**Research Article**
DILI, Autoimmune, Cholestatic and Genetic Diseases

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# Neurological worsening in Wilson disease – clinical classification and outcome

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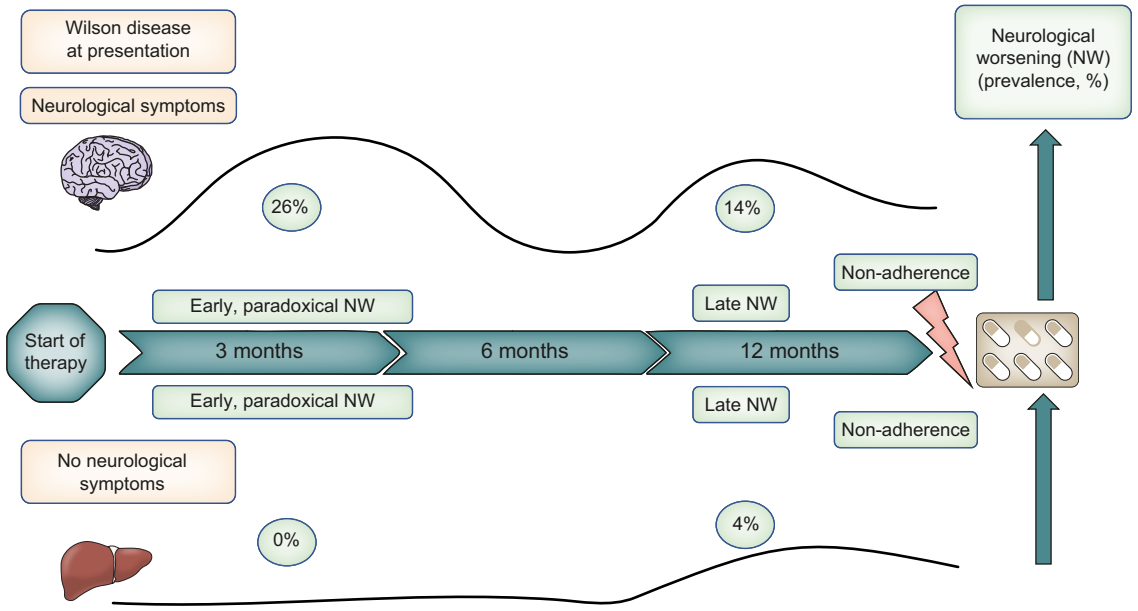
## Graphical abstract




## Highlights

- Neurological worsening in Wilson disease occurs at two peaks.
- Early neurological worsening is observed within 3 months after treatment initiation.
- Late neurological worsening is observed after 12 months of treatment initiation.
- Late neurological worsening is associated with non-adherence.
- Early “paradoxical” neurologic worsening was not observed in patients without preexisting neurologic symptoms.

## Impact and implications

In patients with Wilson disease, defined as an excess accumulation of copper which can damage the liver, brain and other vital organs, neurological worsening can occur despite chelation therapy. The study identifies different patterns of ‘early’ (<3 months) *vs.* ‘late’ (>12 months) neurological worsening in relation to initiation of chelation therapy and establishes possible causes and the potential for reversibility. These data should be useful for counseling patients and for guiding the optimal management of chelation therapy.In patients with Wilson disease, defined as an excess accumulation of copper which can damage the liver, brain and other vital organs, neurological worsening can occur despite chelation therapy. The study identifies different patterns of ‘early’ (<3 months) *vs.* ‘late’ (>12 months) neurological worsening in relation to initiation of chelation therapy and establishes possible causes and the potential for reversibility. These data should be useful for counseling patients and for guiding the optimal management of chelation therapy.

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# Neurological worsening in Wilson disease – clinical classification and outcome

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**Background & Aims:** Prevention of neurological worsening (NW) under therapy is an unmet need in the management of Wilson disease (WD). In this study, we aimed to characterize the occurrence, associated outcomes and potential reversibility of NW in WD.

**Methods:** From a total cohort of 457 patients with WD, 128 patients with WD and neurological features at any time point (all Caucasian, 63 females, median age at diagnosis 22 years) were identified by chart review at University Hospital Heidelberg and grouped according to initial presentation. The timing and occurrence of NW was assessed following a structured clinical examination during clinical visits.

