Aus der Klinik für Neurologie
der Heinrich-Heine-Universität Düsseldorf
Direktor: Prof. Dr. med. Dr. rer. nat. Dr. rer. h.c. Sven Meuth

Klinisch neurologische Befunde und Komorbiditäten bei 115 langzeitbehandelten Wilson Patienten (Langzeitbehandlung von Patienten mit neurologischen Manifestationen des Morbus Wilson)

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Sara Samadzadeh

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2-Longitudinal changes of retinal morphology in Wilson's disease assessed by optical coherence tomography: Results from 47 patients over 5 years
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English summary

Wilson's disease (WD) is a rare (1:40.000) autosomal-recessive disorder of copper metabolism caused by pathogenic variants in the copper-transporting gene, ATP7B that leads to impaired copper transport and tissue accumulation of toxic copper ions and mostly hepatic and neurologic symptoms consequently.

For the present retrospective longitudinal cohort study, 115 long-term-treated patients with neurological manifestations of Wilson's disease are recruited who were treated at the outpatient department of the neurological clinic of the University of Düsseldorf. The clinical neurological long-term outcome is analyzed as well as a list of available comorbidities and a detailed report of some special cases with rare concurrences.

These 115 WD patients were treated for a mean duration of 26 years. They underwent a detailed clinical investigation and scoring of clinical findings yielding 7 motor and 3 non-motor subscores as well as laboratory testing. Both linear and binomial regression analysis are conducted on clinical scores regarding years from diagnosis, and forward stepwise regression is done to investigate their interdependence. Survival analysis is performed to reveal probabilities of HCC manifestation and mortality. Descriptive statistics of special cases are also provided.

To explain the variance of clinical data gait turned out to be the most relevant symptom, although gait disturbances were not the most frequent findings in the broad spectrum of neurological manifestations of WD. The spectrum of symptoms changes with therapy. Initially, most symptoms improve with the duration of therapy but may worsen again with higher age.

Since WD may affect multiple organs the detection of additional disease entities as comorbidities is difficult. A list of neuropsychiatric, orthopedic, gastroenterological, and cardiovascular comorbidities in the present cohort is determined.

Some comorbidities afford special treatment as immunomodulatory therapies of patients with WD and MS, or botulinum toxin therapy of focal dystonia, hypersalivation, and hyperhidrosis or hip displacement operation in patients with severe joint pain. Special chemotherapy is necessary for patients with hepatocellular carcinoma or leukemia. Special monitoring is necessary for patients with psychiatric symptoms since the clinical outcome and laboratory findings are significantly related to the severity of psychiatric abnormalities.

Furthermore, the present study contributes to the problem of a weak geno/phenotype correlation by the analysis of homozygotic twins. Despite identical genetic disposition, WD symptom

presentations may develop differently in monozygotic twins, and they may need to be placed in a very different therapeutical regimen. The underlying gene-environment interaction is unclear so far.

The Kaplan Meier analysis reveals a reduced survival time after diagnosis in the present cohort in full agreement with a previous study in 33 patients from Finland. In contrast to the previously published assumption that elevated serum copper levels prevent the development of hepatocellular carcinoma in WD 5 patients developed HCC in the present cohort.

This reduction of survival time in WD, the worsening of symptoms with age as well as the frequent development of comorbidities underline the necessity of careful monitoring during long-term treatment of WD.

Deutsche Zusammenfassung

Der Morbus Wilson (WD) ist eine seltene (1:40.000), autosomal-rezessiv vererbte Erkrankung des Kupferstoffwechsels, die durch pathogene Mutanten im kupfertransportierenden Gen ATP7B verursacht wird und zu einer Akkumulation von toxischen Kupferionen in verschiedenen Geweben und insbesondere zu Störungen der Leber und des Hirns führt.

Diese seltenen Patienten werden schwerpunktmäßig in der Ambulanz der Neurologischen Universitätsklinik Düsseldorf betreut, so dass für die vorliegende retrospektive longitudinale Kohortenstudie 115 langzeitbehandelte Patienten mit neurologischen Manifestationen des Morbus Wilson rekrutiert werden konnten. Analysiert werden sollte das Ergebnis der wilsonspezifischen Langzeitbehandlung.

Aus den Akten wurden die demographischen Daten, die Score-Ergebnisse vorausgegangener neurologischer Untersuchungen, Laborergebnisse, therapierelevante Daten und Zusatzerkrankungen extrahiert.

Die Patienten waren im Mittel 26 Jahre behandelt. Es stellte sich heraus, dass sich die neurologischen Symptome der Patienten in den ersten 5-10 Jahren der Therapie bessern, dann eine Zeitlang konstant bleiben und dann wieder verschlechtern. Da besonders nichtmotorische Symptome mit dem Alter zunahmen, gehen wir von zwei sich gegenläufig beeinflussenden Faktoren aus: Die Therapie führt zu einer Besserung über die Zeit, das Alter zu einer Verschlechterung.

Dass die Varianz der klinischen Befunde am besten erklärende Symptom war der Gang, obwohl Gangstörung nicht das häufigste Symptom war. Das Spektrum der neurologischen Symptome änderte sich unter der Therapie. Eine besonders problematische Untergruppe von Patienten waren

diejenigen, die eine psychiatrische Manifestation aufwiesen. Korrelierend mit dem Schweregrad der psychiatrischen Symptomatik war auch die sonstige neurologische Symptomatik und das Ergebnis von Laboruntersuchungen verschlechtert.

Die meisten der hier untersuchten Wilsonpatienten wiesen neurologische oder psychiatrische oder internistische oder orthopädische Zusatzerkrankungen auf. Da Kupfer aber alle möglichen Gewebe negativ beeinflussen kann, ist die Abgrenzung, ob eine umschriebene eigenständige Zusatzerkrankung oder eine Mitbeteiligung im Rahmen des M. Wilson vorliegt, mitunter sehr schwierig.

In dem vorliegenden Kollektiv fanden sich 5 Patienten mit hepatocellulärem Carcinom (HCC). Es war früher behauptet worden, dass Kupfer vor der Entwicklung eines HCC schützen würde. Das bestätigt sich jetzt, nachdem viele Wilsonpatienten länger behandelt werden, offensichtlich nicht.

Die Überlebenszeit nach Diagnosestellung war bei den Wilsonpatienten unseres Kollektivs reduziert. Damit konnten wir früher gemachte Aussagen, dass die Lebenserwartung von Wilsonpatienten normal sei, nicht bestätigen. Unsere Daten bestätigen dagegen die von einer finnischen Arbeitsgruppe beobachtete reduzierte Lebenserwartung eines im Vergleich zu unserem, deutlichen kleineren finnischen Kollektivs von Wilsonpatienten.

Diese reduzierte Lebenserwartung, die Verschlechterungstendenz mit dem Alter, das häufige Auftreten von Komorbiditäten und die Entwicklung von Malignomen legen nahe, die Therapie von Patienten mit Morbus Wilson sorgfältig zu kontrollieren.

List of abbreviations

aboBoNT	Abobotulinum neurotoxin (Dysport®)	**LC**	light chain
APOE	Apolipoprotein E gene	**LTx**	liver transplantation
ART	Automatic Real Time	**mg/d**	milligram/ day
BBB	Blood Brain Barrier	**mg/dL**	milligram/ deciliter
BoNT	Botulinum Neurotoxin	**MotS**	Motor score
CHE	Cholinesterase	**MRI**	Magnet Resonance Tomography
cm	Centimeter	**MTHFR**	Methylenetetrahydrofolate reductase gene
COMMD1	copper metabolism domain-containing 1 gene	**N/A**	Not applicable
DPA	D-Penicillamine	**n.m.**	Not mentioned
EDSS	Expanded Disability Status Scale	**Non-MotS**	Non-Motor score
incoBoNT	incobotulinum neurotoxin (Xeomin®)	**NR**	Normal range
g/d	gram/ day	**OCT**	Optical Coherence Tomography
GGT	Gamma-glutamyl Transferase	**onaBoNT**	onabotulinum neurotoxin (Botox®)
GOT (AST)	Aspartate Aminotransferase	**PEG**	percutaneous endoscopic gastrostomy
GPT (ALT)	Alanine aminotransferase	**PNP**	Polyneuropathy
HCC	Hepatocellular Carcinoma	**PNPLA3**	Patatin- like phospholipase domain-containing protein 3 gene
kg	Kilogram	**PPMS**	primary progressive multiple sclerosis

PPT	Thromboplastin time	**TID**	Three-time a day
pRNFL	peripapillary retinal nerve fiber layer	**TRI**	Trientine Hydrochloride (Trientine)
PTSD	Post-traumatic stress disorder	**TIA**	Transient ischemic attack
rCGM	the cerebral glucose metabolism	**TTM**	bis-choline tetrathiomolybdate
RLS	Restless Leg Syndrome	**TS**	Total score
RRMS	Relapsing-remitting MS	**tx- j mouse**	the toxic-milk mouse from the Jackson laboratory
SAH (SAHH) (AHCY)	S-adenosylhomocysteine	**U**	Unit (s)
SNAP-25	synaptosomal-associated protein 25	**U/L**	units/liter
SNARE	the soluble NSF attachment protein receptor	**WD**	Wilson's disease
SPMS	Secondary progressive MS	**WTX101**	Tetrathiomolybdate

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1 Introduction

1.1 A brief history of Wilson's disease

Wilson's disease (WD, MIM 277900) was first described by the British neurologist Samuel Alexander Kinnier Wilson as a "progressive lenticular degeneration accompanied by cirrhosis in the liver" (Wilson, 1912). The manuscript included twelve cases that presented with involuntary movements, spasticity, dysarthria, psychiatric disturbances, and advanced cirrhosis. In the first half of the 20th century, several researchers made important contributions to the knowledge of the pathophysiological mechanisms of the disease. Rumpel reported excess copper in the liver of a patient with WD for the first time, and Cumings corroborated it and established that copper accumulation in the liver and the basal ganglia was the etiological basis of the disease (Rumpel, 1913, Cumings, 1948). Less than 50 years after its discovery, WD became the first chronic hereditary liver disease that had a specific treatment, which made it possible to stop the devastating progression of the disease and prevent the eventual death (Fig. 1) (Sánchez-Monteagudo et al., 2021, Blanc, 2018).

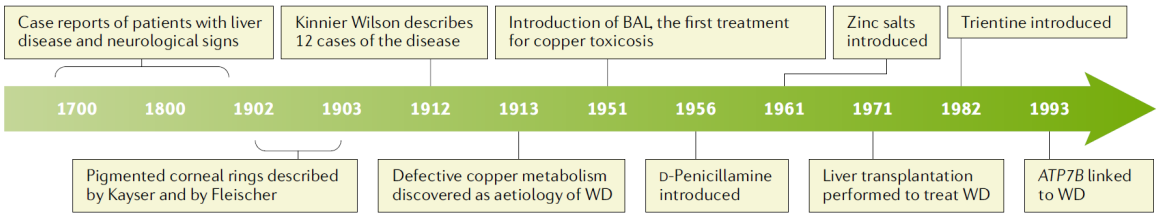


Fig. 1: A timeline of key discoveries in WD; Reprinted by permission from [Copyright Clearance Center]: [Springer] [Nature] [(Członkowska et al., 2018)], [License Number 5198441172348] (2018).

1.2 Pathogenesis of Wilson's disease

1.2.1 Mechanism/pathophysiology

Wilson disease is an inherited disorder of copper metabolism (Bandmann et al., 2015). The disease is caused by homozygous or compound heterozygous mutations (the presence of two different mutant alleles) in ATP7B, which encodes transmembrane copper-transporting ATPase 2 (widely known as ATP7B), which mediates the excretion of copper into bile and delivers copper for the functional synthesis of ceruloplasmin (the major copper-transporting protein in the blood) (Ferenci, 2006). The liver is the site of metabolism for dietary copper; in WD, defective ATP7B function leads to copper overload in hepatocytes, which is associated with liver pathology. Excess non-

ceruloplasmin-bound copper is also released into the circulation, with secondary pathological accumulation in other tissues, particularly the brain, which can lead to neurological symptoms and psychiatric disturbances (Członkowska et al., 2018).

1.2.2 Genetics

Mutations in ATP7B:

ATP7B is located on the short arm of chromosome 13 and contains 20 introns and 21 exons. More than 700 mutations have been described in ATP7B according to the Human Gene Mutation Database (Stenson et al., 2017), although ever some variants with high allele frequencies probably exhibit low penetrance (Wallace and Dooley, 2020); patients with WD can be homozygous for one disease-causing mutation or carry two different disease-causing mutations as compound heterozygotes. WD- associated mutations can affect almost all 21 exons and are frequently missense and nonsense (Członkowska et al., 2018), Table 1 gives some examples accordingly.

Table 1: Some examples of ATP7B mutations (Ferenci, 2006)

missense mutation H1069Q in exon 14	Very common in central, eastern, and northern Europe
missense mutation M645R	southern Europe (Mainland Spain)
R778L mutation in exon 8	southeastern Asia (China)

Several studies have attempted the challenging task of correlating ATP7B genotype with the WD phenotype. In vitro experiments demonstrated that different ATP7B variants present different functional properties with varying copper transporter activity (Huster et al., 2012). For example, in a small sample of patients with WD, it was suggested that certain mutations (named 'truncated mutations') are associated with patients presenting with acute liver failure and an earlier age of disease onset. However, overall, results from studies attempting genotype-phenotype correlations have not been conclusive, partly owing to the poor phenotypic characterization of patients with WD, late diagnosis and overlapping neurological, psychiatric and hepatic signs and symptoms of various severities (Ferenci, 2014, Członkowska et al., 2018).

Other genes:

Studies have explored the role of proteins and mutated genes other than ATP7B as contributors to the phenotype of WD (Członkowska et al., 2018, Shribman et al., 2021). Table 2 gives some examples accordingly.

Table 2: Some examples of other genes

Patatin- like phospholipase domain-containing protein 3 gene (PNPLA3)	(Pingitore et al., 2014)
apolipoprotein E gene (APOE)	(Schiefermeier et al., 2000)
copper metabolism domain containing 1 gene (COMMD1) (formerly MURR1)	(Stuehler et al., 2004)
copper transport protein ATOX1 gene	(Yu et al., 2017)
Methylenetetrahydrofolate reductase gene (MTHFR)	(Gromadzka et al., 2011)

1.2.3 Epigenetics

The potential role of environmental or nutritional factors on epigenetic mechanisms has been explored in animal models of WD. At the interface between the regulation of gene expression and the environment is methionine metabolism, a metabolic pathway that has regulatory effects on DNA methylation. The enzyme S-adenosylhomocysteine (SAH) hydrolase (SAHH; also known as AHCY) has a crucial role in methionine metabolism as it is responsible for metabolizing SAH to homocysteine. If the expression or activity of SAHH is decreased, the level of SAH, which acts as an inhibitor of DNA methylation reactions, will increase. Importantly, SAHH enzyme activity and gene (AHCY) transcript levels are decreased in the presence of hepatic copper accumulation with consequent downstream changes in methionine metabolism parameters (Bethin et al., 1995, Delgado et al., 2008).

Notably, the toxic-milk mouse from the Jackson laboratory (tx- j mouse), which has a spontaneous point mutation affecting the second transmembrane region of the copper transporter, showed dysregulation of methionine metabolism and global DNA hypomethylation in hepatocytes (Medici et al., 2013), with possible downstream effects on the regulation of genes involved in the development of liver damage. In addition, during embryonic development, the liver (a site of major methylation rearrangements) presented major changes in gene transcript levels related to cell cycle and replication in tx- j mice compared with control animals. The provision of supplemental methyl donor choline to pregnant mice was able to bring gene expression in embryonic mice to the same levels as control animals, indicating that fetal livers are susceptible to nutritional factors with potential lifelong consequences on disease phenotype and progression (Medici et al., 2014).

1.2.4 The evidence of genotype-phenotype discordance in homozygotic twins with clinical phenotypic variability

Aside from animal studies, several case reports of homozygous WD-twins with different disease phenotypes indicate and support the involvement of epigenetic changes in the pathogenesis of WD and its phenotypic presentation (Członkowska et al., 2018).

On the one hand twin studies have shown that patients in the same twin family not only had the same onset and clinical symptoms, but a family even had the same allergic reaction to the same medication on the same day (Cheng et al., 2013).

On the other hand Marco Senzolo et al. described two homozygotic twins, both with liver cirrhosis due to WD, one of them with severe neuropsychiatric involvement, but both underwent liver transplantation and subsequently had very different outcomes despite the same genetic background (Senzolo et al., 2007).

Anna Członkowska et al. examined two pairs of monozygotic twins discordant for WD phenotype and suggested the attributable phenotypic characteristics to epigenetic/environmental factors. The first index case developed severe liver failure followed by depressive symptoms, dysarthria, and tremor at the age of 36. Her sister remained presymptomatic at diagnosis at the age of 39. The second index case presented with dysarthria and tremor at the age of 26. Her sister remained clinically presymptomatic at diagnosis at the age of 28 (Członkowska et al., 2009).

K M Kegley et al. reported a case of one monozygotic twin presenting with acute liver failure requiring emergent liver transplantation while the other twin presented with mild liver disease when both shared an identical genetic mutation (Kegley et al., 2010). Nan Cheng et al. also studied the clinical and genetic characteristics of 5 pairs of twins (Cheng et al., 2013).

Sapuppo et al. also observed a streaking phenotypic variability in two Sicilian sisters carrying the same genotype for the ATB7B gene. Although both started to present signs at age 10 years, onset was characterized by neurological signs in the first (tremors, motor incoordination, language and cognitive impairment), while liver involvement has been the only sign in the other. They started the same chelation therapy. After a 20-year follow-up, the former is severely affected (MRI evidence of basal ganglia copper deposits and hyperechogenic liver, thrombocytopenia), while the latter presents only a moderate liver enlargement (Sapuppo et al., 2020).

1.3 Epidemiology of Wilson's disease

1.3.1 Prevalence

The original prevalence of WD estimate from 1984 of 1:30,000-1:50,000 is still valid, based on epidemiological studies, at least for the United States, Europe (Germany, Table 3), and Asia (Sandahl et al., 2020). In some population-based studies, the genetic prevalence was 3-4 times higher than clinically-based estimates (Fig. 2). the genetic prevalence is proposed to be as high as 1 in 7026 individuals. The genetic prevalence could be higher than the clinical prevalence because of incomplete penetrance or the presence of yet to be identified modifier genes. The apparent discrepancy could also be explained by missed diagnoses because of a lack of clinical diagnostic gold standards (Ferenci et al., 2019).

Table 3: available prevalence for Germany

Eastern Germany 1949-1977	1:34,400	(Bachmann et al., 1979)
Western Germany 1962	1:86,000	(Przuntek and Hoffmann, 1987)

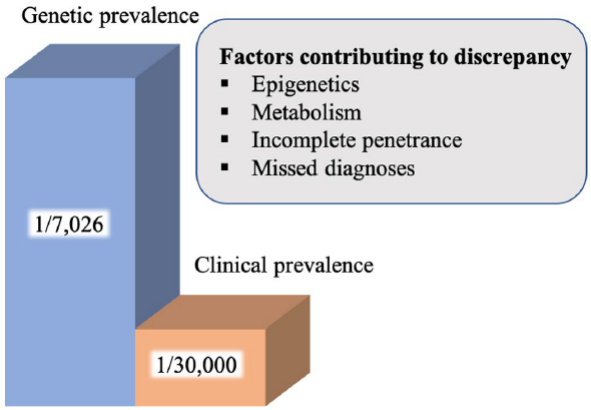


Fig. 2: genetic and clinical prevalence; (Leung et al., 2021) is open access in PMC and available under a Creative Commons or similar license that allows more liberal redistribution and reuse than a traditional copyrighted work.

