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Review of

EAN 2016

Copenhagen, Denmark

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Welcome

Welcome to *EMJ Neurology*, an edition filled with informative and engaging content which spotlights the latest research and developments in the field of neurology. Leading the journal is our in-depth review of one of the most important events on the neurology calendar, the 2nd Congress of the European Academy of Neurology (EAN), which took place in Copenhagen, Denmark, from May 28th–31st 2016. We then present a selection of peer-reviewed articles that offer significant contributions to the advancement of our understanding of neurological disease and provide a perfect springboard for discussion.

EAN 2016 was an excellent showcase of the wide range of neurological research being undertaken to explore the pressing issues and challenges currently facing the field. We have included summaries of a selection of topical research presentations that took place at the congress and which can be found in the abstract reviews. Some of the areas covered here include the treatment of tics and Tourette's syndrome, the rare Fabry's disease, and the impact of bilingualism on cognitive functions, all written by the presenters themselves. Our coverage also highlights the important news and announcements from EAN 2016 which will ensure you stay up-to-date with the latest activity in neurological research. In the congress review section, you can read about the calls for European guidelines to tackle Alzheimer's disease or the emerging treatment for migraine sufferers, as well as many other interesting and important stories.

Following the coverage of EAN 2016 are our insightful peer-reviewed articles which have been written by esteemed and successful neurologists working across Europe and beyond. In this issue we present an impressive selection of articles, including an engaging discourse on the role of apathy as a major factor affecting the quality of life in patients with Parkinson's disease and the clinical challenges faced in overcoming this problem. You will also find an informative review of the rare genetic copper accumulation, Wilson's disease, which highlights the experimental efforts currently underway to address its pathogenesis.

It is our sincere hope that what you find here in *EMJ Neurology* can be used to guide and inform your own efforts in researching and working in the field of neurology. After this year's impressive progress across the field, we cannot wait to see the advancements yet to come and report on them from next year's EAN congress in Amsterdam; we hope to see you there too!

Spencer Gore

Director, European Medical Journal

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Front cover and contents photograph: Copenhagen, Denmark, home of EAN 2016.

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Foreword

Prof László Vécsei

Director of the Department of Neurology, University of Szeged, Szeged, Hungary.

Dear Colleagues,

Welcome to the latest edition of *EMJ Neurology*. This issue features several important fields of clinical neurology, and encompasses a review of the EAN annual congress 2016.

Apathy is a major contributor influencing quality of life in Parkinson's disease, affecting up to 70% of those with the condition. Depression and anhedonia can complicate the diagnosis, but dissociations suggest different pathomechanisms. There is growing data to support the potential role of new drugs; in this issue there is an excellent review of the current understanding of apathy.

Treatment of multiple sclerosis today is an exciting challange. We have several new parenteral and peroral drugs and a review in this issue asks the question: Where do we go from here? Wilson's disease as an inherited silent copper intoxication and haemangioblastoma are also discussed in this issue.

> "The recent 2nd Congress of the European Academy of Neurology (EAN) in Copenhagen, Denmark was very successful."

A future question is the selection of patients for treatment with recanalisation therapies in an extended time window. Another problem is cryptogenic stroke evaluation, including long-term monitoring for paroxysmal atrial fibrillation. Prothrombotic blood alterations could be involved in the left atrial appendage thrombus formation in patients without documented atrial fibrillation and are associated with increased risk of stroke or transient ischaemic attack. Anticoagulation therapy is effective and safe for stroke prevention and decreases mortality in patients with incident atrial fibrillation. Therefore, the aggressive approach to screening for atrial fibrillation and treatment with anticoagulant drugs to prevent stroke and death is essential. These issues and more are tackled in the selection of high-quality articles that follow.

The recent 2nd Congress of the European Academy of Neurology (EAN) in Copenhagen, Denmark was very successful. This major scientific event attracted around 6,000 participants from European and non-European countries. In the course of over 90 scientific sessions, international experts presented interesting trends and highlights of today's neurology and therapy. I hope you enjoy EMJ's coverage of the event in this year's edition of the journal.

László Vécsei

Director of the Department of Neurology, University of Szeged, Szeged, Hungary; Past Regional Vice-President of the European Federation of Neurological Societies (EFNS); General Secretary of Danube Neurology Symposium.

the MENARINI group

MIGRAINE: THE SEVENTH DISABLER[1]

Frovatriptan in the world is:

Allegro®
Auradol®
Dorlise®
Eumitan®
Forvey®
Fromen®
Fromirex®
Frotan®
Frovamen®
Frovamig®
Frovex®
Menamig®
Menatriptan®
Migard®
Migralin®
Migrex®
Perlic®
Pitunal®
Rilamig®
Tigreat®
Triptar®

A MORE SUSTAINED EFFECT ON RELIEF OF MIGRAINE SYMPTOMS[2]

Frovatriptan is indicated for acute treatment of the headache phase of migraine with or without aura[3]

1. Steiner T.J. et al.; J Headache Pain 2013; 14:1 2. Franconi F. et al.; Neurol Sci 2014; 35(Suppl. 1): S99-S105 3. Migard-Summary of Product Characteristics

EFFICACY IN THE TREATMENT OF MIGRAINE

Migraine was ranked seventh highest among specific causes of disability globally (responsible for 2.9% of all years of life lost to disability), and in the top ten causes of disability in 14 of the 21 world regions. [1]

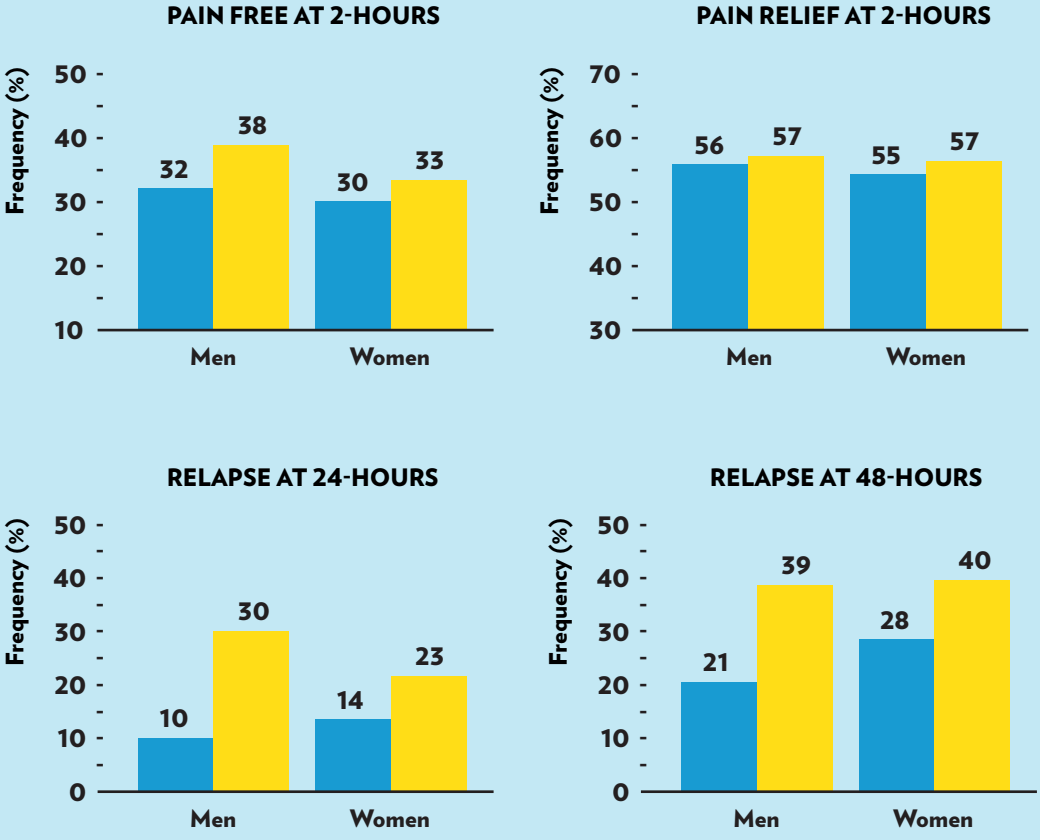
This chronic neurovascular disorder occurs in both genders, although large surveys show higher prevalence of this condition in women, with a female to male ratio in the order of 3:1. [2]

Although migraine acknowledges a complex pathophysiology, involving genetic and psychological factors, the disproportionate number of fertile women with migraine suggests that hormonal factors may indeed play an important role in the pathogenesis of migraine. [2]

In addition, attacks are usually reported to be more severe and difficult to treat in women than in men. [2]

A pooled analysis of three individual randomized, multicenter, double-blind, crossover Italian studies on patients with migraine showed that frovatriptan 2.5 mg had a more sustained effect on relief of migraine symptoms than comparators. In particular, relapse at 24 h was significantly ($p < 0.05$) less likely to be reported in frovatriptan than in comparator-treated patients, with no between-gender difference. This was the case also for rate of relapse after 48 h ($p < 0.05$ between treatments). [2]

Although migraine presents in a more severe form in women, frovatriptan seems to retain its good efficacy and favorable sustained antimigraine effect regardless of the gender. [2]



Proportion (%) of pain free at 2 h, pain relief at 2 h and relapse at 24 and 48 h in the 66 men and 280 women with migraine of the intent-to-treat population.
Data are separately shown for frovatriptan- (blue bars) and comparator-treated patients (yellow bars).
The p value refers to the statistical significance of the between-treatment difference.

Elaboration of Fig. 2 of (2)


ENHEDSPRIS
NIELS BROCK

EAN ANNUAL CONGRESS 2016

THE BELLA CENTER,
COPENHAGEN, DENMARK
28TH-31ST MAY 2016

Welcome to the *European Medical Journal* review of the 2nd Annual Meeting of the European Academy of Neurology Congress

Although history and tradition are hallmarks of Danish culture, recent developments have created a hub of modern Danish trademarks in Copenhagen. Hosted jointly by the European Academy of Neurology (EAN) and the Danish Neurological Society (DNS), the 2nd EAN congress joins the throng of innovative events and projects growing in Ørestad, the rising neighbourhood of Copenhagen. Prof Mads Ravnborg, President of the DNS, perfectly summarised the nature of EAN 2016 in his opening address: "Our purpose is international networking, free exchange of knowledge, mutual understanding, and friendship across national borders and cultures."

A young society no less steeped in tradition than any other, the EAN, with its 47 member societies and 25,000 members, is perfectly situated to offer a platform for multidisciplinary collaboration and debate. This year's congress welcomed almost 6,000 neurologists to share in a combination of scientific and educational excellence, with plenty of opportunity for discussion of research, resource allocation, and healthcare policy, all of which are advocated by the EAN for the benefit of all European healthcare professionals with an interest in neurology.

Alongside oral sessions, poster presentations, and practical tutorials, the congress presented a variety of awards acknowledging the high quality of research emerging in the realm of neurology. Prizes included the Investigator's Award, given to the 20 best presentations across the congress, and the P.K. Thomas Prize, awarded to the best paper on peripheral nerve disorders. In two sessions, 12 young researchers presented their work in competition for the EAN 2016 Tournament prizes. Asya Ekmen, France, went home with first prize in the basic neurology category, for the presentation of 'Zinc release and action at excitatory Schaeffer collateral-CA1 pyramidal cell synapses'.

With regard to clinical neurology, Marte Helene Bjørk, Norway, won the day with a paper entitled 'Low maternal folic acid in women with epilepsy during pregnancy is linked to autistic traits in the child at 3 years of age.' Both of these sessions also awarded two runners up. For clinical neurology, Hans-Georg Wirsching, Switzerland, was awarded for a study of complete glioblastoma resection,

while Simão Cruz, Portugal, was acknowledged for research concerning clinical, biochemical, molecular, and histological features of mitochondrial disorders. In the basic neurology category, Marta Pace, Switzerland, came runner up for investigation of sleep deprivation in pre-ischaemia preconditioning, alongside Tobi van Den Bossche, Belgium, for characterisation of Alzheimer's disease patients with the *SORL1* genetic mutation.

> "Our purpose is international networking, free exchange of knowledge, mutual understanding, and friendship across national borders and cultures."

There was a massive amount of research on display at the event, captivating the rapid rate of progress that has been observed in the field of neurology in recent times. Presentations at EAN displayed research that is certain to increase understanding of conditions such as dementia, Alzheimer's disease, Parkinson's disease, and stroke, amongst many others. There is no doubt that there are many challenges ahead for neurologists in Europe in the years ahead, with certain types of neurological conditions increasing in prevalence, particularly in the context of an ageing population. As such, a lot of presentations at the EAN congress outlined the need for new strategies to cope with, and tackle the increasing demand for care.

In the following review, we bring you breaking news direct from EAN 2016, providing a snapshot of activity at the frontiers of neurological research. We hope you find the stories insightful and fruitful to your own practice. We await another year of neurological progress and the 2017 EAN congress in Amsterdam, Netherlands.

Cultivating a New Paradigm of Stroke Care

SIGNIFICANT developments in stroke care have been seen in recent years and many trials were presented at EAN 2016 looking at specialist centres, imaging options, and treatment for stroke patients, aiming to confirm the effectiveness of these new options. The highlights were reported in a EAN press release dated 30th May 2016. Prof Franz Fazekas, Department of Neurology, Medical University of Graz, Graz, Austria, discussed the need for a change in processes for these new developments to be effectively implemented, saying: "This reorganisation must cover the entire chain of care, from the ambulance ride to the precisely defined use of thrombectomies."

Thrombectomy has been hailed as a novel treatment option in many recent trials, with >60% of patients now surviving a stroke with little or no impairment following the procedure. European organisations have now come together with the aim of providing a treatment consensus paper, defining all aspects of thrombectomy, from when to use it, to what instruments to use, and how to care for the patient postoperatively. Prof Fazekas suggested that many lives will be saved or improved with the implementation of these guidelines.

"This reorganisation must cover the entire chain of care, from the ambulance ride to the precisely defined use of thrombectomies."

Specialised centres for stroke care have been rising recently, providing more specialist staff with more up-to-date knowledge that was hoped to revolutionise care. A recent study of 9,500 patients from the Danish stroke registry has shown that since the change to specialist centres, more patients are receiving thrombolysis within an hour of reaching hospital, more patients are receiving early procedures to treat carotid artery stenosis, and 30-day mortality is reducing. These benefits echo the initial intentions of these centres and give a positive outlook for future improvements in care.

Making the correct decision when treating a stroke is essential and imaging is a key part of the diagnosis, thus confidence in the image presented is vital to choosing the right care in the right time. A recent study of 444 patients showed that doctors are now more confident in magnetic resonance imaging and provide better decisions despite the increased imaging time.

Virtual Support may have Three-Fold Benefit for Epilepsy Patients

COMMUNICATION could be key to bettering the lives of epilepsy patients, suggests a EAN press release dated 29th May 2016. Patients with a variety of chronic diseases have demonstrated great improvements through the use of support networks, however for patients with epilepsy such groups are unfortunately hard to come by. "Networking and mutual support through instant messaging services such as WhatsApp can be a practical and affordable alternative in these cases," highlighted Dr Heather Angus-Leppan, Consultant Neurologist, Royal Free Hospital, London, UK.

With this observation in mind, a group of researchers are investigating the possible outcomes of a virtual discussion group designed to promote mutual support between epilepsy patients. Using the online service WhatsApp to create an instant messaging group, the team created a discussion forum connecting 300 epilepsy patients; this group continued beyond the initial research. Contribution is at the discretion of each individual participant, and important issues such as navigating stigmatism in the workplace are open for conversation. The study aims to examine the effect of the virtual facilitation of communication over a period of 6 weeks.

The study aims to explore the possible benefits that instant messaging groups and the involvement each patient has in them have on three different parameters of patient healthcare: adherence to therapy, length of hospital stay, and quality of life. Moreover, the team are also interested in the notion that increased involvement in the support group and further interpersonal contact may influence the results within these parameters. Dr Angus-Leppan has high hopes for the data: "Based on current findings, we expect to see intensive personal communication improve the patient's adherence to therapy by 20%, lower the frequency of their stays in the hospital, and improve their quality of life by 10%." The conclusion of this study will illuminate the potential of virtual support groups for patient healthcare.

Neurological Expertise will be Vital to Battling Zika Epidemic

"EUROPE certainly needs to get prepared, just as other parts of the world, to cope with the consequences of the fact that the geographical distribution of the [Zika] virus is steadily and rapidly expanding" were the sombre words of Prof John England, Louisiana State University, New Orleans, Louisiana, USA, reported in a EAN press release dated 30th May 2016.

> "Europe certainly needs to get prepared, just as other parts of the world, to cope with the consequences of the fact that the geographical distribution of the [Zika] virus is steadily and rapidly expanding."

Indeed, with 60 countries and territories reporting mosquito-born Zika virus, as well as 10 countries, including Germany and France, reporting person-to-person transmission, the need for formal guidelines is pertinent. Despite lower risk in Europe than other continents, imported cases have already begun to emerge, including a case of Guillain–Barré syndrome (GBS) in the Netherlands. The association of fetal deformations such as microcephaly with the virus is well known amongst the general public, however awareness of the neurological risks for infected patients is low, and the true extent of possible cognitive complications is currently indefinable. "Neurological expertise will be crucial to deal with the consequences of Zika," emphasised Prof Ra'ad Shakir, President of the World Federation of Neurology (WFN).

According to the World Health Organization (WHO), Europe is at a low-to-moderate risk of the mosquito-born Zika virus infection, with the exception of regions populated with the *Aedes aegypti* mosquito. Nonetheless, the epidemic is expected to grow in Europe as a result of person-to-person transmission and travel. "All European countries should put measures in place in order to detect imported cases of Zika virus early and should provide public health advice to travellers to and from affected countries," advised Prof England.

As there are expected to be a high number of travellers to Rio de Janeiro for the 2016 Olympics, this advice is particularly significant as there are currently no particular treatment strategies or vaccines. The WHO and the Pan American Health Organization (PAHO) suggest the use of insect repellent, appropriate clothing, safe sex or abstinence, and air-conditioned accommodation where possible.

Call for European Guidelines to Tackle Alzheimer's Disease

AN APPEAL has been issued for standardised European guidelines for the treatment of Alzheimer's disease and approaches to its early identification in response to concerns for both safety and unsatisfactory care, stated a EAN press release dated 30th May 2016.

Prof Gunhild Waldemar, Neuroscience Centre, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, has called for researchers to work together and draw up guidelines towards effective treatments for Alzheimer's disease, the most common form of dementia. The future human and economic costs of this condition make such a strategy all the more necessary, with experts predicting the number of cases worldwide to rise from 47 million in 2015 to 75 million by 2030.

A better understanding of the condition is thought to be needed alongside more effective treatments and accurate early identification. As a result, Prof Waldemar warns against the assumption that Alzheimer's disease is an inevitability of old age. Modern imaging technology, biomarkers in the blood or spinal fluid, and genetic testing can all be used to support prognoses or early diagnosis of Alzheimer's disease, but it must be remembered that misinterpretation of such tests can have devastating effects. "In future

we will need medical, ethical, and legal guidelines to determine what form these tests should take, when they are appropriate, and to guide pre-biomarker counselling in patients," proposed Prof Waldemar.

The quality and suitability of care provided for dementia patients has also been criticised by Prof Waldemar, who calls for evidence-based guidelines for pharmacological treatment and more training for everyone involved in primary healthcare. A Danish study presented at the EAN congress revealed the unregulated doses and combinations of antipsychotic and other psychotropic medicines being prescribed for Alzheimer's patients despite a lack of evidence on their effectiveness.

> "In future we will need medical, ethical, and legal guidelines to determine what form these tests should take, when they are appropriate, and to guide pre-biomarker counselling in patients."

Additionally, a Swedish study presented evidence of insufficient medical treatment in response to other health problems faced by dementia patients. The research revealed the decreased probability of a patient receiving the drug warfarin to treat the heart rhythm disorder atrial fibrillation which can trigger strokes, in correlation with their cognitive deficits.

Co-ordinated Efforts on an International Scale Needed for Promising Treatment of Alzheimer's

THE PROMISING potential of biomarkers for the early identification and treatment of Alzheimer's disease is not being fully realised on both European and international scales, according to a EAN press release dated 31st May 2016. With the correct priorities in research, biomarkers could be an ideal strategy for the early detection of the disease and treatments tailored to the patient. However, the biomarkers currently being investigated and developed are considered at different stages without an adequate framework.

> "Clear-cut research priorities could accelerate a great many things, for instance, authorisation by regulatory agencies, reimbursement by payers, implementation in clinical practice, and ultimately the development of effective treatments."

Prof Giovanni Frisoni, Department of Mental Health and Psychiatry, University of Geneva, Geneva, Switzerland, highlighted ways to ameliorate the discordance in efforts toward biomarker development across European and international research fields. "Clear-cut research priorities could accelerate a great many things, for instance, authorisation by regulatory agencies, reimbursement by payers, implementation in clinical practice, and ultimately the development of effective treatments," he indicated.

2nd EAN congress

Experts unveiled a five-phase framework at the EAN Congress to help guide biomarker development, intended as a roadmap to help guide researchers, funding agencies, and policy makers. The five sequential phases are designed to speed up the progress of research towards new diagnosis and therapy options. They are: 1) preclinical exploratory studies; 2) clinical assay development for clinical disease; 3) prospective longitudinal repository studies; 4) prospective diagnostic studies; and 5) disease control studies.

Current literature on biomarker research within particular areas such as neuropsychology and amyloid imaging was assessed by experts according to this framework and found to be lacking beyond the first phase. Phases 2 and 3 were considered inconsistently addressed by the literature; preliminary evidence existed for phase 4 in some areas; and the aims of phase 5 had not been satisfied in any case. "This result shows us clearly the areas on which the scientific community must focus next in order to help Alzheimer patients better in the future," Prof Frisoni explained.

Huge Potential for Future Identification of Parkinson's Disease

THIS year's EAN congress yielded a number of progressive studies with regards to identifying the early stage of Parkinson's disease, a EAN press release dated 30th May 2016 reports. Attendees saw a range of new approaches to improving quality of life for those suffering with the disease, with emphasis on early diagnosis as a crucial part of this process.

> "There are still no reliable tests for early diagnosis of Parkinson's, so doctors have had to rely on experience alone. But now we have developed a completely novel neurological approach that links clinical examinations and statistical calculations of probability."

With Parkinson's disease being classed as one of the most common neurodegenerative conditions in Europe, the amount of research concerning it is unsurprising. The rate of sufferers is expected to double by 2030 and it is already ranked fifth in the list of the most expensive neurological conditions facing Europe's health systems. EAN President Prof Günther Deuschl, Faculty of Medicine, Christian-Albrechts University of Kiel, Kiel, Germany, highlighted the significance of the recent studies: "A long line of studies being presented at the EAN Congress confirm the existence of risk markers that are not necessarily associated with Parkinson's disease at first glance, and as such are easily missed." Research has shown that many symptoms emerge long before the onset of typical motor impairment, including hyposmia, constipation, dizziness, urinary dysfunction, and REM sleep behaviour disorder. The studies on this subject brought to light several biomarkers that could be fundamental to identifying Parkinson's disease in its prodromal phase from a reduced sense of smell to muscle weakness, whilst further research indicated that pathological changes are rooted in other nerve cells, not just the brain.