**Results:** Early NW (within the first 3 months of therapy) was observed in 30 out of 115 (26.1%) patients with neurological or mixed presentation and never in patients with a purely hepatic or asymptomatic presentation (0%). Late NW (after >12 months) was seen in a further 23 (20%) with neurological or mixed presentation and in 13 out of 294 (4.4%) patients with a hepatic or asymptomatic presentation. The median time from start of treatment to late NW was 20 months. Only three patients experienced NW between 3 and 12 months. NW was observed with D-penicillamine, trientine and zinc therapy and was reversible in 15/30 (50%) with early NW and in 29/36 (81%) with late NW.

**Conclusions:** In this study, we identified two peaks in NW: an early (≤3 months) treatment-associated peak and a late (>12 months of treatment) adherence-associated peak. Early paradoxical NW was attributed to treatment initiation and pre-existing neurological damage, and was not observed in those with a hepatic or asymptomatic presentation. Late NW is likely to be associated with non-adherence.



## Introduction

Wilson disease (WD) is a genetic disease caused by mutations in the ATP7B protein, which impair biliary copper excretion and result in accumulation of copper in the liver and extrahepatic tissues.[1–3] Depending on the timing of initial diagnosis, the severity of WD can vary widely.[4,5] Almost all patients show evidence of liver disease with additional key diagnostic features including Kayser-Fleischer rings or acute episodes of hemolysis characteristically associated with acute liver failure. Neurological and psychiatric symptoms are common in WD. Neurological symptoms are often the first clinical features leading to the diagnosis of WD; 20–70% of patients presenting at an age of 20–30 years have neurological symptoms.[6] There are a spectrum of neurological signs and symptoms related to WD. The most common neurological features of WD are tremor, dystonia and parkinsonism. Dysarthria, gait and posture disturbances, drooling and dysphagia[2,7–9] might be associated with WD. Psychiatric symptoms occur frequently in the clinical presentation of WD. Related psychiatric features are depression, anxiety, psychosis and behavioral disturbances.[10]

Clinical improvement is usually observed under treatment with chelating agents and/or zinc salts. However, neurological symptoms appear to be less sensitive to therapy in contrast to hepatic symptoms. Neurological worsening (NW) often occurs soon after initiation of WD treatment.[1,11–15] This has been mainly reported in patients starting therapy with chelating agents, but some studies describe similar worsening in patients starting therapy on zinc salts.[14,16,17] The pathophysiology underlying early NW remains poorly understood. The main proposed explantation is that a rapid mobilization of free and toxic copper in the blood and cerebrospinal fluid damages neurological tissues.[11,18,19] Another risk factor for early neurological deterioration might be the use of antidopaminergic drugs.[20] Potential predictors of early neurological deterioration or the association between the applied treatment/dosage with the occurence of NW have not been clearly defined. The aim of our study in patients with WD was to (i) characterize the occurrence

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and outcome of NW, (ii) establish the clinical course and (iii) explore the reversibitily of neurological symptoms.

## Patients and methods

### Study participants

The retrospective data collection was in accordance with the ethical standards of the institutional ethical committee and with the 2013 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local ethics committee. All patients provided informed consent for the analysis of their chart data. Informed consent for the procedure and management was obtained from all individual participants included in the study.

### Statistical correlation analyses

Statistical analysis was performed using SPSS-25 software (IBM, Germany). Graphics were designed with Microsoft Excel (2010). Data were presented as number with percentage or mean with range and standard deviation. Comparisons were made using the Chi-square- test. Statistical significance was set at $p$ value $< 0.05$.

### Methods

We retrospectively studied 163 patients with a definitive diagnosis of WD and neurological symptoms between 2010-2018 (from a cohort of 457 patients with WD) at University Hospital Heidelberg, Germany. Only patients with a Leipzig Score ≥4 and NW were included; 35 of them were lost to follow-up and were excluded from further statistical analysis due to incomplete data. All 128 patients included in this monocentric study were initially diagnosed at Heidelberg University Hospital and were followed-up regularly at Heidelberg every 3 to 6 months by the same physician. Initial therapy was started when referred to our Unit. The choice of treatment was determined by the treatment guidelines for Germany: D-Penicillamine (DPA) is used as the first-line chelating agent. Trientine was prescribed in all patients intolerant to DPA treatment. Moreover, zinc was used for primarily asymptomatic patients or for maintenance therapy after long-term chelation therapy. According to our protocol, we administered DPA in incremental doses of 150-300 mg/day, with the dose increased by 150-300 mg every 4–7 days to a maximum of 1,000–1,800 mg/day in 2–4 divided doses adjusted to weight and phenotype (Table S3). The cohort was divided into the following four phenotypes at time of diagnosis: neurological symptoms, hepatic symptoms, mixed presentation and asymptomatic patients. Phenotypic classification was made upon clincial, laboratory and diagnostic findings. Our standard assessment includes (i) liver function tests, synthetic function, hepatic ultrasound, hepatic elastography and a structured neurological examination and history taking (ii) brain MRI and Unified Wilson disease Rating Scale (UWDRS) at first diagnosis.

