Revista Española de Enfermedades Digestivas
The Spanish Journal of Gastroenterology

Title:
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DOI: 10.17235/reed.2023.9633/2023
Link: PubMed (Epub ahead of print)

Please cite this article as:
Mariño Zoe. Recent advances in the diagnosis and management of Wilson's disease. Rev Esp Enferm Dig 2023. doi: 10.17235/reed.2023.9633/2023.

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Recent advances in the diagnosis and management of Wilson's disease

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In recent years news have not been uncommon from the field of Wilson's disease (WD), a condition deeply studied in books but largely absent from day-to-day practice for most of our colleagues. It is not only that this rare disease is now considered *not-so-rare* according to multiple genome-based studies (1,2) but also that recent advances have been made in terms of biomarkers and therapeutic proposals for upcoming years that should be known and will probably benefit our patients soon (3).

From an epidemiological point of view, a recent paper by the group at Gran Canaria (1) (a recognized hot spot for WD in Spain) estimated a much higher genetic prevalence of WD, based on modelling assays using global genetic databases, among the general population in mainland Spain. The authors estimated a genetic prevalence of 1/6,278 individuals, representing a much higher presence of this disease among the general population than initially thought. However, it remains to be elucidated whether all these potential pathogenic mutations will eventually result in clinically apparent disease or in silent phenotypes that will remain unnoticed by physicians. Indeed, we do not have such big numbers of WD patients in our health system. Even if we consider the most restrictive prevalence estimate scenario (prevalence: 1/30,000 inhabitants) (4), more than 1,500 individuals in Spain would be affected by the disease, and these numbers are far removed from the real-world figures reported in our country (5). The recent development of a National Registry of Wilson's Disease in Spain (https://aeeh.es/registro/registro-de-enfermedad-de-wilson/), supported by the Spanish Association for the Study of the Liver (AEEH), was intended to render WD more visible, and to centralize all WD cases in one place. Understanding these clinically overt WD patients will presumably be helpful to define in which circumstances copper overload may become a problem and prompt patients to seek medical help. After one

year, almost 400 patients with WD have been brought together with the effort of many investigators around Spain (Mariño Z, et al., AEEH 2023, unpublished). This is still far from the expected number of WD-affected individuals but represents an excellent starting point for engaging clinicians, networking, and research proposals. Intriguingly, up to 25 % of patients included in the National Registry so far have not been genetically confirmed. Indeed, the absence of a well-performed genetic study in a proband will hamper family screening around this individual, representing a potential missed opportunity for diagnosing other "silent" cases.

There might be different confluent reasons for the gap between genetic estimations and clinical diagnosis. First, the potential underdiagnosis of an uncertain proportion of WD patients, with probably subclinical or mild disease, especially if such patients have other coexisting hepatic comorbidities able to explain minimal laboratory or clinical disturbances (such as metabolic or alcohol-associated liver disease). Second, the high decentralization of WD patients in Spain, with most physicians being responsible for only 2-5 patients, potentially reducing the capability of suspecting the disease at early stages. And last, the absence of current accurate biomarkers for WD diagnosis hampering the detection of new cases, especially among those with atypical symptoms or in those in whom clinical suspicion remains low.

In this regard, advances in the development of novel biomarkers are desirable and highly expected. This is true mainly for diagnostic purposes but their absence also hampers follow-up of WD patients, in whom we should ascertain a good metabolic copper status over time. None of the current biomarkers is good enough if used in isolation (6). Low ceruloplasmin should not be used as single diagnostic parameter in clinical practice since many clinical conditions other than WD are associated with low levels of this protein (renal protein losses, malnutrition, impaired liver function, or carriers of one single mutation in *ATP7B)*. As an example, the classic cut-off value for low ceruloplasmin (< 0.20 g/L), included in the Leipzig score for WD diagnosis (7,8), was associated with a very low positive predictive value (5.9 %) when used in patients admitted to a liver unit (9). Furthermore, we should remember that normal levels of ceruloplasmin may also be seen in some WD patients, especially those with isolated hepatic phenotypes. Therefore, WD should only be excluded or confirmed by a

combination of 2 different biological parameters, with ceruloplasmin and 24-hour urinary copper being most readily available, to reduce the possibility of misdiagnosis.

Over the last few years new biomarkers have been proposed for WD. Measurement of peripheral ATP7B peptide levels in the serum using mass spectrometry with immunoaffinity enrichment was proposed as a novel approach for WD patients despite their heterogeneous genotypes (10). These peptides would represent surrogate markers of *ATP7B* expression as most pathogenic mutations in WD result in depleted protein levels. In that work ATP7B peptides were shown to be severely deficient in more than 90 % of the WD patients assessed (even in those with normal/intermediate levels of ceruloplasmin), and were associated with an AUC value of 0.98 for WD identification. This highly specialized proteomic technology could be extremely helpful if validated, although costs will probably limit its use in clinical practice.