The Movement Disorder Society recently published criteria for diagnosing Parkinson's

disease, designed to standardise clinical research and provide diagnostic support. Assessment can subsequently factor in environmental and genetic risk information gathered about a patient. Prof Deuschl added: "There are still no reliable tests for early diagnosis of Parkinson's, so doctors have had to rely on experience alone. But now we have developed a completely novel neurological approach that links clinical examinations and statistical calculations of probability."

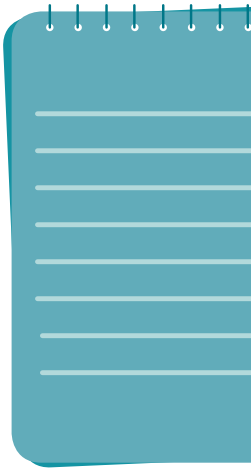
Cognitive Impairments Could Leave People with Chronic Pain at Risk of No Treatment

CHRONIC pain may be overlooked in patients with cognitive impairment and it may not be sufficient to rely on patient self-report, according to a recent Slovenian study. Chronic pain indicates a patient is suffering from pain persistently and possibly for long periods of time. However, research presented at the EAN Congress 2016 indicated that people with cognitive impairments such as dementia often fail to report chronic pain and it therefore goes unnoticed, resulting in treatment not being offered.

> "Cognitively impaired individuals or individuals with dementia evidently articulate their complaints less frequently. We therefore have to do more than just ask them about possible pain; we have to actively examine them to determine whether they are experiencing pain."

Dr Martin Rakuša and colleagues, Department of Neurology, University Medical Centre Maribor, Maribor, Slovenia, conducted a study involving 452 patients with an average age of 65 years who had been diabetic for many years. The aim of the study was to compare the prevalence of chronic pain in the limbs of diabetic patients, a frequent symptom of diabetes, with and without cognitive impairment. This was to evaluate whether a correlation between frequency of chronic pain and cognitive impairment emerged. In this cohort, 199 individuals were cognitively impaired.

Oral sessions, poster presentations, and practical tutorials



Of the 452 patients, 56 (12%) indicated they were suffering from chronic pain in their limbs. Out of those suffering with chronic pain, a minority of 17 people also had a cognitive impairment. Dr Rakuša stated in a EAN press release dated 31st May 2016: "Cognitively impaired individuals or individuals with dementia evidently articulate their complaints less frequently. We therefore have to do more than just ask them about possible pain; we have to actively examine them to determine whether they are experiencing pain."

Failure to receive proper medical advice and treatment for chronic pain could result in the condition persisting for even longer periods of time. This research is important as it has implications for the care of elderly individuals both within and outside of clinical care.

Scandinavian Tradition of Neuroscience Includes Vikings Experimenting with Consciousness

AN HISTORICAL overview presented at the Congress of the European Academy of Neurology reveals a 5,000-year-old Scandinavian tradition of neurological research stretching back to the prehistoric era. A EAN press release dated 29th May 2016 digs deep into this research.

> Prehistoric finds show that skulls were being trepanned in Scandinavia 5,000 years ago, in other words, skulls were being opened up to treat brain diseases or for ritualistic reasons.

Prof Ragnar Stien, Emeritus Professor, Department of Neurology, Oslo University Hospital, Ullevål, Oslo, Norway, pointed to a long and successful tradition in Northern Europe reaching back thousands of years. Prof Stien explained: "Prehistoric finds show that skulls were being trepanned in Scandinavia 5,000 years ago, in other words, skulls were being opened up to treat brain diseases or for ritualistic reasons." Osteological evidence of Viking skeletons indicates a contemporary awareness of neurological diseases and attempts to heal them. The Jomsvikings, a Nordic group existing in the 10th and 11th centuries, were also interested in neuroscience and conducted neurophysiological experiments exploring whether consciousness lingered if the head was removed from the body. According to an old Nordic saga, a warrior was able to test this when his executioner allowed him to hold a knife in his hand while being decapitated. Once beheaded the knife fell to the ground immediately.

During the early modern period in Denmark, notable scholars and physicians made significant discoveries relating to neurological science and greatly advanced what was known about the subject. This tradition can be seen for example with the Bartholin family who were recognised for their anatomical work in the 17th Century; Danish physician and theologian Caspar Berthelsen Bartholin first described the olfactory nerve, while his son Thomas Bartholin was the first to recognise the lymphatic system as an independent organ system. Additionally, scientists from this region were the first to describe neurological diseases that are well known today; one example being Folling's disease, named after the Norwegian Ivar Asbjørn Følling.

The Scandinavian impact on neuroscience continues to gain acknowledgement in more recent years, with several Northern European scientists awarded the Nobel Prize. In 2000, the Swedish pharmacologist Arvid Carlsson received the prize in recognition of discoveries regarding signal transmission in the nervous system. In 2014, Norwegian researchers May-Britt Moser and Edvard Moser were awarded the prize for their studies on spatial orientation and spatial memory.

Pregnant Women Urged to Take Folic Acid in Conjunction with Antiepileptic Drugs

PREGNANT women taking antiepileptic drugs (AEDs) without sufficient nutritional supplements could increase their child's risk of developing autistic traits, according to a press release dated 31st May 2016 from the EAN Congress in Copenhagen.

The Norwegian study, led by Dr Marte Helene Bjørk, Department of Clinical Medicine, Haukeland University Hospital, Bergen, Norway, monitored approximately 58,000 3-year-old children for possible autistic traits in order to examine the effects of AEDs and folic acid in pregnant women. The prospective mothers represented four different cohorts: those with epilepsy that took AEDs with no additional nutritional supplements; those with epilepsy that took AEDs with additional folic acid supplements; those with epilepsy that did not take AEDs; and healthy pregnant women not suffering from epilepsy.

The findings determined that low levels of folic acid in mothers taking AEDs can increase the chance of autism in the unborn child. The women taking AEDs with no additional nutritional supplements were found to bear children with autistic traits 6-times more often than those taking AEDs in conjunction with folic acid. Dr Bjørk commented: "The results clearly show how risky AEDs can be for unborn children. Twelve percent of the children who were exposed to the effects of these drugs during pregnancy ended up exhibiting autistic traits: a rate substantially higher than in the two other groups." Only approximately 3% of children whose mothers did not use medication for their epilepsy presented with autistic traits, and the rate for those whose mothers were healthy was roughly 4%.

Dr Bjørk advised that: "For pregnant women with epilepsy, the early administration of folic acid preparations is therefore an absolute must." However, it should also be noted that women with epilepsy should refer to their treating neurologist to plan their pregnancy, as stopping AEDs holds an even greater health risk for both mother and child than continuing the medication.

New Treatment Options for Migraine Sufferers in Development

MONOCLONAL antibodies targeting neuropeptide calcitonin gene-related peptide (CGRP), a known contributor to primary headache, could provide a new treatment option for sufferers of chronic migraine according to a EAN press release dated 31st May 2016. The discovery is just one of a number of new substances that have shown promising results in recent trials.

Copenhagen

> “Advances in migraine prevention give hope that we will be able to improve quality of life for sufferers in future.”

Migraine is ranked in the top five neurological disorders by the World Health Organization (WHO), affecting 6–8% of men and 10–12% of women. Although several preventative treatments are approved, including propranolol, metoprolol, and flunarizine, little is known about the ways in which these therapies work. Nonetheless, Prof Till Sprenger, Department of Neurology, DKD HELIOS Klinik Wiesbaden, Wiesbaden, Germany, highlighted that: “several randomised, placebo-controlled studies show that these medications can reduce the frequency of migraine attacks, even though the majority of them were originally developed to treat other indications.” The use of botulinum toxin Type A is also shown to effectively shorten the duration of migraine attacks in major studies and clinical practice.

New substances including angiotensin-converting enzyme and angiotensin II receptor antagonists are being developed and have offered new hope to sufferers. Candesartan for example has shown promise in trials: “Candesartan proved effective in two placebo-controlled studies, in which 16 mg of candesartan was compared with a placebo and 160 mg of propranolol. Candesartan was demonstrated to have similar effectiveness to propranolol, and both were superior to placebo,” Prof Sprenger explained. Another optimistic development is in monoclonal antibodies targeting neuropeptide CGRP. Although CGRP receptor antagonists have previously been developed to treat migraine, side effects such as increases in liver enzyme levels have inhibited their approval as treatments by governing bodies. According to recent studies, the new antibodies appear to be better tolerated than the previously used therapies. Prof Sprenger added that: “Advances in migraine prevention give hope that we will be able to improve quality of life for sufferers in future.”

Impairment to Sexuality Underestimated in Neurological Disorders

INFERTILITY, loss of desire, and erectile dysfunction are just some of the effects of neurological disorders, including Parkinson's disease. These can often be underestimated, according to a EAN press release, dated 29th May 2016. Although this can be devastating to an individual's private life and self-esteem, there are steps that can be taken to remedy these, allowing patients and their partners to lead a more fulfilling sex life.

> "The dedicated neurologist can treat typical and simple sexual problems in his patient and reserve urological consultation for the more complex issues and for dysfunctions that prove refractory."

Sexual dysfunction can develop as a result of a whole host of neurological disorders and can affect both men and women. Hypothalamic-pituitary disorders have been shown to decrease sexual desire in men, however, female patients tend to suffer more from amenorrhoea, infertility, lack of sexual desire, reduced vaginal lubrication, and orgasm dysfunction. Lesions in the frontal and temporal lobes, for example in patents who have had a stroke, tend to lead to sexual dysfunction, while epileptic patients can suffer with more serious implications, including hypersexuality and decreased sexual arousal. Parkinson's disease and multiple sclerosis can also have a number of sexual implications; erectile dysfunction, as well as problems with orgasm and ejaculation are reported in male patients, but both men and women can experience reduced sexual desire. Women with polyneuropathy can find it difficult to become sexually aroused, while male diabetic patients may experience erectile dysfunction and/or retrograde ejaculation.

Despite the large number of sexual implications, Prof David Vodušek, Neurology Clinic, University Medical Centre Ljubljana, Ljubljana, Slovenia, encouraged openness and compassion in order to treat the conditions and improve quality of life for sufferers: "The dedicated neurologist can treat typical and simple sexual problems in his patient and reserve urological consultation for the more complex issues and for dysfunctions that prove refractory." He added: "Lubricants help against vaginal dryness, for instance, and oral drugs help to counter erectile dysfunction, especially those based on cGMP phosphodiesterase Type 5 inhibitors."

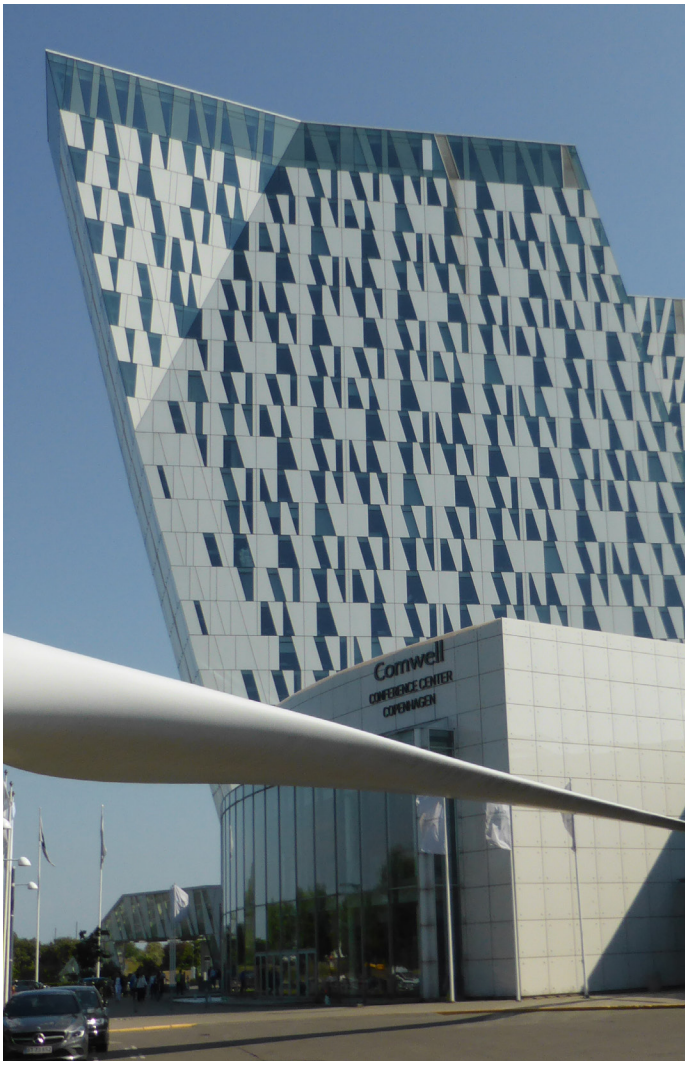
The Gut, the Brain, and Bacteria

THE GUT microbiome contains almost 1,000 different types of bacteria, with up to 100 trillion cells. Recent evidence has suggested a brain–gut microbiome axis and that this may be the basis for some diseases, causing us to rethink therapeutic options. Research detailed in a EAN press release dated 29th May 2016 focussed on this axis and how it can regulate and reform processes in the brain.

A recent study has looked at what effect the micro-organisms might have on behaviour. Dr Patricia Lepage, Institut National de la Recherche Agronomique, Jouy-en-Josas, France, said: "Intestinal microbes can verifiably produce neuromediators that have an effect on the brain. Germ free mice showed less anxiety than their conspecifics whose gut was populated with commensal microbiota. However, there is only scant evidence thus far on how this process works in the human brain." Several processes have been suggested including use of the vagus nerve, the immune system, the enteric nervous system, and microbial metabolic processes.

> "Intestinal microbes can verifiably produce neuromediators that have an effect on the brain. Germ free mice showed less anxiety than their conspecifics whose gut was populated with commensal microbiota."

Whilst it might affect behaviour, the role of the microbiome in regulating brain functions is unclear. One study has demonstrated increased myelination in the prefrontal cortex of germ free mice, compared with those kept under normal conditions.



This finding is particularly interesting when considering disorders such as multiple sclerosis, which can develop as a result of decreased myelin. Dr Gurumoorthy Krishnamoorthy, Max Planck Institute of Biochemistry, Martinsried, Germany, compared the effects of intestinal microbiota on brain inflammation in genetically modified mice to further explore this notion. In contrast to germ free mice, T and B cells activated the immune system of normal mice. He concluded that: "Both trigger inflammatory reactions in the brain, which destroy the myelin sheath in phases, very similar to the way MS unfolds in human beings." This finding suggests that an immune system disorder may be more causative of multiple sclerosis than a nervous system change.

Football Fans Have No Greater Risk of Stroke

GOOD NEWS for football fans; it has been revealed that rooting for a favourite team does not increase the risk of a stroke. Previous studies have suggested that the stress which can be accumulated during matches may be a trigger for heart attacks or strokes in passionate football fans.

However, new evidence has revealed that on match days the number of strokes is not significantly higher than on non-match days, as reported in a EAN press release dated 29th May 2016. A group of researchers in Lisbon examined data in the period from 2012–2015 to determine how high the risk was for enthusiastic football fans in triggering strokes. The study made use of hospital statistics concerning the number of patients admitted on or after matches of the three most popular Portuguese football clubs in order to investigate the risk association. The team discovered that 72 strokes occurred on and after match days compared with 52 on days when no matches were played. The researchers thus noted that although the numbers of strokes or heart attacks were at variance, there was no significant difference when compared to the average.

Almost 6,000 neurologists

Dr Cláudia Borbinha, Department of Neurology, Hospital Egas Moniz, Lisbon, Portugal, explained that: "The absolute number of strokes during football matches may have been higher but it was not an above-average outlier. Our data therefore furnish no significant indications of a correlation between enthusiasm for football and an increased risk of stroke." Football fans can therefore continue to enjoy, be passionate about, and be avid supporters of their teams, as the results reveal that the stress and excitement of match day do not lead to an increased stroke risk or heart attack.

Predicting Whiplash Consequences: Grouping may be the Answer

NEUROLOGICAL symptoms and chronic pain following a car accident, as a result of whiplash, can persist for a number of years. A new study has suggested that dividing individuals into subgroups following an accident can allow better prediction of the long-term effects of their injury according to a EAN press release dated 31st May 2016. It is hoped that this will improve patient management.

A previously developed system for dividing patients was tested in 326 patients who had been involved in an accident 12–14 years ago and then followed-up with a questionnaire. The questionnaire assessed ongoing pain, non-painful complaints, prescriptions and treatments, and any sick leave required. Prof Helge Kasch, The Danish Pain Research Center, Department of Neurology, Aarhus University Hospital, Aarhus, Denmark, said: "It turned out that more than a decade after the fact the people most heavily affected were those who had been assigned to the highest risk groups shortly after the accident."

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Patients in these high risk groups suffered with more neck, head, shoulder, arm, and low back pain. These individuals required more pain relief and had a higher incidence of post-traumatic stress syndromes. It has been confirmed that the categorisation of these patients must take place within 4 days of the accident for accurate assignment. The examination should cover the type and nature of the pain, non-painful complaints, mobility of joints, and emotional state of the patient. Neck pain, headaches, and a negative emotional state were strong indicators of relative ability to work following the accident. Further studies have shown that in Risk Group 1, 4% of patients were unable to return to work within 1 year whilst in the highest-risk group, Group 7, 7.68% of patients were unable to return to work within a year.

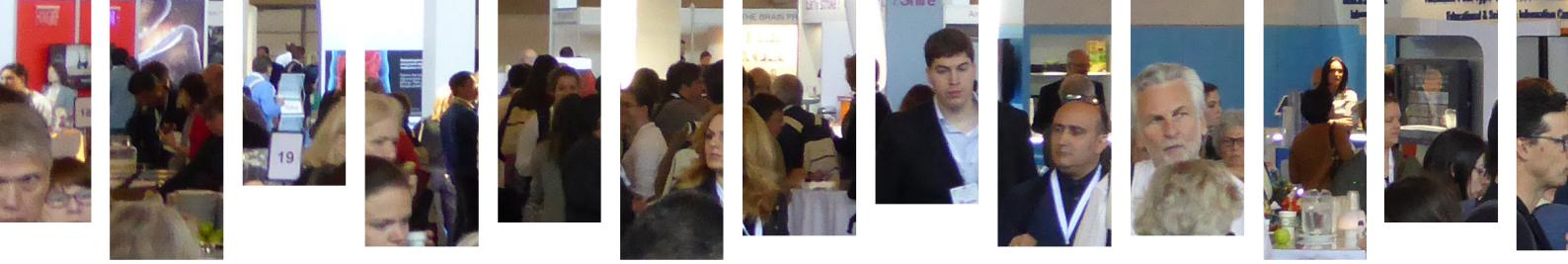
Praise for Scandinavian Research at European Neurology Congress

COMMENDATION for this year's congress host city, Copenhagen, Denmark, imbued a EAN press release dated 30th May 2016. Along with other Scandinavian countries, Denmark continue their long tradition of excellent neurological research, as well as having a highly active neurological community. This year's congress was host to roughly 6,000 participants and 1,574 accepted abstracts with a total of 6,555 authors, 82 scientific sessions, 362 invited speakers, and 100 abstracts presented by Danish researchers alone.

One strong position Danish researchers have is the access to large volumes of highly valuable registry data. Prof Mads Henrik Ravnborg, President of the Danish Neurological Society, commented: "Studies based on systematically collected registry data are of major value. Unlike clinical studies, which are conducted under strictly defined conditions, registries reflect medical practice with all its irregularities. The strengths and weaknesses of therapies are therefore rendered visible with exceptional clarity. For this reason, these studies contribute greatly to the safety of patients. We are definitely among the European frontrunners in several areas with respect to the breadth of neurological indications for which we have registry data."

Numerous examples of these systematic studies were presented at this year's congress including a large-scale study focussing on the abilities of children whose mothers took the antiepileptic drug valproate while pregnant. Migraines were a hot topic at this year's congress, along with research conducted on the first nationwide register-based study of Charcot–Marie–Tooth Disease (CMT), the first of its kind to report estimates of incidence and mortality. CMT is a prevalent heritable neurological disease, and yet prior to the study listed above, the prognosis of such diseases has been based purely on assumption. These studies highlight the importance of using population-based health registries, as well as displaying the need for researchers to have access to patient records.

> "Studies based on systematically collected registry data are of major value. Unlike clinical studies, which are conducted under strictly defined conditions, registries reflect medical practice with all its irregularities."



Valery L. Feigin

Professor of Epidemiology and Neurology, Director of the National Institute for Stroke and Applied Neurosciences, School of Public Health and Psychosocial Studies, Faculty of Health and Environmental Studies, Auckland University of Technology, Auckland, New Zealand.

Q: Stroke is a highly prevalent occurrence in New Zealand; can you tell us about some of the most important risk factors, and the preventability of stroke occurrence?

A: Stroke is a very common disease, not only in New Zealand but in other countries as well, and the prevalence of stroke is increasing throughout the world. Yet, it is one of the most preventable disorders. The most important risk factors for stroke are elevated blood pressure, irregular heart beat (atrial fibrillation), smoking, poor diet, being overweight, sedentary lifestyle, chronic stress/depression, and excess intake of alcohol. There is good evidence that at least half of all stroke events could be prevented from happening by controlling these risk factors, and some evidence suggests that even 90% of strokes could be avoided.

Q: At your institute, you are conducting another Auckland regional community stroke study which, when piloted in 1981, was unprecedented in neurological research. How are the results of this study influencing the direction of research?