### Study groups

*Neurological presentation*

Clinical neurological evaluation was based on a structured interview and examination (see supplementary information). During the focused neurological examination, the patients were examined with respect to the following clinical signs: tremor, dystonia, parkinsonism and fine motor skills. Having at least one of these clinical features classified the patient as having a neurological presentation in the absence of elevated liver enzymes, abnormal synthetic function, abnormalities of hepatic ultrasound, reduced platelets and enlarged spleen.

*Hepatic presentation*

Patients were classified as having a hepatic presentation if they displayed one or more features including transaminases >40 U/L, bilirubin >1.0 mg/dl, international normalized ratio >1.2, radiological/biopsy changes (*e.g.* fibrosis/cirrhosis), hepatic encephalopathy, or features of portal hypertension or decompensation.

*Mixed presentation*

Patients were classified as having a mixed presentation when at least one of the neurological features and hepatic features were found.

*Asymptomatic presentation*

Patients were classified as asymptomatic if there was no evidence of neurological features in the context of normal liver function tests and a confirmed diagnosis of WD.

### Primary endpoint: Neurological worsening

The primary endpoint of this study was the finding of NW following careful history taking and examination as explained above. Both were performed by the same medical rater at different time points from diagnosis: baseline, ≤3 months, >3 to ≤6 months, >6 to ≤ 12 months and >12 months. All 128 patients were categorized into 'neurological worsening' or 'no neurological worsening' compared to baseline with respect to the following clinical signs: ataxia, rigidity or parkinsonism, dystonia, tremor, chorea and disturbances of coordination. Neurological evaluation was based on a structured interview in each patient at baseline and each visit by the same rater. The new onset of at least one of these clinical features (not present at diagnosis) classified the patient as having NW. In case of impairment, the rater classified the symptoms as 'mild', 'moderate' or 'severe' in comparison to the last follow-up. Therefore, patients with worsening of pre-existing symptoms could also be classified as having NW.

### Secondary endpoints

*Reversibility of neurological worsening*

In patients with WD and NW, neurological examination and structured interview (as mentioned above), were assessed by the same medical rater at different times from diagnosis: baseline, ≤3 months, >3 to ≤6 months, >6 to ≤12 months and >12 months. All such patients were categorized as 'worsening reversible' or 'worsening irreversible' compared to baseline. The duration from NW to last follow-up was 5 years. Reversible worsening was defined as a complete improvement of symptoms back to baseline levels. Irreversible worsening was defined as no improvement or a partial improvement of neurological symptoms compared to baseline.

*Treatment association*

Additionally, we listed the use of specific WD medications at any time during follow-up: baseline, ≤3 months, >3 to ≤6 months, >6 to ≤12 months and >12 months (Table 1). The relationship of treatment to NW and its reversibility was assessed.

*Adherence association*

Drug adherence was characterized using a detailed structured self-designed questionnaire at every 3 month visits. Patients were asked in detail how often they forgot medication since the last follow-up, which dose was forgotten or if they overdosed. Final assessment of "adherence" or "non-adherence" was made by the consulting physician. Non-adherence was defined as number of omissions more than twice in a week or more than 6 days in a month. Treatment break was defined as a pause of medication for at least 3 months.

## Results

### Patient demographics

This single-center study included 163 patients (n = 87 males *vs.* n = 76 females) with a proven diagnosis of WD and neurological symptoms at any time between 2010-2018 out of a total cohort of 457 patients with proven WD at the University Hospital Heidelberg. Of the 163 patients with a median age of 24 at diagnosis, 35 were lost to follow-up and were excluded due to missing data. In total, 128 patients with WD were included and were divided into the following study groups, according to initial presentation: neurological (n = 57; 44.53%), hepatic (n = 11; 8.59%), mixed (n = 58; 45.31%) and asymptomatic (n = 2; 1.56%). Mean age of patients at diagnosis was 22 years, 49% of the patients were males. The sex and age distributions across the four study groups at diagnosis are detailed in Table 1. Cirrhosis was described in 32% of patients (diagnosed based on impaired laboratory liver function and cirrhosis on biopsy or imaging [ultrasound, Fibroscan®, MRI or CT]). Kayser-Fleischer rings were found in 55% of patients, by slit lamp examination, predominantly in the neurological or mixed phenotype group. The three most common neurological symptoms at diagnosis were tremor, dysarthria and dysphagia (Table 2).