1.3.2 Age and gender distributions

The presentation of WD varies according to age and gender. The mean age of symptom onset was reported as 15.5 years for those with hepatic and 20.2 years with neurologic involvement. Neurologic presentations were more common at diagnosis in men than women (60% vs. 39%) and hepatic presentations were more common in women than men (58% vs. 41%).

These patterns may help inform our understanding of the pathophysiological basis for phenotypic variation but should not influence decisions to investigate WD. While the majority of cases present between the age of 5 and 35 years old, the range is wide for both hepatic and neurologic presentations. A diagnosis of WD has been reported in a 3-year-old child and two individuals in their eighth decade of life (Shribman et al., 2019).

1.4 Clinical manifestation and classification of Wilson's disease

Symptoms vary widely and are present most commonly between 5 and 35 years of age.

1.4.1 Hepatic presentation

Hepatic manifestations range from acute presentations, acute hepatitis to acute (fulminant) liver failure, and chronic presentations from steatosis and chronic hepatitis to compensated and decompensated cirrhosis. Several clinical features are relevant to all hepatic presentations and should immediately raise the suspicion of WD (for details see Table 4 and (Shribman et al., 2019).

1.4.2 Neurologic presentation

In most publications from gastroenterology groups, the neurological symptoms and their frequencies and also their importance may differ from original terminology in the neurology field and that causes discrepancies and controversies.

The majority of neurologic presentations consist of a movement disorder associated with bulbar symptoms. The movement disorder is usually characterized by tremor, dystonia, or parkinsonism. These ‘core’ movement disorders often occur in combination and may initially be subtle. Bulbar symptoms consist of dysarthria, drooling and/or dysphagia. There are a range of additional neurologic features, including cerebellar dysfunction, chorea, hyperreflexia, seizures and cognitive impairment, which can also co-exist, in addition to psychiatric features

The presence of bulbar symptoms, a mixed movement disorder, cognitive impairment, and associated psychiatric features are therefore all red flags for WD (Table 4) (Shribman et al., 2019, Ortiz et al., 2020).

Table 4: A summary of the common hepatic and neurologic presentations and associated red flags of WD (Shribman et al., 2019, Ortiz et al., 2020)

Presentation	Red flags	
	History and Examination Examination	Investigation
Related to Gastrologic and hepatologic field		
Acute liver failure Cirrhosis Compensated Decompensated Chronic hepatitis Steatosis	Previous episodes of jaundice Family history of unexplained liver disease Splenomegaly	Jaundice (Bilirubin) High AST to ALT ratio (Aspartate aminotransferase, Alanine aminotransferase.) Low alkaline phosphatase Unexplained anemia (hemolysis) Low platelets Abnormalities in liver biopsy
Related to neurologic/psychiatric field		
Tremor Dystonia Parkinsonism	Dysarthria Drooling Changes in handwriting Poor scholastic performance Executive dysfunction Behavioral or personality changes Family history of unexplained neurologic symptoms	MRI brain abnormalities Kayser-Fleischer ring
Common first-line screening examinations of WD in adults: (Mulroy et al., 2021) Liver enzymes, Serum copper, caeruloplasmin, and Urinary copper. Slit-lamp examination Wilson's disease		
Core movement disorders	Bulbar symptoms	Additional neurologic features
Tremor Dystonia Parkinsonism	Dysarthria Drooling Dysphagia	Cerebellar dysfunction Chorea Hyperreflexia Seizures Cognitive impairment

1.4.3 Classification and form of progression by clinical dominating symptoms

Wilson's disease is characterized by various manifestations as a result of numerous qualitative and quantitative symptoms, which emerge at different times (Hermann, 2019). But there is no solid classification for symptom progression in long-term and forms of WD regarding the course of disease (Table 5). The terminology used in Table 5 (as pseudoparkinsonism or pseudosclerotic) nicely illustrates the above-mentioned discrepancy when gastroenterologists classify neurological symptoms.

Table 5: Classification of Wilson's disease by clinical dominating symptoms (Hermann, 2019)

Form of progression (clinical variant)	Key clinical symptoms
Non-neurological forms of progression	
Preclinical (asymptomatic)	None, diagnosis prior to the onset of symptoms
Hepatic (abdominal)	Acute and chronic decompensated liver disease; cirrhosis of the liver
Neurological forms of progression	
Pseudoparkinsonism	Bradykinesia/hypokinesia, rigidity, hypomimia, minor resting tremor, postural/intention tremor, dysarthria, hypersalivation
Pseudosclerotic	Postural, flapping and intention tremor, cerebellar ataxia, acroataxia, dysarthria (scanning)
Mixed form (arrhythmic-hyperkinetic)	Choreatic, athetotic, and torsion dystonia hyperkinesia, in part Parkinson's disease
Psychiatric form of progression	

1.5 Comorbidities of Wilson's disease

So many different comorbidities and co-occurrences with Wilson's disease were observed and reported in studies, this can be seen mostly in patients with long duration of follow-up. In general it is easy to distinguish WD and a comorbidity when the onset of symptoms can clearly be distinguished. If WD and comorbidity cause similar symptoms and become clinically manifest around the same time as will be demonstrated for WD and Multiple Sclerosis or WD and psychosis diagnosis of both disease entities may become really difficult.

1.5.1 Psychiatric comorbidities

A high frequency of psychiatric disorders was confirmed in WD that may both present as psychic disturbances in previously asymptomatic individuals, and as psychiatric symptomatology in the

course of the disease. A pure psychiatric presentation is rare, confounding, and often characterized by mild psychic disturbances, such as poor scholastic performance, or changes in behaviors. More frequently, some neurological signs can be traced together with psychiatric disorders.

Psychiatric comorbidity in WD is characterized by a high prevalence of mood disorders, and particularly interesting is the association with bipolar spectrum disorders. Such an association explained the frequent report of psychopathological features like mood swings, irritability, over-activity, and aggressiveness (Mura et al., 2017). Table 6 summarizes reported psychiatric comorbidities for WD patients in the literature.

Table 6: psychiatric comorbidities in WD (Mura et al., 2017)

depression, mania, psychosis, adjustment disorder, personality disorder/suicide attempt, catatonia, obsessive-compulsive disorder, anxiety, attention deficit hyperactivity disorder (ADHD), conduct disorder, bipolar disorder, irritability/suicidal ideation, schizophrenia-like psychosis, change in mood/aggressive behavior, behavior disorder, phobias, delusional disorder, alcohol abuse

1.6 Neurologic comorbidities

In larger series of WD patients very rare neurological comorbidities of the central and the peripheral nervous systems have been observed such as Multiple Sclerosis (MS) and Polyneuropathy (PNP), respectively.

1.6.1 Multiple sclerosis and Wilson's disease

The occurrence of both MS and WD is rare, and it has previously been described in three patients. Although WD and MS are very different disease entities, there is some overlap in the symptoms, the age of onset as well as medical findings. This can explain the common historical background and the differential diagnostic difficulties that sometimes arise in differentiating between the two clinical pictures.

Case 1: a 38-year-old male patient was diagnosed with a hepatic form of WD at age 12 and with MS at age 38. Intravenous radio copper test confirmed the diagnosis of WD, MS was diagnosed after the occurrence of transient sensory disturbances in both legs, based on typical changes on MRI over the course of 2 years, in the cerebrospinal fluid and in multimodal evoked potentials (Günther et al., 2010).

Case 2 and 3: one female and one male were diagnosed with WD and MS. In both cases, the diagnosis of MS preceded the diagnosis of WD. Both patients exhibited neurological signs typical of MS (internuclear palsy, optic neuritis). The first one displayed the typical sign of WD (hypomimia, sialorrhea, behavioral changes). The second patient had liver injury without any neurological symptoms. In both cases, the clinical course of MS was very mild (17 and 12 years of observation) which was explained by the possible immunosuppressive effect of free copper (Dzieżyc et al., 2014).

1.6.2 Sensory impairment and Wilson's disease

Although there are so many complaints of hypoesthesia, loss of sensation, and neuropathic pain in patients with WD, there is no case report of polyneuropathy (PNP). There is one report on impaired cold/warm sensation and possible C-fiber damage (von Giesen et al., 2003). Furthermore, there is only one case series describing thirteen patients with WD clearly fulfilled the five diagnostic criteria of Restless Leg Syndrome (RLS); in eight of them, the burden of RLS was clinically significant. The RLS was of moderate severity, equally distributed among sexes, manifested mainly in the evening and before falling asleep, and had developed mostly after clinical manifestation of WD, still at a young mean age. The known RLS-associated features were absent (normal iron and kidney parameters) or rare (positive family history, polyneuropathy). Compared to WD patients without RLS, patients with RLS were significantly elder and had suffered longer from WD. WD-specific RLS mimics as well as RLS confounding motor comorbidities (dystonia, tremor, chorea) were frequent and a diagnostic challenge; in difficult cases, the differentiation was reached by clinical observation of the motor behavior in the evening or at nighttime. In this study, one RLS patient had responded to L-dopa, which had been administered to treat tremor years before; all the other patients were treatment naïve as regarding RLS. It is suggested that patients with neurological manifestations of WD are examined for the presence of RLS, and if RLS is confirmed and deemed to be of clinical significance, dopaminergic treatment is certainly an option. (Trindade et al., 2017).

1.7 Treatment and long-term management of Wilson's disease

1.7.1 Current and future treatment

The long-term management of WD can be divided into an initial phase and a maintenance phase (Table 7). Patients with prevalent hepatic manifestations are almost always started on chelating agents (penicillamine or trientine) in the initial phase, with the goal of achieving negative copper balance (Leung et al., 2021, Hedera, 2019).

For most cases with acute liver failure related to WD, liver transplantation is the best option. The initial anti-copper agent for neurological manifestations is controversial. Zinc salts may be considered in asymptomatic patients, but as soon as neurological symptoms appear chelator therapy with D-Penicillamine (DPA) or Trientine (TRI) has to be initiated. In the future, bis-choline tetrathiomolybdate (TTM) with the ability to cross BBB could become the first-line agent for patients with predominantly neurological phenotypes of WD (Fig. 3) (Weiss et al., 2017), however the side-effect profile of TTM remains to be elaborated.

The lifelong maintenance phase attempts to preserve copper balance without resulting in copper deficiency. This is usually achieved by providing a low dose of the chelating agent and/or zinc. It is important to remember that in case of neurological symptoms probably because of the Blood-Brain Barrier (BBB) higher doses should be administered (Hefter et al., 1993). There is limited research and knowledge regarding the timing and effective methods of transitioning between the various agents (Leung et al., 2020). Current clinical practice includes frequent monitoring of liver enzymes and 24-hour urine copper for a few months after the switch to other therapy.

Table 7: Current medications for Wilson disease (Mulligan and Bronstein, 2020)

Drug	Mechanism	Dosing	Side Effects	Potency
D-Penicillamine	Chelates copper and promotes urinary excretion	Titrated up to 1 g/d, divided into 2–4 doses Must be taken 30 min before meals or 2 h after meals	Fever, rash, anemia, bone marrow suppression, lymphadenopathy, lupus-like syndrome, worsening neurologic symptoms	Very effective at attaining negative copper balance, 5–10 mg/d in early therapy
Trientine	Chelates copper and promotes urinary excretion	Titrated up to 1 g/d, divided into 2–4 doses Must be taken 30 min before meals or 2 h after meals	Proteinuria, bone marrow suppression, Autoimmune reactions, worsening neurologic symptoms	Less effective attaining negative copper balance, 2–3 mg/d in early therapy
Zinc salts	Induces metallothionine in gastrointestinal	50 mg TID Must be taken at least 1 h before or after meals	Gastrointestinal upset	Weak

	epithelium and inhibits copper absorption			
Bischolinetetra-thiomolybdate (Under phase III clinical trial)	Complexes free copper with albumin	15 mg/d	Hypertransaminasemia, bone marrow suppression spectrum of side-effects has to be determined	
TID= 3 times a day mg/d= milligram/ day g/d= gram/ day				

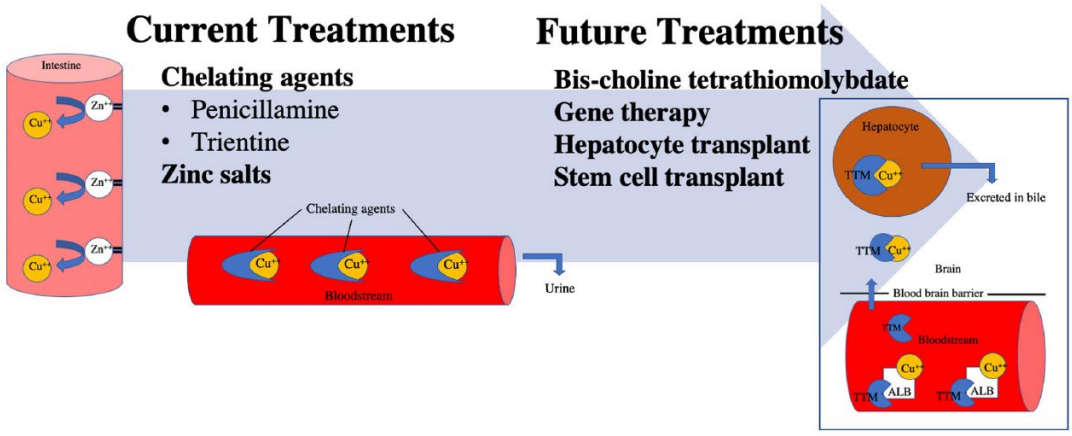


Fig. 3: Current and future treatment; (Leung et al., 2021) is open access in PMC and available under a Creative Commons or similar license that allows more liberal redistribution and reuse than a traditional copyrighted work.

Patients with WD must remain on lifelong maintenance therapy, which is also a challenge due to potential adverse effects of anti-copper agents, drug interactions with other medications, and financial constraints (Leung et al., 2021). For example, massive proteinuria was seen in some cases secondary to treatment with D-penicillamine (Borrego García et al., 2020).

1.7.2 Treatment of Neurological Symptoms with Botulinum Neurotoxin

Although dystonia is a frequent symptom in WD surprisingly few patients have been reported who are treated with botulinum neurotoxin (BoNT). Interestingly, a major interaction between the light chain of the BoNT molecule and copper has been described (see below).

The action of BoNT/A results from the binding of the heavy chain to a cell membrane, endocytosis, and translocation of the light chain (LC) into the cytosol of the cell. There, the LC zinc

metalloprotease cleaves synaptosomal-associated protein 25 (SNAP-25), a component of the soluble NSF attachment protein receptor (SNARE) complex responsible for docking vesicles at the presynaptic membrane (Pirazzini et al., 2013, Pirazzini et al., 2015, Schiavo et al., 1992). Therefore, the majority of anti-BoNT/A compounds target LC because of their key role in exocytosis. However, as long as such substances act only extracellularly as antibodies, for example, the enzymatic activity of the LC cannot be stopped. This seems to be different for metal complexes, providing the rationale for the development of a novel class of LC inhibitors. Copper and mercury are highly significant LC inhibitors. A key binding interaction between copper and Cys165 in the BoNT/A LC has been analyzed and described. Extracellularly applied ligand–copper complexes at low concentrations effectively reduce intracellular LC cleavage of SNAP-25. Administration of copper complexes in life-threatening BoNT/A-treated rodents effectively delayed BoNT/A mediated lethality (Bremer et al., 2017).

Both cell-based and animal experiments suggest that the efficacy of botulinum toxin treatment may be reduced in patients with Wilson's disease, who suffer from deficient copper transport and elevated copper storage. In Wilson's disease, the biliary copper excretion from the liver into the stool is disturbed, leading to a copper overload primarily of the liver and secondarily of the entire organism (Bandmann et al., 2015). BoNT/A has been suggested for symptomatic treatment in WD (Litwin et al., 2017, Liver, 2012, Roberts and Schilsky, 2008, Hölscher et al., 2010). However, reports on the symptomatic treatment in WD with botulinum neurotoxin are rare. One possibility might be that BoNT/A is not used by gastroenterologists who mainly treat patients with WD. Whether copper reduces the effect of BoNT/A on symptoms of WD has been discussed by our team (Hefter and Samadzadeh, 2021).

Table 8 provides an overview of the cases presented in the literature to date. Unfortunately, no doses are presented, with only one exception. Teive et al. reported that 35 U was injected per lateral pterygoid and 30 U in the submentalis muscle complex (Teive et al., 2012). Based on our experience, this is not an unusually high dose. Patients responded with a mild-to-moderate improvement, which is within the range of responses reported in the literature for this difficult-to-treat type of dystonia (Hassell and Charles, 2020). For the patient with hand dystonia, it is only reported that a series of injections with aboBoNT/A had been performed and that the hand recovered completely. In this case, the effect of BoNT/A cannot be distinguished from the effect of withdrawal of the dystonia-inducing medication.

Table 8: Case reports in the literature.

Parameter	(Joan et al., 2008)	(Litwin et al., 2013)	(Hölscher et al., 2010)	(Teive et al., 2012)
N	4	1	3	5
Severity of WD	Severe dystonia, 3 patients to be transplanted	Hand dystonia induced by Antidepressants	2 with dystonia, 1 with tremor	General dystonia
Indication	Pain Limb dystonia	Hand dystonia	Dystonia Tremor	Jaw opening dystonia
Preparation	n.m.	aboBoNT/A	n.m.	onaBoNT/A
Dose	n.m.	n.m.	n.m.	100 U
Efficacy	some functional recovery	complete normalization	n.m.	Mild to moderate improvement
Side effects	n.m.	n.m.	n.m.	3/5 mild dysphagia
n.m. = not mentioned				

1.8 Aim of this study

The adverse impact of WD on quality and expectancy of life has been minimized by introduction of the copper chelating therapy around 1960 about 60 years ago (Walshe, 1956). In 1990, research revealed that WD patients may have a normal life expectancy as long as they are compliant to take the copper chelating agents (Stremmel et al., 1991). And now that even more than 30 years have passed, there is still little information on the long-term survival of WD patients and on WD symptoms' resistance to treatment.

Furthermore, among abundant literature reviewed about the treatment of WD and its progression, there is still a lack of long-term quantitative and semiquantitative studies.

We benefit from retrospective cohort data on the outcome of 115 long-term treated WD whose neurological symptoms have been semi-quantitatively characterized using a scoring system.

The analysis of outcome is split up into the following 7 questions:

1) Do neurological symptoms in WD improve with therapy?

2) Do all symptoms respond equally to therapy?

3) Does age have an influence on the spectrum of symptoms in long-term treated WD?

4) Do subgroups of WD patients exist with a worse outcome than the rest of the patients?

5) What is the spectrum of comorbidities in WD and what is their influence on the outcome?

6) Do special symptoms and/or comorbidities need specific treatment?

7) Do patients with WD have normal life expectancy under standard copper chelating therapy?

2 Patients and methods

2.1 General aspects

The present study is a retrospective longitudinal (cohort) analysis of clinical data documented in the charts of the patients. The permission of the ethics committee of the University of Düsseldorf is obtained for this dissertation, which allows the retrospective analysis of clinical data of Wilson patients. (Studiennummer:2018-20-Retrospektive Datenerhebung und -analyse Retrospektive Analyse der klinischen Verläufe von Wilsonpatienten)

2.2 Patients

In the outpatient department of the University hospital in Düsseldorf (Germany), patients with neurological Wilson´s disease from all parts of Germany are seen on a regular basis every 3 to 12 months. For the present dissertation, demographical and clinical parameters of 115 WD patients are collected retrospectively.