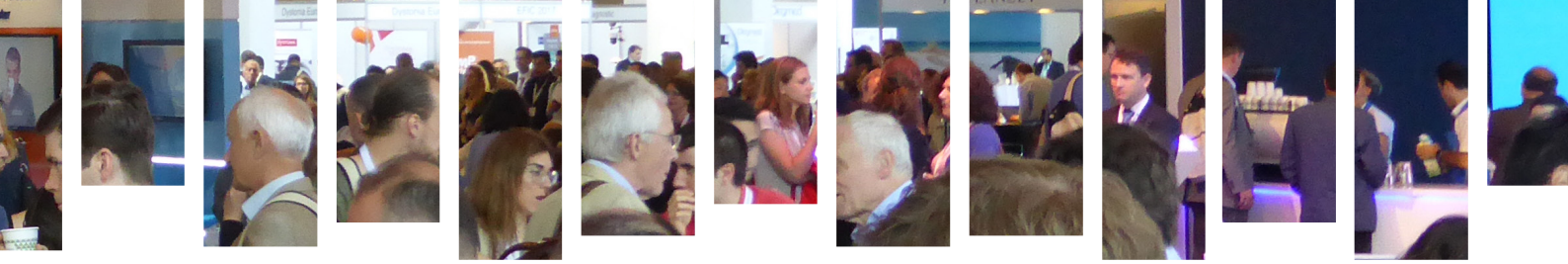
A: This was a ground-breaking study, indeed, that gave rise to the next three Auckland regional community stroke studies 10 years apart (1991–1992, 2002–2003, and 2011–2012) on a much bigger scale, with the last one covering the population of Auckland of 1.2 million residents. Our research findings showed the long-term trends in stroke incidence and outcomes in New Zealand and also showed that we have large ethnic disparities in stroke incidence and outcomes, with Māori and Pacific people having a 2 to 3-times greater risk of stroke and significantly worse stroke outcomes compared to New Zealand Europeans. Unfortunately, the burden of stroke in New Zealand is getting bigger and bigger, although the last study showed an encouraging trend in reducing ethnic disparities. This data formed the backbone of healthcare planning in New Zealand, and it is crucial to continue these studies 10 years apart.

> “Stroke is a very common disease, not only in New Zealand but in other countries as well, and the prevalence of stroke is increasing throughout the world. Yet, it is one of the most preventable disorders.”

Q: Throughout your career, you have been involved in numerous research projects. Which project did you find most exciting, and why?

A: It is really hard to separate out the most exciting projects, they were all very exciting to me. Of the most recent ones, two stand out. The first is the Global Burden of Disease (GBD) Study. I am particularly involved in the GBD stroke burden estimates, and we have published a number of papers in top medical journals. These articles provide estimates of stroke burden, its trends, and factors influencing stroke burden on global, regional, and country specific levels that are absolutely crucial for evidence-based healthcare planning and priority setting for stroke management and prevention.

The second is the Stroke Riskometer project, which utilises our unique Stroke Riskometer™ app for both practice and research. With the app being translated into 18 of the world’s most spoken languages, potentially covering >90% of the world’s population, this project may become the largest epidemiological and primary stroke prevention project in the world. We already have



>200 investigators from 61 countries involved in the app-based epidemiological study. The project is supported and endorsed by the World Stroke Organization, World Federation of Neurology, World Heart Federation, and the European Stroke Organisation. In 2014, American physicians voted the Stroke Riskometer™ the number one app in the Medical Conditions category of all 100,000+ health-related apps worldwide. We are currently doing a randomised controlled trial to prove the efficacy of the app for primary stroke and cardiovascular disease prevention.

Q: At your institute, clinical research is a high priority. What key advantage for neurology, in your opinion, does clinical study have over laboratory research?

A: Laboratory research is important for informing clinical research but laboratory research findings cannot be directly applied to clinical practice. By definition, clinical research is research on human subjects, therefore findings from such projects, if proven valid and generalisable, can be directly translated into clinical practice. At the Auckland University of Technology National Institute for Stroke and Applied Neurosciences, we are conducting primarily population-based observational epidemiological studies and randomised clinical trials that have the potential to be widely applicable to the worldwide population, and to improve current medical practice in areas such as stroke, traumatic brain injury, dementia, and muscular dystrophy.

Q: How significant is classification to the accurate diagnosis and management of traumatic brain injury? How has this process changed during recent years?

A: Accurate classification of traumatic brain injury (TBI) is important for diagnosis and adequate management of this condition. While the diagnosis of TBI and its classification into three broad categories of TBI severity (mild, moderate, and severe) are well established, the practice of routine coding of TBI in hospitals and other medical practices is far from ideal. About 30% of TBI cases, especially mild TBI cases, are not admitted to hospitals and many mild TBI cases are not really mild by their consequences. In addition, our recent research showed that almost none of the currently used classifications of mild TBI cases have sufficiently good predictive abilities. Therefore it is very difficult, and actually not possible in most countries, to get accurate estimates of TBI burden from official statistics.

Q: What aspect of being involved in neurological research do you find most fulfilling?

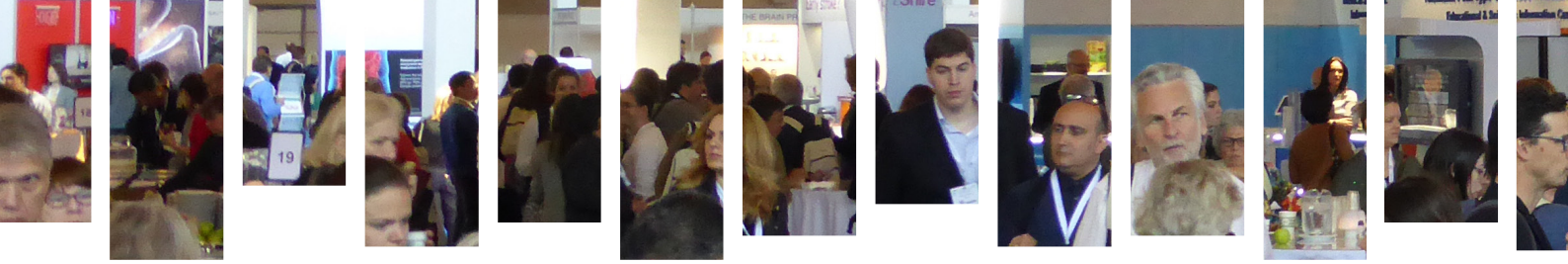
A: Seeing appreciation from peers and the academic community for the research I have done is probably one of the most fulfilling aspects of being involved in such research. And then of course when I see my research findings implemented into practice, and the positive results they bring to people, it is the most rewarding experience I could dream of.

Q: Are there any areas of research you have not yet had the chance to explore, but that you hope to in the future?

A: I hope to embark on large international trials in primary prevention of stroke and other major non-communicable diseases (NCDs). I also hope to initiate research projects that untangle ethnic disparities in stroke burden and identify ways to reduce that burden, as well as conducting additional epidemiological and clinical research into stroke and other major neurological disorders, research that will inform healthcare policy changes needed to reduce the burden of neurological disorders around the world. Many of the research projects we have recently initiated will lead to future research of this kind, and will be informed by findings already gained through previous research projects.

Q: In what innovative ways do you think healthcare costs can be reduced in the field of neurology? Are these issues more pressing on a global or national scale?

A: The most cost-effective way to reduce healthcare costs is primary prevention. So far, most of the emphasis in neurological research has been on improving care and rehabilitation of people



already suffering from these disorders. However, the reality is that due to ageing of the population and improvements in acute care, the number of people who suffer from neurological disorders is increasing across the globe. This puts additional pressure on the already stretched resources and services available for people with neurological disorders. Therefore, the number of people who have access to such services has decreased over time and will be decreasing in the future if effective primary prevention strategies are not developed and implemented into practice.

Q: If you had to give one piece of advice to a medical student considering specialising in neurology, what would it be?

A: I have found neurology to be a most fascinating field that contributes to the people's health and knowledge about the most sacred organ of the body, the brain. Exploring the most unexplored of body functions has always been and remains to be for me the driving force of my interest in neurology.

It is universally accepted that we are currently experiencing a global epidemic of stroke and other NCDs. This epidemic began mid-last century, triggered largely by changes in our lifestyle: smoking, excessive intake of salt, sugar, alcohol, poorly balanced diet, and sedentary lifestyle. There is evidence that reducing exposure to these lifestyle risks leads to reduced incidence of stroke, cardiovascular disease, cancer, and many other NCDs.

Taxation has been proven to be the most effective strategy in reducing exposure to smoking and excessive intake of salt, sugar, and alcohol. If these risks take a toll on our health, and taxation is the best way to reduce exposure to these risks, it logically follows that governments should introduce such taxation and reinvest the resulting revenue back into the health of the population by funding much needed preventative programmes and research in primary prevention and health. All it takes is recognition of the urgent need to improve primary prevention, and the good will of the governments to act.

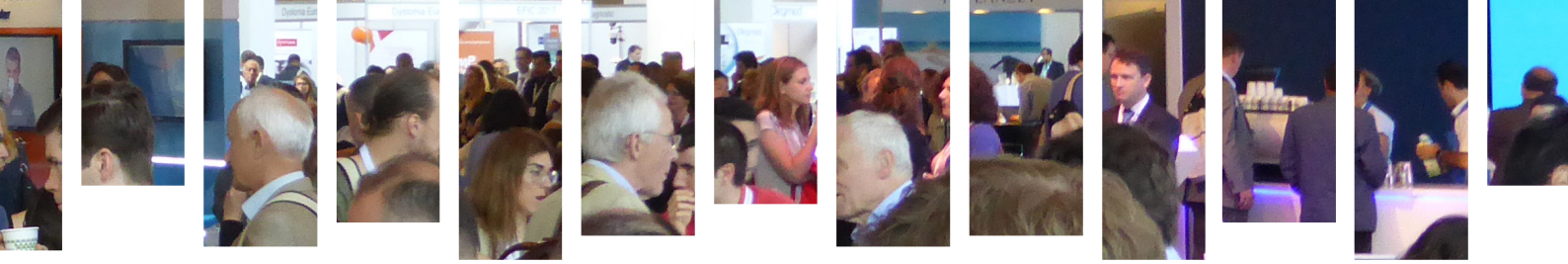
> “...the reality is that due to ageing of the population and improvements in acute care, the number of people who suffer from neurological disorders is increasing across the globe.”

Amos D. Korczyn

Professor Emeritus of Neurology and Pharmacology, Tel Aviv University, Tel Aviv, Israel; Chairman, Controversies in Neurology Annual Conference; Chairman of the Medical and Scientific Advisory Board of the Israeli Alzheimer's Disease Society (EMDA).

Q: How would you describe the overall rate of progress in neurological research in recent times?

A: The neurological sciences have made enormous progress in various directions. These very impressive achievements are largely due to better understanding of basic biological mechanisms and processes using molecular tools. One outstanding example has been in neurogenetics, in which the new tools that have been developed allow us now to identify genetic mutations and polymorphisms associated with neurological disorders. Thus we have been able to move from syndromic definitions to real disease entities, such as in epileptic disorders, where we have been successful in identifying rare mutations responsible for the disease in some families. This, in turn, can shed light on the mechanisms underlying seizures. While



in theory this should allow for the development of specific therapies, we are only beginning to see this happening. In common disorders like Parkinson's disease and senile dementia, genome-wide association studies have indicated the involvement of inflammatory processes. But it is not clear to what extent these indicate pathogenic or compensatory processes. Another important example of progress is immunology, where clarification of basic mechanisms allowed us to identify new diseases such as autoimmune encephalitis, which resulted in new therapies. Of course the development of imaging methods was able to shed light on ongoing brain processes and identify anatomical substrates. On the other hand, several areas are still in stagnation. We do not understand the basic processes causing late onset neurodegenerative diseases, and this is not due to lack of trying. We are also unable to provide substantial help in recovery from lesions or in rehabilitation after trauma.

Q: One of your particular research interests is dementia. What drew you to specialise in this area, and how has our understanding of the condition developed in recent decades?

A: Over the years, I became more interested in many aspects of old age neurology, however cognitive changes are among the most intriguing to me and attract my attention. Dementia is one of the more important disorders which have to be better understood in order to find the responsible pathways and see whether they can be managed. The high prevalence of the disorder and its effect on weak members of society are targets which need to be tackled. In particular, it has become clear over the past few decades that there is an overlap between dementing disorders and other neurodegenerative diseases both in terms of clinical presentations and underlying mechanisms. For example, we are far off from the idea that Parkinson's disease is just a motor disorder or that in frontotemporal dementia it is just the cognition which is affected. Therefore, the separation between 'memory clinics' and 'movement disorders centres' seems to be obsolete.

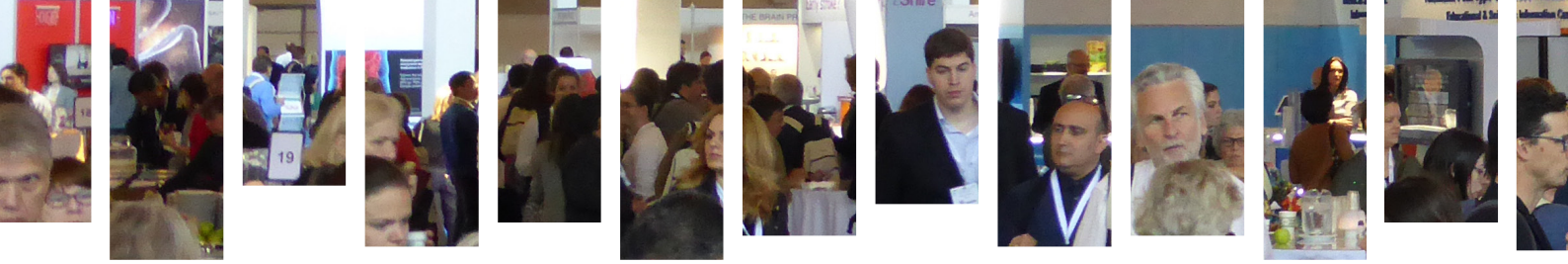
“Over the years, I became more interested in many aspects of old age neurology, however cognitive changes are among the most intriguing to me and attract my attention.”

Q: What more do you think needs to be done in order to advance our understanding of this and other degenerative neurological conditions? How can patients improve their understanding of the diseases and their prevention or treatment?

A: What has become clear to me is that the traditional definitions of diseases need to be modified, as these may hinder progress in the field. The stress of diagnosing a person as having either Alzheimer's disease or vascular dementia and the attempts to define and redefine each are counterproductive since most elderly subjects with cognitive deterioration may not have just one single brain pathology but rather several coexistent disorders. The strict division overshadows the need to look at the interaction between neurodegeneration and ischaemia for example. Thus I do not believe that the term Alzheimer's disease is scientifically valid anymore, although it can be useful for other purposes, such as raising funds. We should go back to the term 'senile dementia' and attempt to identify, in individual patients, the multiple responsible factors leading to it. Senile dementia is a syndrome, not a disease, and using the term Alzheimer's disease is therefore misleading when we talk about elderly people with cognitive failure.

Q: Has there been any noticeable change in the type or prevalence of neurological conditions that affect patients across Europe, or has this remained fairly similar since you started your career?

A: The changes that were seen reflect changes in the population, with ageing leading to a huge increase in the number of cases with stroke, senile dementia, and movement disorders. This trend is not going to reverse any time soon. The increase in the number of old people will add severe



strains on our society. Another impressive change is in the prevalence of disorders like multiple sclerosis, where the introduction of magnetic resonance imaging has led to earlier and more precise diagnosis.

Q: What advice would you give to young medical professionals about to begin a career in neurology?

A: The progress made in science should not lead to a feeling of omnipotence since there is much more that we do not understand. Young neurologists should internalise the old Aristotelean wisdom "cure rarely, comfort always" as the cornerstone of medical practice. For those with a curious mind, who wish to pave and develop new avenues I can say that there are so many basic questions that we do not have even the faintest idea how to approach. Thus it is important to be modest and to keep an open mind, and at the same time try to bridge the gulf between basic science and clinical medicine.

Q: Have you found that there is a particular area of neurology that you feel requires more research and funding?

A: There are several areas where we do not even know how to ask the right questions. For me the most basic problem facing science today is the mind body problem. What is the mode of interaction between the mental sphere and the physical world? The attempt to solve this riddle has been ongoing for millennia without any progress.

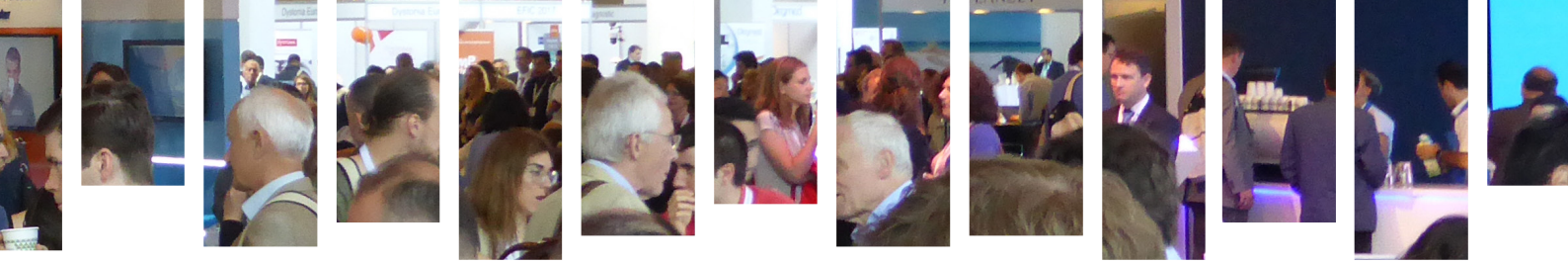
In practical terms, of course the more common disorders require most attention, particularly the debilitating ones. Unfortunately, in many cases the emphasis is laid by the industry rather than by pure scientific considerations. Thus the investments in dementia research over the past two decades have been directed by the pharmaceutical firms rather than by rational thinking. For example, it is illogical to direct 90% of the investments in dementia therapy targeting amyloid which may turn out to be a false signal or epiphenomenon.

> "It is important to be modest and to keep an open mind..."

Q: Can you tell us a bit more about your work with the Controversies in Neurology Annual Conference (CONy)? How do congresses like this one and the European Academy of Neurology (EAN) assist with the advancement of the field?

A: The EAN, like the European Federation of Neurological Societies (EFNS) and the European Neurological Society (ENS) before, is very successful. They mainly encourage and promote authoritative reviews of different fields of neurology, formulating guidelines for diagnosis and treatment. This is very well done in the plenary lectures and the excellent educational courses, and the results are very helpful to the practicing clinician. But I feel that they largely concentrate on the known and accepted, and pay too little attention to the unknown. They refrain from asking questions, but to me only raising such issues will lead to progress. In CONy we have just the opposite approach, and we try to illuminate areas of uncertainty, and always ask, "Is it really true?" and "How do we know?"

We sometimes quote René Descartes as saying "*cogito ergo sum*" and forget that what he really said was "*dubito ergo cogito; cogito ergo sum*." We have to doubt everything and debate accepted ideas. Only by asking questions can we reach better understanding. In CONy debates, experts in the field expose weaknesses in old and new dogmas. Is amyloid really a relevant target in treating Alzheimer's disease? Does the prion theory of neurodegenerative diseases really hold water? And so on and on, in the various neurological disciplines. Asking questions, rather than accepting old 'truths', is the way to progress. Much of what we accept for granted are really assumptions or dogmas. But as Karl Popper pointed out, progress in science is made by disproving old theories, thus allowing new ones to be advanced. The idea of CONy is that everything that can be questioned, should be questioned. Our upcoming congress in Athens next March (www.comtecmed.com/cony) will be done in collaboration with the Hellenic Neurological Society (HNS). To honour the host country, we shall add two special symposia, one on Adamantiades-Behçet's disease and the



other on the 'Brain and Mind in Ancient Greek Medicine and Philosophy'.

Q: In your opinion, what are the three biggest obstacles facing neurologists today and how can they be overcome?

A: The biggest obstacle I see is the division between psychiatry and neurology. Philosophers have been arguing for millennia about the 'brain-mind question'. Some are monists, others dualists. Of course we see patients all the time thought to have mental diseases in whom an organic cause is discovered, and who can be cured by removing a brain tumour, etc. But this still leaves the majority of mental disorders unaccounted for. Will they all be eventually found to be 'organic', and the field of psychiatry will eventually disappear as we learn more about physical events leading to mental derangements? I doubt it.

Another important question is of phenotypic heterogeneity. Why is it that patients having a certain genetic mutation present with different clinical features, in different ages, even within the same family? The explanations given so far are largely theoretical and speculative and we need better understanding in order to prognosticate and to modify disease course.

A third issue is of neurodegeneration where we still cannot differentiate between changes which are causative, compensatory, or just epiphenomena. Without better ways to differentiate those it will be difficult to decide where and how to intervene. Are inflammatory responses in neurodegeneration a defence mechanism or a Trojan horse causing even more damage? Are there 'good' and 'bad' subtypes of neuroinflammation?

Q: What research are you currently working on? Are there any areas of neurology you would like to explore in the future which you have not had a chance to research thus far?

A: Much of my research efforts focus on the neurodegenerative diseases. One project is TABASCO, the Tel Aviv Acute Brain Stroke Cohort where we follow stroke survivors looking for factors associated with, and hopefully responsible for, cognitive deterioration in these patients. What is the role of stress, of inflammation, of accelerated degeneration of cells, synapses, or tracts?

Q: What do you consider to be the greatest achievement in your career?

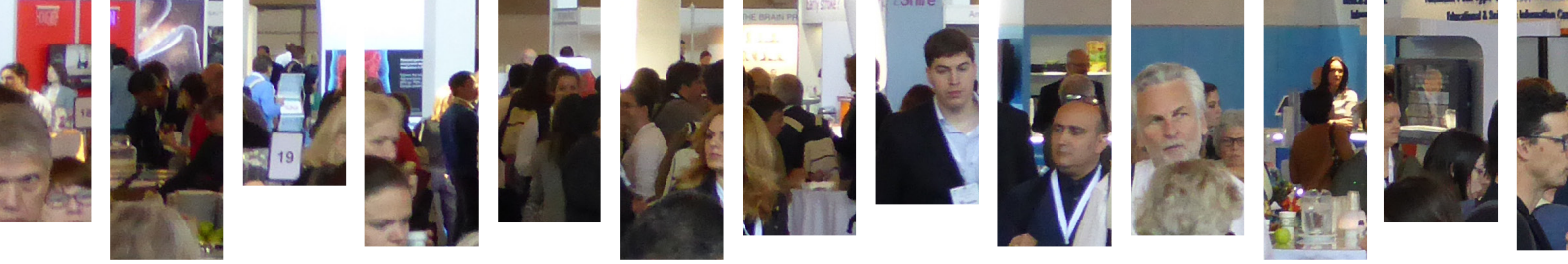
A: I consider myself a researcher, a physician, and a teacher. I cannot say that I had greater achievements in research or in treating patients than my colleagues. What I hope I was able to achieve was to educate a large number of pupils, both in basic neuroscience and particularly in clinical neurology. Many of them achieved high positions and I am proud of having had a chance to contribute to their success.

Rita Krishnamurthi

Senior Research Fellow, Programme Leader, Cerebrovascular Diseases Research Team, National Institute for Stroke and Applied Neurosciences, Auckland University of Technology, Auckland, New Zealand.

Q: You are a research fellow at the National Institute for Stroke and Applied Neurosciences (NISAN); what makes this institution unique within the neurology research landscape?

