sex, as shown in the results section. We considered this not only a proof of independence of

clinical phenotypes from these two demographical parameters, but also a proof of homogeneity of the group.

The presence of hepatic and of neurological affectation, respectively, proved to correlate with age in our group. It is obvious and pathogenically plausible that the hepatic involvement appears at a younger age, as copper accumulates in the liver. Later, with the release of free copper in blood flow, neurological manifestations become clinically apparent. Our contribution was to set 36.5 years as a cut-off for the age to delineate the predominance of hepatic manifestations. This comes to give a statistical basis to the clinical observation that hepatic impairment is predominant in young WD patients, while neurological one is predominant in older patients. Merle et al (2007) found out, on a large German cohort, that the average age of patients presenting with hepatic signs is about 5 years lower than that of patients with neurological presentation.

The independence of the diagnosis score (Leipzig score) from age, sex, onset modality, presence of hepatic involvement and severity of the hepatic disease (cirrhosis/chronic hepatitis) shows that the difficulty of diagnosis is equivalent in these sub-groups. We were able to show in a previous paper, in agreement with the results of other authors, that the presence of hepatic involvement shortens the delay of the diagnosis (Merle et al 2007; Militaru et al 2011). This seems to be independent from the numeric value of Leipzig score, as the value of the score did not correlate either with the diagnosis delay or with presence of the hepatic involvement. It would be interesting to study, in order to assess the practical importance of Leipzig score, if its use in setting the diagnosis shortens the delay. We could not do this in our group because Leipzig score was always used and we had no control group.

In conclusion, the presence of hepatic manifestations at the diagnosis of WD decreases with age, while the neurological ones increase. The distribution on clinical (Leipzig) phenotypes is not influenced by age or sex. There is no relation between the diagnostic score and other factors such as age, sex, onset modality and hepatic involvement.

Acknowledgements. The authors wish to express their gratitude towards Prof. Peter Ferenci and his co-workers of Innere Medizin III – Medical University of Vienna for their generous help with the genetic testing.

References

Bugbee D., Cox D. W. (curators), Wilson Disease Mutation Database – University of Alberta – Canada (http://www.wilsondisease.med.ualberta.ca/database.asp) (accessed 6th August 2011).

de Bie P., Muller P., Wijmenga C., Klomp L. W. J., 2007 Molecular pathogenesis of Wilson and Menkes disease: correlation of mutations with molecular defects and disease phenotypes. J Med Genet **44**:673-688.

Ferenci P., Caca K., Loudianos G., Mielli-Vergani G., Tanner S., Sternlieb I., Schilsky M., Cox D., Berr F., 2003 Diagnosis and phenotypic classification of Wilson disease. Liver Int **23**:139-142.

Mak C. M., Tam S., Fan S. T., Liu C. L., Lam C. W., 2006 Wilson's disease: a patient undiagnosed for 18 years. Hong Kong Med J **12**(2):154-158.

Merle U., Schaefer M., Ferenci P., Stremmel W., 2007 Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. Gut **6**:115-120.

Militaru V., Miu N., Pop T. L., Burac L., Stefanescu A, Crisan S., Duncea C. R., Vesa S., 2011 The delay of the diagnosis in Wilson's disease. Clujul Medical **84**(2):274-277.

Roberts E. A., Schilsky M. L., 2008 Diagnosis and Treatment of Wilson Disease: An Update. Hepatology **47**(6):2089-2111.

Prashanth K. L., Taly A. B., Sinha S., Arunodaya G. R., Swamy H. S., 2004 Wilson's disease: diagnostic errors and clinical implications. J Neurol Neurosurg Psychiatry **75**:907-909.

Tanner S., 1999 Disorders of Copper Metabolism. In: Diseases of the Liver and Biliary System in Children. Kelly D. (ed), pp. 167-185, Blackwell Science Ltd., Oxford.

Walshe J. M., Yealland M., 1992 Wilson's disease: the problem of delayed diagnosis. J Neurol Neurosurg Psychiatry **55**:692-696.

Received: 22 June 2011. Accepted: 05 July 2011. Published online: 22 August 2011.
Authors:
Valentin Militaru, 5th Medical Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, 11 Tăbăcarilor Street, 400139 Cluj-Napoca, România, EU, email: Valentin.Militaru@umfcluj.ro.
Nicolae Miu, 2nd Pediatric Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, 5 Crişan Street, 400177, Cluj-Napoca, România, EU.
Tudor L. Pop, 2nd Pediatric Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, 5 Crişan Street, 400177, Cluj-Napoca, România, EU; tudor.pop@umfcluj.ro.
Lucia Burac, 2nd Pediatric Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, 5 Crişan Street, 400177, Cluj-Napoca, România, EU; lucabu@hotmail.com.
Ana Ştefănescu – "Iuliu Haţieganu" University of Medicine and Pharmacy – 2nd Pediatric Clinic, 5 Crişan Street, 400177 Cluj-Napoca, România EU; anilucka@yahoo.co.uk.
Sorin Crişan, 5th Medical Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, 11 Tăbăcarilor Street, 400139, Cluj-Napoca, România, EU, email: crisan.sorin@gmail.com.
Ştefan Vesa – 5th Medical Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, 11 Tăbăcarilor Street, 400139, Cluj-Napoca, România, EU, email: stefanvesa@gmail.com
Liliana Rădulescu – 5th Medical Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy, 11 Tăbăcarilor Street, 400139, Cluj-Napoca, România, EU, lili_m_radulescu@yahoo.com.
Caius R. Duncea – 5th Medical Clinic, "Iuliu Haţieganu" University of Medicine and Pharmacy 11 Tăbăcarilor Street, 400139, Cluj-Napoca, România, EU, email: crduncea@yahoo.com.
How to cite this article:
Militaru V., Miu N., Pop T. L., Burac L., Ştefănescu A., Crişan S., Vesa Ş., Rădulescu L., Duncea C. R., 2011 Diagnostic particularities in Wilson's disease as related to age, sex and clinical presentation. HVM Bioflux **3**(2):119-125.