2.3 Neurological examination and clinical scoring system

At each visit of the outpatient department of the University hospital in Düsseldorf, patients underwent a detailed clinical neurological investigation. Seven motor items (dystonia, dysarthria, bradykinesia (reduced frequency of alternating finger movements or alternating tongue movements), tremor, gait disturbance, oculomotor deficits, cerebellar abnormalities (during the finger/nose test or the knee/heel test or rebound testing)), as well as three non-motor abnormalities (reflex abnormalities, sensory abnormalities, neuropsychological and psychiatric abnormalities such as anxiety, depression, hallucinations and cognitive impairment), are scored whether these abnormalities are absent (0) or only mildly (1), moderately (2) or severely (3) present. The motor sub-scores are summed up to yield a Motor Score (MotS: 0 – 21), the three non-motor sub-scores are summed up to a Non-Motor Score (N-MotS: 0 – 9) and the sum of MotS and N-MotS yields the Total Score (TS: 0 – 30) (Table 9). These scores have been used in our department to monitor therapy in WD for more than 25 years now. The purpose of this score is to characterize and compare degrees of symptom severity in long-term treated WD patients. It will also be investigated whether changes can be detected at short or long periods of therapy.

Table 9: Scoring system description

Motor Score (MotS)	
-dystonia	-Dystonia describes a neurological condition characterized by involuntary sustained or intermittent muscle contractions producing repetitive movements and abnormal postures.
-dysarthria	-Dysarthria is a motor speech disorder characterized by imprecise, slow, weak, and uncoordinated speech.
-bradykinesia	-Bradykinesia means slowness of voluntary movements of fingers or hands.
-tremor	-Tremor is defined as an involuntary, rhythmic, oscillatory movement of a body part.
-gait disturbance	-Gait disturbances (namely ataxia) describe signs and symptoms resulting from cerebellar dysfunction, the affectation of cerebellar pathways, or corticospinal pathways (spasticity).
-oculomotor deficits	-Oculomotor disturbances include gaze impairment, or nystagmus (periodic, mostly involuntary eye movements)
-cerebellar abnormalities	-Dysmetria and ataxia of extremities are rated including finger/nose and heel/shin test.
Non-Motor score (N-MotS)	
-reflex abnormalities	- Abnormal responses (elevated or reduced) of muscle reflexes and presence or absence of pyramidal tract signs
-sensory abnormalities	-Abnormal sensations
-neuropsychological and psychiatric abnormalities	-any psychiatric symptom which deserves treatment: depressive mood, psychosis, mania, impulse-control disorder etc.…
Total score (TS)	
Motor Score (MotS) = Range from 0 to 21 Non-Motor score (N-MotS) = Range from 0 to 9 Total score (TS) = Range from 0 – 30	

2.4 Laboratory findings

After clinical investigation and scoring of symptoms and discussion of adjustments of medication and treatment of special symptoms, blood samples were taken for routine monitoring of copper elimination therapy in WD. The following parameters were selected: liver enzymes (GOT, GPT, GGT), parameters of copper metabolism (serum level of copper, serum level of ceruloplasmin, copper concentration in the 24h-urine), Quick´s test, thromboplastin time (PPT), platelet counts and

creatinine. At the previous visit, it was discussed with the patient whether the next 24h-urine had to be collected with or without medication. In case that the treating physician was interested in analyzing the remaining copper over-load, the patient had to discontinue the medication for 3 days and during the day of collection. Usually, the copper content of the 24h-urine collected under medication was analyzed. The determination of all laboratory parameters was carried out in the laboratory of the University Clinic Düsseldorf.

2.5 Optical Coherence Tomography

Optical coherence tomography (OCT) is a fast and non-invasive technique and the latest generation of OCT devices is capable of depicting retinal changes at nearly the cellular level (Saidha et al., 2011, Albrecht et al., 2012b, Lambe et al., 2020).

We used a Spectralis OCT device (Heidelberg Engineering, Heidelberg, Germany) with image alignment eye tracking software (TruTrack, Heidelberg Engineering Heidelberg, Germany) for only some patients (Twins and WD+MS). We obtained perifoveal volumetric retinal scans consisting of 25 single vertical axial scans (scanning area: 6x6 mm^2, centered at the fovea). To assess the peripapillary retinal nerve fiber layer (pRNFL), a circular scan with a diameter of approximately 3.4 mm was performed after manually positioning the center on the middle of the optic disc. Furthermore, we performed high-resolution horizontal scans through the middle of the fovea. All of the scans were performed using the eye-tracking system. The RNFL measurements and high-resolution single horizontal scans were averaged from 100 images and measurements for volumetric calculations were averaged from 10 scans (Automatic Real-Time, ART). All of the scans were of sufficient or good quality (>20 DB) and fulfilled OSCAR-IB quality control criteria for OCT scans (Tewarie et al., 2012). While the results of the RNFL- and paramacular volumetric measurements were automatically analyzed by the Heidelberg Eye Explorer software, the paramacular retinal layers were manually segmented (Albrecht et al., 2012a).

2.6 Treatment of WD: Wilson specific medication, treatment of special symptoms and comorbidities

After the clinical investigation, laboratory findings of the previous visit were discussed with the patient. If a patient presented with high 24h-urinary copper excretion, the copper chelating medication was increased. Also, when the cholinesterase (CHE) revealed a systematic decline over 3 investigations the dose was increased. In the case of normal copper excretion after a medication

break for 3 days, the dose of copper chelating drugs was carefully reduced. In case of adverse reactions, medication was switched. Switches of medication were extracted from the charts.

Patients with severe disabling symptoms (Table 8) were treated with botulinum neurotoxin (BoNT). Injection scheme of BoNT-therapy (with onaBoNT/A (Botox®), aboBoNT/A (Dysport®), and incoBoNT/A (Xeomin®) were extracted from charts. Details of BoNT treatment are presented in the result section.

2.7 Statistical analysis

All statistical calculations in this study are conducted in RStudio (version 1.4.11)[1], and considering that R software is modular and package dependent, for some specific calculations or graphing particular packages are used. All graphs are made by "ggplot2" (version 3.3). survival analysis (Kaplan Meier curve) regarding death event or HCC manifestation is done by "survival package" (version 3.2). Either linear or polynomial regression models regarding clinical score variation with years from diagnosis are simulated by "basic stats package", and stepwise regression for the interdependence of clinical score benefits from "Olsrr package" (version 0.5.3). Other simple statistical methods such as correlation (Pearson or Spearman) and mean comparison (Student's t-test or Wilcoxon-Mann-Whitney-Test) are conducted by means of "stats package" (version 4.0.5) and are mentioned in context. Finally, the p-value <0.05 is considered statistically significant.

[1] https://www.rstudio.com/

3 Results

In this chapter, the demographical characteristics and treatments duration is reported which provide a proper outline of the population under study, and afterward, the trends associated with the progression of disease with regards to treatment years are investigated. Finally, the rare special cases with comorbidities are described which are very notable case reports.

3.1 Characteristics of the study population

3.1.1 Demographical characteristics

In the outpatient clinic of the University hospital in Düsseldorf, patients with neurological Wilson´s disease from all parts of Germany are seen, and in this retrospective study, this valuable data accumulated during a long duration of follow-up, decades of visits, is exploited.

After data preparation, cleaning, and aggregation, a total of 115 WD patients were included in the dissertation including 47 males and 68 females respectively. As the duration of treatment is considerable, the age structure of the population under study varies considerably, and therefore, the age parameter is always reported as age at a definite time, for example, age at the first visit or even diagnosis. As summarized in Table 10, the mean values of age at diagnosis and final visits are considerably different and equal to 22.6 and 43.2 respectively. The treatment duration will further stress this fact.

Among many WD patients from almost the entire Germany, there are few special patients that have rare comorbidities and therefore they are worth mentioning. These invaluable cases included comorbidities such as multiple sclerosis, orthopedic and psychiatric conditions which are reported in the comorbidities chapter.

Table 10: Demographical characteristics of the population under study

Sex Distribution	47 males	68 females
	Mean Value	Standard deviation
body height (cm)	176.12	10.97
body weight (kg)	74.62	15.07
age at diagnosis (years)	22.61	10.79
age at final visit (years)	43.22	12.61
cm = centimeter, kg = kilogram		

3.1.2 Duration and scheme of treatment and follow-up visits

As the duration of treatment is considerable, the related parameters and follow-ups require a little clarification. First of all, age at diagnosis indicates the age at which WD symptoms manifest themselves and the disease is diagnosed. It is worth mentioning that the data from diagnosis up to now is not necessarily available for all patients in our database.

Another parameter is the age at the final visit, it is actually the most recent age state of our population under study, and if we subtract from age at diagnosis, the result is treatment duration whose data is mostly available.

Fig. 4 shows the age distribution and histogram associated with 115 WD patients. The vertical line on the histograms are mean values reported in Table 10

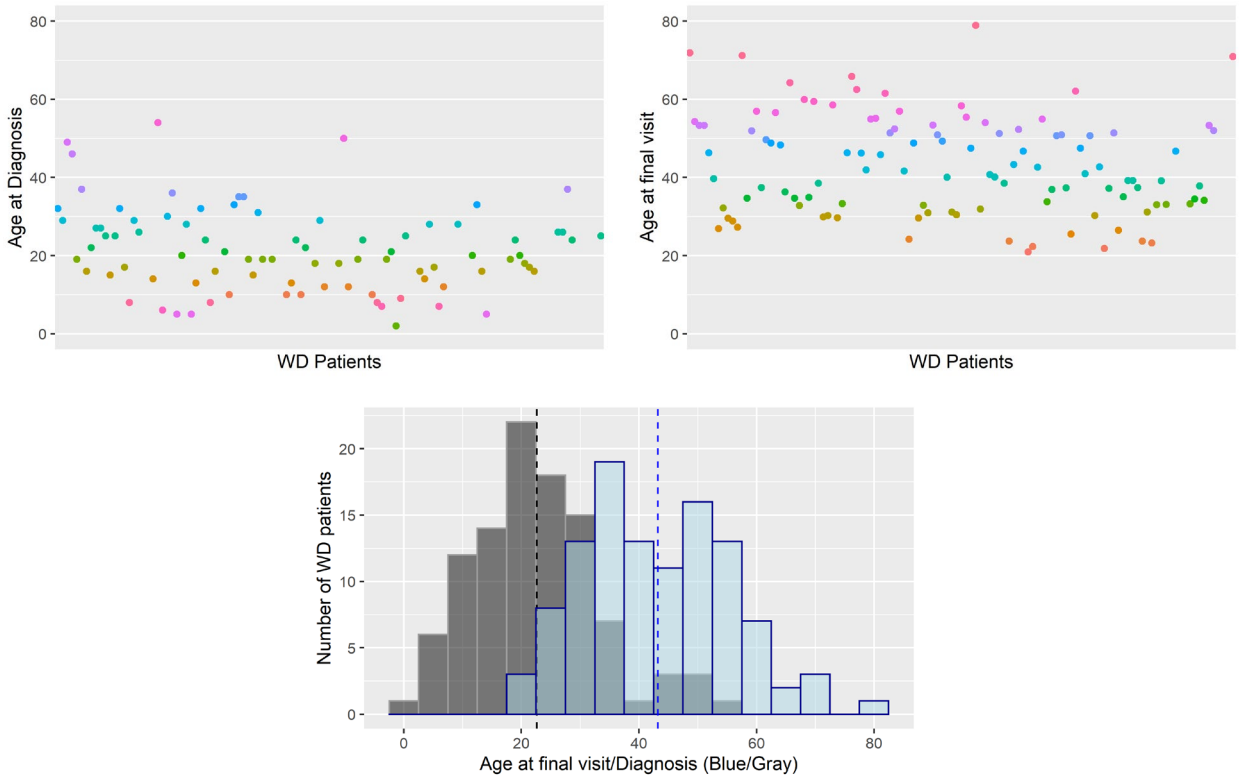


Fig. 4: Age distribution (scatterplots above) and histogram (below) at final visit and diagnosis.

Another parameter to consider is the number of visit sessions available for each patient. As follow-up visits are on a regular basis, therefore analyzing longitudinal variation and progression of the disease is possible. The histogram depicted in Fig. 5 shows the frequency of sessions in our dataset with a mean of 5.5 sessions.

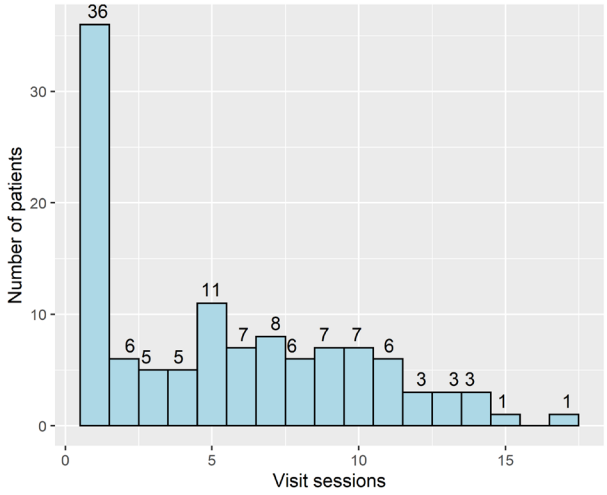


Fig. 5: Histogram of visit sessions

And Fig. 6 shows the distribution of consecutive visits available for each patient. The distribution of visits' dates is colored by patients' ID and the sequence of visits regarding the patients' age as well.

Fig. 6: Distribution of follow-up visits colored by patients' ID (left) and regarding patients' age (right)

Finally, the last related parameter to treatment is duration which is depicted in Fig. 7. The mean value for duration is 26.8 years.

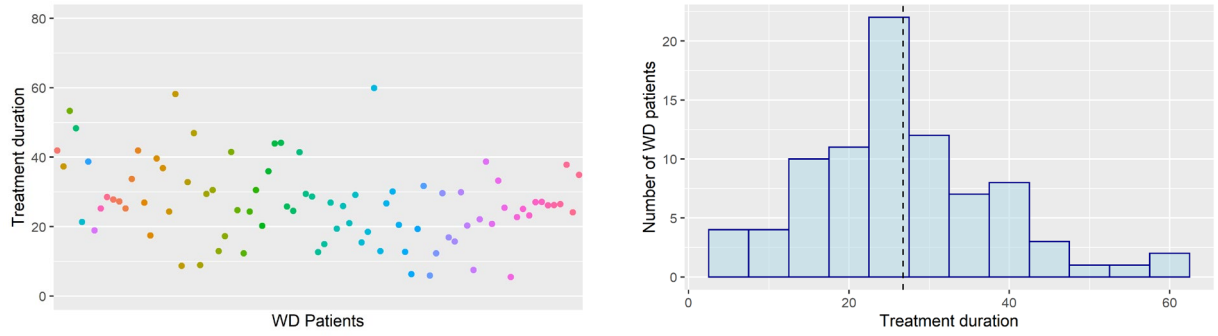


Fig. 7: Treatment duration colored by patients' ID (left) and histogram (right)

Over decades DPA was the only licensed copper chelating medication for WD. This is the reason why most of our WD patients started their therapy with DPA and why most of our patients remained on DPA (Table 11).

In other centers, zinc is used to initiate treatment in WD. It may be that zinc can prevent clinical manifestation in asymptomatic patient and maintain the level of impairment in mildly affected patients. But to our experience, it may happen that asymptomatic patients develop neurological symptoms under zinc monotherapy with even high doses of zinc (Hartmann and Hefter, 2014). Therefore, zinc monotherapy is used only in very exceptional cases in our center (section 3.4.3). Many patients used zinc as an additional medication to DPA (Table 11). In that case intake of medication becomes difficult because zinc has to be taken before the meals and not together with a chelating drug.

Trientine was used as a second-line treatment for decades. In case of adverse reactions to DPA, patients were switched to DPA. In difficult to treat patients as patients with mental retardation Trientine was even used as first line treatment (Table 11). A switch from Trientine to Cuprior or Metalite (Trientine) became necessary when the product of the UniVar® company was not available. In some cases, a switch to DPA became necessary.

About 4 years ago Cuprior® was licensed for the treatment of WD without a license study. By law, this new license situation implied a switch of all patients from Trientine® to Cuprior®. The higher potency of Cuprior® and missing comparison study provided WD patients and treating physicians a hard time. Some patients succeeded to stay on Trientine®.

About 2 years later Trientine was licensed under the name CuFence® and most patients were switched back from Cuprior® to CuFence®.

In general, we avoid switching of medication since we are interested in high compliance of the WD patients. Switching of preparation is a deviation from routine treatment and always irritates the patients.

Table 11: WD treatment scheme of 115 patients

Mono therapy	88	Combination therapy	15
Trientine Hydrochloride	22	Trientine Hydrochloride +Zinc	7
D-penicillamine	65	D-penicillamine + Zinc	8
Zinc	2	No medication / N/A	2/9
One time Switch		More than one Switch	7
Trientine Hydrochloride -> D-penicillamine	2	D-penicillamine -> Trientine Hydrochloride -> Cufence	1
D-penicillamine -> Trientine Hydrochloride	5	Zink + D-penicillamine -> Trientine Hydrochloride ->Cufence	1
Trientine Hydrochloride -> Cuprior	7	Trientine Hydrochloride -> Cuprior -> Cufence	2
Trientine Hydrochloride -> WTX101(Tetrathiomolybdate)	1	D-penicillamine -> Cuprior ->Cufence	1
D-penicillamine ->Cufence	1	Trientine Hydrochloride -> D-penicillamine -> Trientine Hydrochloride -> Cufence	1
D-penicillamine -> Cuprior	2	Trientine Hydrochloride + Zinc -> Cuprior + Zinc	1
Side effects: Two patients with nephrotic Syndrome under D-penicillamine, one switched to Cuprior, the other one switched to Trientine Hydrochloride Medication break: Two patient Trientine (Cuprior®)/ Trientine (Cufence®) N/A=Not applicable			

3.2 Progression of Clinical scores

3.2.1 The frequency of neurological symptom occurrence

The neurological symptoms associated with clinical scores are reported in Table 12. It reports the presence of symptoms regardless of their severity for both initial and final available visits as well as its occurrence during follow-ups. Although, there are some differences between reported percentages representing ratios of symptom-positive to symptom-negative, however closer investigation in the next section does not lead to a statistical significance difference.

Table 12: The frequency of neurotological symptom occurrence in all patients with more than one session (79 patients)

Symptom	At first available visit			During follow-up			At final visit		
	positive	negative	percent	positive	negative	percent	positive	negative	percent
Bradykinesia	55	19	70%	67	6	84%	48	24	61%
Dystonia	34	40	43%	45	28	60%	30	42	38%
Tremor	30	44	38%	44	29	56%	22	50	28%
Neuropsychological and psychiatric abnormalities	28	46	35%	42	31	53%	20	52	25%
Gait disturbance	27	47	34%	40	33	51%	19	53	24%
Dysarthria	25	49	32%	37	36	47%	17	55	22%
Cerebellar abnormalities	20	54	25%	37	36	47%	17	55	22%
Reflex abnormalities	14	60	18%	37	36	47%	17	55	22%
Oculomotor deficits	13	61	16%	31	42	39%	17	55	22%
Sensory abnormalities	5	69	6%	24	49	30%	11	61	14%

3.2.2 Severity of clinical score at first visit versus final visit (non-motor /motor and total score)

In order to investigate differences between first and final visits, the associated clinical scores are plotted in Fig. 8. The data points are jittered and overlaid by boxplot to make it more discernible. However, mean comparisons by t-tests do not reveal any significant differences except for tremor symptom whose mean reduction is statistically significant; $t(132)=2.36$, $p=0.02$, 95% CI[0.04 0.4].