A: NISAN is uniquely placed to conduct studies in public health/neuroepidemiology and neuropsychology, with a particular focus on stroke, traumatic brain injury (TBI), and neuromuscular disorders. As such the institute has successfully conducted some of the world's most well-known population-based incidence and outcome studies in stroke (ARCOS studies) and TBI (BIONIC). The demographic makeup of New Zealand allows studies to be conducted in ethnically diverse



populations, another unique feature of the research conducted at NISAN. The Institute is also known for innovative research, for example with the development of the Stroke Riskometer™ mobile phone app, designed to measure the risk of stroke and to provide educational information about stroke and its risk factors. This research has gained worldwide attention and won several awards for its contribution to stroke prevention.

Q: Your doctoral research explored animal models of molecular neuroprotection for Parkinson's disease. How far has our understanding, and thus capability in treating, Parkinson's disease advanced since then?

A: Since my doctoral studies, I have shifted my focus from Parkinson's disease to stroke, from a public health perspective. However, research conducted in my lab continues to explore novel growth factors for their potential neuroprotective effect. While no effective neuroprotective agent has yet been found, efforts continue to discover bioactive agents, which may be neuroprotective and aid brain neuroplasticity.

Q: Tell us about the innovative ways in which you are raising awareness of the risks and symptoms of stroke in the general population?

A: There are several; I am involved in several community groups where I am often invited to present information about stroke: symptoms, risk factors, and ways to prevent stroke. These include senior citizens groups and specific ethnic groups (Chinese and Sri Lankan community groups, for example). I also provide articles to local community media (radio, newspapers). The other way is via the Stroke Riskometer™, which is an educational tool that is available internationally and is being translated into the world's most spoken languages.

Q: What other interesting neurological research is currently emerging from NISAN?

A: Currently we are investigating the effectiveness of Health and Wellness Coaching (HWC) in a randomised clinical trial, in those people who have been identified as having a moderate-to-high risk of cardiovascular disease. It is known that just providing education about health and lifestyle is not adequate for disease prevention, and people lose motivation over time. HWC is a psychological intervention which is a goal-oriented, client-centred partnership with a trained coach that is health-focussed. It is a process of client enlightenment, empowerment, and ownership in the form of purposeful conversation, whereby the client is moved towards his or her desired outcomes. Should this intervention be successful, we aim to seek government support to implement HWC in primary care practice for stroke prevention.

Q: How does neurological healthcare differ internationally? Is there anything specific that you think could be done to improve the application of treatment strategies globally?

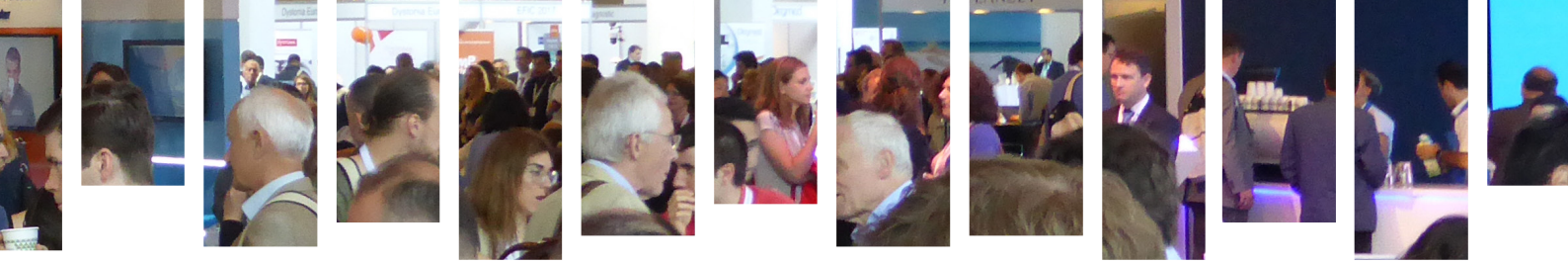
A: Neurological care does differ internationally largely due to economic and social disparities. One of the strategies to reduce these differences in healthcare would be the increased utilisation of technology, for example, telehealth, mobile technology for disease diagnosis, for increased access to information/education, or for self-management of risk factors and chronic conditions such as diabetes.

Q: Have there been any recent developments in technology that have significantly altered the direction of neurological research?

A: There have been many, particularly advances in neuroimaging. In our field, the use of mobile technology is changing the way information is accessed and research is conducted. For example, we are able to conduct international research through the Stroke Riskometer™.

Q: What do you look forward to the most about scientific meetings, such as the 2nd Congress of the European Academy of Neurology (EAN)?

A: I look forward to witnessing and learning from presentations by some of the the world's most well-known and respected scientists and clinicians, as well as the opportunity to meet them and form collaborations with our team in New Zealand.



Q: Have you had any mentors during your career that have influenced your work and/or perspectives?

A: Yes, I have had several. The most recent and perhaps the most influential mentor has been Prof Valery Feigin, a neurologist and a world-renowned neuroepidemiologist. He has been instrumental in guiding my career in stroke epidemiology at an international level and opening my mind to many possibilities for the future of stroke research particularly in terms of stroke prevention.

Q: Take us back to your decision to move from Fiji to Auckland; what led you to medicine, and neurology in particular, and why?

A: The move from Fiji to Auckland was for personal reasons (I had recently married and my husband was settled in New Zealand). Soon after moving to New Zealand, I was offered a research position at the University of Auckland (AUT) in the field of cardiovascular research. In the next few years, our research centre started conducting research into novel neuroprotective molecules for neurodegenerative diseases such as Parkinson's disease, and I was offered a scholarship to do my PhD in this field. I then upskilled myself in the field of neuroscience, and continued to work in the area of neurodegenerative disorders. In 2009 a senior research fellow position came up at AUT University, which I was fortunate to be offered. It was here that I joined Prof Valery Feigin's team and have remained there since. I have developed a keen interest in stroke prevention research and in particular, identifying the causes of ethnic disparities in stroke burden. There is much work to be done in this area and I hope my work along with that of the greater NISAN team will make a real difference to people's lives.

BRAIN HEALTH: TRANSLATING SCIENTIFIC EVIDENCE INTO CLINICAL PRACTICE IN MULTIPLE SCLEROSIS

This satellite symposium took place on 29th May 2016, as part of the 2nd Congress of the European Academy of Neurology (EAN), Copenhagen, Denmark

Chairperson

Per Soelberg Sørensen[1]

Speakers

Heinz Wiendl,[2] Andreas Lysandropoulos,[3] Andrew Chan[4]

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MEETING SUMMARY

Brain volume loss (BVL) progresses more rapidly in patients with multiple sclerosis (MS) than in healthy individuals, and brain atrophy begins early in the course of the disease. The objective of this symposium was to emphasise the importance of care and preservation of the brain within treatment protocols for MS so that early and appropriate management can be initiated to preserve brain volume and function.

Prof Per Solberg Sørensen chaired the symposium and welcomed the speakers. Prof Heinz Wiendl gave a presentation on BVL in MS and described its underlying pathophysiology. Dr Andreas Lysandropoulos illustrated how information on BVL is clinically relevant and can be taken from clinical studies to assist clinical practice and decision-making. The final presentation was given by Prof Andrew Chan who highlighted the important role of brain atrophy in decision-making for early treatment and presented recent data for two treatments for MS: teriflunomide and the monoclonal antibody alemtuzumab. The symposium was concluded by a short question and answer session.

Brain Volume Loss in Multiple Sclerosis: Underlying Pathophysiology

Professor Heinz Wiendl

MS has traditionally been regarded as a white matter disease and identifying lesions using conventional magnetic resonance imaging (MRI) has become essential in the early diagnosis and management of the disease.[1] More recently, grey matter disease has emerged as an important component of MS and has been suggested to correlate better with specific disability outcomes.[1] Healthy individuals lose between 0.1% and 0.3% of brain volume per year.[2] BVL in people with MS, however, is typically 3-times this rate throughout the course of the disease.[3] The rate of grey matter volume loss increases with the stage of the disease, from 3.4-fold in relapsing-remitting MS (RRMS) to 14-fold in secondary progressive MS.[3]

The application of different technologies can be useful in identifying loss of white and grey matter. Cortical extrinsic curvature (CEC) is a novel measure of white matter loss and is a more sensitive indicator of 'pure' white matter loss than conventional MRI methods.[4] Based on CEC measures, white matter atrophy appears to occur early in MS and may occur as early as clinically isolated syndrome (CIS). An increase in CEC in patients with MS correlates significantly with loss of white matter integrity in the corpus callosum as assessed by fractional anisotropy, supporting the validity of CEC.[4] There is increasing interest in nuclear regions of the brain such as the thalamus, which are affected early in the course of MS. A recent study evaluating early thalamic atrophy in patients with CIS and RRMS demonstrated that loss of relative thalamic volume correlated significantly with disease duration.[5] Patients with RRMS and CIS with disease duration ≤24 months already had a reduced relative thalamic volume versus healthy controls. The alterations in white matter in these patients could not be explained by the distribution of white matter lesions and it was concluded that early thalamic atrophy is mainly due to silent microstructural thalamic alterations.[5]

Pathological processes that underlie BVL include demyelination, gliosis, and axon loss. Focal loss of myelin tissue, particularly within overt white matter lesions, is a contributing factor of brain atrophy.[6] Demyelination is facilitated, at least in part, by autoreactive antibodies via several well-defined mechanisms including complement activation, phagocytosis by macrophages, and recruitment of autoreactive T cells.[7,8] Remyelination is not uncommon in early lesions, although it fails in later stages of MS.[9] Other mechanisms that may contribute to demyelination include the activities of cytotoxic $CD8^+$ T lymphocytes and activated macrophages, hypoxia-like injury leading to oligodendrocyte apoptosis, and primary oligodendrocyte degeneration.[8] As an MS plaque progresses from acute to chronic, early inflammation and oedema resolve and astrocytes produce a glial scar.[10] Gliosis may confound assessments of BVL in multiple ways including maintenance of volume in the event of axonal injury[9] and contraction of astrocyte volume as the lesion matures.[11] The volume of astrocytes is reduced, which represents a physical and chemical barrier to axon regeneration.[12] Perhaps the most destructive mechanism of MS is axonal loss/neurodegeneration. Axonal loss has been demonstrated in both acute (new active) and chronic active (older reactivated) lesions in MS patients with disease duration from 2 weeks to 27 years.[13] Axonal density is reduced up to 80% in chronic plaques.[10] Axonal pathology has been demonstrated in normal-appearing white matter as well as demyelinated lesions, indicating that factors other than inflammation can contribute to neurodegeneration.[10,14] Potential mechanisms include Wallerian degeneration as well as retrograde and anterograde transneuronal degeneration.[9,14] Different mechanisms contribute to this degeneration, including cytotoxic $CD8^+$ T cells, which may directly destroy axons,[14,15] possibly via granule-mediated death.

Recent *in vivo* microscopy studies evaluating the dynamics of neuronal reaction in response to an inflammatory trigger demonstrate that acute axonal transection is an early indicator of neurodegeneration in MS.[15-17] Neuronal reactions can occur in the absence of visible demyelination in which acute neuroinflammation induces a pervasive state of reversible axonal dysfunction.[16] Ultrastructural axonal damage may occur with or without demyelination and may recover spontaneously.[17] Transition from RRMS to secondary progressive MS occurs when the central nervous system (CNS) can no longer compensate for neuronal loss, resulting in progressive disability.[15] This important information demonstrates that any type of inflammatory mechanism

could eventually lead to neuronal dysfunction. This has also been demonstrated in studies in which it was shown that chronically demyelinated axons lacking Na^+/K^+ ATPase are incapable of nerve transmission.[18] Thus, demyelination is one possibility for axonal destruction or dysfunction but it is not the only one. Chronic CNS inflammation therefore involves several different pathological mechanisms that lead to neuroaxonal damage by apoptosis and necrosis. These mechanisms include reactive oxygen and nitrogen species, hypoxia, cytokines, and glutamate, which trigger mechanisms that include oxidative stress, mitochondrial damage and dysfunction, Ca^{2+} influx, and demyelination.[19]

Other adaptive immune cells involved in inflammation in MS include $CD3^+$ T cells. Numerous studies have shown that T cells, in addition to being directly destructive, act as orchestrators for other immune cells. Antibodies contribute to demyelination via opsonisation and complement deposition, and dendritic cells serve as antigen-presenting cells to autoreactive T cells during the inflammatory phase.[7,20] Pro-inflammatory cytokines released by autoreactive T cells, such as interferon (IFN)-γ, interleukin (IL)-23, IL-17, and tumour necrosis factor alpha, further activate immune cells to ultimately contribute to demyelination and CNS damage.[7] Over the past few years, it has emerged that cells of the innate immune system are also involved in neurodegeneration. Cells that form a normal part of the CNS glial cell population, such as macrophages, can cause injury by releasing proteases and reactive oxygen species.[20] Mast cells release enzymes that can lead to demyelination and destruction of oligodendrocytes and neurons.[7] Initial activation of the adaptive immune response triggers an inflammatory response that may decrease over time; however, it may be the activation of the innate immune response that results in the sustained inflammation of MS.

To summarise, BVL in MS is a combination of inflammatory and neurodegenerative events, with pathological correlates that include demyelination, axonal loss, and gliosis. Inflammation involves both the adaptive and the innate immune system, from outside and within the CNS. These events work together to result in the focal or diffuse brain atrophy of MS.

Lost in Translation? Brain Atrophy from Clinical Studies to Clinical Practice

Doctor Andreas Lysandropoulos

Evidence has shown that BVL is correlated with, and is predictive of, disability worsening in MS.[21] Popescu et al.[21] showed that early BVL is strongly correlated to clinical outcomes 10 years later. Interestingly, in RRMS, the correlation is stronger for central (periventricular) atrophy; whereas in primary progressive MS, whole brain volume and grey matter volume are better correlated with Expanded Disability Status Scale (EDSS) and MS Severity Scale scores.[21] In a 20-year longitudinal study, Fisniku et al.[22] showed that grey matter volume loss is significantly correlated with disability measures. In a meta-analysis of 13 randomised controlled trials of disease-modifying treatment of 2-year duration, Sormani et al.[23] showed that there was a significant correlation between treatment effects on BVL and disability progression, demonstrating that the effect of a drug on disability is also related to its effect on BVL. Cognition is a central issue in MS as more than half the patients will have serious cognitive disability. Studies have shown that cognitive impairment is correlated with parenchymal volume loss (but not T1 and T2 lesions)[24] either with whole or grey matter loss.[25] Brain atrophy is related to quality of life (QoL) through physical and cognitive disability and other outcomes such as emotional well-being and fatigue, which are correlated to grey matter, white matter, and parenchymal volume.[26]

Assessment of brain volume changes is important for measuring the efficacy of a disease-modifying treatment and may reflect neuroprotective effects. Brain volume can be reliably measured using MRI-based methods.[2] Several different approaches have been developed to assess BVL including direct segmentation or indirect registration techniques.[27] Segmentation defines brain tissue based on variation in signal intensity, whereas registration defines brain tissue based on edge displacement at two time points.[27] Accuracy in the techniques assessing changes in brain volume is important because atrophy is a slow process, with corresponding small volume changes.[27] MRI acquisitions should also be performed at the same imaging site using the same equipment and conditions for accurate longitudinal follow-up.[27] The phenomenon of pseudoatrophy during the first year on treatment is also a consideration as it may be associated with anti-inflammatory

treatments and may be more apparent in white matter than grey matter.[11]

Different clinical trials cannot be directly compared due to differences in patient demographics, disease stage, mechanism of drug action, and probably MRI techniques. However, if we accept that brain volume is a reflection of neurodegeneration, we can rely on clinical trial data to indicate the effect of a disease-modifying treatment on brain volume.

Case Study

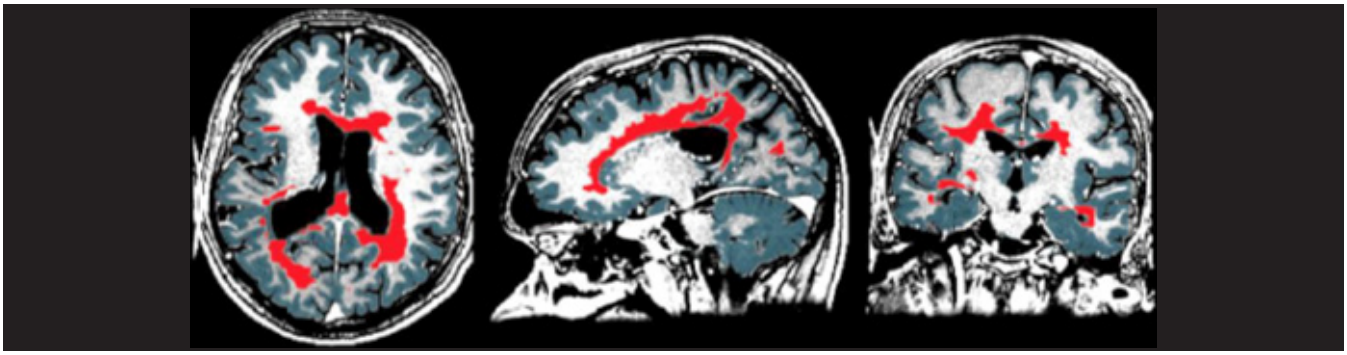
A young patient who had been on first-line disease-modifying treatment for 2 years presented a mild relapse with double vision, a new T2 gadolinium-enhancing lesion on MRI, and an EDSS of 2.0 during the relapse. The patient had recovered completely from his relapse.

As the EDSS, relapse rate, and lesion load were low, it was unclear whether or not second-line treatment should be commenced.

1. QC

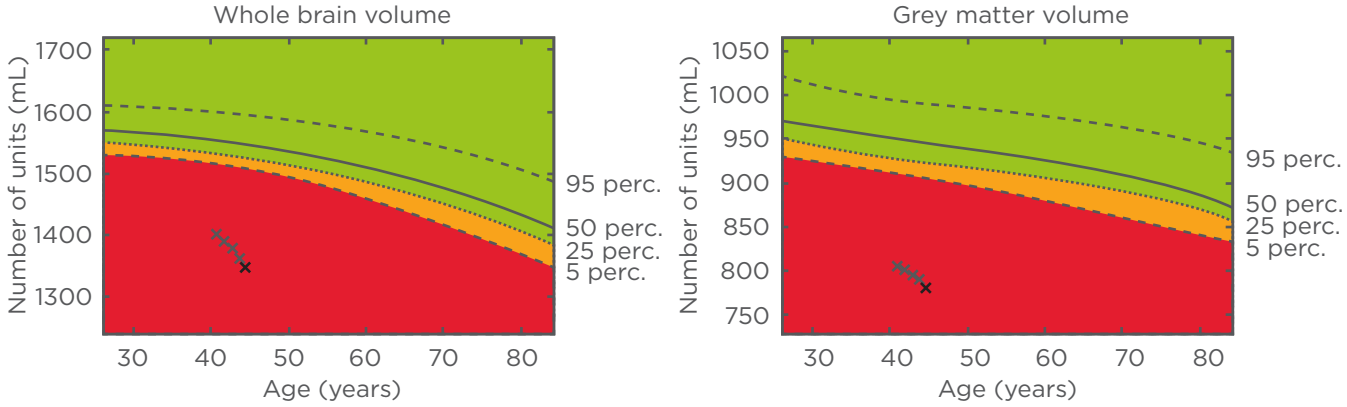
QC status	Remarks
Approved	No remarks

2. Visual results



3. Brain volumes

Brain structure	Volume (mL*) (current MRI)	Normal range (5th and 95th perc)	Normative perc	Atrophy (compared to previous MRI)
Whole brain volume	1345.8 mL*	1505-1591 mL*	<1	-0.97%
Grey matter volume	780.3 mL*	906-990 mL*	<1	-1.15%



*Displayed brain volumes are normalised for head size. The normalisation factor for this patient equals 0.87.

4. Lesion load

Type	Lesion volume (mL) (current MRI)	Lesion volume change (mL) (compared to previous MRI)
FLAIR lesions	74.45 mL	1.70 mL
Gd enhanced lesions	0.07 mL	-0.04 mL

Figure 1: Case study showing reductions in whole brain and grey matter volume in a young patient.
Visual representation of T2 lesion load is shown as red areas (visual results, upper section of figure). Volume reduction over time (age of patient) in whole brain and grey matter is indicated by crosses (brain volumes, lower section of figure).
QC: quality control; MRI: magnetic resonance imaging; Gd: gadollinium; perc: percentile; FLAIR: fluid attenuated inversion recovery.

However, measurements of brain volume (whole brain and grey matter volume) identified an ongoing important degenerative process. Measurements indicated that whole brain volume and grey matter volume were far below normal for a patient of this age (Figure 1), providing an indication for the need of immediate escalation to second-line treatment.

To summarise, measurement of BVL correlates with physical and cognitive disability as well as QoL in patients with MS, and slowing BVL is an emerging goal of therapy. Although not routinely used in clinical practice, the potential impact of disease-modifying treatment on BVL should be considered and incorporated into clinical decision-making.

The Role of Brain Atrophy in Decision-Making for Early Treatment

Professor Andrew Chan

Early treatment of MS is important to slow disease progression and improve prognosis. Several agents are now available, each demonstrating different mechanisms of action and efficacy and safety profiles. Teriflunomide and alemtuzumab, two available treatments for MS, demonstrate different efficacy and tolerability profiles which can allow treatment to be somewhat individualised according to disease risk and patient considerations.