### Neurological worsening, reversibility and treatment association

In total, 69 (53.91%) patients in our cohort suffered from NW. The three predominant neurological symptoms of worsening were dysarthria, tremor and ataxia (Table 2). The study cohort was divided into subgroups based on initial clinical presentation and was further split into three subgroups: (i) early, (ii) late, or (iii) no NW (Fig. 1): Fig. 1 presents NW according to initial presentation at diagnosis. After occurrence of NW, patients were followed-up for 5 years. The main features of irreversible NW were dysarthria, ataxia and dysphagia (Table 2), whereas tremor was mostly reversible. The initial treatment received by patients with NW is listed in Table 3; most patients (85%) were under DPA as first-line therapy. A minority of patients were under trientine dihydrochloride or zinc therapy at the time of NW. NW was totally reversible in 65% of patients and was classified as irreversible in the remaining third (Table 4). When considering both the initial presentation of WD and NW, more than 80% of patients can be classified as having a neurological or mixed phenotype (see Table 1). In relation to the total cohort of patients with WD (N = 457) followed-up from initial diagnosis and treatment at University Hospital Heidelberg, the true overall prevalence of NW (n = 69) is 15%. Comparing clinical subgroups, only 13 patients (4.4%) from the hepatic or asymptomatic subgroups (n = 294 within the total cohort) demonstrated NW after >12 months of treatment. NW after >12 months of treatment was therefore very rarely observed in patients with these baseline phenotypes and NW at ≤3 months was not detected. In patients with neurological symptoms (n = 163 within the total cohort), 56 (34.4%) developed NW, of whom 23 patients suffered from NW after >12 months (14.1%) and 30 patients (18.4%) from NW at ≤3 months. Three patients (1.8%) developed NW between 3 and 12 months.

When comparing time of NW, two peaks can be described: the first cumulated occurrence of NW after treatment initiation occurred at ≤3 months, the second peak occurs after >12 months (Fig. 2). Categorizing NW by therapeutic agents, this effect can be shown either for DPA, trientine and zinc (see Table 5). Regarding the subcohort of 115 patients with a neurological or mixed presentation in which most cases of NW were observed, 56 showed NW: 42 were treated with DPA, five with trientine, three with zinc and six paused their medication due to non-adherence. Most cases of NW were observed under DPA since most patients received DPA. Overall, NW had no significant impact on overall survival (supplementary information).

### Adherence association

We further studied whether adherence to WD therapy was associated with the peaks of NW described above

**Table 1. Patient baseline characteristics.**

| | | Initial presentation | | | |
|---|---|---|---|---|---|
| | Total | Hepatic | Hepatic + neurological | Neurological | Asymptomatic |
| Initial presentation | 457 | 290 (63.46) | 90 (19.69) | 73 (15.97) | 4 (0.87) |
| Included in the study | 128 | 11 (8.59) | 58 (45.31) | 57 (44.53) | 2 (1.56) |
| Sex distribution | | | | | |
| Female | 63 | 7 (11.11) | 26 (41.27) | 29 (46.03) | 1 (1.59) |
| Male | 65 | 4 (6.15) | 32 (49.23) | 28 (43.08) | 1 (1.54) |
| Cirrhosis at diagnosis | 41 (32.0) | 5 (45) | 36 (62) | — | — |
| Kayser-Fleischer ring | 71 (55.5) | 4 (36.40) | 58 (62.07) | 31 (54.39) | 0 (0) |
| Initial therapy | | | | | |
| D-Penicillamine | 109 (85.2) | 10 (9.17) | 49 (44.95) | 48 (44.04) | 2 (1.83) |
| Trientine | 14 (10.9) | 0 (0) | 8 (57.14) | 6 (42.86) | 0 (0) |
| Zinc | 5 (3.9) | 1 (20) | 1 (20) | 3 (60) | 0 (0) |

Baseline characteristics of the study cohort with regard to sex, age and initial clincal presentation and phenotype. Data presented as n (%).