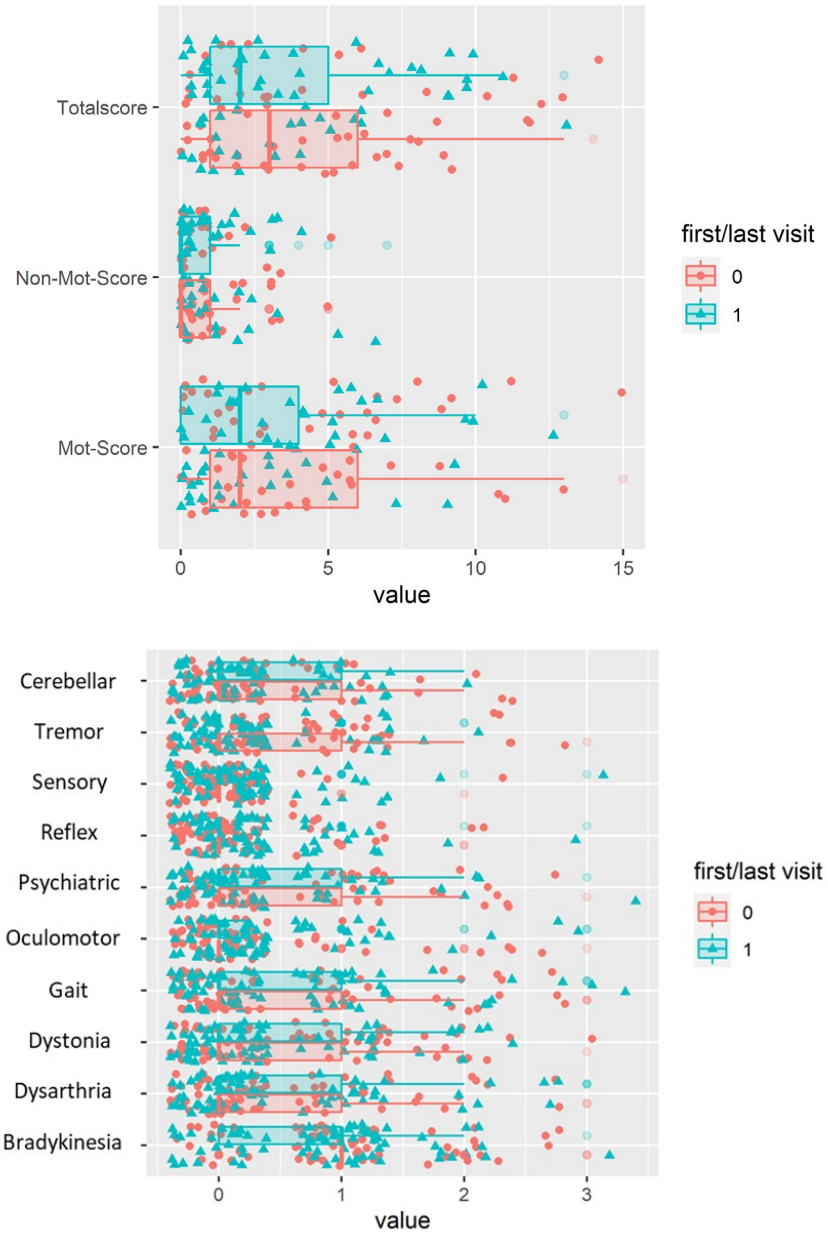


Fig. 8: Distribution of clinical score at first and last visits. Summation scores (above) and individual scores (below)

3.2.3 The spectrum of symptoms according to age groups

Considering the fact that accumulated data belongs to patients with varying degrees of disease progression and periods from disease manifestation, therefore it is more appropriate to categorize them into groups based on years from diagnosis. Six groups of 0 to 2, 2 to 7, 7 to 12, 12 to 19, 19 to 25 and 25 to 56 (maximum) were considered. Each group has at least 30 observations, and the first group only has 2 years period to resolve initial variations better whereas others cover 5 to 7 years, however, the last group includes 25 years and more, holding the rest of the data.

This noble categorization made the visualization and interpretation much easier. Fig. 9 depicts means and its confidence intervals for different scores based on the mentioned classification. An initial look at the variation of the total, motor and non-motor scores reveals that firstly, the variation of score over time is not a linear function of time but rather polynomial. Secondly, since the magnitude of the non-motor score is negligible in comparison with the motor score, therefore total score which is the summation follows closely the motor score.

By studying other figures, it becomes evident that polynomial relation seems to be a rule rather than an exception, and interestingly statical inference confirms it. Every score shows polynomial relation in this grouping setup except for the oculomotor score which shows almost no variation at all.

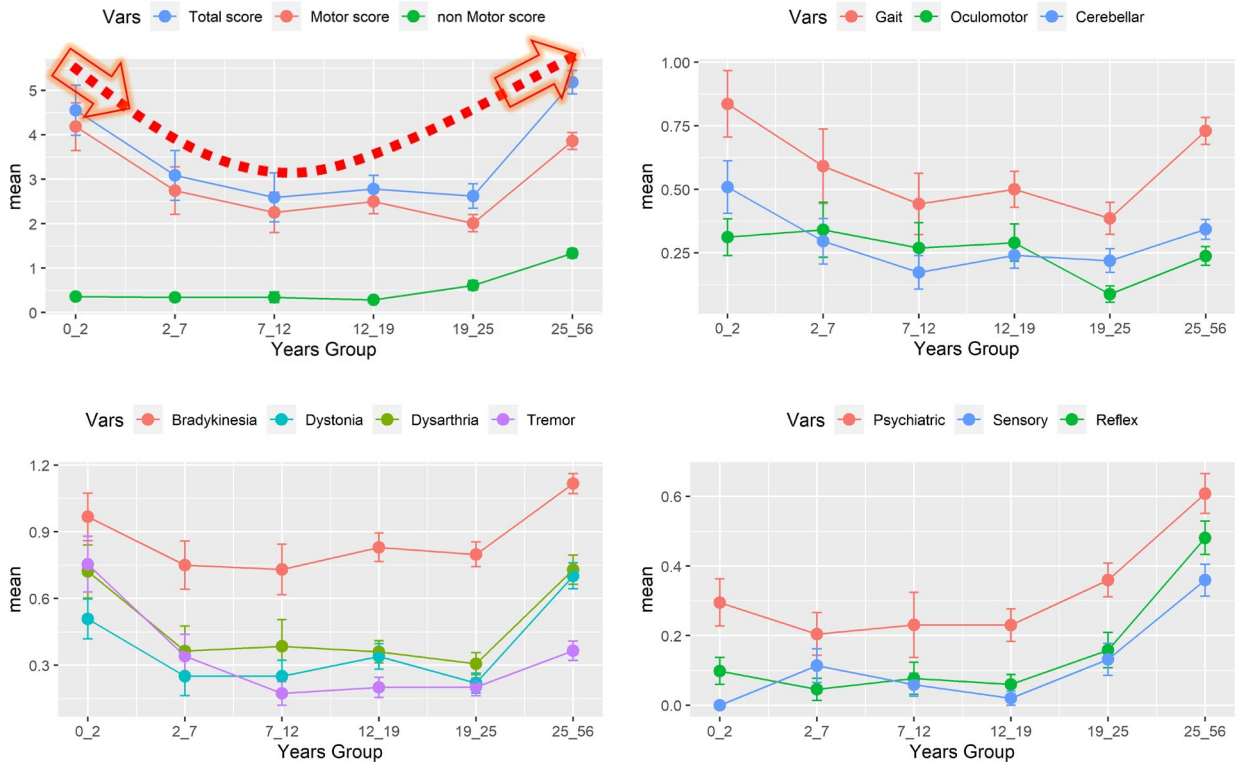


Fig. 9: Mean variation of clinical scores according to proposed year groups. year groups are ordinal numbers from one to six that represents increasing intervals of treatment from diagnosis. Summation scores (above left); gait, oculomotor and cerebellar scores (above right); bradykinesia, dystonia, dysarthria and tremor scores (below left); psychiatric, sensory, and reflex scores (below right)

The statistical result of the polynomial model ($Y=c+b*x+a*x^2$) is reported in Table 13. The year groups are actually ordinal numbers from one to six that represents increasing intervals of treatment from diagnosis, and the associated p-values for x = year-groups and x^2 = year-groups squared test whether time elapsed from treatment, which is expressed in terms of interval and squared interval,

has a meaningful contribution to the model or not. And according to the result, it is statically related to changes in the response variable.

It asserts that the trend that scores decrease initially reaching a minimum and increasing afterward. This is maybe due to the fact that the treatment may promote the patients' status initially reaching a stabilized condition and afterward aging or other contributing factors exacerbate the general status.

Table 13: Clinical score as a polynomial model of year groups (regression analysis between scores as response variables (Y) and year groups as predictors)

Y=	Intercept +				Year Groups +				Year Groups * Year Groups			
	Est.	Std. Er	t val.	P val.	Est.	Std. Er	t val.	P val.	Est.	Std. Er	t val.	P val.
Dysarthria	1.14	0.16	7.02	0.00	-0.49	0.10	-4.99	0.00	0.07	0.01	5.33	0.00
Dystonia	0.87	0.14	6.25	0.00	-0.41	0.08	-4.90	0.00	0.06	0.01	5.65	0.00
Bradykinesia	1.22	0.14	8.49	0.00	-0.31	0.09	-3.54	0.00	0.05	0.01	4.17	0.00
Tremor	1.15	0.12	9.25	0.00	-0.49	0.07	-6.51	0.00	0.06	0.01	6.02	0.00
Gait	1.23	0.17	7.33	0.00	-0.43	0.10	-4.31	0.00	0.06	0.01	4.33	0.00
Oculomotor	0.42	0.12	3.39	0.00	-0.07	0.07	-1.00	0.32	0.01	0.01	0.66	0.51
Cerebellar	0.73	0.12	6.14	0.00	-0.27	0.07	-3.80	0.00	0.03	0.01	3.66	0.00
Motor score	6.17	0.62	9.95	0.00	-2.26	0.37	-6.06	0.00	0.31	0.05	6.25	0.00
Reflex	0.33	0.10	3.14	0.00	-0.24	0.06	-3.86	0.00	0.04	0.01	5.29	0.00
Sensory	0.18	0.10	1.85	0.07	-0.15	0.06	-2.54	0.01	0.03	0.01	3.80	0.00
Psychiatric	0.49	0.13	3.73	0.00	-0.22	0.08	-2.83	0.00	0.04	0.01	3.85	0.00
Non-Motor	0.90	0.22	4.01	0.00	-0.55	0.14	-4.08	0.00	0.10	0.02	5.73	0.00
Total score	7.08	0.75	9.40	0.00	-2.82	0.45	-6.20	0.00	0.41	0.06	6.84	0.00

3.2.4 Clinical scores of patients just undergoing treatment

Age grouping according to years from diagnosis in the previous section showed that the trend with patients whose treatment is just beginning is different from others which is reasonable due to the effect of treatment. Therefore, in our database, we have chosen a subset whose treatment data are available almost from the beginning of the treatment.

Fig. 10 marked 9 patients with blue triangles whose data within the first 5 years of diagnosis are available, and Fig. 11 shows the corresponding total, non-motor and motor scores across different visits regarding age at visit and years from diagnosis. Although years from diagnosis is more appropriate, however, its resolution is not suitable that is a yearly basis, thereby within-year variations are not shown appropriately and drawn as a vertical linear sequence.

By looking at total or motor scores which are similar, a dramatic decreasing trend for some cases is very easy to notice that is in line with previous results. There is, however, one special case (shown as green) that suffered from MS comorbidity reported in section 3.4.3. As a result, its summation scores did not show an overall decrease trend.

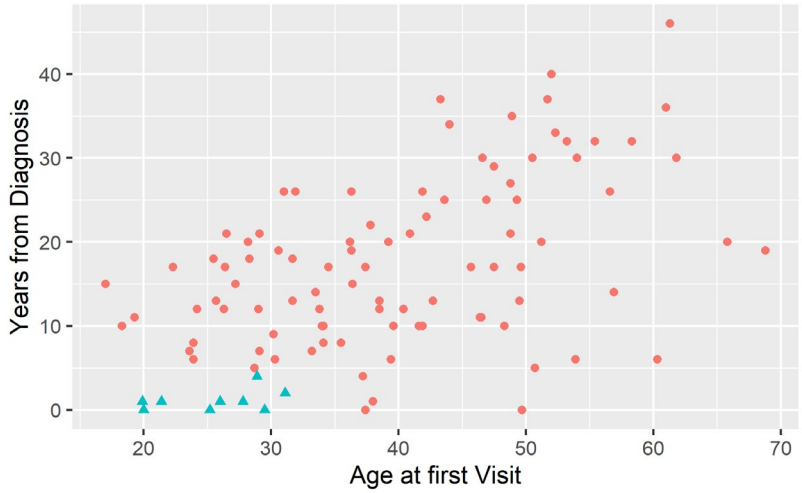


Fig. 10: Distribution of available WD patients regarding age at visit and years from diagnosis. Available data on WD patients with early WD manifestation (<5 years) are colored blue.

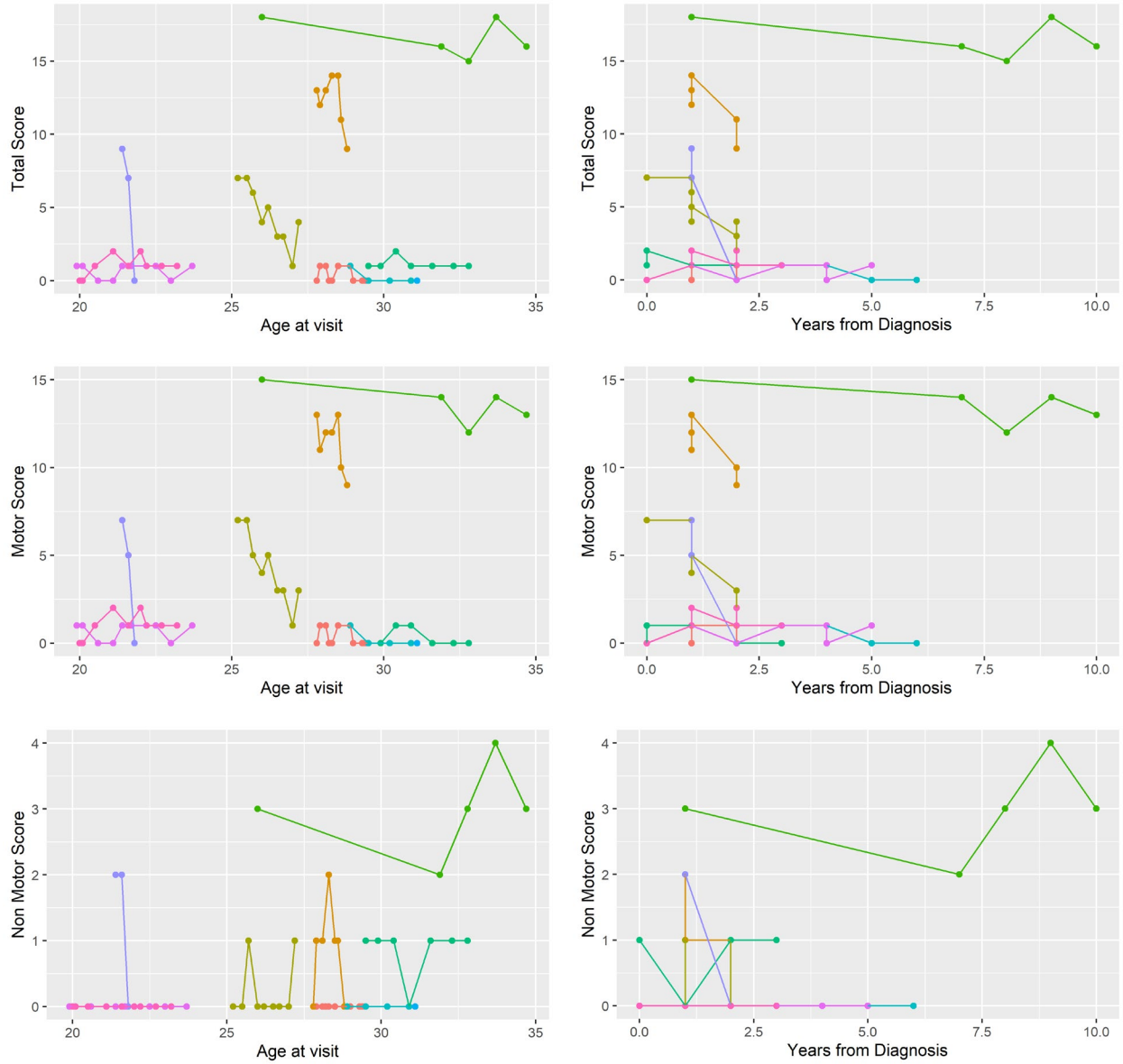


Fig. 11: variation of summation scores for patients whose early WD manifestation (<5 years) is available. The right side from top to bottom depicts total, motor, and non-motor scores respectively, regarding age at visits and the left side shows similarly the same values but regarding years from diagnosis.

3.2.5 Late diagnosis of Wilson's disease in a case without the onset of symptoms

A 60-year-old woman patient had noticed mild icterus at the age of 11 which disappeared without specific treatment. She had suffered from uncharacteristic skin irritation for two years (which was treated with vitamins and injections of calcium), frequent bleeding of the nose, and migraine. At age 22 she developed mild gastrointestinal symptoms (gastritis, meteorism, nausea, impairment of the bowl motility), claimed to be exhausted and anxious, and was subsequently treated with tranquilizers in low dosage. Dysmenorrhea was present at age 24 and 25 and 34 to 37. A

hysterectomy was performed because of a myoma. At age 37, her weight decreased by more than 11 kg for unknown reasons.

Twenty years later, after the death of her father, a further, sudden decrease of bodyweight occurred from 53 kg to 47 kg (body height 172 cm). At age 49, the gastrointestinal complaints (abdominal pain more on the left than on the right side, nausea, and dizziness) were analyzed in detail. Abdominal ultrasound investigation revealed a normal-sized spleen but a slightly increased size of one liver lobe. Laparoscopic inspection of the liver revealed some fibrotic changes. Histological analysis demonstrated fatty vacuoles in the cytoplasm of one-fourth of the liver cells, and iron pigments in the periphery of some lobules. The final statement of the laparoscopic investigation and histological analysis was that she had had virus hepatitis. Though copper staining did not reveal copper deposition in the lobule, the quantitative analysis of the liver biopsy revealed significantly elevated copper content in the liver (356 pg/g wet weight; normal range: 5.2-13.2 pg/g ww). On the basis of this result and the laboratory finding of decreased serum copper and ceruloplasmin levels Wilson's disease was diagnosed. She was treated with a rather low dose of 600 mg D-penicillamine, although liver dysfunction, neurological symptoms, and a Kayser-Fleischer-ring were missing, and liver enzymes were normal.

Six years later the question was again raised whether the patient had Wilson's disease. Consequently, DPA-treatment was stopped. The serum level of free copper again increased to pathological values, 24-h urinary copper excretion decreased to slightly elevated values, but it could be increased to 8 times the normal level with 900mg DPA. The ceruloplasmin level remained below 10.8 mg/dl on average. From the time when WD was first diagnosed the patient underwent at least nine independent slit-lamp examinations. In none of them could a Kayser-Fleischer ring be detected.