Teriflunomide is a selective and reversible inhibitor of dihydroorotate dehydrogenase which reduces proliferation of activated T cells and B cells, while leaving resting and slowly-dividing cells relatively untouched,[28-31] allowing lymphocytes to remain available for immune surveillance.[32] Teriflunomide tablet once-daily has been investigated across the spectrum of relapsing MS, in patients with early MS and those with chronic progressive disease.[33-36] The Teriflunomide Multiple Sclerosis Oral (TEMSO) and Teriflunomide in Patients With Relapsing Multiple Sclerosis (TOWER) randomised Phase III trials evaluated the safety and efficacy of teriflunomide versus placebo in patients with relapsing MS.[35,37]

In a *post hoc* analysis of TEMSO, teriflunomide 14 mg reduced relapses by 30.6% versus placebo (p=0.0001) at 2-year follow-up.[38] Subgroup analysis of TEMSO according to baseline disease activity demonstrated significant effects with teriflunomide in reducing BVL versus placebo in patients with both high and low disease activity at baseline.[39] Similar results were observed in another *post hoc* analysis of the same trial performed according to confirmed disability progression (CDP; defined as an increase from baseline of ≥1.0 point on EDSS [or ≥0.5 points for patients with a baseline EDSS score >5.5] that persisted for at least 12 weeks). Teriflunomide slowed BVL in patients with 12-week CDP (43.9% reduction, p=0.0043) and without 12-week CDP (23% reduction, p=0.0129) versus placebo at 2 years.[40] These results demonstrate the clinical utility of teriflunomide early in treatment, before CDP is observed. Both of the Phase III trials for teriflunomide have shown consistent benefit on disability worsening.[35,37] The long-term extension of TEMSO (currently at 9-year follow-up) has shown that assessments of disability scores have remained relatively stable throughout.[41] In a pooled analysis of four placebo-controlled trials, the most common adverse events included hair thinning (13.5% for teriflunomide 14 mg versus 5.0% for placebo), diarrhoea (13.6% versus 7.5%), alanine aminotransferase increase (15.0% versus 8.9%), and nausea (10.7% versus 7.2%),[42] with no increased risk of serious infections.[43] In extensions studies with up to 13 years follow-up, no new safety signals have been identified.[42-45]

Alemtuzumab is a monoclonal antibody specific for CD52, a protein that is expressed on the surface of B and T lymphocytes and other immune cells.[46] Binding of the antibody to CD52 results in a depletion of CD52-expressing cells. However, within weeks of treatment, a distinct pattern of repopulation occurs that is believed to rebalance the immune system to a more anti-inflammatory state.[46,47]

Two Phase III trials have evaluated the safety and efficacy of alemtuzumab versus subcutaneous IFN-β1a in patients with RRMS; CARE-MS I, conducted in treatment-naïve patients[48] and CARE-MS II, conducted in patients who had relapsed after first-line treatment.[49] In both CARE-MS I and CARE-MS II, patients receiving alemtuzumab were treated at baseline with 12 mg of antibody daily for 5 consecutive days, followed by 12 mg of antibody daily for 3 consecutive days 12 months later. In CARE-MS I, alemtuzumab produced a 42% slowing in BVL versus subcutaneous IFN-β1a at 2 years (Figure 2).[50]

In CARE-MS II, alemtuzumab produced a 24% slowing in BVL versus subcutaneous IFN-β1a at 2 years.[50] Following completion of CARE-MS I or CARE-MS II, patients were offered the

opportunity to enter extension studies.[50-52] In CARE-MS I, median rate of BVL decreased progressively over 4 years and remained low in Year 5 (Year 1: -0.59%, Year 2: -0.25%, Year 3: -0.19%, Year 4: -0.15%, Year 5: -0.20%). The median rate of BVL progressively slowed over 3 years in CARE-MS II and remained low in Years 4 and 5 (Year 1: -0.48%, Year 2: -0.22%, Year 3: -0.10%, Year 4: -0.19%, Year 5: -0.07%).[50] In an evaluation of BVL in patients who were switched from subcutaneous IFN-β1a to alemtuzumab upon entering the extension studies, BVL at 2 years (prior to switching) was -0.50% and -0.33% in CARE-MS I and CARE-MS II, respectively. After switching, median BVL at years 1, 2, and 3 was -0.07%, -0.13%, and -0.09% in CARE-MS I and 0.02%, -0.05%, and -0.14% in CARE-MS II, respectively.[52] Patients continued to show improvements in pre-existing disability, with 33% and 43% of patients with 6-month confirmed disability improvement at 5 years for CARE-MS I and CARE-MS II extensions, respectively.[53,54] Importantly, 68% and 60% of patients, respectively, did not receive re-treatment with alemtuzumab after the second course at Month 12.[53,54] Significant improvements in QoL were demonstrated for alemtuzumab versus IFN-β1a at 1-year and 2-year follow-up in CARE-MS II as measured by disease-specific (Functional Assessment of Multiple Sclerosis) and general (EQ visual analogue scale) measures.[55,56]

During the alemtuzumab clinical development programme, infections were predominantly mild-to-moderate and decreased over time to an incidence of 42% at 5 years (Genzyme, data on file). Immune thrombocytopaenic purpura developed in approximately 2% of patients, most of whom had a sustained and durable response to treatment with first-line therapy for immune thrombocytopaenic purpura.[57]

To conclude, teriflunomide has significant efficacy for BVL versus placebo, shows long-term benefits with continuous treatment, and has a well-established safety profile, with up to 13 years of data. The monoclonal antibody alemtuzumab has a consistent safety profile and has shown durable efficacy of >5 years in reduction of BVL that is associated with reductions in disability worsening and improvements in QoL.

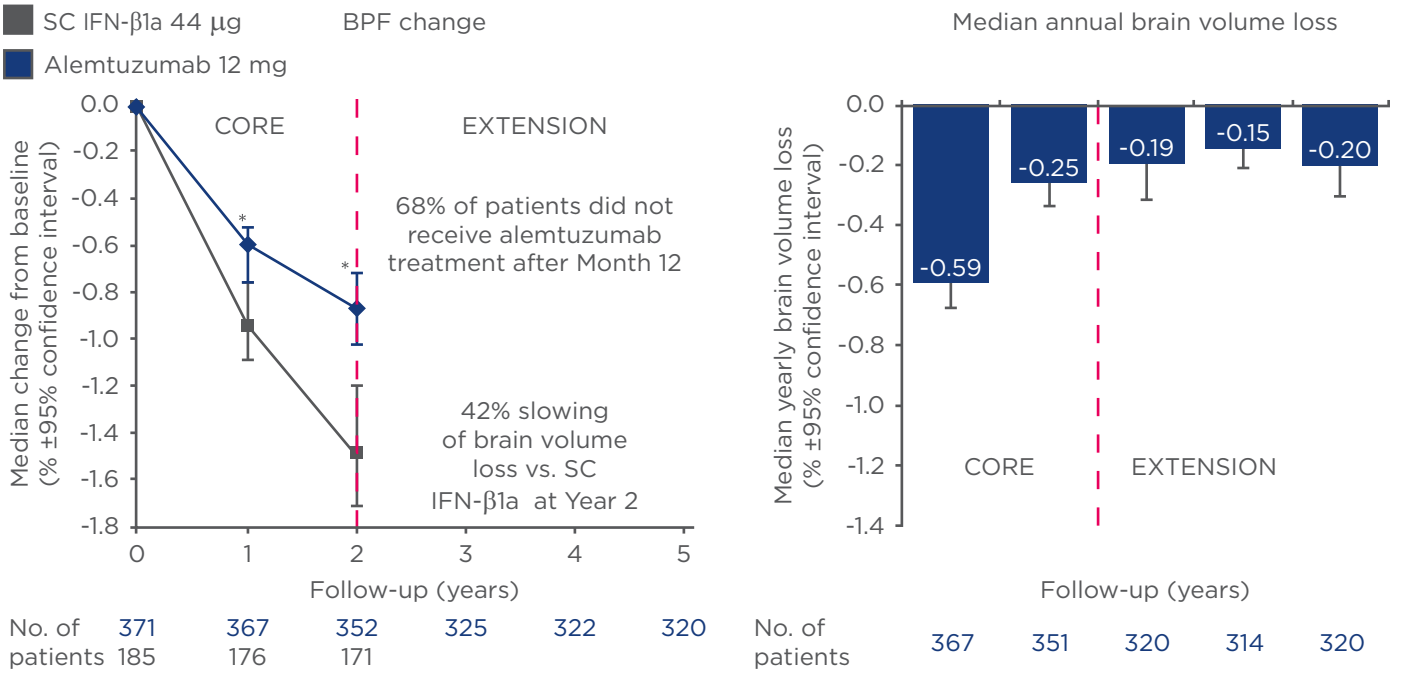


Figure 2: Slowing of brain volume loss over 5-year follow-up with alemtuzumab in treatment-naïve patients in CARE-MS I.[50]

Left: Change from baseline in BPF over time in the core study (0–2 years) for patients treated with alemtuzumab and SC IFN-β1a.

Right: Median annual change in brain volume in the core and extension studies for patients treated with alemtuzumab.

*p<0.0001 for alemtuzumab versus SC IFN-β1a.

SC: subcutaneous; IFN-β1a: interferon beta-1a; BPF: brain parenchymal fraction.

QUESTION AND ANSWER SESSION

Prof Sørensen noted that in RRMS, clear MRI parameters have been set to characterise activity and asked the panel whether MRI parameters should be defined to allow characterisation of disease worsening.

Prof Wiendl responded that there is a critical need for objective measures of disease worsening other than the 'clinical impression' that is currently used. Consensus criteria needs to be defined based on clinical studies with clear methodology that can be applied to a real-world setting.

Prof Sørensen noted the difficulties in measuring whole brain or grey matter atrophy and asked the panel if they thought alternative measurements, such as thalamic volume, would be more practical for use in clinical practice.

Dr Lysandropoulos thought that working together with radiologists and hospital directors to ensure that the same MRI machine is used under consistent conditions, together with the use of appropriate software, should allow accurate enough brain measurement.

Prof Sørensen mentioned the impressive data for alemtuzumab showing that 60–70% of patients did not require further treatment after the two courses for up to 5 years and asked whether patients could be re-treated with alemtuzumab if necessary.

Prof Chan responded that re-treatment appears to be safe and no new safety signals have been observed so far.

Prof Sørensen asked Dr Chan if it is safe to use any of the first-line treatments for MS, e.g. IFN, after treatment with alemtuzumab.

Prof Chan said that, theoretically, it would be safe, although clinical experience is limited. Most of the patients in the extension trials were re-treated with alemtuzumab; only 2–3% were re-treated with alternative medications.

A member of the audience asked the panel whether there were any data available on switching patients from rituximab to alemtuzumab.

The panel responded that there was not a lot of data but in principle, switching from rituximab to alemtuzumab would be possible. To date, only anecdotal evidence is available on the efficacy and safety of this sequence.

A member of the audience asked the panel if there was any anecdotal evidence of patients treated with alemtuzumab that did not need to be re-treated with any drug.

The panel responded that an important question for immunologists is 'what is the difference in the re-programmed immune system between patients that need another course of treatment and those that become stable without secondary autoimmunity'. There is no clear answer. Studies are underway to investigate the development of T cell receptor repertoire profiling to provide some insight.

REFERENCES

1. Jacobsen CO, Farbu E. MRI evaluation of grey matter atrophy and disease course in multiple sclerosis: an overview of current knowledge. Acta Neurol Scand Suppl. 2014;(198):32-6.
2. De Stefano N et al. Clinical relevance of brain volume measures in multiple sclerosis. CNS Drugs. 2014;28(2):147-56.
3. Fisher E et al. Gray matter atrophy in multiple sclerosis: a longitudinal study. Ann Neurol. 2008;64(3):255-65.
4. Deppe M et al. Increased cortical curvature reflects white matter atrophy in individual patients with early multiple sclerosis. Neuroimage Clin. 2014;6:475-87.
5. Deppe M et al. Early silent microstructural degeneration and atrophy of the thalamocortical network in multiple sclerosis. Hum Brain Mapp. 2016;37(5):1866-79.
6. Bermel RA, Bakshi R. The measurement and clinical relevance of brain atrophy in multiple sclerosis. Lancet Neurol. 2006; 5(2):158-70.
7. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol. 2005;23:683-747.
8. Reynolds R et al. The neuropathological basis of clinical progression in multiple sclerosis. Acta Neuropathol. 2011;122(2): 155-70.
9. Simon JH. Brain atrophy in multiple sclerosis: what we know and would like to know. Mult Scler. 2006;12(6):679-87.
10. Popescu BF et al. Pathology of multiple sclerosis: where do we stand? Continuum (Minneap Minn). 2013;19(4 Multiple Sclerosis):901-21.
11. De Stefano N, Arnold DL. Towards a better understanding of pseudoatrophy in the brain of multiple sclerosis patients. Mult Scler. 2015;21(6):675-6.
12. Zhang D et al. Astrogliosis in CNS pathologies: is there a role for microglia? Mol Neurobiol. 2010;41(2-3):232-41.
13. Trapp BD et al. Axonal transection in the lesions of multiple sclerosis. N Engl J Med. 1998;338(5):278-85.
14. Siffrin V et al. Multiple sclerosis - candidate mechanisms underlying CNS atrophy. Trends Neurosci. 2010;33(4): 202-10.
15. Trapp BD, Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder?

Annu Rev Neurosci. 2008;31:247-69.

16. Sorbara CD et al. Pervasive axonal transport deficits in multiple sclerosis models. Neuron. 2014;84(6):1183-90.

17. Nikić I et al. A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. Nat Med. 2011;17(4):495-9.

18. Young EA et al. Imaging correlates of decreased axonal Na+/K+ ATPase in chronic multiple sclerosis lesions. Ann Neurol. 2008;63(4):428-35.

19. Friese MA et al. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. Nat Rev Neurol. 2014;10(4):225-38.

20. Wu GF, Alvarez E. The immunopathophysiology of multiple sclerosis. Neurol Clin. 2011;29(2):257-78.

21. Popescu V et al. Brain atrophy and lesion load predict long term disability in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2013;84(10):1082-91.

22. Fisniku LK et al. Gray matter atrophy is related to long-term disability in multiple sclerosis. Ann Neurol. 2008;64(3):247-54.

23. Sormani MP et al. Treatment effect on brain atrophy correlates with treatment effect on disability in multiple sclerosis. Ann Neurol. 2014;75(1):43-9.

24. Zivadinov R et al. A longitudinal study of brain atrophy and cognitive disturbances in the early phase of relapsing-remitting multiple sclerosis. J Neurol Neurosurg Psychiatry. 2001;70(6):773-80.

25. Calabrese M et al. Cortical lesions and atrophy associated with cognitive impairment in relapsing-remitting multiple sclerosis. Arch Neurol. 2009;66(9):1144-50.

26. Mowry EM et al. Quality of life in multiple sclerosis is associated with lesion burden and brain volume measures. Neurology. 2009;72(20):1760-5.

27. Durand-Dubief F et al. Reliability of longitudinal brain volume loss measurements between 2 sites in patients with multiple sclerosis: comparison of 7 quantification techniques. AJNR Am J Neuroradiol. 2012;33(10):1918-24.

28. Gold R, Wolinsky JS. Pathophysiology of multiple sclerosis and the place of teriflunomide. Acta Neurol Scand. 2011;124(2):75-84.

29. Oh J, O'Connor PW. Teriflunomide in the treatment of multiple sclerosis: current evidence and future prospects. Ther Adv Neurol Disord. 2014;7(5):239-52.

30. EMC. AUBAGIO 14 mg film-coated tablets. Dec 2015. Available at: https://www.medicines.org.uk/emc/medicine/28533. Last accessed: 5th July 2016.

31. Bruneau JM et al. Purification of human dihydro-orotate dehydrogenase and its inhibition by A77 1726, the active metabolite of leflunomide. Biochem J. 1998;336(Pt 2):299-303.

32. Bar-Or A et al. Teriflunomide and its mechanism of action in multiple sclerosis. Drugs. 2014;74(6):659-74.

33. Miller AE et al. Oral teriflunomide for patients with a first clinical episode suggestive of multiple sclerosis (TOPIC): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Neurol. 2014;13(10):977-86.

34. O'Connor PW et al. A Phase II study of the safety and efficacy of teriflunomide in multiple sclerosis with relapses. Neurology. 2006;66(6):894-900.

35. O'Connor P et al. Randomized trial of oral teriflunomide for relapsing multiple sclerosis. N Engl J Med. 2011;365(14):1293-303.

36. Freedman MS. Long-term safety and efficacy of teriflunomide in patients with relapsing forms of multiple sclerosis in the TEMSO extension trial. Poster 544. ECTRIMS, Copenhagen, Denmark 2-5th October 2013.

37. Confavreux C et al. Oral teriflunomide for patients with relapsing multiple sclerosis (TOWER): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Neurol. 2014;13(3):247-56.

38. Radue E et al. Teriflunomide slows brain volume loss in relapsing MS: a SIENA analysis of the TEMSO MRI dataset. Abstract 229. Presented at: The European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS) Congress, 10th October 2015.

39. Wuerfel J. Teriflunomide Slows Brain Volume Loss: Subgroup Analysis of the SIENA TEMSO MRI Dataset. P3 052. Poster presented at American Academy of Neurology Congress, 18th April, 2016.

40. Sprenger T. Teriflunomide Significantly Slows Brain Volume Loss in MS Patients Irrespective of Disability Progression. P3 047. Poster presented at American Academy of Neurology congress, 18th April, 2016.

41. Vucic S. Long-term clinical and MRI outcomes from teriflunomide extension studies. Poster P34. Seoul, Republic of Korea, 19-21 November 2014.

42. Comi G et al. Pooled safety and tolerability data from four placebo-controlled teriflunomide studies and extensions. Mult Scler Relat Disord. 2016;5:97-104.

43. Singer B et al. Teriflunomide Treatment Is Not Associated With Increased Risk of Infections: Pooled Data From the Teriflunomide Development Program. P2.194. Poster presented at American Academy of Neurology congress, 29th April, 2014.

44. Confavreux C et al. Long-term follow-up of a phase 2 study of oral teriflunomide in relapsing multiple sclerosis: safety and efficacy results up to 8.5 years. Mult Scler. 2012;18(9):1278-89.

45. Freedman MS et al. Safety and Efficacy of Teriflunomide for up to 9 Years in Relapsing Forms of Multiple Sclerosis: Update of the TEMSO Extension Trial. P3.150. Presented at the American Academy of Neurology congress, 29th April, 2014.

46. Hu Y et al. Investigation of the mechanism of action of alemtuzumab in a human CD52 transgenic mouse model. Immunology. 2009;128(2):260-70.

47. Zhang X et al. Differential reconstitution of T cell subsets following immunodepleting treatment with alemtuzumab (anti-CD52 monoclonal antibody) in patients with relapsing-remitting multiple sclerosis. J Immunol. 2013;191(12):5867-74.

48. Cohen JA et al. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. Lancet. 2012;380(9856):1819-28.

49. Coles AJ et al. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. Lancet. 2012;380(9856):1829-39.

50. Barkhof F et al. Alemtuzumab slows brain volume loss over 5 years in patients with active relapsing-remitting multiple sclerosis with most patients not receiving treatment for 4 years: CARE MS I and II extension study. Abstract 151. Oral presentation at ECTRIMS, 23rd September, 2015.

51. Arnold DL et al. Alemtuzumab demonstrates durable reduction of MRI activity over 5 years in CARE-MS I with the majority of patients treatment-free for 4 years. P1100. Poster presented at ECTRIMS, 23rd September, 2015.

52. Barkhof F. RRMS Patients Switching from SC IFNB-1a to Alemtuzumab in the CARE-MS I and II Extension Study Have a Reduced Rate of Brain Volume Loss. P6.183. Poster presented at American Academy of Neuology congress, 21st April, 2016.

53. Arroyo Gonzalez R et al. Treatment-naïve patients with active relapsing-remitting multiple sclerosis at baseline demonstrate continued disability improvement over 5 years with alemtuzumab: the CARE-MS I extension study. P22099. Poster presented at European Academy of Neurology congress, 7th June, 2016.

54. LaGanke C et al. Patients with Active Relapsing-Remitting Multiple Sclerosis and Inadequate Response to Therapy at Baseline Show Durable Disability Improvement over 5 Years

with Alemtuzumab: CARE-MS II. DX02. Presented at CMSC, 3rd June, 2016.

55. Moreau T et al. Alemtuzumab improves quality of life in relapsing-remitting multiple sclerosis patients who relapsed on prior therapy: 3-year follow-up of CARE-MS II. P044. Poster presented at ACTRIMS-ECTRIMS, September 2014.

56. Steinman L et al. Defining clinical meaning of patient-reported outcomes with disability assessment in multiple sclerosis: an analysis of the CARE-MS II study. P802. Poster presented at ACTRIMS-ECTRIMS, September, 2014.

57. Cuker A et al. Detection and management of immune thrombocytopenia in alemtuzumab-treated patients in the multiple sclerosis clinical development program. P 590. Poster presented at ECTRIMS, 23rd September, 2015.

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FROM CHEMOTHERAPY TO TARGETED THERAPY IN LOW-GRADE GLIOMAS

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Low-grade gliomas represent a group of primary brain tumours that cause disabling symptoms (mainly epileptic seizures), grow slowly but continuously, and have a high risk of malignant transformation. The best initial treatment includes as much surgical resection as possible. In cases of incomplete resection and/or persisting seizures and/or older age and/or progression on magnetic resonance imaging (MRI) after initial observation, adjuvant treatments (radiotherapy, chemotherapy) are needed. The precise role of each modality is still under discussion.

A recent Phase III randomised trial from the United States (RTOG 9802), published in early 2016[1] has demonstrated that radiotherapy followed by alkylating chemotherapy with procarbazine, lomustine (CCNU), and vincristine is superior over radiotherapy alone in terms of improving survival. We are waiting for the final results of another Phase III trial (EORTC 22033-26033), that has compared radiotherapy to chemotherapy as the best initial treatment. A reason for using upfront chemotherapy alone is that it could avoid or delay late cognitive deficit from radiation. In the case of patients with pharmacoresistant seizures, both radiotherapy and chemotherapy can be useful. A critical question is how best to assess the response to treatments using neuroimaging, as standard MRI is insufficient. New advances are represented by volumetry, MRI perfusion, magnetic resonance spectroscopy, and positron emission tomography with amino acids. Recently, molecular alterations such as the *isocitrate dehydrogenase 1/2* mutation and 1p/19q co-deletion have been shown to be able to subdivide low-grade gliomas into subgroups with significantly different prognosis,[2] which has resulted in the ability to treat these subgroups with different approaches. New trials are now being designed with molecular inclusion criteria instead of conventional histologic diagnosis.

REFERENCES

1. Buckner J et al. Radiation plus procarbazine, CCNU, and vincristine in low-grade glioma. N Engl J Med. 2016;374:1344-55.
2. Louis DN et al. The 2016 World Health Organization classification of tumors of the central nervous system: A summary. Acta Neuropathol. 2016;131(6):803-20.

STROKE AND FABRY'S DISEASE: COMMON MANIFESTATION OF A RARE DISEASE

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Fabry's disease (FD) is an X-linked lysosomal storage disorder, characterised by decreased or absent activity of the lysosomal enzyme alpha-galactosidase A and it has been identified as an underdiagnosed aetiology of stroke in the young. The prevalence of cerebrovascular accident in FD patients based on the Fabry Outcome Survey is 11% in males and 16% in females, a frequency 12-times higher than expected in a comparable non-Fabry population. In the Fabry Registry, 6.9% of males and 4.3% of females with FD suffered strokes both ischaemic and haemorrhagic. Moreover, a large proportion suffered their stroke before the diagnosis of FD was made. In addition, we identified that in our patients with FD and no history of cerebrovascular accident or transient ischaemic attack, 44% had asymptomatic ischaemic lesions on brain magnetic resonance imaging. Rolfs et al.[1] reported that 4.9% males and 2.4% females

with cryptogenic stroke <55 years of age suffered from FD and this corresponds to ~1.2% of young stroke patients. Several studies screening for FD in young patients with stroke were performed in Europe and North America with conflicting results.