Other new biomarkers are based on the copper fraction not bound to ceruloplasmin (NCC = non-ceruloplasmin-bound copper, considered toxic or bioavailable), which is typically high among patients with WD. Indirect estimations by mathematical extraction of total serum copper levels and ceruloplasmin have shown significant methodological limitations, and yield uninterpretable results in up to 20 % of patients (3). In this context, direct NCC measurement has been proposed. The first method is based on exchangeable copper (ExchCo) measurement (11), representing the copper that is easily mobilized from ceruloplasmin and other transporting proteins in the presence of EDTA. Relative ExchCo (REC) has been shown to be useful in different clinical scenarios of WD with a diagnostic cut-off at ≥ 18.5 % (12). REC was able to discriminate patients from carriers and normal individuals, and WD from other chronic liver diseases (13,14). In Spain, several tertiary hospitals used this EDTA methodology within the last year, offering a valuable and complementary tool for unclear cases. During the follow-up course of the disease ExchCo has also been proposed as a good biomarker, as it was shown to decrease after starting therapy (15) and was significantly reduced among compliant patients (16). However, ideal target ranges for exchangeable copper during follow-up have not been set yet, and caution is mandatory. Another strategy for direct NCC measurement was proposed, called the copper-speciation assay, which is based on the determination of the different copper

species in the human body using anion-exchange high-performance liquid chromatography combined with inductively coupled plasma mass spectrometry (17). This technology has also been shown to be very accurate for NCC evaluation, and suggests that the EDTA method may underestimate NCC levels. This methodology has received the support of the FDA and has been included in clinical trials. However, validation in real-life scenarios is needed, and potential costs might reduce applicability.

The last biomarker that should be mentioned is radiolabeled copper [^{64}Cu] as used for positron emission tomography (PET). Reduced incorporation of ^{64}Cu into ceruloplasmin identified WD patients in the past (18) by showing radioactivity accumulated in the liver and absent from the blood, and might still be considered an accurate diagnostic method today (19). This classic methodology has become novel again by allowing to directly visualize disturbed biliary excretion of copper in WD patients (20). Current pharmacological therapies for WD promote copper depletion but are unable to restore the physiological pathway of copper in the body (3). In contrast, the novel gene therapy developed for WD is expected to enterally restore copper metabolism as has already been shown in murine models (21). The value of ^{64}Cu-PET studies has thus become attractive again.

Finally, we should also review some of the therapeutical advances for WD in the last few years. On top of the current classical proposals with chelators and zinc salts, a new formulation of bis-choline tetrathiomolybdate (TTM) —an oral stabilized form of the previous ammonium tetrathiomolybdate used for very severe neurological cases and able to rapidly decrease copper levels in the blood— has been developed. The mechanism of action of TTM is still unclear. This copper-protein-binding molecule has been proposed as a once-daily oral therapy for WD patients, and has been shown to significantly reduce NCC levels and to improve disease-related disability in a phase-II trial including 28 patients with WD (22). Recent data coming from the phase-III trial with 214 patients enrolled was presented in the last International Liver Meeting (23) supporting positive results in terms of copper control and UWDRS score reduction at 48 weeks, when compared to the standard of care. Safety was good, with transaminase elevations being the most frequent adverse event (in up to 14.6 % of the

cohort). Future data regarding the long-term extension results of TTM are expected soon as a prelude to TTM approval in real life. Whether the use of TTM will be expanded for all patients or limited to some subgroups still needs to be clarified. In this process, efficacy and safety results will need to be considered together. The favorable dosage of this drug favors compliance and improves quality of life. On the other hand, molybdenum accumulation in tissues because of the TTM formulation and its potential deleterious effects will need to be strictly monitored in patients, considering the effects on the murine WD models treated with TTM (24).

But probably the most novel approach to WD is gene therapy. Liver-directed adeno-associated vector (AAV)-based gene therapy was shown to represent a big step forward in a murine model of WD ((25)) some years ago —treatment with the vector reduced copper concentration in the liver and urine, restored fecal copper excretion, and normalized ceruloplasmin activity and transaminase levels in the circulation. Moreover, this therapeutic effect was shown to be sustained for at least one year, as seen by liver histology preservation at sacrifice. The first-in-human clinical trials of AAV-based gene therapy for WD were developed soon after (NCT04537377 and NCT04884815), and have been actively recruiting patients since 2022; first results are expected within the next months. Hopefully, gene therapy will potentially cure WD patients by restoring copper metabolism, thus preventing patients from lifelong therapy.

In the meantime, WD treatment as based on chelators or zinc represents the rule and still comprises some challenges. Together with the current suboptimal biomarkers for ensuring copper metabolic control, female WD patients in childbearing age will ask their physicians about pregnancy and the safety profiles of their anti-copper drugs. In Spain, up to 48 % of female WD patients claimed they were afraid of getting pregnant mainly for the potential effects these drugs might have on their babies (Romero M, et al, AEEH 2023, unpublished). In the largest multicenter study regarding this issue (26), well-treated disease was associated with normal gestation and a low risk of birth defects. Copper overload as a result of treatment discontinuation or uncontrolled disease is associated with higher rates of complications. On the other hand, chelator doses should be reduced during pregnancy to limit their potential teratogenic effects

(3,7), which have been described at high doses in animal models but are not expected if used within therapeutic ranges.

In conclusion, we are facing exciting advances both in biomarkers and novel therapies that will presumably change Wilson's disease scenario for the next years. Therefore, adequate copper monitoring and genetic confirmation will be desirable in all our patients. Sharing clinical data within the National Registry may also constitute a good collaborative scientific platform for solid networking and a potential contribution of Spain in the field of rare genetic liver diseases.

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