**Table 2. Neurological symptoms in patients with NW.**

| | At diagnosis | At time of neurological worsening | Irreversible at time of neurological worsening |
|---|---|---|---|
| Tremor | 59 | 19 | 2 |
| Dysarthria | 53 | 21 | 13 |
| Dysphagia | 53 | 8 | 4 |
| Ataxia | 33 | 18 | 9 |
| Dystonia | 13 | 7 | 3 |
| Dysgraphia | 12 | | 2 |
| Bradykinesia | 10 | | |
| Rigor | 4 | 2 | |
| Chorea | 2 | 1 | |

NW, neurological worsening. Data presented as n.

(Table 4). Overall, we observed non-adherence to therapy (≥2 omissions per week) in 18 patients (14.07%), of whom 15 (83.3%) developed NW (6 [40%] with NW at ≤3 months; 9 [60%] with NW after >12 months). Division into the subgroups 'neurological or mixed' *vs*. 'hepatic or asymptomatic' showed the following distribution for non-adherence to therapy and NW: Among 115 patients with a 'neurological or mixed' presentation, non-adherence was noticed in 12, of whom seven developed early NW and two late NW. Thus, in patients with a 'neurological or mixed' presentation, non-adherence was seen in 7/30 (23.3%) with early NW and 2/23 (8.7%) with late NW. Since similar data on adherence in the total cohort of hepatic and asymptomatic patients were not available, we can infer that non-adherence was seen in 6/13 (46.2%), suggesting its importance for late NW in these patients. All six patients with non-adherence in the 'hepatic or asymptomatic' subgroup developed NW after >12 months.

NW after >12 months was observed at a median of 18 months, with a range from 12 to 60 months. The majority of cases of NW after >12 months were observed between 12 and 24 months (68%), with a median of 20 months in hepatic and asymptomatic patients.

## Discussion

The aim of our study was to review the occurrence and outcome of NW and correlate the association to specific treatments and phenotypes in patients with WD. Our Intention was to (i) characterize and define 'early' and 'late' neurological deterioration and (ii) examine the potential reversibitily of neurological symptoms whilst under therapy.

The study cohort reflects real world characteristics of patients with WD, with a mean age of 22 years at diagnosis.[1,21] Specifically, only patients with a Leipzig Score ≥4 and/or with genetic confirmation of WD were included, which increased the study validity. Moreover, the distribution of clinical presentations (53% with a mixed or hepatic presentation and 44% with a neurological presentation) is similar to that reported by other studies.[9,20,22] The sex ratio was nearly equally distributed in our cohort with a slight predominance of males, which accords with a Polish case series[23]. Concerning initial therapy, the majority of patients were under DPA as first-line therapy, according to the standard of care outlined in the German National Guidance for the treatment of WD. Moreover, the fact that most patients with NW were on DPA may just reflect that this was the most commonly used drug. As we do not have data on the distribution of treatments in those who did not develop NW, the present study cannot elucidate whether the risk of NW is related to treatment. Our study demonstrates for the first time that neurological deterioration in WD occurs at two frequency peaks: ≤3 months (43.47%) and >12 months (52.17%), which are independent of the type of initial anti-copper treatment. Early NW appears to be at least partly therapy associated. It might also be related to the natural course of WD itself. Late neurological deterioration appears to be adherence associated and was reversible in 65.22% of all cases. Previous studies suggest that deterioration is irreversible in up to 50%, particularly after treatment initiation with DPA in WD.[1–3,11,13] In contrast, a study by Litwin *et al.* concurs with our results concerning the reversibility of neurological deterioration, describing complete and partial reversibility in 53% and 13% of