Recently, the Stroke in Young Fabry Patients study, a multicentre observational study that included 5,023 young patients with stroke, identified definitive FD in 27 patients (0.5%). From January 2011, we enrolled 311 young stroke patients in a similar screening study in Argentina (53% of participants were male, mean age: 41 years). We identified a patient with a pathogenic mutation: c.888G>A/p.Met296Ile/Exon 6 (0.3%). Moreover, through family pedigree we identified 10 additional family members with FD. All of them had mild symptoms of FD corresponding to late onset, monosymptomatic, or oligosymptomatic forms of the disease different from the classical phenotype. Moreover, 22 females in our study had non-pathogenic mutations or mutations of unclear significance, emphasising the importance of differentiating these patients from FD patients to avoid unnecessary treatments. There is emerging evidence that enzyme replacement treatment may not only improve or stabilise cardiac and nephrologic manifestations of FD but also reduce the burden of ischaemic lesions in these patients. For this reason early identification and treatment of these patients is essential.

REFERENCES

1. Rolfs A et al. Prevalence of Fabry disease in patients with cryptogenic stroke: A prospective study. Lancet. 2005;366(9499):1794-6.

SCHWABE SYMPOSIUM ON BEHAVIOURAL AND PSYCHOLOGICAL SYMPTOMS ASSOCIATED WITH DEMENTIA: PREVALENCE, CLINICAL IMPORTANCE, AND TREATMENT OPTIONS

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Auguste suffered from pre-senile Alzheimer's disease. She was hospitalised in Frankfurt in 1901 due to severe psychotic and behavioural manifestations associated with Alzheimer's disease. Behavioural and psychological symptoms of dementia (BPSD) are very heterogeneous features of dementia patients, often grouped into clusters of psychotic, affective, and apathetic disorders as well as agitation. There is currently no definite classification of BPSD. The prevalence of BPSD is almost as high as dementia itself as most patients at some point develop a number of BPSD in the course of their lifetime. Besides the tremendously high frequency of BPSD, the importance of these symptoms is underlined by the consequences both on the patient and their caregivers, which include: profound subjective distress, social isolation or ostracism, physical or chemical restraint, and neglect. As a result, BPSD are a major cause of institutionalisation, caregiver burden, and treatment costs.

Although cognitive deficits and concomitant functional deterioration are the hallmarks of dementia, BPSD are nearly universal across dementias and dementia stages. This trend is arguably more devastating than cognitive decline and is inversely related to quality of life. BPSD are of paramount importance and special care and treatment of BPSD is mandatory.

The premise of treatment is firstly, to screen for BPSD possibly using general questionnaires (Neuropsychiatric Inventory; The Behavioural Pathology in Alzheimer's Disease Scale, etc.) or tools targeting BPSD more specifically (Cornell Scale for Depression in Dementia,

Cohen–Mansfield Agitation Inventory, etc.). Secondly, to investigate the possible causes. Indeed, dementia is a vulnerable state for, and the *conditio sine qua non* of, BPSD, but is not necessarily the direct cause. Pathoaetiological factors to be considered are the biological changes occurring in the dementia (cerebral lesion, type and stage of dementia, neuromodulatory changes), somatic disease, and pain. However, brain or systemic changes challenge the organism and the mind and some have observed that BPSD may be rather attributable to overwhelmed coping strategies and failing resilience. This also depends on genetic predisposition and epigenetic adaptations resulting in complex monitoring systems such as personality traits. The brain is in essence a means for an organism to adapt to environmental constraints; thus, the physical or social environment, that is not adapted to the needs of a demented patient with analytic or communication deficits, may induce or aggravate BPSD. A thorough assessment of the possible direct or indirect causes of BPSD will suggest a number of treatment options including somatic, psychotropic, psychological, and behavioural treatments as well as environmental and systemic (caring system) adaptations. As the dynamics of BPSD, with or without treatment, usually evolve over time, reassessing patients and caregivers on a regular basis is mandatory.

Somatic disorders must be identified and treated aetiologically or symptomatically where pain treatment is often centre stage. Cholinergic and glutamatergic neuromodulation of behaviours suggests the use of acetylcholinesterase inhibitors (depression, irritability, apathy) or memantine (agitation, aggressiveness, psychosis). Ginkgo biloba extract (Egb-761®) may be useful for anxiety, depression, or apathy. However, most studies on pro-cognitive drugs have been conducted in well-selected patients with low levels of BPSD and its use in highly disruptive BPSD may not be helpful. Some serotonin reuptake or monoamine oxidase inhibitors may be useful in the depressed as well as against agitation and aggression even in the absence of depression. Atypical antipsychotics may help in the case of psychotic or agitated BPSD although in a number of countries some of them may be off-label if used in this indication. Haloperidol can be an option in highly disruptive BPSD. In any case, side effects must be monitored carefully. Other psychotropic medications may at times be useful (anti-epileptic drugs, sleep medication, etc.).[1]

Grounded in vulnerability-stressor models, psychosocial therapies have been developed over the years and are widely considered to be pivotal. They precede drug treatment whenever possible. Sensory-motor and cognitive approaches such as mobility programmes, snoezelen, and aroma therapy may be helpful for some having intermediate-to-high evidence levels of efficacy. Personalised theories aim at forging the intervention according to a life-history perspective (specialised psychotherapeutic treatment, self-maintenance, and reminiscence therapy etc.). A negative behavioural loop between BPSD and caregiver stress prompts caring for the carer (psycho-education, social counselling, etc.) which has a good scientific evidence level. Adapting and structuring the environment to make it safe for the patient is part of a global approach to BPSD treatment. Case discussions and supervision of professional caregivers is most likely useful. However, no single medical or psychosocial treatment may be successful when used in isolation.

Other available treatments may sometimes be useful while others are still under development and desperately awaited to increase the panoply of approaches so as to fit the individual needs of patients with BPSD (light therapy, electroconvulsive therapy, repetitive transcranial magnetic stimulation, new pharmacological treatments, technology-assisted treatments, etc.) In the absence of disease-modifying treatments of the different dementias, the focus on BPSD treatment and research can hardly be over-emphasised.

REFERENCES

1. Savaskan E et al. [Recommendations for diagnosis and therapy of behavioural and psychological symptoms in dementia (BPSD)]. Praxis (Bern 1994). 2014;103(3):135-48.

THE IMPACT OF BILINGUALISM ON COGNITIVE FUNCTIONS ACROSS LIFESPAN AND IN BRAIN DISEASES

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The last decades have witnessed fundamental changes in our understanding of both brain and language. In cognitive neuroscience, the static, localisationist view of a 1:1 correspondence between circumscribed brain areas and specific cognitive functions gave way to a dynamic interaction of multiple networks, in which the same function can be distributed among many areas while the same area can be part of different networks. In the neuroscience of language, the concept of language as an autonomous, informationally-encapsulated module, as advocated in the 1980s, has been superseded by the notion of widely distributed language-related brain networks, going well beyond the traditional language areas and interacting with other aspects of cognition as well as with motor functions.[1]

These advances in the neuroscience of language are of particular importance in developing our understanding of bilingualism. Firstly, we have come to realise that different languages of a multilingual person cannot be reduced to static representations in isolated brain areas but are subject to parallel activation, inhibition, switching, and monitoring within the same brain networks. Secondly, the tension between this parallel activation and a selective output necessary for successful communication constitutes a permanent training for frontal-executive functions. Accordingly, the cognitive effects of bilingualism transcend language itself, leading to a better performance on many executive tasks,[2] particularly those requiring inhibition and switching. In contrast, given the complex nature of a bilingual (or even multilingual) vocabulary leading to multiple interactions, lexical access in bilinguals tends to be slower.[3] These theoretical considerations are supported by converging empirical evidence comparing systematic performance of monolingual and bilingual subjects on a range of cognitive tests, particularly on those involving frontal-executive functions. A better performance in the bilingual group has been documented across the lifespan, in young[4] as well as elderly[5] subjects. Bilinguals have been shown to develop dementia 4–6 years later than monolinguals[6] and to be twice as likely to recover their cognitive functions after stroke.[7] This suggests that bilinguals are able to build up a stronger 'cognitive reserve', offering some protection against cognitive ageing and the effects of different brain pathologies.

As the discussion at the EAN congress in Copenhagen, Denmark, showed, many questions such as the role of the age of acquisition remain open and will require further research. Traditionally, bilingualism research has focussed on what was considered to be its ideal case: early acquisition and perfect command of more than one language. In contrast, recent studies suggest that the effects of bilingualism are possibly even stronger in those who acquire a second language later in life.[5] Even a short intensive language course can improve attentional switching and the effect is maintained 9 months after in those who practice 5 hours per week or more.[8] Future studies will need to go beyond a simple comparison of monolinguals and bilinguals as distinct, dichotomous groups and determine the 'dose-response curve', linking language learning, knowledge, and use to their cognitive effects. This includes not only the beneficial effects of bilingualism but also its potential side effects, such as slower lexical access.[3]

REFERENCES

1. Bak TH. The neuroscience of action semantics in neurodegenerative brain diseases. Curr Opin Neuro. 2013;26(6):671-7.

2. Bak TH. The impact of bilingualism on cognitive ageing and dementia: Finding a path through a forest of confounding variables. Linguist Approaches Biling. 2016;6(1-2):205-26.

3. de Bruin A et al. The effects of language use on lexical processing in bilinguals. Lang Cogn Neurosci. 2016:1-8.

4. Vega-Mendoza M et al. The impact of late, non-balanced bilingualism on cognitive performance. Cognition. 2015;137:40-6.

5. Bak TH et al. Does bilingualism influence cognitive aging? Ann Neuro. 2014;75(6):959-63.

6. Alladi S et al. Bilingualism delays age at onset of dementia, independent of education and immigration status. Neurology. 2013;81(22):1938-44.

7. Alladi S et al. Impact of bilingualism on cognitive outcome after stroke. Stroke. 2016;47(1):258-61.

8. Bak TH et al. Novelty, challenge, and practice: The impact of intensive language learning on attentional functions. PloS One. 2016;11(4):e0153485.

CHALLENGES IN DIAGNOSING TICS, TOURETTE'S SYNDROME, AND COMORBIDITIES

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DEFINITION AND EPIDEMIOLOGY

Diagnostic categories within Tic disorders (inclusive of Tourette's syndrome [TS]) include transient and chronic conditions. Tic disorders are characterised by the presence of tics, which are involuntary contractions of muscles or vocal expressions, but may also compromise more complex behaviours. A tic is defined as a "sudden, rapid, recurrent, non-rhythmic motor movement or vocalization,"[1] a definition applied consistently across all entities of tic disorders. The elimination of the term 'stereotyped' in the recent version of Diagnostic and Statistical Manual of Mental Disorders[1] is an attempt to differentiate tics from typical symptoms in the autistic spectrum.[2] TS typically manifests between 3 years and 8 years of age, tic severity increases and in most children then attenuates before or during puberty, so that at 18 years of age, tic severity has decreased in the majority of individuals with TS.[3]

CLINICAL PICTURE

Tics may present as simple motor or vocal tics, or as more complex behaviours. Based on recent studies that measure the influence of reward or the environmental cues on tic frequency, the general perception of tics has moved from regarding those symptoms as automatic/unconscious to half-willful behaviours. Along with this perspective, premonitory urges, physical perceptions emerging just before the advent of a tic, have attracted attention in the literature. It is an important area and its exploration with patients of all ages helps to raise the individual's awareness of the processes taking place in their body before, during, and after the performance of a tic. This emerging awareness has become part of the psychoeducation with parents, children, and adults with TS, but also represents a crucial starting point for cognitive behavioural therapy. The clinical picture of pure TS is often complicated by self-mutilating behaviours and comorbidities, which are the rule rather than the exception, with attention deficit hyperactivity disorder (ADHD) and obsessive-compulsive disorder (OCD) representing at high frequencies in children and young adults with TS.[4]

DIAGNOSTIC WORK-UP

The European guidelines for TS and other tic disorders[5] emphasise the complexity of these neuropsychiatric disorders that require interdisciplinary co-operation between medical professionals, but also patients, parents, and teachers for both diagnostic work-up and planning of treatment. The guidelines describe standardised instruments used to evaluate tic severity, and

specifically highlight the Yale-Global Tic Severity Scale,[7] the instrument most widely used in the evaluation of patients with tics, both in clinical settings and for research purposes. The guidelines further recommend the use of a structured diagnostic instrument (such as the K-SADS) to screen for other psychiatric diagnoses and specific instruments allowing for a dimensional mapping of tics and comorbid conditions (such as ADHD and OCD, based on the diagnostic interview).

EMERGING AREAS OF FOCUS

The burden of emotional dysregulation[8] and the frequent presence of mood disorders, especially in young adults with TS,[9] has been identified as an area lacking substantial evidence and is thus of major interest in the years to come. The main reason for this renewed focus is the fact that an individual's recovery and social function are often determined by their comorbidities and their ability to regulate emotions.

REFERENCES

1. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, (2013) 5th Edition, Arlington, Virginia: American Psychiatric Publishing.

2. Plessen KJ. Tic disorders and Tourette's syndrome. Eur Child Adolesc Psychiatry. 2013;22 Suppl 1:S55-60.

3. Leckman JF. Tourette's syndrome. Lancet. 2002;360(9345): 1577-86.

4. Freeman RD et al. An international perspective on Tourette syndrome: selected findings from 3,500 individuals in 22 countries. Dev Med Child Neurol. 2000;42(7):436-47.

5. Cath DC et al. European clinical guidelines for Tourette syndrome and other tic disorders. Part I: assessment. Eur Child Adolesc Psychiatry. 2011;20(4):155-71.

6. Kaufman J et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 1997;36(7):980-8.

7. Leckman JF et al. The Yale Global Tic Severity Scale: initial testing of a clinician-rated scale of tic severity. J Am Acad Child Adolesc Psychiatry. 1989;28(4):566-73.

8. Budman CL et al. Explosive outbursts in children with Tourette's disorder. J Am Acad Child Adolesc Psychiatry. 2000; 39(10):1270-6.

9. Gorman DA et al. Psychosocial outcome and psychiatric comorbidity in older adolescents with Tourette syndrome: controlled study. Br J of Psychiatry. 2010;197(1):36-44.

TICS AND TOURETTE'S SYNDROME: MOVEMENT DISORDERS OR BEHAVIOURAL CONDITIONS?

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First described by French physician Georges Gilles de la Tourette in 1885, Tourette's syndrome is a tic disorder characterised by a degree of complexity and symptomatic heterogeneity that crosses the traditional boundaries between neurology and psychiatry.[1] Tics are relatively common symptoms of hyperkinetic movement disorders and are defined as involuntary, sudden, rapid, recurrent, non-rhythmic movements (motor tics: eye blinking, facial grimacing, shoulder shrugging), or vocalisations (phonic tics: grunting, sniffing, throat clearing). The most commonly reported complex tics include coprolalia (swearing as a tic), echolalia (repeating other people's words), and paliphenomena (repeating own actions or words). Both simple and complex tics are characteristically modulated by psychological and environmental factors. Moreover, converging evidence from large studies conducted in multiple centres,[2] single centres,[3] and community populations[4] have shown that 90% of patients with Tourette's syndrome fulfil diagnostic criteria for at least one comorbid psychiatric disorder. The concept of a 'Tourette's syndrome spectrum' encompasses conditions characterised by tics only ('pure Tourette's syndrome'), tics and complex symptoms such as self-injurious behaviours ('full-

blown Tourette's syndrome'), tics and comorbid psychiatric disorders such as obsessive-compulsive disorder, attention-deficit and hyperactivity disorder, affective disorders, and impulse control disorders ('Tourette's syndrome-plus'). Practical implications of a better understanding of the behavioural spectrum of Tourette's syndrome include the differential diagnosis between complex tics, compulsions, and hyperactivity, and the choice of the most appropriate treatment approach (both behavioural and pharmacological). Most importantly, research from the last decade on health-related quality of life using disease-specific measures has informed clinicians about the differential impact of tics and behavioural symptoms on patients' wellbeing, highlighting the importance of a holistic approach to a quintessentially neuropsychiatric condition.

REFERENCES

1. Cavanna AE, Seri S. Tourette's syndrome. BMJ. 2013;347:f4964.

2. Freeman RD et al. An international perspective on Tourette syndrome: Selected findings from 3,500 individuals in 22 countries. Dev Med Child Neurol. 2000;42(7):436-47.

3. Cavanna AE et al. Dissecting the Gilles de la Tourette spectrum: A factor analytic study on 639 patients. J Neurol Neurosurg Psychiatry. 2011;82(12):1320-3.

4. Khalifa N, von Knorring AL. Prevalence of tic disorders and Tourette syndrome in a Swedish school population. Dev Med Child Neurol. 2003;45(5):315-9.

TREATMENT OF TICS AND TOURETTE'S SYNDROME: CANNABIS, DEEP BRAIN STIMULATION, AND BEYOND

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Gilles de la Tourette's syndrome (GTS) is a complex neuropsychiatric disorder. According to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition, GTS is defined by the presence of multiple motor tics and at least one vocal tic, with childhood onset lasting for >1 year. A substantial number of patients (up to 90%) suffer from additional psychiatric comorbidities, including attention deficit hyperactivity disorder (ADHD), obsessive-compulsive disorder, depression, anxiety, and impulsivity. In the majority of patients, comorbidities impair patients' quality of life more than tics. Therefore, inmany patients treatment of comorbidities is paramount. Treatment of tics should be suggested to those patients who suffer from severe and disabling tics. Since all available treatment strategies do not influence the underlying cause of GTS or the prognosis of the disease, patients' decision against treatment of tics should be accepted.

According to most national and European clinical guidelines, in any case treatment should be started with psychoeducation of patients and, with regards to minors, their parents. Thereafter and only in cases where indicated, treatment with either behavioural therapy or pharmacotherapy should be offered. Most experts recommend behavioural therapy with habit reversal training (HRT) or exposure and response prevention training (ERP) as first-line treatment. However, in most European countries there is a tremendous lack of well-trained psychotherapists. Thus, most patients have no access to this effective and established treatment. According to well-powered randomised controlled trials (RCTs), HRT and ERP result on average in a tic reduction of 30–40%. To overcome this restriction, in some centres behavioural therapy is alternatively offered via telemedicine or e-health systems. In addition, a current RCT is in preparation that aims to investigate the efficacy of internet-based HRT completely independently of a psychotherapist.

With respect to pharmacotherapy, there is general agreement that dopamine receptor blocking drugs

(antipsychotics) are the most effective substances for the treatment of tics. However, antipsychotics are not effective in all patients; on average they result in a tic reduction of ~50%, but not in a complete tic remission. Although well-powered RCTs are still missing, most European experts recommend aripiprazole as first-line treatment in both children and adults with GTS. From clinical experience it is suggested that aripiprazole is better tolerated than other first and second-generation antipsychotics. The most common side effects are sedation, restlessness, and sleeping problems. As second-line treatment tiapride (in children), sulpiride, and risperidone are recommended. However, none of these newer drugs are officially licensed for the treatment of tics and GTS, respectively. Until today, in most countries only the first-generation antipsychotics haloperidol and pimozide are formally approved for this indication. However, haloperidol and pimozide should be used only when other drugs are ineffective.

If different antipsychotic drugs do not result in a significant tic improvement or they cause intolerable side effects, only a few other treatment options can be recommended. In carefully selected patients, local injections with botulinum toxin can be taken into consideration, in particular for the treatment of simple motor tics located in the face, head, and neck. Furthermore, in some patients tetrabenazine can be used. However, since there is evidence that tetrabenazine may cause depression in a substantial number of patients, patients should be screened for depression and be informed about this side effect before treatment is initiated. In patients who suffer from comorbid ADHD, noradrenergic drugs such as clonidine and guanfacine can be used for the treatment of milder tics. According to a recent meta-analysis, these drugs are not effective in the absence of ADHD.

In adult patients, treatment with cannabis-based medicine can be taken into consideration. According to case reports and two small RCTs, cannabinoids such as delta-9-tetrahydrocannabinol might be effective not only in the treatment of tics, but also of psychiatric comorbidities. A substantial number of patients use cannabis (illegally) as a kind of a self-medication. A large number of other substances have been suggested in the treatment of tics, among them are drugs that influence the serotonergic, GABAergic, histaminergic, and glutamatergic systems. Due to lack of data, no final recommendation can be given. Presently, antibiotics and immune-modulating drugs cannot be recommended for the treatment of tics. However, within the next few months, results will be released from a large EU-wide multicentre clinical trial investigating the role of infections in GTS. This will also provide important new data with respect to immune-based treatment strategies in patients suffering from GTS.

In otherwise treatment-resistant and severely affected patients, surgical treatment with deep brain stimulation (DBS) can finally be taken into consideration. According to data obtained from an open uncontrolled case series and small RCTs (including 3–13 patients) DBS causes a tic reduction of 50% (on average). However, while in some patients DBS results in a marked tic improvement, in others only slight or no improvement occurs. So far, no predictors for response to DBS have been identified. It is unclear which of the eight different targets suggested so far should be used. In most patients, DBS has been performed in the thalamus and globus pallidus internus. While in recent years it has been suggested to use DBS only in adult patients, according to the latest clinical guidelines it can also be taken into account in extremely affected and otherwise treatment-resistant children.

Although there are different therapeutic strategies available, including behavioural, pharmacological, and surgical treatments, treatment of tics is unsatisfactory in a large number of patients. New treatment options are urgently needed, resulting not only in better tic reduction and less adverse effects, but ideally also in improvement of psychiatric comorbidities.

FURTHER READING

1. Verdellen C et al. European clinical guidelines for Tourette syndrome and other tic disorders. Part III: behavioural and psychosocial interventions. Eur Child Adolesc Psychiatry. 2011;20;20(4):197-207.

2. Roessner V et al. European clinical guidelines for Tourette

syndrome and other tic disorders. Part II: pharmacological treatment. Eur Child Adolesc Psychiatry. 2011;20(4):173-96.

3. Müller-Vahl KR et al. European clinical guidelines for Tourette syndrome and other tic disorders. Part IV: Deep brain stimulation. Eur Child Adolesc Psychiatry. 2011;20(4):209-17.

4. Zheng W et al. Aripiprazole for Tourette's syndrome: A systematic review and meta-analysis. Hum Psychopharmacol. 2016;31(1):11-8.

5. Bloch MH et al. Meta-analysis: treatment of attention-deficit/hyperactivity disorder in children with comorbid tic disorders. J Am Acad Child Adolesc Psychiatry. 2009;48(9):884-93.

6. Müller-Vahl KR. Treatment of Tourette syndrome with cannabinoids. Behav Neurol. 2013;27(1):119-24.

7. Baldermann JC et al. Deep brain stimulation for Tourette-syndrome: A systematic review and meta-analysis. Brain Stimul. 2016;9(2):296-304.

8. Schrock LE et al. Tourette syndrome deep brain stimulation: A review and updated recommendations. Mov Disord. 2015;30(4):448-71.