During the next two years, which were without treatment, she remained neurologically asymptomatic. She still had various gastrointestinal complaints but did not develop hepatic dysfunction. Her MRI scan was normal, and detailed electrophysiological investigations did not reveal any subclinical impairment. Compared to conventionally treated WD-patients, she is among the four least impaired WD-patients. The definitive diagnosis of WD resulted from the radio copper test.

While her husband's values were in the normal range, and those of her daughter lay in the heterozygotic range, her values lay in the homozygotic range, because she did not incorporate copper into ceruloplasmin at all. In addition, her excretion of radioactive copper via urine was elevated (Hefter et al., 1995).

3.2.6 Forward stepwise regression of clinical scores

The forward stepwise regression model facilitates understanding how variations of summation scores namely Total, motor and non-motor are explained by the other scores. The p-value is set to 0.05, and the model is simulated. The ranking of the winner's clinical scores is reported in Table 14 to Table 16 accordingly.

About 70% of variations of Total score is explained only by gait disturbances which are almost the same for Motor score, however, the next contributing factor is Bradykinesia for Total score whereas Dysarthria is the second for Motor score. On the other hand, about 65% of the variance of the non-motor score is explained through reflex abnormalities, and at the second position comes psychiatric abnormalities.

Table 14: Forward stepwise regression of total clinical score based on all individual clinical scores

Step	Variable Entered	R-Square	Adj. R-Square	C(p)	AIC	RMSE
1	Gait disturbance	0.690	0.69	59085.38	2513.03	2.15
2	Bradykinesia	0.80	0.80	38538.80	2272.64	1.74
3	Reflex abnormalities	0.85	0.85	28510.46	2104.53	1.51
4	Dysarthria	0.90	0.89	19834.64	1903.06	1.26
5	Cerebellar abnormalities	0.93	0.93	13293.17	1683.04	1.04
6	Neuropsychological and psychiatric abnormalities	0.95	0.95	9719.63	1513.70	0.90
7	Tremor	0.97	0.97	6031.84	1260.48	0.72
8	Dystonia	0.98	0.98	3788.63	1023.36	0.58
9	Oculomotor deficits	0.99	0.99	1549.98	609.15	0.41
10	Sensory abnormalities	1.00	1.00	11.00	-145.01	0.21

Table 15: Forward stepwise regression of motor score based on all individual clinical scores except non-motor related scores

Step	Variable Entered	R-Square	Adj. R-Square	C(p)	AIC	RMSE
1	Gait disturbance	0.68	0.68	61944.48	2279.20	1.76
2	Dysarthria	0.83	0.83	33599.85	1935.03	1.30
3	Cerebellar abnormalities	0.90	0.90	19954.60	1644.87	1.01
4	Bradykinesia	0.93	0.93	13404.38	1426.47	0.84

5	Tremor	0.96	0.96	8198.30	1161.10	0.66
6	Dystonia	0.98	0.98	4076.36	798.44	0.48
7	Oculomotor deficits	1.00	1.00	4.09	-409.50	0.17

Table 16: Forward stepwise regression of total non-motor score based on all individual clinical scores except motor related scores

Step	Variable Entered	R-Square	Adj. R-Square	C(p)	AIC	RMSE
1	Reflex abnormalities	0.65	0.65	18546.32	1268.81	0.73
2	Neuropsychological and psychiatric abnormalities	0.91	0.91	4622.67	522.50	0.38
3	Sensory abnormalities	0.99	0.99	0.82	-744.06	0.13

3.2.7 Relation of psychiatric symptoms and other parameters

The relation between psychiatric abnormalities and the summation scores is of importance. The psychiatric score is added to both Total and non-motor scores, by subtracting it we can investigate how the neuropsychological aspect of the disease may correlate with all non-psychiatric symptoms.

Fig. 12 shows that increasing psychiatric score correlates well with increasing subtracted summation scores, and Table 17 asserts a significant linear regression model for which every unit increase of psychiatric score is equivalent to 2.3, 0.3 and 1.9 unit increase in subtracted total and non-motor and motor score respectively.

By further investigation of the psychiatric score and laboratory findings, it is revealed that there are as well non-linear relations for laboratory findings with years from diagnosis. Fig. 12 shows also mean variations of psychiatric and the laboratory parameter cholinesterase (CHE) across different year groups. Closer examination shows that while psychiatric score follows a convex curve, the CHE parameter is following a concave path which is an indication of negative correlation. In this regard, the Spearman correlation indicates a statistically significant value, r=-0.49, p=0.001.

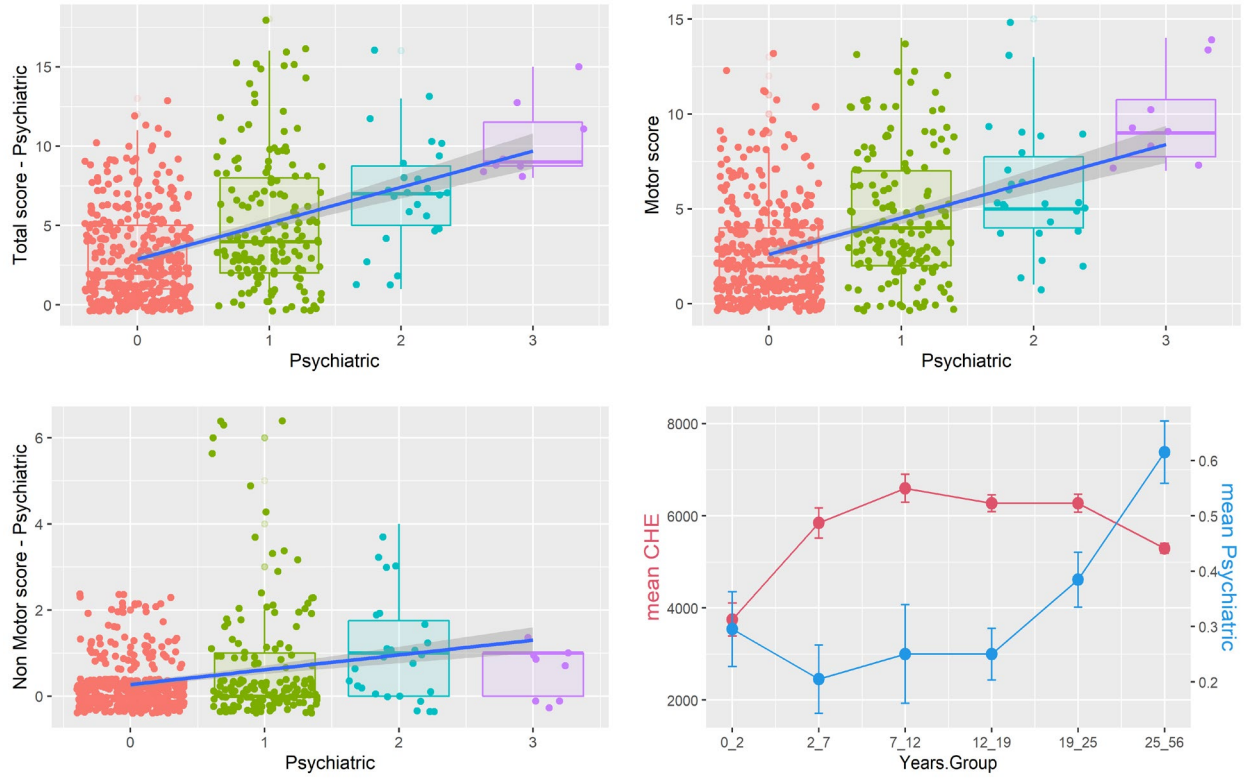


Fig. 12: Psychiatric relation with summation scores and with the laboratory parameter CHE. Correlation of the subtracted total score (above left), motor score (above right) and subtracted non-motor score (below left) with the psychiatric score. Correlation of mean psychiatric subscore and mean CHE across different years group.

Table 17: Linear regression model between subtracted summation scores and psychiatric score

		Total - psych.	Motor	Non-Motor - psych.
Intercept	Est.	2.88	2.61	0.27
	std. Er.	0.16	0.14	0.04
	t value	17.89	18.38	6.24
	P value	0.00	0.00	0.00
Psychiatric	Est.	2.27	1.93	0.34
	std. Er.	0.21	0.19	0.06
	t value	10.69	10.34	6.04
	P value	0.00	0.00	0.00

3.3 Genotype-phenotype discordance in homozygotic twins with clinical phenotypic variability

The two young women presented themselves for the first time in 2/2019 in our Wilson outpatient clinic. A few months earlier, both had been genetically tested at the same time and showed identical mutation patterns.

Human genetic test result for twins: Wilson's disease (MIM 277900) (ATP7B-gene) Heterozygotic mutation of c.2304dupC (p.Met769His*fs**26) and c.3207C>A (p.His1069GIn)

After the diagnosis of Wilson's disease for both, it was recommended that they should be seen in practice with experience in the treatment of Wilson's disease without initiating any therapy. After a significant time lag, the two patients finally presented themselves.

The two sisters grew up together in their parents' house. Both went through normal school and vocational training. They both married at the age of 22.

The severely affected sister developed unclear health problems due to Wilson's disease during her marriage, which brought them to divorce after 2 years, and she returned to the parents' house. She then developed further neurological symptoms such as tremor, writing and gait disorders, which ultimately led to an inpatient evaluation without a conclusive explanation of the health status of this sister. The human genetic examination recommended following the inpatient stay finally led to the diagnosis of Wilson's disease.

At the first presentation, this patient was almost anarthric, had a severe juvenile Parkinson's syndrome with considerable slowing of movement and a severe stance and gait ataxia, so that she was not able to walk alone but had to rely on an accompanying person or a wheelchair. In addition, there was vertical gaze palsy and hypersalivation. Sensory abnormalities were not found, the pyramidal signs could not be found. The writing was not possible. There was only a mild anxiety disorder, probably on the basis of helplessness, with no evidence of an intellectual deficit.

The patient researched on the internet that Wilson's disease could be treated with D-penicillamine, so she started DPA with a dose of 450mg without consult. Due to symptom severity, the daily dose was increased over 4 weeks in 300mg from 450 mg to 3 times 600 mg DPA.

Initially, the patient responded well to the DPA therapy, the liver values improved significantly, the copper excretion was initially very high and then decreased as expected. The neurological

symptoms improved in the first few weeks of therapy. Then after about 2 months, the patient developed weight gain and dyspnea and a further deterioration in her general condition. The improvement in the neurological symptoms stagnated. In view of the multiplication of protein excretion, the nephrotic syndrome was diagnosed as a drug side effect on the DPA. After switching from DPA therapy to Trientine (Cuprior®), the protein loss, the general condition and the neurological symptoms improved.

With the increasing mobility of the patient - the patient was finally able to walk a few steps - unfortunately, she had experienced a fall accident with a broken shoulder on the right and two necessary operations as a result, which unfortunately delayed the rapid neurological improvement. After all, she was able to go on a long-distance trip for two months to visit her grandparents.

Upon her return, the patient developed a fever and cough and was admitted to a peripheral hospital. This happened at the time of the 3rd wave of the Covid-19 pandemic. Allegedly, the patient should have tested negative, we have no documentation of the Covid-19 test result, an autopsy was not carried out due to the natural death of the patient. In the differential diagnosis, bronchopneumonia should be considered as the cause of death in asphyxia despite percutaneous endoscopic gastrostomy (PEG) application.

After the marriage, the second sister ran her household and became the mother of a healthy child. At the introduction, there was a wish for a second child.

Clinically and neurologically, the second sister was completely asymptomatic at the first presentation. Since both sisters had a bodyweight of less than 50 kg, the DPA dose in the asymptomatic sister was increased to only 600 mg over 2 weeks.

Although this patient was clinically asymptomatic, she already had abnormal liver values, which worsened even further in the initial phase of the dose increase. After a few weeks, however, there was a clear improvement in the liver values, which are currently still improving, even if not at the same rate as at the beginning. During the ongoing chelating therapy, this asymptomatic sister remained asymptomatic, so that nothing stands in the way of having a second child. The course of summation scores is depicted in Fig. 13.

The OCT exam in twins revealed a normal size optic disc with almost normal retinal nerve fiber layer (RNFL) in both eyes of the two patients, the specific pattern of change in WD with the reduction in diameter of RNFL, GCIP and INL cannot be seen clearly in segmented layers. There

are slight differences between OCT parameters of two patients but still in the normal range in comparison with normal control (Table 18).

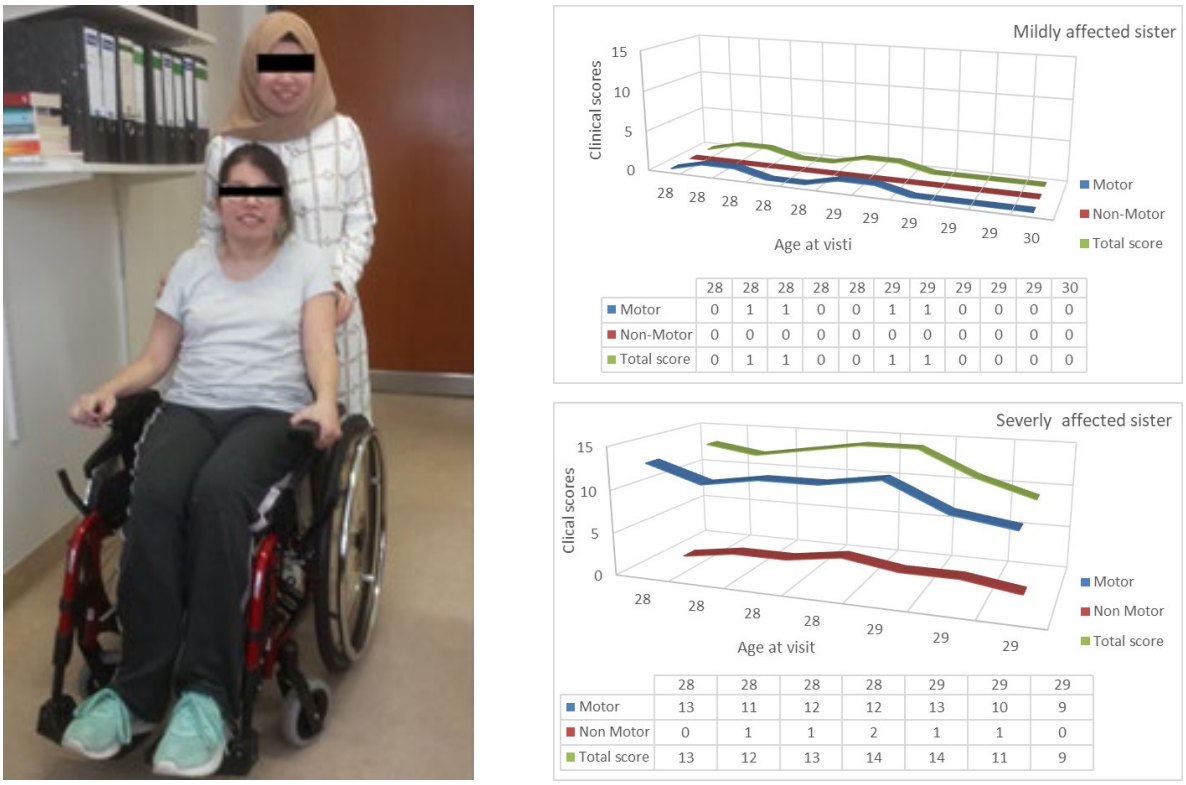


Fig. 13: These two identical twins are clinically affected in completely different ways: one sister is wheelchair-bound, the other sister is completely asymptomatic (see summation scores variations on the right side). Both patients responded to DPA therapy, however, the severely affected patient developed a nephrotic syndrome during DPA therapy.

Table 18: OCT result of homozygotic twins in comparison to healthy control

		Severely affected patient		Mildly affected patient	
	Healthy Control	Right eye	Left eye	Right eye	Left eye
pRNFL (µm)	101.07±7.61	98	100	98	100
RNFL (mm^3)	1± 0.12	1.12	1.11	1.07	1.13
GCIPL (mm^3)	2.02±0.13	2.10	2.10	2.12	2.10
INL (mm^3)	0.96±0.06	1.07	1.04	1.06	1.04
OPL (mm^3)	0.81±0.07	0.84	0.87	0.79	0.78
ONL (mm^3)	1.78±0.17	1.78	1.78	1.86	1.81
pRNFL= peripapillary retinal nerve fiber layer; GClP = combined ganglion cell and inner plexiform layer; INL, inner nuclear layer; OPL= outer plexiform layer; ONL = outer nuclear layer					

3.4 Comorbidities in study population

In this section, we reported all the comorbidities documented in patients' charts and their frequencies among them regarding neurologic, psychiatric, and orthopedic concurrences.

3.4.1 Frequency of co-occurrences (comorbidities)

We stratified all co-occurrences in Table 19. Overall. 28 patients with casually related or not related neurological concurrences were seen in our population. Psychiatric problems such as depression and bipolar disorder were diagnosed for 18 patients based on psychiatric criteria. Some age-related comorbidities such as hypertension and also fluctuated problems like obesity were not included in this table.

Table 19: Frequency of all co-occurrences (comorbidities)

Neurologic	Psychiatric	Orthopedic	Rheumatologic	Cardiovascular and Pulmonary
PNP (6) Stroke/TIA… (5) MS (4) Spasticity (6) Epilepsy (3) Chorea (3) Myasthenia Gravis (1)	Depression (15) Bipolar disorder (3) Psychotic episodes (1) Impulse control disorder (1) Mild mental retardation (3) alcohol and nicotine addiction (2)	knee, hip, and shoulder Arthrosis (13) Total hip replacement (4) Cervical and lumbar spine syndrome (7) Hallux Valgus (1)	Seropositive chronic polyarthritis (1) Systematic sclerosis (Scleroderma) + Eosinophilic Fasciitis (1)	Cardiomyopathy (1) Severe arrhythmia (1) Pericarditis (1) COPD (1)
Gastroenterological	**Endocrinological**	**Hematologic / Oncologic**	**Tumor and Malignancies**	**Others**
Gilbert syndrome (3) Cholinesterase deficiency (1) Cholecystitis (3) Hemochromatosis (2)	Hypothyroidism (11) Hashimoto's thyroiditis (2) Nodular goiter (1)	Acute myeloid leukemia (1) non-Hodgkin lymphoma (1)	Hepatocellular carcinoma (5) Ependymoma tumor (1)	Allergy and allergic reaction (4)

3.4.2 WD-related or not related neurologic Co-occurrences (disease /symptoms)

In this section, more detailed information was prepared for neurologic concurrences regarding medication and disease type. The dosage of WD medications was not indicated in Table 20 due to changes regarding symptom severity during follow-up.