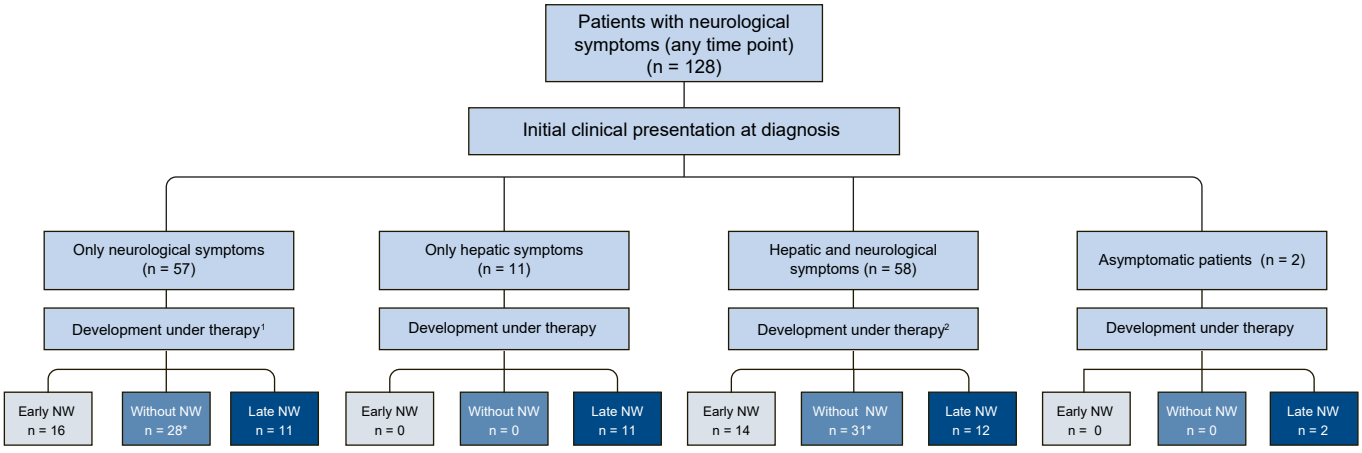


**Fig. 1. Classification of study cohort at initial diagnosis and subsequent characterization by NW.** The study cohort was divided into four subgroups of initial clinical presentation: hepatic, neurological or hepatic and neurological symptoms in contrast to asymptomatic patients. The subgroups were then divided by (i) early, (ii) late, or (iii) no NW counted from initial diagnosis. *Stable neurological symptoms under therapy. [1]NW, n = 1 at 3 to ≤6 months; NW, n = 1 at 6 to ≤12 months. [2]NW, n = 1 at 6 to ≤12 months. NW, neurological worsening.

**Table 3. Association of different treatments with neurological worsening.**

| | D-Penicillamine | Trientine | Zinc | Pause |
|---|---|---|---|---|
| Initial treatment in patients with NW | 62 (89.8%) | 6 (8.7%) | 1 (1.5%) | – |
| Treatment at time of NW | 43 (62.4%) | 9 13.0%) | 8 (11.6%) | 9 (13.0%) |
| Treatment duration at time of NW (calculated from most recent treatment switch) | | | | |
| ≤3 months | 21 | 5 | 1 | |
| 3 to ≤6 months | 2 | 0 | 0 | |
| >6 to ≤12 months | 1 | 2 | 0 | |
| >12 months | 19 | 2 | 7 | 9 |

NW, neurological worsening. Data presented as n (%).

**Table 4. Reversibility of neurological worsening and association with adherence.**

| | Reversible | Irreversible | Total |
|---|---|---|---|
| **All NW** | 45 (65.2%) | 24 (34.7%) | 69 |
| Adherent patients with NW | 32 | 22 | 54 |
| ≤3 months | 10 | 15 | 25 |
| 3 to ≤6 months | 0 | 0 | 0 |
| >6 to ≤12 months | 0 | 1 | 1 |
| >12 months | 22 | 6 | 28 |
| Non-adherent with NW | 13 | 2 | 15 |
| ≤3 months | 5 | 0 | 5 |
| 3 to ≤6 months | 1 | 0 | 1 |
| >6 to ≤12 months | 0 | 1 | 1 |
| >12 months | 7 | 1 | 8 |

NW, neurological worsening. Data presented as n (%).

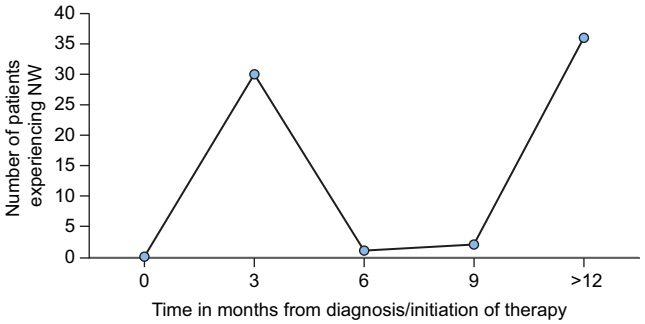


**Fig. 2. Proposed schematic clinical classification of paradoxical treatment-associated and adherence-associated neurological worsening.** Two peaks of neurological worsening occur: the first occurs after treatment initiation (≤3 months), the second occurs after 12 months. The paradoxical treatment-associated neurological worsening at ≤3 months and the adherence-associated neurological worsening after >12 months are schematically presented. NW, neurological worsening.