DEFINITION OF EPILEPSY AND CLASSIFICATION OF SEIZURES

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The International League Against Epilepsy (ILAE) has recently presented important new contributions, which have been prepared by international task forces as draft manuscripts and then presented for comment on the ILAE website. After taking into consideration the comments made by the epilepsy community the final ILAE position paper is published in Epilepsia, the official journal of ILAE. This process ensures that these proposals of key aspects of epilepsy are conceptually sound, acceptable, and clinically meaningful.

According to the traditional definition, epilepsy is a disorder characterised by two or more unprovoked seizures occurring >24 hours apart. This definition was concise, easy to apply, and known to many, but there were several inherent limitations involved: 1) a person can never outgrow epilepsy; 2) some people are now treated as if they have epilepsy after one seizure; 3) a person can have an epilepsy syndrome, but not epilepsy; and 4) those with photic or reflex seizures are not defined as having epilepsy. 'A practical clinical definition of epilepsy' was published in 2014.[1] The comments made by the epilepsy community have had a major impact on the final version. The task force proposed that epilepsy be considered a disease of the brain defined by any of the following conditions: 1) at least two unprovoked seizures occurring >24 hours apart; 2) one unprovoked seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the subsequent 10 years; or 3) diagnosis of an epilepsy syndrome. Epilepsy is considered to be resolved for individuals who had an age-dependent epilepsy syndrome but are now past the applicable age or those who have remained seizure-free for the previous 10 years, with no seizure medicines in the last 5 years.

In April 2016, a draft version of 'Operational Classification of Seizure Types'[2] was posted on the ILAE website to recognise that some seizure types can have either a focal or generalised onset, to allow classification when the onset is unobserved, to include some missing seizure types, and to adopt more transparent names. Changes include: 1) 'partial' becomes 'focal'; 2) seizures of unknown onset can still be classified; 3) awareness is used as a classifier of focal seizures; 4) the terms 'dyscognitive', 'simple partial', 'complex partial', 'psychic', and 'secondarily generalised' are eliminated; and 5) 'bilateral tonic-clonic seizure' replaces 'secondarily generalised' seizure. The new classification does not represent a fundamental change, but allows greater flexibility and transparency in naming seizure types. Comments for this proposal were open until the beginning of June 2016 and the final version is due to be published during the final quarter of 2016.

REFERENCES

1. Fisher RS et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia. 2014;55(4):375-82.

2. Fisher RS et al. Operational Classification of Seizure Types by the International League Against Epilepsy. 2016. Available at: http://www.ilae.org/visitors/centre/documents/ClassificationSeizureILAE-2016.pdf. Last accessed: 6 July 2016.


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EDITORS PICK

The significant health burden that apathy creates for patients with Parkinson's disease is revealed in the following article by Muhammed and Husain; also highlighted are the difficulties of a therapeutic approach towards tackling a lack of motivation. This is an important issue for clinical neurologists who must be careful not to mistake the characteristics of apathy with those that emerge from neurodegenerative disease, while also tackling its many other diagnostic challenges. Meanwhile, no gold standards have been agreed upon for treatment and caution must be advised to those tasked with treating this poorly understood yet common neuropsychiatric syndrome. This paper explores these themes.

CLINICAL SIGNIFICANCE OF APATHY IN PARKINSON'S DISEASE

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ABSTRACT

Apathy, or lack of motivation, is increasingly recognised as a major factor affecting quality of life and prognosis in Parkinson's disease (PD). Impacting every stage of the disease, including *de novo* cases, reports have suggested it can affect up to 70% of patients. Despite the pervasiveness of apathy in PD, challenges remain in its detection, clinical assessment, and treatment. Strong overlap with depression and anhedonia can complicate diagnosis, and although common features exist between all of these neuropsychiatric conditions, dissociations may be suggestive of different underlying brain mechanisms. Several lines of evidence have implicated frontostriatal reward and effort-related neural pathways in the genesis of apathy, but the precise processes remain to be fully elucidated. The mainstay of current approaches in the treatment of apathy rely on dopamine replacement, although there is growing evidence that support a potential role for other agents. This paper reviews the current understanding of this important non-motor complication of PD.

Keywords: Apathy, Parkinson's disease (PD), depression, anhedonia, clinical significance, diagnosis, prognosis, mechanisms, treatment, quality of life.

INTRODUCTION

Apathy is a common neuropsychiatric syndrome often characterised by lack of motivation, and is gaining appreciation as a major problem in Parkinson's disease (PD).[1] Its importance has become more apparent over the past two decades as awareness of its clinical impact in many different neurodegenerative disorders has grown.[2] As well as being a debilitating non-motor symptom, apathy also poses a significant health burden for patients and their caregivers. It is associated with several adverse outcomes and reduced quality of life,[3] yet its underlying mechanisms are poorly understood. This is further complicated by the overlap between apathy and

other neuropsychiatric illnesses, such as depression and anhedonia, which often occur concurrently.[4] This review will discuss the clinical significance of apathy in PD, focussing on its clinical impact, differentiation from other neuropsychiatric disorders, and potential treatment options. Possible mechanisms of apathy, which may help combat the aforementioned diagnostic challenges, will also be suggested.

EPIDEMIOLOGY

The reported prevalence of apathy in PD ranges from 7–70%.[5,6] This large variability is a result of the assessment tools used, and also likely reflects the heterogeneity of the PD population. On average ~40% of PD cases,[1] and up to one-quarter of newly diagnosed drug-naïve patients,[7] are affected. Dividing PD patients further into phenotypic subgroups demonstrates that both tremor-dominant and akinetic–rigid variants of PD suffer from apathy. The rates however are not equal; akinetic-rigid phenotypes are more strongly affected[8] and the addition of apathy contributes to poorer patient outcomes normally observed in this subpopulation. Age is also an associated factor: patients with apathy tend to be older,[1] and those given a PD diagnosis at an older age may also have an increased risk of developing the disorder.[5] Other demographic differences such as gender, disease duration, and Hoehn and Yahr score do not seem to have a definite association, but increased motor-symptom severity is related.[1] Similarly, PD cases with more advanced motor disease progression at 4-year follow-up are also more likely to develop the condition.[9] Importantly, the evidence suggests that this motor association is not a secondary psychological reaction to the physical disability, but instead is linked to neurodegeneration,[10] implying distinct pathological processes in the development of apathy.

Table 1: Proposed diagnostic criteria for apathy in clinical practice in neurodegenerative disorders.[15]

A	Loss of or diminished motivation in comparison to the patient's previous level of functioning and which is not consistent with his/her age or culture. These changes in motivation may be reported by the patient or by the observations of others.	
B	Presence of at least one symptom in at least two of the three following domains for a period of at least 4 weeks and present most of the time.	
Domain B1 (behaviour)	Loss of, or diminished, goal-directed behaviour as evidenced by at least one of the following:	• Initiation symptom: loss of self-initiated behaviour (e.g. starting conversation, doing basic tasks of day-to-day living, seeking social activities, communicating choices) • Responsiveness symptom: loss of environment-stimulated behaviour (e.g. responding to conversation, participating in social activities)
Domain B2 (cognition)	Loss of, or diminished, goal-directed cognitive activity as evidenced by at least one of the following:	• Initiation symptom: loss of spontaneous ideas and curiosity for routine and new events (e.g. challenging tasks, recent news, social opportunities, personal/family and social affairs) • Responsiveness symptom: loss of environment-stimulated ideas and curiosity for routine and new events (e.g. in the person's residence, neighbourhood, or community)
Domain B3 (emotion)	Loss of, or diminished, emotion as evidenced by at least one of the following:	• Initiation symptom: loss of spontaneous emotion, observed or self-reported (e.g. subjective feeling of weak or absent emotions, or observation by others of a blunted affect) • Responsiveness symptom: loss of emotional responsiveness to positive or negative stimuli or events (e.g. observer reports of unchanging affect, or of little emotional reaction to exciting events, personal loss, serious illness, emotion-laden news)
C	These symptoms (A–B) cause clinically significant impairment in personal, social, occupational, or other important areas of functioning.	
D	The symptoms (A–B) are not exclusively explained or due to physical disabilities (e.g. blindness and loss of hearing), motor disabilities, diminished level of consciousness, or the direct physiological effects of a substance (e.g. a non-prescription drug, a medication).	

For a diagnosis of apathy, the patient should fulfil the criteria A, B, C, and D.
Adapted from Mulin et al.[15]

CHALLENGES IN DIAGNOSIS

There are many overlapping descriptions of apathy but no clear consensus on an exact definition or formal diagnostic criteria.[11] The syndrome is generally defined as a disorder of motivation with central common features. These include a reduction in goal-directed behaviour encompassing cognitive, emotional, and self-initiated motor domains. One challenge that gives rise to the difficulty in its classification is the distinction of apathy as a syndrome in itself or as a symptom of the underlying disease with which it is associated.[12,13]

In PD, it is often difficult to recognise and distinguish between symptoms of apathy and the phenomenology of the neurodegenerative condition, as there are many features common to PD and apathetic syndromes. For example, indifference and lack of effort caused by apathy can overlap with the masked facial expression and paucity of movement observed in PD.[14] The need for reliable and reproducible criteria is therefore paramount for accurate diagnosis and appropriate treatment. Diagnostic criteria of apathy in Alzheimer's disease (AD) and other neuropsychiatric disorders have been proposed but they have yet to be implemented formally in the current Diagnostic and Statistical Manual of Mental Disorders or International Classification of Disorders classification systems and there are no definite standards for diagnosing apathy specifically in PD.[11,13,15] The criteria currently suggested for apathy are summarised in Table 1.

DETECTION AND MEASUREMENT OF APATHY

A variety of clinical questionnaires have been designed to assess apathy, but no gold standard has been agreed upon for clinical practice. The Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) attempts to identify apathy in patients through a single questionnaire component, however this should only be considered for screening purposes.[16] More detailed methods for the assessment of apathy in PD take the form of self-reporting clinical questionnaires and semi-structured interviews. Perhaps the most extensively used questionnaire, from a research perspective, is the Lille Apathy Rating Scale,[17] which examines different subdomains and may be the most thorough assessment of apathy in PD.[16] For everyday clinical practice, an abbreviated short form of the Lille Apathy Rating Scale[18] has shown reliability in evaluating apathy in PD; a caregiver version also exists.[19] The Apathy Scale,[20] Apathy Evaluation Scale,[21] and the Apathy Inventory[22] have also been validated for use in PD. These instruments, when combined with other objective behavioural measures, give rise to a more accurate and better diagnosis of apathy in PD.

HOW DOES APATHY AFFECT PROGNOSIS?

Mixed opinions exist regarding the clinical impact that apathy has in PD,[23] but overwhelming evidence points towards a negative prognosis. The difficulty lies in attributing causality. Few longitudinal studies have compared the outcomes associated with PD and concurrent apathy directly, but an association with more rapid disease progression and cognitive decline has been suggested in those that have.[24] Other cross-sectional studies have found apathy in PD to be associated with a number of adverse outcomes, including communication difficulties,[25] increased motor symptoms, and increased physical disability.[1] Perhaps the most significant and consistent finding is the association with cognitive decline in PD.[23] Apathy may in fact herald the onset of dementia[26] and therefore represent an important prognostic factor, particularly as dementia increases mortality in PD.[27]

IMPACT OF APATHY ON QUALITY OF LIFE

The presence of apathy in the elderly correlates with a reduction in satisfaction with life and in general health status.[28] This clearly reflects the importance of motivation as well as the negative consequences of its absence. In AD and other dementias, apathy is accompanied by increased functional disability, reduced rehabilitation success, and a larger burden and stress on caregivers.[29]

Similar findings also hold true in PD, with lower quality of life scores reported in patients who suffer from apathy.[3,30] Results, however, do vary, and the distinction between the effects of apathy and coexisting neuropsychiatric disorders, such as depression or cognitive impairment, can lead to differences in measured outcomes.[31] Mood disturbance and apathy together appear to have the greatest negative impact on quality of life.[32]

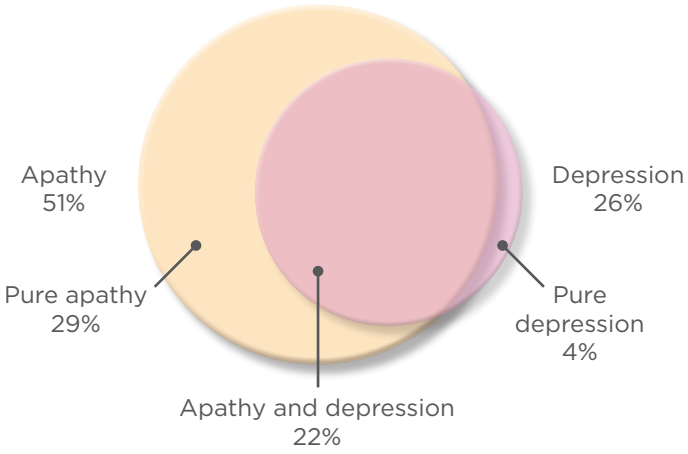

Figure 1: Example of overlap and prevelance of apathy and depression in a cohort of Parkinson's disease patients.
Adapted from Kirsch-Darrow et al.[40]

Indeed, non-motor symptoms in PD are a frequent cause of institutionalisation and increased cost of care.[33] Caregivers are also negatively affected by the presence of apathy in PD. This is apparent in the early stages of the disorder as the care burden becomes significantly greater, even in comparison to other neuropsychiatric illnesses such as depression.[34] Activity of daily living scores are also lower, so a functional impact on day-to-day life is also apparent in those suffering from apathy.[1] These reduced activity of daily living scores seem to improve with treatment of apathy in PD, as does the extent of caregiver burden, but quality of life does not recover.[35] Consequently, therapies to improve motor function are only one part of restoring quality of life for patients, and neuropsychological disorders like apathy should also be addressed in order to improve outcomes.[14]

OVERLAP WITH DEPRESSION AND ANHEDONIA

PD patients can suffer from a large range of neuropsychiatric symptoms. A report suggests that 61% of PD patients have at least one psychiatric symptom, and 45% demonstrate two or more.[24] Depression is frequently described, occurring at approximately 2-times the frequency of the general population,[36] and also appears to be the most important association with apathy in elderly non-demented PD patients.[31] Patients suffering with depression often have symptoms of apathy; indeed, loss of motivation forms part of the diagnostic criteria for depression. Thus, given the strong relationship there has been much debate in the past as to whether apathy is a distinct entity in PD or just a symptom of depression. In a number of other neurological disorders including AD, Huntington's disease, and frontotemporal dementia, apathy is deemed to be a specific neuropsychiatric syndrome, distinct from depression.[37]

Similarly, consensus is moving toward a distinction between depression and apathy in PD,[30,38] as approximately half of PD patients with apathy do not suffer from concomitant depression (Figure 1).[39] These findings suggest that both disorders are likely to be frequent features of PD, but that they can exist independently of one another.[4,40] Neuroimaging studies have also revealed differences between apathy and depression.[41,42] This distinction is important clinically as misdiagnosis can result in inadequate treatment and also worse patient outcomes.[43,44] Depression and apathy are also strongly associated with fatigue, which is another disabling non-motor symptom in PD.[45]

Anhedonia also has considerable overlap with apathy and is a key component of various neuropsychiatric conditions including depression, where it can be a diagnostic feature. Anhedonia is traditionally defined as reduced ability to experience pleasure[46] but more recently it has been conceptualised as a disorder that affects motivation mechanisms, including anticipatory 'wanting' (the desire to obtain a reward) and consummatory 'liking' (the satisfaction obtained when consuming a reward).[47] This can be likened to the desire for food when hungry versus the sensation of satisfaction when you finally eat it, respectively. These components of pleasure may have separate neural pathways. 'Wanting' is associated with frontostriatal dopaminergic circuits, which have also been linked with motivation and apathy,[48,49] while 'liking' has been associated with the nucleus accumbens, ventral pallidum, and projections to the orbitofrontal cortex (Figure 2).[50]

Although these two systems are interrelated, they are associated with dissociable behavioural features. Some suggest that apathy might be more closely related to the anticipatory, dopamine-responsive component of anhedonia, while the consummatory component is more associated with depression.[51,52] The similarities between apathy and anhedonia are seemingly

closer than those between apathy and depression; anhedonia accompanies both and might be associated with deficits in 'liking' and 'wanting',[4] although this is a controversial area. In PD, anhedonia, like apathy, appears to be independent of motor symptoms, has been attributed to frontal lobe dysfunction,[53] and is also responsive to PD therapies.[54]

MECHANISMS OF APATHY IN PARKINSON'S DISEASE

Given the heterogeneity of PD and the overlap of apathy with other neuropsychiatric conditions, there is likely to be a range of different underlying mechanisms leading to reduced motivation. It is unlikely that there is one simple cause of apathy; as a result, more targeted and personalised objective methods for the diagnosis and treatment of apathy are needed. Surprisingly, despite the clinical significance, the mechanisms underlying apathy in PD patients are poorly understood. Some theories of goal-directed behaviour conceptualise the processes underpinning motivation as a sequence, starting with the generation of options for behaviour, followed by selection of the goal, initiation or inhibition of an action, and then subsequent learning from the result.[5] Dysfunction in any of these components might potentially lead to the manifestations of the different subtypes of apathy. To break down the components further, it is possible that the reduced ability to select goals in apathy is also dependent on several factors. For example, patients might become less sensitive to reward or hypersensitive to effort.[55] These effects may vary between individuals due to differing levels of neurotransmitter dysfunction or degeneration in different frontostriatal circuits.

Disparities in how frontostriatal brain regions are affected by degenerative processes in PD might lead to different emotional, cognitive, and behavioural manifestations of apathy.[49] Regions within the limbic system have been most frequently associated with the neural correlates of apathy in PD. Position emission topography (PET) studies suggest a mesolimbic rather than a nigrostriatal cause,[56] and structural and functional imaging implicate frontal and subcortical brainareas, all of which are linked with reward processing.[57,58] Other important reward-related areas involved in apathy include the ventral striatum and specifically its frontal connections with dopamine as the principal neurotransmitter.[49]

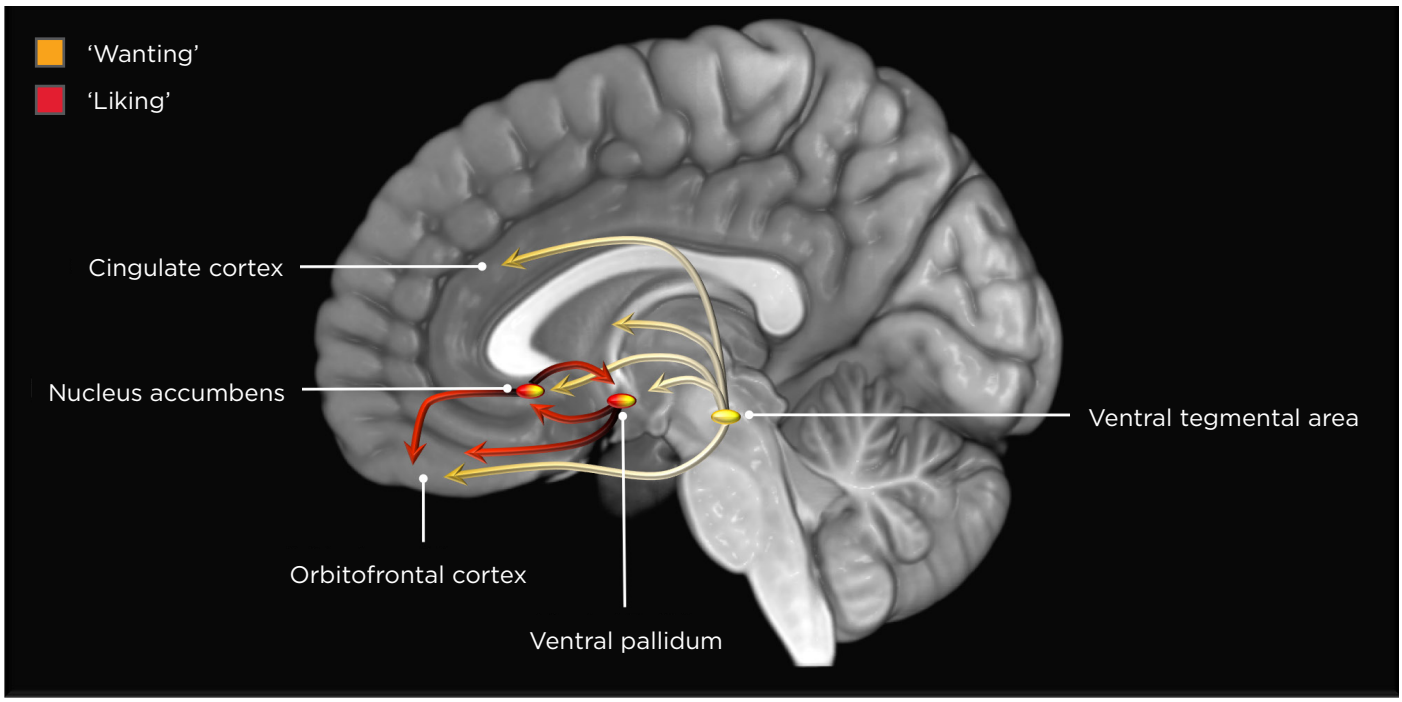

Figure 2: The proposed neural projections involved in the 'wanting' and 'liking' brain circuits, which are implicated in motivation and pleasure.

Hedonic hotspots include the nucleus accumbens and the ventral pallidum, with strong associations to the orbitofrontal brain areas and reward processing areas.

Correspondingly, reward circuits and dopamine dysfunction appear to be key in the development of apathy, as discussed forthwith.