Table 20: WD-related or not related neurologic Co-occurrences (disease /symptoms)

Fall No.	Gender	WD Medication	Age at First Diagnosis	Type	Medication
MS+WD					
1	Female	D-penicillamine +Zinc	19	SPMS	Mitoxantrone
2	Female	Trientine	18	SPMS	See section 3.4.3
3	Female	D-penicillamine	N/A	PPMS	N/A
4	Female	Zinc	N/A	RRMS	No medication
Epilepsy + WD					
1	Male	Trientine	N/A	Mild mental retardation + Epilepsy	Carbamazepine 1600mg
2	Female	N/A	N/A	Petit mal Epilepsy	N/A
3	Male	D-penicillamine	N/A	Structural Epilepsy	Lamotrigine 250mg
Stroke/ Traumatic Brain Injury, Cerebral hemorrhage (Spasticity)					
1	Female	Trientine (->D-penicillamine)	N/A	Traumatic Brain Injury	Baclofen
2	Male	D-penicillamine (->Cuprior)	2019	Stroke + Epilepsy	Aspirin +Levetiracetam + Lamotrigine
3	Male	Trientine (->WTX101 (Tetrathiomolybdate)	1993	Cerebral hemorrhage	No Medication
4	Male	D-penicillamine	2016	Transient ischemic attack (TIA)	Aspirin
5	Female	Trientine (->D-penicillamine)	2004	Cerebral hemorrhage	N/A
Chorea					
1	Male	D-penicillamine + Zink	N/A	Generalized chorea	Tetrabenazine 75mg + Tiapridex 300mg

2	Male	D-penicillamine	N/A	Mild generalized chorea	Clonazepam 0.5mg
3	Female	D-penicillamine	N/A	Generalized chorea	Tiapridex 300mg
PNP					
1	Male	Trientine (->Cuprior)	N/A	Lower extremities	Primidone 125mg
2	Male	D-penicillamine	N/A	Lower extremities	No medication
3	Male	D-penicillamine + Zink	N/A	Lower extremities	Metamizole 3000mg
4	Male	D-penicillamine	N/A	Lower extremities	No medication
5	Female	D-penicillamine	N/A	Lower extremities	Primidone 250mg + Tilidin (Opioid) 300mg
6	Male	D-penicillamine	N/A	Induced by chemotherapy Upper and Lower extremities	N/A
SPMS= secondary progressive multiple sclerosis PPMS= primary progressive multiple sclerosis RRMS= relapsing-remitting multiple sclerosis N/A=Not applicable					

3.4.3 Multiple sclerosis and Wilson's disease (4 cases)

In our dataset, there were 4 cases of WD patients with MS, and regarding the rare occurrence of such comorbidity, two of them are reported as follows.

Case 1: A female patient with SPMS since 1980 when she was 19 years old and Wilson's disease since 1985 when she was 24 years old.

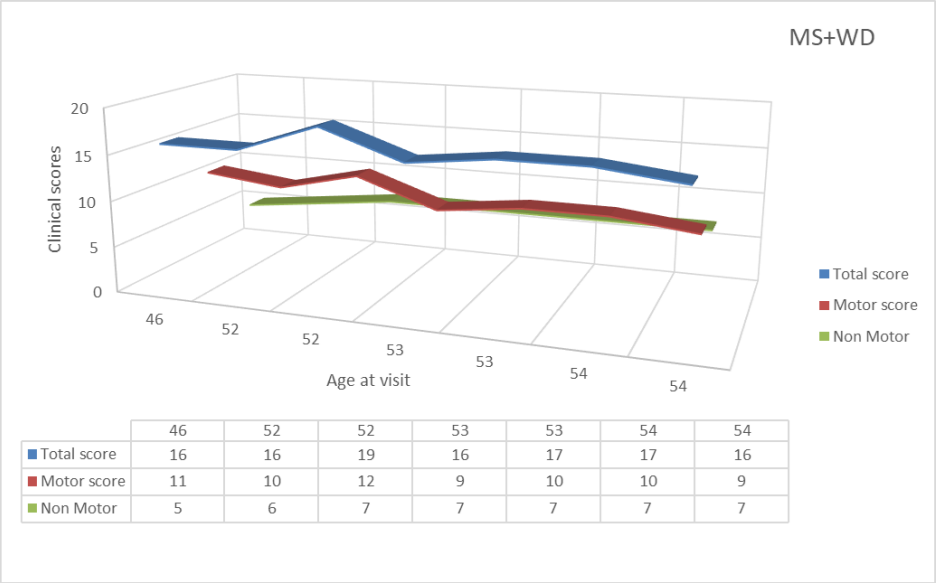
The character of MS was relapsing until 1994 and it has been progressive since 1994 so that she has been wheelchair-bound since 2003 with an Expanded Disability Status Scale (EDSS) score of 7. (Findings of neurological examination at the time of EDSS 7: tetraspasticity with a clear paraparesis, increased patellar and ankle reflexes, 4/8 pallesthesia on the hands, 2-0 / 8 pallesthesia on the legs and feet, gaze nystagmus up- and down-beat nystagmus, sensory disturbance, severe oculomotor disturbance (more relevant to MS signs than WD neurological signs))

The last treatment with interferon was from 2003 to 2005, and in 2003 a Baclofen pump was implanted, since the spasticity, which is mainly present in the lower extremities, with oral

medication could no longer be treated well. Due to the secondary progression of MS, the treatment with Mitoxantrone was started in 2006 after cardiovascular evaluation. Mitoxantrone was administered with a standard dose of 12 mg / m^2 body surface area every 3 months during the first year of treatment and was decreased to 12 mg / m^2 body surface every 3 months in the second year. The therapy with 900 mg D-penicillamine was initiated for her and was increased to 1200 mg after a sign of mild progression due to WD and Zinc was also added. In addition, she has suffered from detrusor-sphincter dyssynergia of the bladder for several years and a suprapubic urinary catheter has been placed many times for him by the urologist.

Case 2: A female patient with secondary progressive multiple sclerosis (SPMS) since 1982 when she was 18 years old and Wilson's disease since 1992 when she was 28 years old.

MS first manifestation was hypoesthesia and paresthesia in both lower extremities in 1978 and the diagnosis of MS was made in 1982. The course of the disease was relapsing-remitting until the mid-80s, then the chronic progressive course was started. She was completely disabled, wheelchair-bound with an EDSS of 7 and needed help for daily routine activities. The course of summation scores is depicted in Fig. 14.



	46	52	52	53	53	54	54
Total score	16	16	19	16	17	17	16
Motor score	11	10	12	9	10	10	9
Non Motor	5	6	7	7	7	7	7

List of Medications

2012-13 (3 cycles Cyclophosphamide), From 2009 (Azathioprin), 2005-08 (Mitoxantron 94mg/m2),

2005 (Rebif), 2004 (Copaxone); Trientine

Fig. 14: Summation scores variation of MS+WD patient (case 2) and the prescribed list of medications

The OCT exams in patients with MS and WD revealed a reduction in peripapillary retinal nerve fiber layer (pRNFL) thickness over time, the reduction can be seen in GCIP as well. The alteration of other layers is shown in Table 21 and Fig. 15 in comparison with healthy control.

Overall, both patients experienced the burden of MS more than WD and WD was better under control than SPMS regarding the nature of those diseases.

Table 21: OCT result of a patient with MS+WD in comparison to healthy control (right eye) (baseline and 3 follow-up visits)

	Healthy Control	Baseline 2010	Visit 2 2012	Visit 3 2015	Visit 4 2016
pRNFL (µm)	101.07±7.61	58	59	64	56
RNFL (mm^3)	1± 0.12	0.59	0.58	0.53	0.57
GCIPL (mm^3)	2.02±0.13	1.42	1.40	1.36	1.37
INL (mm^3)	0.96±0.06	0.91	0.92	0.93	0.93
OPL (mm^3)	0.81±0.07	0.80	0.81	0.84	0.79
ONL (mm^3)	1.78±0.17	2.04	2.09	2.07	2.04
pRNFL= peripapillary retinal nerve fiber layer; GClP = combined ganglion cell and inner plexiform layer; INL, inner nuclear layer; OPL= outer plexiform layer; ONL = outer nuclear layer					

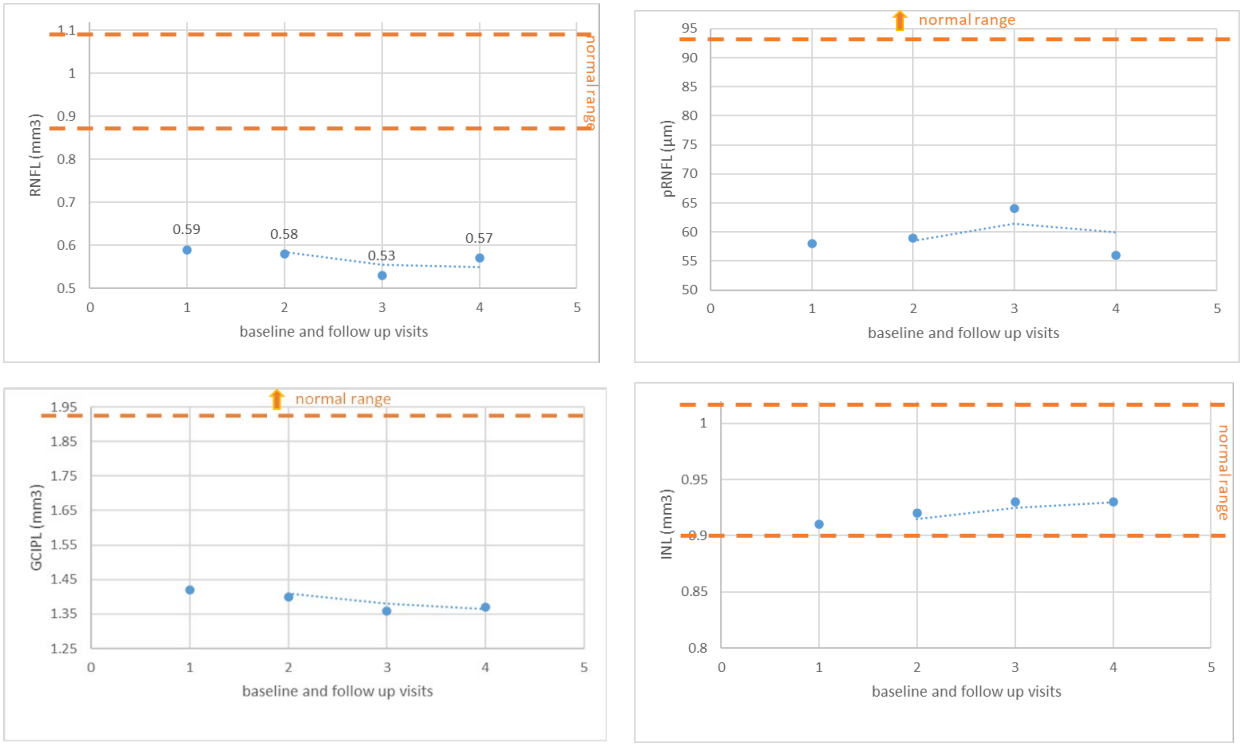


Fig. 15: OCT result of a patient with MS+WD (right eye) (baseline and 3 follow-up visits); RNFL (right above), pRNFL(left above), GCIP(right below), INL(left below)

3.4.4 Psychiatric comorbidities

In this section, more detailed information was prepared for psychiatric concurrences regarding medication and disease type. The dosage of WD medications was not indicated in Table 22 due to changes regarding symptom severity during follow-up. There are some patients with more than one comorbidity for example in case number 11 and 12.

Table 22: Psychiatric Co-occurrences

Fall No.	Gender	WD first Medication	Valid diagnosis based on psychiatric criteria	Medication
1	Male	Trientine	Depression	Mirtazapine 45mg
2	Male	Trientine (->D-penicillamine) (->Trientine) (->Cufence)	Depression	Fluoxetine 40mg
3	Male	D-penicillamine	Depression	Escitalopram 10mg
4	Male	D-penicillamine	Depression	Fluoxetine 20mg
5	Male	D-penicillamine + Zink	Depression	Mirtazapine 15mg
6	Female	Trientine (->Cuprior)	Depression	No Medication
7	Female	Metalcaptase	Depression	Venlafaxine 225mg
8	Female	D-penicillamine	Depression	Fluoxetine 10mg
9	Female	Trientine (->Cuprior) (->Cufence)	Depression	Fluoxetine 20mg Mirtazapine 30mg
10	Female	D-penicillamine	Depression	Opipramol Dihydrochloride 100mg
11	Female	D-penicillamine	Depression + Myasthenia Gravis	Citalopram 40mg
12	Male	D-penicillamine	Depression + Drug addiction	Mirtazapine 30mg
13	Female	D-penicillamine	Depression + Impulse control disorder	Mirtazapine 30mg

14	Female	N/A	Depression + RRMS	N/A
15	Female	Trientine	Post-traumatic stress disorder (PTSD) + Depression	Mirtazapine 30mg
16	Female	Trientine	Reactive Depression + Myotonia Congenita Type Becker	Venlafaxine 300mg Naproxen 500mg
17	Male	Trientine (->Cuprior)	Depression + Drug and alcohol addiction	Naproxen 500mg
18	Male	D-penicillamine	Depression + Drug addiction (Cannabis)	No Medication
19	Female	D-penicillamine	Psychotic Episodes Psychosis	Quetiapine 100mg Venlafaxine 175mg
20	Male	D-penicillamine	Bipolar Disorder	No Medication
21	Male	D-penicillamine	Bipolar Disorder	Topiramate 50mg
22	Female	D-penicillamine	Bipolar Disorder	Duloxetine 60mg
N/A=Not applicable				

3.4.5 Orthopedic comorbidities

Orthopedic comorbidities were also frequent in our population due to the aging process as well as the musculoskeletal effect of some of WD neurological symptoms like gait abnormalities, the presence of other comorbidities such as MS and generalized dystonia can also play a role in orthopedic problems Table 23.

Table 23: Orthopedic Co-occurrences (disease /symptoms)

Fall No.	Gender	WD Medication	Type	Medication
Cervical spine syndrome – Lumbar spine syndrome				
1	Male	Trientine (->Cuprior)	Lumbar + knee arthrosis	No medication
2	Female	D-penicillamine	Hallux Valgus	Operation
3	Male	D-penicillamine	Knee arthrosis	No medication
4	Male	Trientine	Cervical and Lumbar spine syndrome	No medication

5	Male	Trientine (->Cuprior)	Cervical and Lumbar spine syndrome	Naproxen
6	Male	Trientine Hydrochloride -> WTX101(Tetrathiomolybdate)	Knee arthrosis	No medication
7	Male	D-penicillamine + Zink	Total hip replacement + knee arthrosis	Metamizole 3000mg
8	Female	D-penicillamine	Total hip replacement	No medication
9	Female	D-penicillamine	Lumbar spine syndrome	Primidone 250mg
10	Female	Trientine Hydrochloride	Knee and Hip arthrosis	Primidone 250mg
11	Male	Trientine Hydrochloride	Knee and Hip arthrosis	No medication
12	Male	D-penicillamine	Knee and Hip arthrosis	No medication
13	Female	Trientine Hydrochloride (->Cuprior)	Total hip replacement	Diclofenac -Metamizole
14	Male	D-penicillamine (->Cuprior)	Cervical spine syndrome	Operation + BoNT therapy
15	Male	D-penicillamine	Cervical and Lumbar spine syndrome	No medication
16	Male	D-penicillamine	Shoulder and knee arthrosis	No medication
17	Female	D-penicillamine	Shoulder and knee arthrosis	Primidone 250mg + Tilidin (Opioid) 300mg
18	Female	D-penicillamine (->Trientine)	Total hip replacement	
19	Female	D-penicillamine	knee arthrosis	No medication
20	Female	D-penicillamine + Zinc	knee arthrosis + Lumbar spine syndrome	No medication
21	Female	Trientine (->Cuprior)	Hip and knee arthrosis + Lumbar spine syndrome	Ideos 500mg + Calcium
22	Female	D-penicillamine	knee arthrosis	No medication

3.4.6 Special cases

There are some very special co-occurrences in our dataset that are reported in Table 24. One of them as an example with two different diseases in addition to WD is reported as follows.

Table 24: Summary of medications for WD patients with special comorbidities

Fall No.	Age at WD First Diagnosis	WD Medication	Other Co-occurrence	Medication
1	25 Female	D-penicillamine (->Cuprior)	Juvenile Parkinson's Syndrome	L-dopa 3x125mg
2	14 Female	Trientine	Myotonia Congenita Type Becker (CLCN1-Gen, c. 1437_1450del homozygote)	Mexiletine 200mg
Myotonia Congenita Type Becker (CLCN1-Gen, c. 1437_1450del homozygote) Wilson's disease (ATP7B-Gen c.3207C>A homozygote)				
3	46 Female	N/A	Systematic sclerosis (Scleroderma) + Eosinophilic Fasciitis	L-dopa 3x250mg
4	24 Female	D-penicillamine	Hemochromatosis	Phlebotomy
5	26 Male	D-penicillamine (->Trientine)	Hemochromatosis + Superficial siderosis (SS)	Phlebotomy
6	14 Male	Zinc	Myasthenia Gravis + non-Hodgkin lymphoma	Pyridostigmine 330mg
N/A=Not applicable				

Superficial siderosis (SS) + Hemochromatosis and Wilson's disease:

At the age of 26, our patient presented himself to the neurological outpatient department for the first time with the symptoms of progressive dysarthria, bradydiadochokinesis, and fine motor disorder. Due to the typical laboratory constellation (low serum copper, low serum ceruloplasmin, increased Copper excretion in the 24-hour urine) was the diagnosis of Wilson's disease was made. Therapy

with the chelating agent D-Penicillamine resulted initially in a considerable reduction of the neurological symptoms. In the further course, the patient came once a year for control of the DPA therapy.

An unusually high ferritin level was recorded after 5 years from WD diagnosis, which led to a detailed investigation of the iron metabolism. Hereditary hemochromatosis was diagnosed with HLA-HFE homozygous positive results (C282Y). The hemochromatosis was after the diagnosis is regularly treated by phlebotomy.

After that, the 55-year-old patient complained of increasing hearing loss, vertigo, and gait disturbance as new symptoms that appeared without a history of an accident or a change in medication. Clinical examination confirmed hearing loss, revealed cerebellar syndrome and bilateral pyramidal tract disturbances. Neurophysiology confirmed pathological findings on clinical examination. Cerebral magnetic resonance imaging (MRI) disclosed deposition of hemosiderin suggestive for superficial siderosis of the central nervous system (Fig. 16). Cerebrospinal fluid findings were normal. Finally, the triad of hearing loss, cerebellar syndrome, and pyramidal tract disturbances associated with typical findings on MRI led to the diagnosis of superficial siderosis. Etiology, in this case, remained unclear; no source of bleeding was detected. Thus, no causal therapeutic option was available (Warnke et al., 2011).

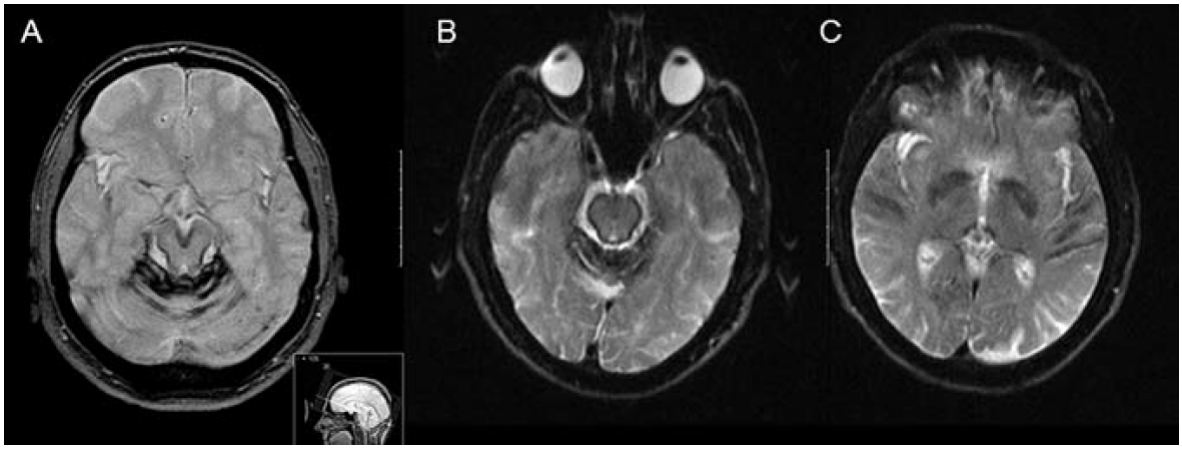


Fig. 16: T2-weighted sequences; Hemosiderin deposition around the midbrain and in the folia cerebelli (A), around the brain stem (B) as well as in the sulci of the temporal lobe (C). In addition, there are changes in the basal ganglia, that are typical for a patient with long-term treated Wilson's disease (C). (Warnke et al., 2011)

3.5 Specific treatment and long-term management in the study population

3.5.1 Treatment of Neurological Symptoms with Botulinum Neurotoxin in Wilson's Disease: Six Cases

With the present series of six patients (Table 25), which is the largest one to date for the symptomatic treatment with botulinum toxin of a broad spectrum of symptoms in WD, we summarize our experience with a special focus on the doses and efficacy of BoNT/A in WD.