patients, respectively, over 9.2 ± 5.2 months.[20] Similarly, an early deterioration was also observed under trientine and zinc therapy, indicating that NW is not necessarily linked to the specific type of initial therapy.[24,25] That said, paradoxical neurological deterioration after the initiation of therapy remains an unexplained unfavorable disease course. Due to the concept of relatively mobile "free" copper, copper toxicity and resulting early NW could be caused by chelating agents in WD.[20,26] De-coppering agents cause a release of free copper, inducing oxidative stress and potentially damaging the central nervous system.[9,13,25,27–29] Evidence for different copper pools and such a shift under early therapy were noted in previous studies.[30] Some studies measured copper concentrations in the cerebral fluid under treatment in humans or the copper levels in the brain in animal models of WD.[29,31] Data in these studies is consistent with the association of increased "free" copper and neurological deterioration. In Brewer's study from 2009, NW was associated with significant relative spikes in serum free copper levels.[3,31] The mobilization of free copper secondary to chelation is not the only mechanism to explain neurological deterioration. Increased copper in the cerebrospinal fluid was also found at initial diagnosis and before treatment.[32–34] In a French study of liver transplantation for patients with WD who were already under chelation, exchangeable copper was normal at the time of NW (just before liver transplantation).[35–37] Therefore, other mechanisms may also explain neurological deterioration: For example, iron accumulation in the neurons, the direct toxic effect of copper or impairment of astrocytes.[32–34,37,38]

Patients who did not worsen neurologically generally did not show significant spikes in free copper.[3,31,39,40] We therefore interpret early (treatment-associated) worsening as a consequence of mobilization of free copper, *i.e.* copper not bound to ceruloplasmin or chelating agents. In contrast to treatment-associated NW, 'late' (adherence-associated) NW is defined as deterioration >12 months after initiation of therapy, *i.e.* the second frequency peak (Fig. 2). Non-adherence to therapy seems to be concomitant with the peak of late NW. Data might show a tendency for late NW in non-adherent patients with a 'hepatic or asymptomatic' presentation (n = 6; 46.2%) *vs.* non-adherent patients with a neurological presentation (n = 2; 6%). With early NW, non-adherence is more common in patients with a neurological presentation (n = 7; 21%) and is not observed in patients with a 'hepatic or asymptomatic' presentation (n = 0). Nevertheless, non-adherence is often underestimated. Therefore, correlation to parameters of copper metabolism is an essential tool to detect under treatment. In cases of non-adherence, exchangeable copper increases and non-ceruloplasmin-bound copper may also be elevated (>25 μg/dl).[41]

Moreover, the underlying etiology might similarly be the relatively increased amount of incorporated copper, which cannot be bound to chelating agents. Important factors such as interruption of daily routines to avoid taking medication with food is a significant lifestyle issue for some patients as already described in previous studies by Walshe *et al.* 1986 and Schilsky *et al.* 2019.[42,43] A study by Jacquelet *et al.* showed that patients (n = 139) with an asymptomatic presentation have more difficulty with adherence.[44] Moreover, adherence was lower in patients who had problems with storage or delivery of medications.[44] NW between 3-12 months might be the result of a mixture of factors including treatment, non-adherence, and

**Table 5. Neurological worsening: treatment association.**

| Characteristic | n (%) |
|---|---|
| Medication switch after neurological worsening | 19 (100) |
| D-Penicillamine to Trientine | 9 (47) |
| Trientine to D-Penicillamine | 0 |
| Trientine to Zinc | 0 |
| Zinc to Trientine | 4 (21) |
| D-Penicillamine to Zinc | 1 (5)* |
| Zinc to D-Penicillamine | 5 (26) |

Reflects the medication switch after occurrence of neurological worsening.
*NW due to overdosing.

unknown environmental etiologies, for example weight gain (adjusted therapeutic dose needed), nutrition, adolescence or concomitant treatment with agents blocking dopamine neurotransmission.[9,45–47] We also assumed sex-related differences concerning neurological deterioration, which has been shown to present more commonly in men.[20,23] This hypothesis was not confirmed in our study. Another association of overall NW seems to be an initial neurological presentation at diagnosis due to cerebral lesions. Independent of initial presentation, all patients may develop neurological symptoms later due to a lack of adherence. Patients with pure neurological or mixed (hepatic and neurological) presentation suffered more often from neurological deterioration than patients with presentations classified as hepatic or asymptomatic. Nevertheless, the definition of a mixed phenotype cannot be used with certainty and should only be used as a temporally based definition, which requires correlation with clinical findings. Patients can switch phenotype classification if new symptoms occur. However, our observations are in line with previous reports of a worse prognosis for patients with WD who have neurological signs and symptoms at diagnosis.[20,27] One such interpretation might be that relative "free" toxic copper is a second hit to the nervous system resulting in NW or that the disease is already a systemic disease involving the brain with diffuse copper overload.