Table 25: Demographical and treatment-related data of WD and botulinum neurotoxin A (BoNT/A) therapy.

Parameter	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Demographical data						
Age	30	66	64	50	52	35
Sex	FEMALE	FEMALE	FEMALE	MALE	MALE	MALE
Age at first diagnosis (years)	10	12	15	18	18	5
WD therapy	Trientine®	DPA	Trientine®	DPA	DPA	DPA
Clinical score *						
MotS	2	5	8	9	9	5
Non-MotS	0	0	3	0	2	1
TS	2	5	11	9	11	6
Laboratory findings ** (of the last visit before recruitment into this study: grey windows indicate values out of the normal range)						
Cerulo (mg/dL)	<7	<7	14	10	<7	<7
CU (mg/dL)	0.02	0.21	0.49	0.40	0.60	0.05
AST (U/L)	25	21	41	34	52	15
CHE (U/L)	6079	5194	5149	6744	4290	3870
24 h-CU mg/d	0.041	0.045	0.092	0.037	0.059	0.377
BoNT/A Therapy (data of the last visit before recruitment: treatment-related data and laboratory findings result from the same visit)						
Indication	Palmar hyperhidrosis	Segmental dystonia	Hypersalivation	Generalized dystonia	Cervical dystonia hypersalivation	Spasmodic dysphonia
Preparation	ona BoNT/A	Abo BoNT/A	inco BoNT/A	inco BoNT/A	inco BoNT/A	ona- or inco BoNT/A

Dose of BoNT/A	100 U/hand	500–1000 U for neck	100 U/gland	200 U for trunk 200 U for left arm	200 U for CD 100 U/gland	5–10 U/ side
Recommended dose range	100 U/hand	500–1000 U for CD	50–100/gland	Off-label	200 U for CD 100 U/gland	5–10 U/ side
Efficacy	Good	Moderate	Moderate	Good	Mild/ Moderate	Very good
Side effects	Pain during injection	None	None	None	None	None
** Cerulo, Ceruloplasmin: normal range in our clinical laboratory (NR): 20–60 mg/dL; CU, serum copper: NR: 0.7–1.5 mg/L; AST, alanine aminotransferase: NR: <35 U/L; CHE, cholines-terase: NR: 5120–12.920 U/L; 24 h-CU, 24-h urine copper excretion: NR: < 0.04 mg/d mg/dL =milligram/ deciliter U/L = units/liter						

3.5.2 WD and Palmar Hyperhidrosis (Patient 1)

This 30-year-old woman was diagnosed with WD at the age of 10 years. She was asymptomatic when treatment was initiated. Due to a severe skin reaction, she was switched from a low dose (600 mg) of D-penicillamine to 1200 mg Trientine®. After puberty, she developed progressive general hyperhidrosis and extreme speech hastening, which is her main symptom. She did not accept therapy with a higher dose of Trientine®. She has normal intelligence but did not finish her education during the last ten years. She receives an injection with 100 U of onabotulinumtoxin A (onaBoNT/A; Botox®) per hand because of palmar hyperhidrosis once or twice a year. She reports a good (about 60% improvement during the first 3 months after the injection) response to BoNT/A; the effect lasts for months.

3.5.3 WD and Segmental Dystonia (Patient 2)

This 66-year-old woman and her younger sister were diagnosed with WD at the ages of 12 and 10 years, respectively, when a family screening was performed. A metabolic disorder was suspected because both sisters had elevated liver enzymes and she had developed a tremor and impaired handwriting. About 6 years later because of hip dysplasia, she had to be operated on and a hip replacement was performed. After the operation, a prolonged wake-up phase was noticed. During the next years, she developed complex segmental dystonia involving the neck and upper trunk muscles. However, she refused to increase the dose of DPA beyond 900 mg. She was treated with

500 to 1000 U of abobotulinumtoxin A (aboBoNT/A; Dysport®) and experienced moderate improvement, but was satisfied with this treatment. Under treatment with 900 mg of DPA and about 750 U of aboBoNT/A in the meantime, her situation was stable over years. She was a smoker and had chronic coughs. When she complained of general weakness and intensive coughs, lung cancer was detected, but she refused to undergo chemotherapy or surgical intervention. She died within 7 months Fig. 17.

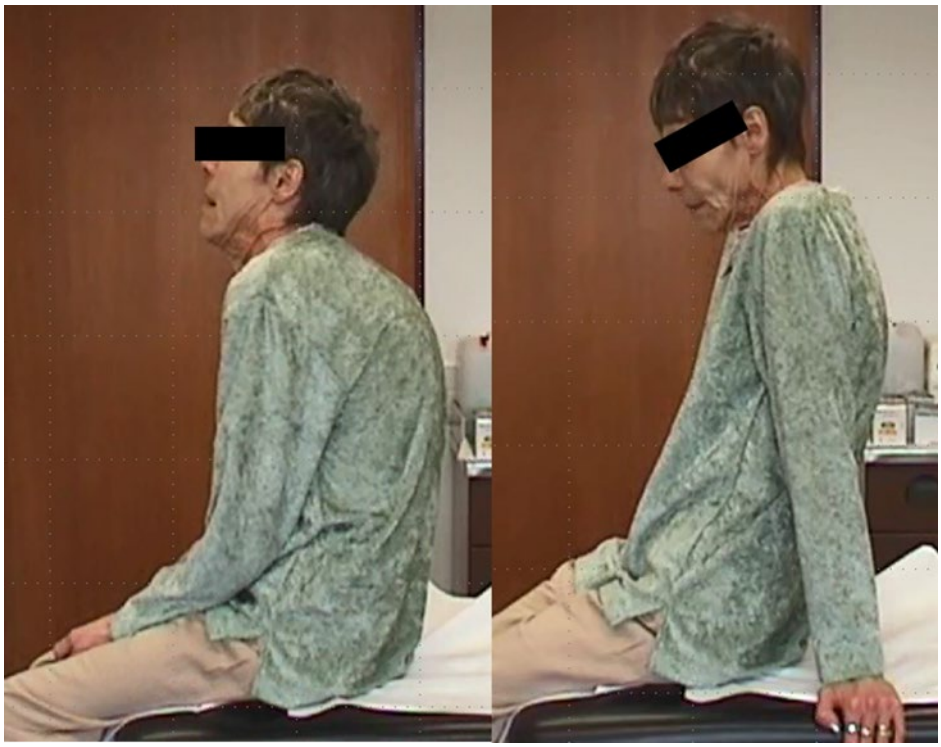


Fig. 17: Wilson's disease and segmental dystonia (patient 2). During upright sitting, shoulders were pulled forward, and the head was drawn back and downwards (left side). When she leaned back, her shoulders could be moved back and the retrocaput component disappeared (right side).

3.5.4 WD and Generalized Dystonia and Hypersalivation (Patient 3)

This 64-year-old woman was diagnosed with WD when she was 15 years old. In 1988, she presented for the first time in our department with generalized dystonia. The dose was increased from 900 mg of DPA up to 2700 mg without any relevant change in the dystonia. During the next years, dysarthria became so severe that she had to use a computer-assistive device. Swallowing became difficult and she refused to be switched to Trientine®. With progressive dysarthria and difficulties in swallowing, she also developed severe hypersalivation, which was treated from time to time with 200 U of incobotulinumtoxin A (incoBoNT/A; Xeomin®). During the first 3 months after the injection, she had a good response. Thereafter, the effect slowly declined, but she did not want to attend the botulinum toxin outpatient clinic more frequently (Fig. 18)

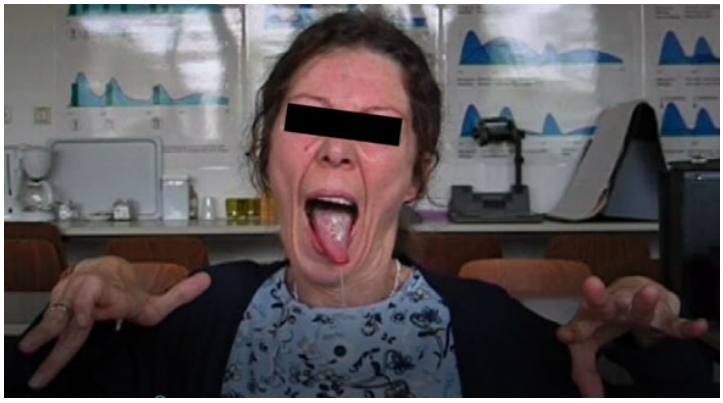


Fig. 18: WD and generalized dystonia (Meige syndrome, oromandibular dystonia, cervical dystonia, trunk, and limb dystonia) and hypersalivation (patient 3).

3.5.5 WD and Generalized Dystonia (Patient 4)

The history of this patient has already been mentioned in two articles (Meyer et al., 1991a, Meyer et al., 1991b). In short, he was diagnosed at the age of 18 years and treated with 600–900 mg of DPA. Hyperkinesia and long tract involvement improved under medication, but dysarthria progressed. During the following years, the patient moved to another city, studied computer sciences, and became an information technology (IT) specialist. He did not increase the medication although he became so dysarthric that only his wife was able to understand and translate him. When he returned to a nearby city and presented again in our institution, he had developed severe generalized dystonia. Due to a severe pain syndrome with pain in the lower back more on the left than on the right side and in the left arm, he had (as an IT specialist) looked for help on the Internet and therefore presented to be treated with BoNT/A, not for control of WD. He is highly cooperative concerning his BoNT/A injections but not WD. He is injected every 3 months with 200 U of incoBoNT/A into the back and 200 U into the left arm, with a good response (Fig. 19).

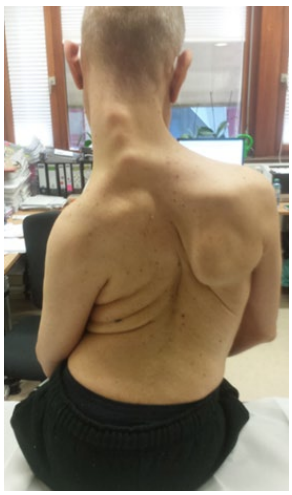


Fig. 19: WD and generalized dystonia (patient 4).

3.5.6 WD and Multifocal Dystonia and Hypersalivation (Patient 5)

This 52-year-old man was diagnosed after high school when he started to study medicine. He developed writing difficulties, tremors, and fatigue, as well as a lack of concentration. Finally, he developed juvenile Parkinsonian syndrome with drooling. He was listed for liver transplantation, but liver dysfunction and neurological symptoms recovered under treatment with 900 mg of DPA. He gave up studying medicine and became a physiotherapist. The Parkinsonian syndrome improved, but multifocal dystonia persisted with dysarthria, cervical dystonia, and foot dystonia. Due to a disc prolapse, he was operated on the cervical spine when he was 47 years old. He tended to treat his motor problems with physiotherapy and remained on the low dose of 900 mg of DPA. Over the years, he gradually worsened: his cervical dystonia and dysarthria became severe. When he was 50 years old, he experienced a left hemispheric stroke probably due to a dissection of the left carotid artery, but an open foramen oval with a paradoxical embolus might have also been the cause of the stroke. Before the stroke, the patient had already been treated with 500 U of aboBoNT/A or 200 U of incoBoNT/A to improve neck pain. After the stroke, the patient became anarthric and started to drool again. In addition to the treatment of the cervical dystonia, the patient was also treated because of the hypersalivation with 100 U of incoBoNT/A into the parotid glands with success (Fig. 20).

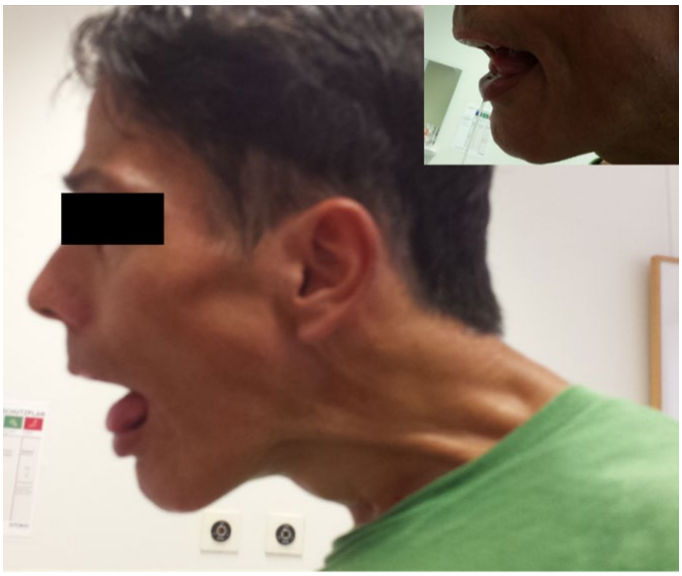


Fig. 20: WD and multifocal dystonia and hypersalivation (patient 5).

3.5.7 WD and Spasmodic Dysphonia (Patient 6)

The diagnosis of WD was made when the patient was 5 years old. Development and childhood were normal under continuous medication. When the patient was married, he left his parents and decided

to stop the medication. Even after the first visit to the department of gastroenterology in Düsseldorf, he continued cessation of medication. In the following 3 years, he became dysarthric and suffered from an impulse control deficit. He was divorced and decided to live with his parents again. In 2014, he survived an embolic infarction of the lungs and decided to start with a low dose of DPA again. Due to increasing difficulties in swallowing and loss of 14 kg body weight, he was referred to be analyzed for the presence of malignancy, which was not confirmed. During that time, he became anarthric and used his mobile phone for communication. The consulting neurologist and psychiatrist both agreed on the diagnosis of a psychogenic speech disorder because of the severe psychosocial problems and recommended treatment with Seroquel®. The speech therapist insisted on the diagnosis of organic dysarthrophonia. Five months later, the patient presented in our institution. He was completely anarthric and had a juvenile Parkinsonian syndrome, which rapidly improved after the withdrawal of Seroquel®. Dose of DPA was increased up to 2700 mg and dysarthria was treated with 5 U of onaBoNT/A or 10 U of aboBoNT/A per cricoarytenoideus muscle. Injections were performed from the outside. Clinical score, speech, and laboratory findings slowly but continuously improved excellently (Fig. 21; right side). After 2 years, BoNT/A injections could be discontinued. Laboratory findings yielded a normal copper excretion after 3 days of no medication. Thereafter, the patient reduced and withdrew medication a second time and worsened rapidly again. Two more injections of BoNT/A became necessary and the dose was increased again to the former level. In the following months and years, the patient stabilized, did not need further BoNT/A injections, and his impulse control deficit considerably improved. Nevertheless, the patient is unable to perform regular work (Fig. 21).

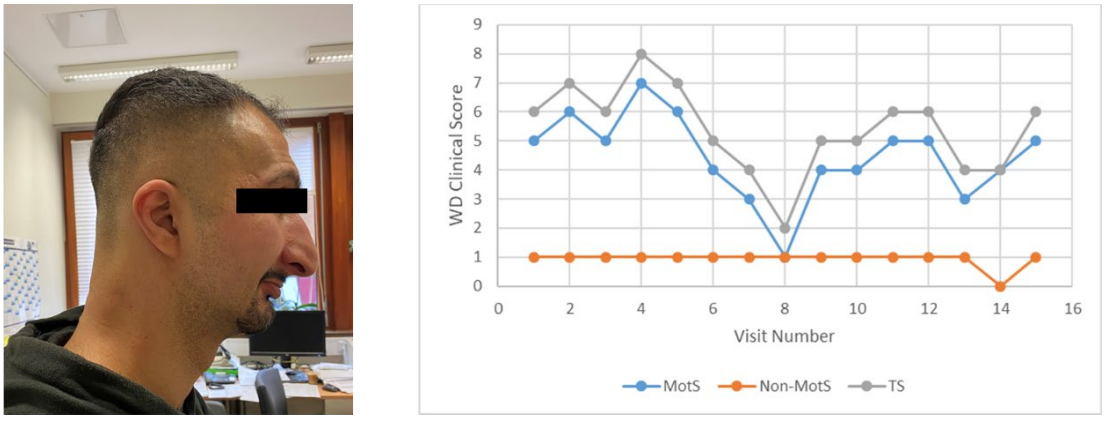


Fig. 21: WD and spasmodic dysphonia (patient 6; left). (right) Development of clinical scores (for details, see Table 1), demonstrating excellent improvement after the onset of copper chelating therapy. After a second withdrawal medication, the patient worsened again. He did not reach the level of improvement again that he had had before the second withdrawal of the medication.

3.6 Survival analysis (Kaplan Meier survival curve) in the study population

In order to show the probability of the events such as HCC manifestation or mortalities from Wilson disease, Kaplan Meir curves are plotted in Fig. 22. From a total of 115 WD patients, 12 patients passed away and 5 patients manifest HCC, and no other patients dropped out of the study before the end of observation.

The gray zone in the figures is the confidence interval of survival probabilities calculated. Since the number of events in our data set is relatively few, particularly with HCC manifestation, therefore the interval width is considerable wide, and even after 60 years from WD diagnosis, the survival probabilities may indicate at best values of more than 90% and at worst as low as 60%. On the other hand, there is no healthy control or stratification possible to compare mortality between groups.

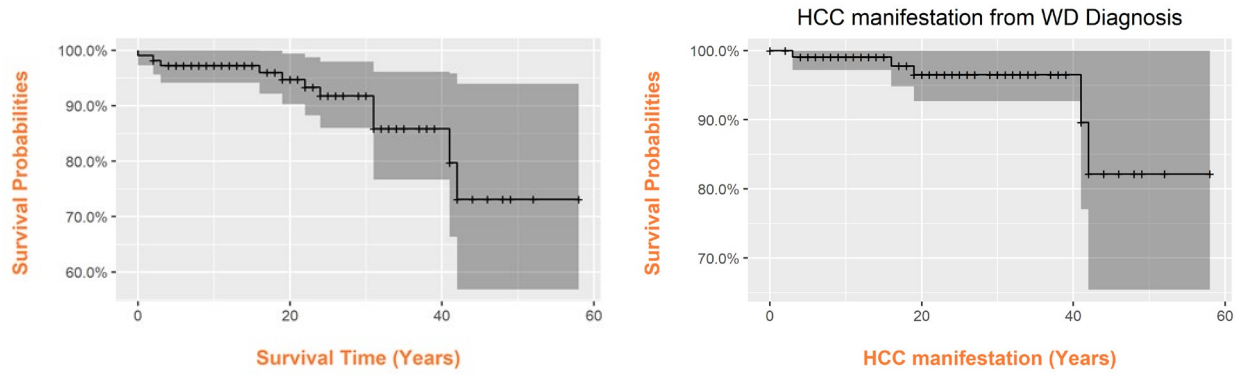


Fig. 22: Kaplan Meier Curve (Death (left side) and hepatocellular carcinoma (right side) as event)

4 Discussion

In section 1.8 “Aim of the study” we raised 7 different questions, which will be discussed and answered step by step in the following section.