Due to its retrospective and single-center design, this study has notable limitations. The clinical classification of neurological deterioration as well as reversibility are at risk of bias. Bias was limited by multiple measures including using the same rater to evaluate patients at baseline and follow-up visits. Laboratory (copper) assessments and brain MRI analysis would[44] have been useful to support our observations of NW. We do not routinely perform sequential brain MRI examination of patients with WD at our Liver Centre, particularly if the diagnosis of WD is confirmed. Thus, we compared baseline data and follow-up results based on a center specific, standardized structured interview and focused clinical examination to detect NW. Additionally, we cannot exclude bias due to non-random selection of patients. Whilst we recognize the use of adherence scales, such as the Morisky scale, there is currently no validated standard for measuring compliance to anti-copper treatment. However, as a center of excellence for WD, we utilized standardized methods to identify cases of potential non-compliance. Although adherence scores for chronic diseases exist, the Morisky scale was used only once in WD.[44] Further extended studies including UWDRS, brain imaging and laboratory measurement of free copper are needed to objectively validate our findings. Another interesting correlation would be the measurement of exchangeable copper (which is a direct function of free copper) and neurological deterioration in association with urinary copper excretion. Additionally, similar subgroups of WD presentation would be needed, to outline or identify phenotype classification as a potential confounder. As deterioration is not directly linked with type of therapy, there might be a predisposition for early worsening, reflecting the natural course of the disease. The differentiation between early treatment-associated NW and the natural course of the disease remains a diagnostic dilemma and cannot yet be clearly defined. This distinction cannot even be made by evaluating brain MRI data or UWDRS. As mentioned above, the mobilization of free copper secondary to chelation is not the only mechanism to explain NW. Nevertheless, the temporal association with treatment initiation as well as the reversibility in most cases of early NW would support the concept of treatment-associated NW caused by the sudden mobilization of free copper secondary to chelation, which is already recognized.

Despite study limitations, it is most noteworthy that this is the first study that attempts to define the exact time of occurrence for early and late neurological deterioration in WD and which therefore further identifies potential risk factors. Moreover, the concept of late NW and its association to adherence is a novel key finding. Nevertheless, pediatricians are more often confronted with non-adherence especially in pubescent patients. Further studies in the pediatric cohort might contribute to the understanding of adherence-associated NW.

In summary, the study shows two peaks of frequency of NW (Fig. 2). Whereas treatment-associated NW appears mainly at ≤3 months, we defined adherence-associated NW as deterioration after >12 months. The previous guiding principle for chelating agents "start low and go slow" could be further extended to "start low, go slow and *maintain it*". Of equal importance is surveillance of compliance, especially concerning maintenance therapy and NW.

## Conclusion

In general, our study results document that there are two relative peaks of NW: an early peak at ≤3 months (treatment-associated NW), which seems to be related to therapy initiation and a late peak after >12 months (adherence-associated NW), which appears to be associated with patient adherence but has no significant impact on overall survival. Early paradoxical NW was attributed to treatment initiation and pre-existing neurological disease and was mostly reversible; it was not observed in patients with a hepatic presentation. The specific type of WD treatment does not seem to influence this clinical paradigm. Late NW might very well be associated with non-adherence and was observed in 4% of primarily hepatic or asymptomatic cases. Notwithstanding study limitations, this is the first study characterizing the timing of 'early' and 'late' NW in treated WD and therefore delivers important preliminary data that can guide the management of WD and future prospective studies.

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## Abbreviations

DPA, D-Penicillamine; NW, neurological worsening; UWDRS, Unified Wilson disease Rating Scale; WD, Wilson disease.

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## Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

## Authors' contributions

IM and KHW conceived the project and designed the study. EE collected the data. IM analyzed study data and delivered statistical output. JP, UM, AP, AA and KHW reviewed and revised the final manuscript.

## Data availability statement

Data are available from the authors with the permission of corresponding author. The data that support the findings of this study are available from the corresponding author [KH Weiss] upon reasonable request.

## Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2023.04.007.

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