4.1 Do neurological symptoms in WD improve with therapy?

To answer this question, we compared the presence of symptoms at the first and the last visit in patients who had at least two control visits (n=79). Compared to the first visit, the frequency of occurrence was slightly lower during the last visit, but the statistical analysis revealed no significant differences except for the subscore tremor.

At first glance, this result appears to be at variance with a variety of reports that neurological symptoms respond to therapy (Burke et al., 2011) and with our observation that the total neurological score clearly declines during the first 5 years of treatment except that another neurological comorbidity as MS (Fig. 11) hides the improvement.

We, therefore, performed a more refined analysis of age by determining the mean intensity of a symptom per patient of all visits within a small age range. This analysis clearly shows that most symptoms improve continuously with age up to the age of 40. Beyond 40 most symptoms were observed more frequently again.

This type of dependence of neurological symptoms on age or duration of treatment has not been demonstrated previously. Hefter et al. have shown an increase of non-motor symptoms with age (Hefter et al., 2018). But the clear improvement over years of treatment followed by more symptoms in elderly patients has not been described before.

A second-order polynomial fits excellently to the dependence of neurological symptoms on age ranges (Table 13).

4.2 Do all symptoms respond equally to therapy?

This is true not only for the summation scores (total score, motor score, non-motor score; see Fig. 9) but also for most individual symptoms (Table 13). But there are at least two exceptions that have a less good fit with a second-order polynomial: the oculomotor symptoms and the sensory symptoms (Table 13). Ocular motor abnormalities belong to the less frequent findings in the spectrum of neurological symptoms, but may be very severe and very disabling (Shribman et al.,

2019, Ortiz et al., 2020). Ocular gyric crisis has been observed in two of our patients. Fortunately, in both cases, this impossibility to shift the gaze to a point of interest in the visual field disappeared in both cases during the first two years of treatment. Gaze nystagmus is also observed more frequently during the first than during the last visit Table 12. In contrast, sensory symptoms were rare at the first visit and more frequently seen during the last visit.

We, therefore, conclude that the neurological symptoms underly two completely different influences: 1) the improvement due to therapy and 2) the worsening with age. This is demonstrated in the following scheme Fig. 23.

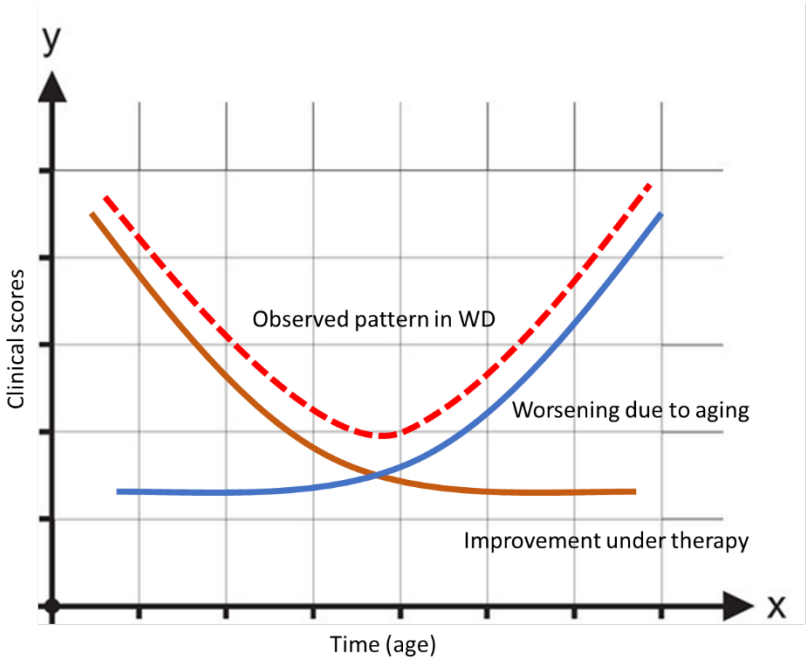


Fig. 23: schematic drawing of the aging and therapy effect on the course of WD disease

As a result, a typical u-shape of the results of the curve shows an excellent fit with a second-order polynomial Fig. 9.

4.3 Does age have an influence on the spectrum of symptoms in long-term treated WD?

Although the analysis showed the influence of age on symptom worsening, other significant confounding factors should be considered. Therefore, It had been demonstrated years ago that cerebral glucose metabolism (rCGM) is disturbed in WD (Kuwert et al., 1992). With the initiation of treatment in WD rCGM recovers in most brain regions. However, it could be demonstrated that with a too low maintenance dose rCGM worsens again. Thus, it may be that the u-shaped

symptom/age dependence curves simply result from treatment with a too low maintenance dose. Another similar interpretation is that those patients who are about 60 years now have been treated with too low doses initially so that the recovery of symptoms was incomplete. In the following years, although attempts had been made to reduce the symptoms by means of higher doses no further improvement occurred. To solve this difficulty of interpretation longitudinal studies are recommended which will clearly show the temporal development of symptoms.

However, the difference between the oculomotor and the sensory symptom curves may be used as an argument for a relevant influence of age or duration of treatment.

4.4 Do subgroups of WD patients exist with a worse outcome than the rest of the patients?

It has been reported over and over again that WD with psychiatric symptoms has a worse outcome than patients without psychiatric impairment. The Italian task group for orthotopic liver transplantation has also reported that WD patients with psychiatric symptoms do not respond to liver transplantation (LTx) as well as WD patients without psychiatric symptoms (Medici et al., 2005). Another group even recommended avoiding LTX in WD patients with clear psychiatric symptoms (Senzolo et al., 2007).

This is confirmed by our analysis of clinical long-term outcomes. The more affected the WD patients with psychiatric symptoms were the more impaired was also liver function indicating a possible compliance problem (Fig. 12). Patients with anxiety, psychosis, and/or mania tend to reduce or discontinue psychiatric specific as other relevant medication.

But side effects of psychiatric-specific drugs may worsen special neurological symptoms as wells as liver function. Therefore, knowing and observing patients carefully, is necessary to get the right feeling of whether the patient is compliant or not.

It is difficult to decide whether psychotic symptoms or mania are possible symptoms of neuropsychiatric WD or are separate disease entities. Since psychosis and bipolar mood disturbances may persist in excellently treated patients, we tend to assume that these patients suffer from WD and another psychiatric disease entity.

The subgroup of patients with orthopedic symptoms also tends to have a worse outcome compared to patients with pure WD. Gait is among the most relevant symptoms in WD (Table 14). However, gait is severely influenced by arthrosis, cervical and/or lumbar spine syndrome as well as bone

deformities. Before copper eliminating drugs became available bone deformities were a frequent finding (Bhatnagar et al., 2017) in WD. Nowadays bone deformities do not occur in sufficiently treated WD patients. However, the frequent pain in large joints, the high frequency of total hip replacements, and the frequent cervical as well as lumbar spine syndrome in WD (for comparison see also B Andersen Dissertation) indicate that orthopedic problems are still a relevant clinical problem in WD.

Whether these orthopedic problems indicate insufficient treatment or side effects of copper elimination therapy is completely unsolved. In the future, treating physicians should be more aware of these symptoms.

Four of our patients suffered from WD and MS. If these disease entities were completely independent a frequency of occurrence of less than 1/7.000 x 1/40.000 = 1/280.000.000 had to be expected. 4 patients in Germany yield a prevalence of 4/80.000.000=1/20.000.000 which is about 14-fold higher than expected from independent inheritance. Therefore, the relationship between inflammation and elevated serum levels of free copper has to be analyzed in more detail.

4.5 What is the spectrum of comorbidities in WD and what is their influence on the outcome?

Because of the widespread influence of copper on enzymatic chemical reactions in the human body, it is very difficult to decide whether separate comorbidities exist or symptoms are enhanced because of WD. But the persistence of symptoms despite sufficient copper elimination indicates the presence of a separate disease entity.

In our cohort of 115 WD patients, the majority of patients had at least one comorbidity. In case the comorbidity is diagnosed first it may obscure the diagnose of WD. For example, WD was diagnosed in the patient with myotonia by chance, when the patient was seen by ophthalmologist for other reason, Kayser-Fleischer-Ring was examined and reported.

On the other hand, WD may obscure the manifestation of rare comorbidity as hemosiderosis for example in section 3.4.6. The rare combination of WD and hemochromatosis had already been diagnosed years before the hemosiderosis was detected. The gait disturbance in this patient was interpreted as a symptom of WD in this patient. Only after the development of atypical aspects as pyramidal tract signs and elevated muscle reflexes an additional MRI scan was performed.

It has been mentioned by Walshe that elevated copper levels in WD may prevent the occurrence of a hepatic cell carcinoma (HCC) (Walshe, 1956). This has been repeated by others in literature as well. As time went by several WD patients with HCC have been reported. In a larger series of WD patients ((Pfeiffenberger et al., 2015); n=1186) only 8 patients (=0.67%) with HCC were detected. In our cohort of 115 patients, 5 patients (=4.3%) had developed HCC. We think that HCC is not a separate disease entity but is the result of long-standing liver cirrhosis which is part of WD. In all five patients with HCC, the time to diagnose was longer than usual. Therefore, all five patients remained untreated for a longer time than most other patients of our cohort.

4.6 Do special symptoms and/or comorbidities need specific treatment?

We were surprised to realize that despite its frequent occurrence focal dystonia was treated only in a few patients with botulinum toxin worldwide (1.7.2). Six of the 13 WD patients being described in the literature to be treated with BoNT/A were participants of our cohort. Worldwide most WD patients are treated by gastroenterologists with little or no experience in the application of BoNT/A. We, therefore, think that a large number of WD patients with focal dystonia remain insufficiently treated although it is known that dystonia may respond less well to the standard medication of WD (Burke et al., 2011) and that there is a need for alternative treatment options.

BoNT/A is Effective in Normal Doses in WD

The number of WD patients including our case series reported to be treated with botulinum neurotoxins is fairly small (n = 19). Unfortunately, the preparation and dose are often missing in the reports. None of the reports indicated inefficacy of BoNT therapy or response only to unusually high doses. In our case series, mild to very good efficacy was observed during the treatment of all six patients. The spectrum of indications for treatment with BoNT in the 19 WD patients was broad (Table 8, Table 25). In those indications where no effectiveness would have been noticed immediately (dysarthria in patient 6 and hand function in (Litwin et al., 2017)) and for which low doses were used, efficacy was the best. In summary, the present and other cases with clinical data reported in the literature to date do not provide any hint that normal doses of BoNT are not effective in WD. The reason why this is the case is not obvious. The mechanism of how the LC of BoNT/A and copper interact intracellularly is well-known; extracellular administration of copper complexes effectively reduces LC-mediated cleavage of SNAP-25 (Bremer et al., 2017). Thus, for the interaction between copper and the LC of BoNT/A, sufficiently high levels of copper have to be available intracellularly. As long as copper is irreversibly bound intracellularly to metallothionein

(Bandmann et al., 2015), which is usually the case in long-term-treated WD patients, this copper accumulation does not influence BoNT action. However, the normal response to BoNT/A treatment of muscles and glands in WD, as demonstrated in the present study, indicates that the crossly fluctuating and not always elevated levels of free copper in the serum of not optimally cooperating WD patients, as in our series, are not high enough to reduce BoNT activity to a clinically relevant extent.

BoNT/A Is Only Used in Severely Affected Patients:

The WD treatment strategy is to diagnose and treat it and eliminate copper as early and as possible. With this strategy, the majority of WD patients can be kept in an asymptomatic state. If neurological symptoms have become manifest, the use of higher doses is necessary to reduce the neurological symptoms (Hefter et al., 1993, Kuwert et al., 1992). A mild slowness or bradykinesia may persist, but in most WD patients, symptoms can be reduced to such a degree that employment is preserved (Hefter et al., 2018). Therefore, in most WD patients, there is no need for symptomatic treatment with botulinum neurotoxin or other oral medication than copper chelating drugs (Litwin et al., 2017). Therefore, it does not matter that, worldwide, WD patients are treated mainly by hepatologists with little experience with BoNT/A; however, in exceptional or incompliant patients, a clear indication for treatment with BoNT/A may exist. This is demonstrated by our case series. All these six patients were not optimally compliant (Table 25) for different reasons. These few patients can be referred to movement disorder specialists for BoNT/A treatment.

In the Majority of WD Patients, There Is No Indication for BoNT/A Treatment:

In the majority of WD patients, neurological symptoms respond quite well to copper elimination therapy (Hefter et al., 2018, Burke et al., 2011). Only in a small percentage of WD patients do severe neurological symptoms persist. If these patients undergo liver transplantation, neurological symptoms often respond quite well so that BoNT/A may be performed to bridge to transplantation and provide the patient some relief, as described by Joan et al (Joan et al., 2008). Even when a WD patient is newly diagnosed, there is no urgent need to initiate BoNT therapy as well. However, for patient 6, who became anarthric after the withdrawal of medication, or patient 5, who suffered from severe drooling after a stroke, it may greatly benefit the patient to be treated with BoNT transiently.

In cases of an additional neurological disease like MS, polyneuropathy or myasthenia gravis these disease entities have to be treated according to the therapeutical guidelines for these diseases. This is also the case for non-neurological comorbidities. Severe joint pain and arthrosis have to be treated

by replacement operations, malignancies by specific chemotherapies and cardiovascular disease entities by specific medication or operation.

4.7 Do patients with WD have normal life expectancy under standard copper chelating therapy?

In 1990 Stremmel et al. published a study on long-term outcomes in WD. They demonstrated that the life expectancy of WD patients is normal up to the age of 35 years (Stremmel et al., 1991). Years later Sipilä et al. reported on the long-term outcome of 33 patients in Finland (Sipilä et al., 2020). In their cohort of WD patients' life expectancy was significantly reduced compared to the normal population. Especially when WD patients reached an age above 40 survival time significantly declined.

We compare these two available Kaplan-Meier survival curves with the survival curve presented in Fig. 24 (below). The death rate in our cohort is even lower than in the Finnish cohort. We observed 12 out of 115 deaths (= 10.4%), in the Finnish cohort 5 patients died (= 15.1%). However, 2 patients in our cohort died early around the time of diagnosis and 6 patients because of a malignancy. Therefore, the survival curve of our cohort declines slightly more steeply than the survival curve of the finish cohort Fig. 24 (left).

In summary, the analysis of survival time in our cohort revealed an at least as steep decline with age than that presented for the Finnish cohort Fig. 24 (left). Therefore, our results support the Finnish observation that life expectancy is not normal in WD. Up to the age of 40 and a treatment duration of 20 years, survival time seems to be normal when the initial manifestation of WD is survived. But beyond the age of 40 the risk to develop a malignant decompensation of liver cirrhosis rapidly increases. This is the major reason for a reduced life expectancy in WD.

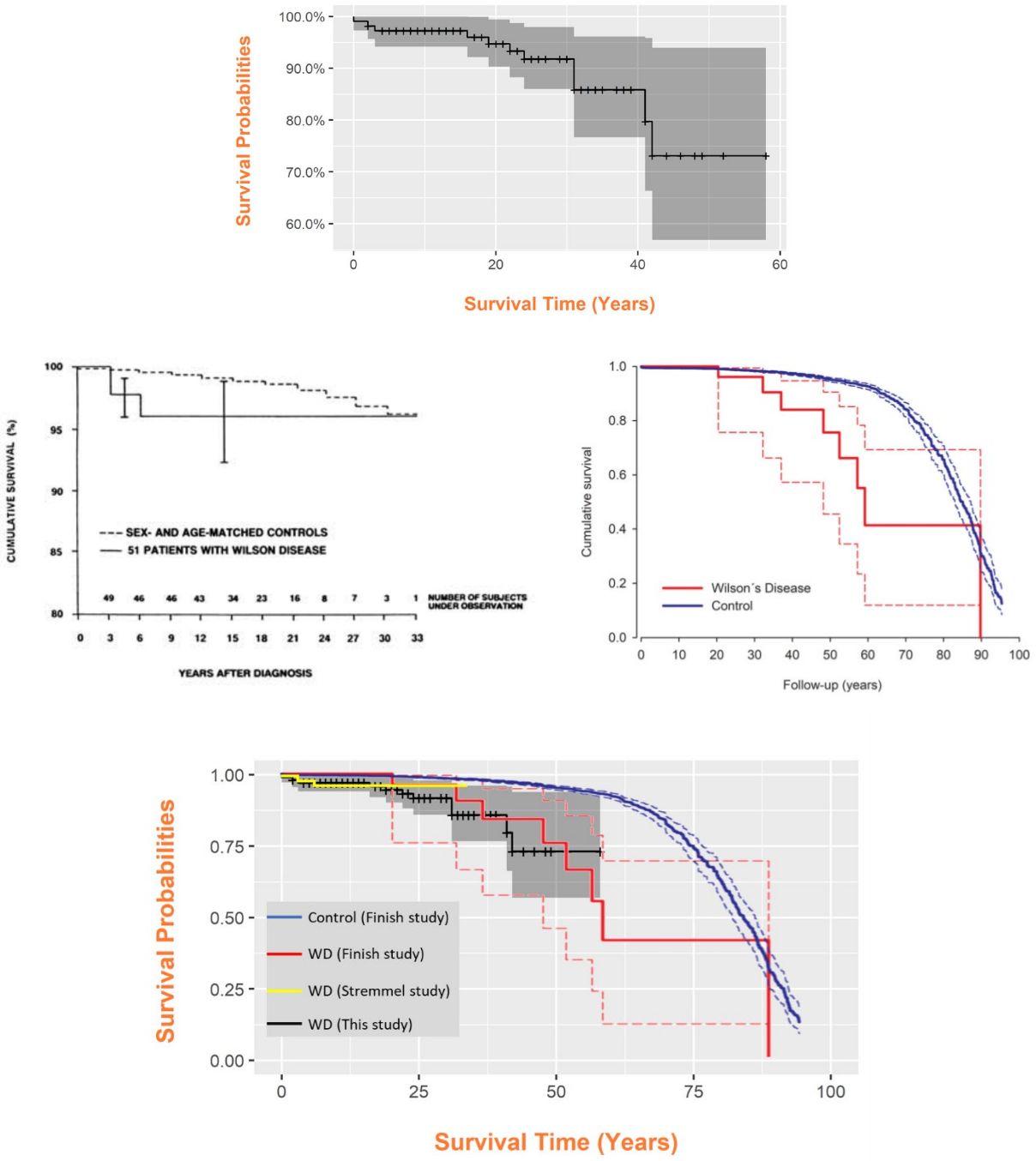


Fig. 24: survival analysis comparison among different studies with the current study (below); our survival analysis (above); Stremmel et al. study (right) (Stremmel et al., 1991); Finnish study(left) (Sipilä et al., 2020); (Stremmel et al., 1991) is open access available under a Creative Commons or similar license that allows more liberal redistribution and reuse than a traditional copyrighted work; (Sipilä et al., 2020) reproduced with permission from (scientific reference citation), Copyright Massachusetts Medical Society.

4.8 Recommendations for the treatment of patients with WD

In summary, WD in itself is a complex disease entity because of its possible impairment mainly of the central and mildly of the peripheral nervous system as well as a variety of organs including the blood. Specific therapy systematically improves symptoms with WD, but with age and duration of treatment a secondary worsening may occur. The high frequency of comorbidities that may become manifest before or after the manifestation of WD affords careful clinical monitoring. The possible development of HCC is a further important argument for careful monitoring. Early treatment as well as the use of sufficiently high doses are recommended to treat neurological WD to reduce symptoms and increase the quality of life. Comorbidities should be detected early and treated adequately since survival time is reduced in WD mainly because of late onset of specific therapy and the development of malignancies or other comorbidities.

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