THERAPIES

Given the different domains of apathy and the variety of potential mechanisms contributing to its clinical phenotype, it may be necessary to adopt an individualised, symptom-specific approach in the treatment of each patient.[59] A variety of pharmacological approaches have been used,[60] each with varying success, however at present there are no formally approved drugs.[61]

The severity of apathy level in PD tends to be lower in patients treated with higher doses of dopaminergic drugs.[1] Dopamine agonists including pramipexole[62] and piribedil[63] can have beneficial effects on apathy, as well as mood, in PD.[64] Pramipexole can also lead to improvements in associated anhedonia and depression symptoms.[54] Likewise, the ability of dopamine receptor agonists to improve motivation has been observed in other patients with prefrontal or basal ganglia lesions.[65] Apathy in PD is not always dopamine responsive; indeed, it has been suggested that there are two types of syndrome: dopamine-sensitive and dopamine-resistant apathy. The former is often reported to occur following deep brain stimulation (DBS) a number of months after dopamine withdrawal following the procedure, and it can be reversed after reintroduction of the medication.[56,66] Dopamine-resistant apathy, on the other hand, seems to be related to the progression of PD and may be due to structural atrophy in deep brain structures, such as the nucleus accumbens and the caudate nucleus.[67] Therefore, which class a patient falls into may have significant repercussions on drug responsiveness and subsequent prognosis. Dopamine therapies with reported beneficial effects on apathy include: levodopa,[68] the dopamine agonists pramipexole,[62] piribedil,[63] ropinirole,[66] and rotigotine,[69] and the monoamine oxidase inhibitor rasagiline for patients with early untreated PD.[70]

Although dopamine dysfunction is likely to be contributory it is not necessarily the sole cause. Dysfunction of non-dopaminergic systems has also been shown to predict the development of apathy[9] and acetylcholine is a possible candidate implicated in the process. A double-blind, placebo-controlled trial studied the cholinesterase inhibitor rivastigmine. The trial included PD patients suffering from apathy, who were neither depressed nor demented, and yielded marked motivational improvements. This highlights the potential for its use as a therapeutic intervention.[35] Other drug therapies such as methylphenidate (which increases brain dopamine and noradrenaline levels) have also been trialled in the treatment of neurological conditions such as AD.[71] Although there have been no controlled studies specific to PD, there is a case report of methylphenidate improving apathy in a PD patient,[72] as well as a report of a notable improvement in apathy severity from a trial that compared gait hypokinesia and freezing.[73]

The use of antidepressant therapy in apathy is controversial. There is evidence to suggest that antidepressants can lead to apathy in some individuals,[44,74] however this may be agent-specific, with selective serotonin reuptake inhibitors faring worse. At the same time, antidepressants with a dopamine component, such as bupropion, have been more effective,[75] but like most therapies for apathy, further validation is needed.

No firm evidence for DBS as a treatment of apathy has been found. DBS has been reported to lead to apathy directly in some studies[76] but improve motivation in others,[77] all with no apparent association to stimulator location.[78] As previously noted, apathy associated with DBS may be secondary to weaning off of dopamine medication as motor symptoms improve with stimulation. This theory is particularly plausible as apathy is associated with reduced striatal metabolism in PET imaging prior to subthalamic nucleus DBS.[58]

Non-pharmaceutical interventions such as exercise and cognitive training have also been proposed,[79] but current evidence is limited. Some studies have reported results suggesting that increased physical activity in PD is associated with lower levels of apathy,[80] however whether this is due to greater motivation to perform activity in the first place remains to be established. Caregivers should be advised to encourage and prompt patients with apathy to engage in tasks and remain active as patients often have little insight into their disorder.

CONCLUSION

Apathy in PD greatly impacts upon the quality of life of patients and their caregivers. Although much headway has been made in recent years, it is a challenging disorder to study and treat.

Associations with other neuropsychiatric conditions make diagnosis difficult and lack of mechanistic understanding has made choosing appropriate therapies a challenge. Although there are positive prospects, more objective assessment methods, better understanding of underlying mechanisms, and greater rigor in trial design are required to advance the field and improve management of these patients.

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REFERENCES

1. den Brok MG et al. Apathy in Parkinson's disease: A systematic review and meta-analysis. Mov Disord. 2015;30(6):759-69.

2. Duffy J. Apathy in neurologic disorders. Curr Psychiatry Rep. 2000;2(5):434-9.

3. Benito-León J et al. Impact of apathy on health-related quality of life in recently diagnosed Parkinson's disease: The ANIMO study. Mov Disord. 2012;27(2): 211-8.

4. Kaji Y, Hirata K. Apathy and Anhedonia in Parkinson's Disease. ISRN Neurol. 2011;2011:219427.

5. Sinha N et al. Impulsivity and apathy in Parkinson's disease. J Neuropsychol. 2013; 7(2):255-83.

6. van Reekum R et al. Apathy: why care? J Neuropsychiatry Clin Neurosci. 2005;17(1): 7-19.

7. Pedersen KF et al. Apathy in drug-naïve patients with incident Parkinson's disease: the Norwegian ParkWest study. J Neurol. 2010;257(2):217-23.

8. Moretti R et al. Apathy: A Complex Symptom Specific to the Clinical Pattern of Presentation of Parkinson's Disease? Am J Alzheimers Dis Other Demen. 2012;27(3): 196-201.

9. Pedersen KF et al. Occurrence and risk factors for apathy in Parkinson disease: a 4-year prospective longitudinal study. J Neurol Neurosurg Psychiatry. 2009;80(11): 1279-82.

10. Pluck GC, Brown RG. Apathy in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2002;73(6):636-42.

11. Robert P et al. Proposed diagnostic criteria for apathy in Alzheimer's disease and other neuropsychiatric disorders. Eur Psychiatry. 2009;24(2):98-104.

12. Starkstein SE et al. The nosological position of apathy in clinical practice. J Neurol Neurosurg Psychiatry. 2008;79(10): 1088-92.

13. Marin RS. Apathy: a neuropsychiatric syndrome. J Neuropsychiatry Clin Neurosci. 1991;3(3):243-54.

14. Shulman LM. Apathy in patients with Parkinson's disease. Int Rev Psychiatry. 2000;12(4):298-306.

15. Mulin E et al. Diagnostic criteria for apathy in clinical practice. Int J Geriatr Psychiatry. 2011;26(2):158-65.

16. Leentjens AF et al. Apathy and anhedonia rating scales in Parkinson's disease: critique and recommendations. Mov Disord. 2008;23(14):2004-14.

17. Sockeel P et al. The Lille apathy rating scale (LARS), a new instrument for detecting and quantifying apathy: validation in Parkinson's disease. J Neurol Neurosurg Psychiatry. 2006;77(5): 579-84.

18. Dujardin K et al. Assessing apathy in everyday clinical practice with the short-form Lille Apathy Rating Scale. Mov Disord. 2013;28(14):2014-9.

19. Dujardin K et al. The Lille Apathy Rating Scale: Validation of a caregiver-based version. Mov Disord. 2008;23(6):845-9.

20. Starkstein SE et al. Reliability, validity, and clinical correlates of apathy in Parkinson's disease. J Neuropsychiatry Clin Neurosci. 1992;4(2):134-9.

21. Marin RS et al. Reliability and validity of the Apathy Evaluation Scale. Psychiatry Res. 1991;38(2):143-62.

22. Robert PH et al. The Apathy Inventory: assessment of apathy and awareness in Alzheimer's disease, Parkinson's disease and mild cognitive impairment. Int J Geriatr Psychiatry. 2002;17(12):1099-105.

23. Bogart KR. Is apathy a valid and meaningful symptom or syndrome in Parkinson's disease? A critical review. Health Psychol. 2011;30(4):386-400.

24. Aarsland D et al. Range of neuropsychiatric disturbances in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry. 1999;67(4):492-6.

25. McKinlay A et al. A profile of neuropsychiatric problems and their relationship to quality of life for Parkinson's disease patients without dementia. Parkinsonism Relat Disord. 2008;14(1): 37-42.

26. Dujardin K et al. Apathy may herald cognitive decline and dementia in Parkinson's disease. Mov Disord. 2009; 24(16):2391-7.

27. Hughes TA et al. Mortality in Parkinson's disease and its association with dementia and depression. Acta Neurol Scand. 2004; 110(2):118-23.

28. Vallerand RJ et al. Motivation in later life: theory and assessment. Int J Aging Hum Dev. 1995;41(3):221-38.

29. Teixeira AL Jr, Caramelli P. Apathy in Alzheimer's disease. Rev Bras Psiquiatr. 2006;28(3):238-41.

30. Oguru M et al. Apathy and Depression in Parkinson Disease. J Geriatr Psychiatry Neurol. 2010;23(1):35-41.

31. Skorvanek M et al. Apathy in Elderly Nondemented Patients With Parkinson's Disease: Clinical Determinants and Relationship to Quality of Life. J Geriatr Psychiatry Neurol. 2013;26(4):237-43.

32. Martinez-Martin P et al. The impact of non-motor symptoms on health-related quality of life of patients with Parkinson's disease. Mov Disord. 2011;26(3):399-406.

33. Chaudhuri KR et al. Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol. 2006;5(3):235-45.

34. Leiknes I et al. Caregiver distress associated with neuropsychiatric problems in patients with early Parkinson's disease: the Norwegian ParkWest study. Acta Neurol Scand. 2010;122(6):418-24.

35. Devos D et al. Rivastigmine in apathetic but dementia and depression-free patients with Parkinson's disease: a double-blind, placebo-controlled, randomised clinical trial. J Neurol Neurosurg Psychiatry. 2014;85(6):668-74.

36. Lieberman A. Depression in Parkinson's disease -- a review. Acta Neurol Scand. 2006;113(1):1-8.

37. Levy ML et al. Apathy Is Not Depression. J Neuropsychiatry Clin Neurosci. 1998;10(3):314-9.

38. Kirsch-Darrow L et al. Apathy

and Depression: Separate Factors in Parkinson's Disease. J Int Neuropsychol Soc. 2011;17(6):1058-66.

39. Brown RG, Pluck G. Negative symptoms: The 'pathology' of motivation and goal-directed behaviour. Trends Neurosci. 2000;23(9):412-7.

40. Kirsch-Darrow L et al. Dissociating apathy and depression in Parkinson disease. Neurology. 2006;67(1):33-8.

41. Hollocks MJ. Differential relationships between apathy and depression with white matter microstructural changes and functional outcomes. Brain. 2015;138(Pt 12): 3803-15.

42. Benoit M. Imaging correlates of apathy and depression in Parkinson's disease. J Neurol Sci. 2011;310(1-2):58-60.

43. Drijgers RL et al. Pharmacological Treatment of Apathy in Neurodegenerative Diseases: A Systematic Review. Dement Geriatr Cogn Disord. 2009;28(1):13-22.

44. Barnhart WJ et al. SSRI-induced apathy syndrome: a clinical review. J Psychiatr Pract. 2004;10(3):196-9.

45. Skorvanek M et al. The associations between fatigue, apathy, and depression in Parkinson's disease. Acta Neurol Scand. 2015;131(2):80-7.

46. Fawcett J et al. Assessing Anhedonia in Psychiatric Patients. Arch Gen Psychiatry. 1983;40(1):79-84.

47. Rømer Thomsen K et al. Reconceptualizing anhedonia: novel perspectives on balancing the pleasure networks in the human brain. Front Behav Neurosci. 2015;9:49.

48. Berridge KC. Motivation concepts in behavioral neuroscience. Physiol Behav. 2004;81(2):179-209.

49. Levy R, Dubois B. Apathy and the Functional Anatomy of the Prefrontal Cortex-Basal Ganglia Circuits. Cereb Cortex. 2006;16(7):916-28.

50. Berridge KC, Kringelbach ML. Affective neuroscience of pleasure: reward in humans and animals. Psychopharmacology (Berl). 2008;199(3):457-80.

51. Loas G et al. Anhedonia in Parkinson's Disease: An Overview. J Neuropsychiatry Clin Neurosci. 2012;24(4):444-51.

52. Jordan LL et al. Hedonic and behavioral deficits associated with apathy in Parkinson's disease: Potential treatment implications. Mov Disord. 2013;28(9): 1301-4.

53. Assogna F et al. Anhedonia in Parkinson's disease: a systematic review of the literature. Mov Disord. 2011;26(10): 1825-34.

54. Lemke MR et al. Anhedonia, depression, and motor functioning in Parkinson's disease during treatment with pramipexole. J Neuropsychiatry Clin Neurosci. 2005;17(2):214-20.

55. Chong TT et al. Dopamine enhances willingness to exert effort for reward in Parkinson's disease. Cortex. 2015;69:40-6.

56. Thobois S et al. Non-motor dopamine withdrawal syndrome after surgery for Parkinson's disease: predictors and underlying mesolimbic denervation. Brain. 2010;133(Pt 4):1111-27.

57. Baggio HC et al. Resting-state frontostriatal functional connectivity in Parkinson's disease-related apathy. Mov Disord. 2015;30(5):671-9.

58. Robert GH et al. Preoperative factors of apathy in subthalamic stimulated Parkinson disease: A PET study. Neurology. 2014;83(18):1620-6.

59. Pagonabarraga J et al. Apathy in Parkinson's disease: clinical features, neural substrates, diagnosis, and treatment. Lancet Neurol. 2015;14(5): 518-31.

60. Chase TN. Apathy in neuropsychiatric disease: diagnosis, pathophysiology, and treatment. Neurotox Res. 2011;19(2): 266-78.

61. Santangelo G et al. Apathy in Parkinson's disease: diagnosis, neuropsychological correlates, pathophysiology and treatment. Behav Neurol. 2013;27(4): 501-13.

62. Guttman M, Jaskolka J. The use of pramipexole in Parkinson's disease: are its actions D(3) mediated? Parkinsonism Relat Disord. 2011;7(3):231-4.

63. Thobois S et al. Parkinsonian apathy responds to dopaminergic stimulation of D2/D3 receptors with piribedil. Brain. 2013; 136(Pt 5):1568-77.

64. Castrioto A. Emotional manifestations of PD: Neurobiological basis. Mov Disord. 2016. [Epub ahead of print].

65. Adam R et al. Dopamine reverses reward insensitivity in apathy following globus pallidus lesions. Cortex. 2013;49(5): 1292-303.

66. Czernecki V et al. Apathy following subthalamic stimulation in Parkinson's disease: a dopamine responsive symptom. Mov Disord. 2008;23(7):964-9.

67. Carriere N et al. Apathy in Parkinson's disease is associated with nucleus accumbens atrophy: a magnetic resonance imaging shape analysis. Mov Disord. 2014;29(7):897-903.

68. Czernecki V et al. Motivation, reward, and Parkinson's disease: Influence of dopatherapy. Neuropsychologia. 2002; 40(13):2257-67.

69. Ray Chaudhuri K et al. Rotigotine and specific non-motor symptoms of Parkinson's disease: post hoc analysis of RECOVER. Parkinsonism Relat Disord. 2013; 19(7):660-5.

70. Smith KM. Combined rasagiline and antidepressant use in Parkinson disease in the ADAGIO study: effects on nonmotor symptoms and tolerability. JAMA Neurol. 2015;72(1):88-95.

71. Rosenberg PB et al. Safety and efficacy of methylphenidate for apathy in Alzheimer's disease: a randomized, placebo-controlled trial. J Clin Psychiatry. 2013; 74(8):810-6.

72. Chatterjee A, Fahn S. Methylphenidate treats apathy in Parkinson's disease. J Neuropsychiatry Clin Neurosci. 2002;14(4): 461-2.

73. Moreau C et al. Methylphenidate for gait hypokinesia and freezing in patients with Parkinson's disease undergoing subthalamic stimulation: a multicentre, parallel, randomised, placebo-controlled trial. Lancet Neurol. 2012;11(7):589-96.

74. Wongpakaran N et al. Selective serotonin reuptake inhibitor use associates with apathy among depressed elderly: a case-control study. Ann Gen Psychiatry. 2007;6:7.

75. Corcoran C et al. Bupropion in the management of apathy. J Psychopharmacol. 2004;18(1):133-5.

76. Drapier D et al. Does subthalamic nucleus stimulation induce apathy in Parkinson's disease? J Neurol. 2006;253(8): 1083-91.

77. Czernecki V et al. Does bilateral stimulation of the subthalamic nucleus aggravate apathy in Parkinson's disease? J Neurol Neurosurg Psychiatry. 2005;76(6): 775-9.

78. Kirsch-Darrow L et al. The trajectory of apathy after deep brain stimulation: From pre-surgery to 6 months post-surgery in Parkinson's disease. Parkinsonism Relat Disord. 2011;17(3):182-8.

79. Stanton BR, Carson A. Apathy: A practical guide for neurologists. Pract Neurol. 2016;16(1):42-7.

80. van der Kolk NM, King LA. Effects of exercise on mobility in people with Parkinson's disease. Mov Disord. 2013; 28(11):1587-96.

DEVELOPMENT AND TRANSLATION OF THERAPIES FOR SPINAL MUSCULAR ATROPHY

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ABSTRACT

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterised by widespread loss of lower motor neurons from the spinal cord, leading to progressive weakness and muscle atrophy. SMA is largely caused by homozygous loss of the *survival motor neuron (SMN) 1* gene, resulting in reduced levels of full-length SMN protein. Although no approved treatment is currently available for SMA, several clinical trials investigating different approaches to increase SMN levels are showing promising early results. Trials investigating the use of therapies targeting muscle strength and neuroprotective pathways are also in progress, generating the possibility of delivering combination therapies utilising both SMN-dependent and SMN-independent targets. Due to an increased understanding of the cellular and molecular consequences of SMN depletion, a second wave of therapies targeted at pathways downstream of SMN are currently undergoing preclinical development. As these therapies move forward towards the clinic, new treatment options are likely to become available, raising the potential to generate an effective 'cure' for SMA.

Keywords: Spinal muscular atrophy (SMA), motor neuron, therapy, treatment, clinical trials.

INTRODUCTION

Spinal muscular atrophy (SMA) is a hereditary motor neuron disease characterised by loss of motor neurons from the anterior grey horn of the spinal cord.[1] With an incidence of 1 in ~10,000 live births and a carrier frequency of 1 in ~35–50, this autosomal recessive disease is the most common genetic cause of infant mortality.[2,3] In 95% of cases, SMA is caused by homozygous deletion of the *survival motor neuron (SMN) 1* gene.[4] In humans, there are two *SMN* genes, a telomeric *SMN1* copy and an almost identical, centromeric *SMN2* copy.[4] The *SMN2* gene has a C to T substitution that results in the exclusion of exon 7 and the production of a truncated protein product, which is rapidly degraded.[4,5] However, exclusion of exon 7 is incomplete, resulting in approximately 10–15% of the protein produced from *SMN2* being full-length SMN.[6] As complete loss of SMN protein is embryonically lethal,[7] SMA patients are dependent on SMN protein produced by the *SMN2* gene,[8] with variability in protein levels occurring as a result of differences in *SMN2* copy number; individuals with a higher *SMN2* copy number have a less-severe disease phenotype.[9] Thus, *SMN2* copy number represents the primary determinant of disease severity in SMA.[1]

The degeneration of lower motor neurons that occurs in SMA leads to a progressive decline in motor development, manifesting as muscle atrophy and weakness, primarily affecting proximal muscle groups.[9] The profile of disease progression can vary substantially between patients with some phases of plateau in the decline of motor development.[10] Based on the age of onset, motor function achieved, and typical age of death, SMA can be classified into as many as five distinct clinical subgroups with varying severity.[11] In all subtypes, molecular genetic analysis is now the gold standard for diagnosis.[12] Type 0 onset occurs *in utero* with life not extending beyond the first few weeks after birth.[1] Type 1 SMA, otherwise known as Werdnig–Hoffmann's disease, is the

most common type of SMA.[11] Disease onset occurs by 6 months and patients are unable to sit without support and cannot control head movement.[2] Poor bulbar function and weak intercostal muscles lead to difficulties in feeding and breathing, resulting in death within the first 2 years of life in the absence of palliative care.[12] Patients with Type 2 SMA (Dubowitz's syndrome) have an intermediate phenotype, being able to maintain a sitting position unaided, but without the ability to walk independently.[12] Onset occurs between 7 months and 18 months of age and death frequently occurs during adolescence due to respiratory problems.[2] Type 3 SMA (Kugelbery–Welander's disease) shows marked symptom heterogeneity; some patients are able to walk independently and have some muscular weakness, while others begin to walk but require wheelchair assistance in childhood.[2] Type 4 SMA disease onset typically occurs in the second or third decade of life, with patients suffering from muscular weakness without respiratory problems.[12] For both SMA Type 3 and Type 4, life expectancy is often comparable to that of the general population.[11] Several rare forms of SMA also exist, caused by mutations in genes other than *SMN1*. These include X-linked SMA, SMA with respiratory distress, and spinal and bulbar muscular atrophy (Kennedy's disease).[12]

Following the identification of the disease-causing gene, it has become possible to model SMA in various animal systems.[13,14] Studies in these animal models have led to an increased understanding of the molecular pathogenesis of SMA and have indicated that SMN replacement therapies may be a viable therapeutic strategy to treat SMA.[1,2,12]

THE SURVIVAL MOTOR NEURON PROTEIN

In order to successfully target the SMN protein as a therapeutic approach for SMA, it is necessary to understand the functions of the SMN protein and the downstream effects of SMN reduction. SMN protein is a ubiquitously expressed, multifunctional protein that forms a macromolecular complex, essential for the splicing of pre-messenger RNAs (mRNAs).[15-17] SMN associates with Gemins 2–8 and Unrip to form a complex that enables Sm core proteins and uridine-rich small nuclear ribonucleic acids to form small nuclear ribonucleoproteins (snRNPs).[15,16] During pre-mRNA splicing, snRNPs are essential for the excision of introns from mRNA precursors in the nucleus.[17] Reduced SMN levels lead to a tissue-specific decrease in snRNP assembly that correlates with phenotypic severity in mouse models of SMA.[18] Moreover, widespread splicing defects have been found in SMA tissues with a wide diversity of genes being affected,[19] including genes encoding splicing regulators and proteins required for motor circuit function.[20,21] However, it has also been suggested that splicing defects may represent a late feature of SMA indicating that alternative splicing events could simply represent a consequence of disease progression in SMA, rather than the primary cause.[22]

Aside from its housekeeping function in snRNP assembly, SMN has been shown to have additional, non-canonical roles that may contribute to disease pathogenesis in SMA. During axonogenesis and axonal sprouting there is a progressive shift of SMN towards an axonal localisation in the human spinal cord, suggesting an axonal function of SMN.[23] Indeed, SMN localises to axonal transport granules that deliver mRNAs to the synapse, where local translation can occur.[24] SMN is also involved in axonal elongation, with loss of SMN leading to defects in axon outgrowth.[14,25,26] Through interaction with mRNA binding proteins, SMN is involved in the localisation of beta-actin and *beta-actin* mRNA to growth cones of developing motor neurons, which leads to axonal elongation and growth cone size regulation.[26] Interestingly, while SMN deficient motor neurons have reduced growth cone size,[25,26] mice that lack beta-actin in motor neurons do not,[27] indicating that other pathways contribute to the defective axonal elongation phenotype in SMA. Indeed, inappropriate activation of the Rho A/Rho-kinase (ROCK) pathway has been shown to lead to defects in neuritogenesis in SMA.[28,29] Moreover, it has been demonstrated that insulin-like growth factor 1 (IGF-1) is essential for enhancing axonal outgrowth of motor neurons. Intriguingly, circulating IGF-1 levels are also reduced as a consequence of the SMN reduction in SMA.[30,31]

Several other cellular and molecular pathways are also dysregulated in SMA due to reduced SMN levels. For example, ubiquitin homeostasis is altered in SMA whereby SMN depletion in mouse models of SMA leads to downregulation of ubiquitin-like modifier-activating enzyme 1 (UBA1) and accumulation of its downstream targets.[32,33] UBA1 activates ubiquitin as the first step of the ubiquitin conjugation process to mark proteins for

degradation by the proteasome.[34] The proteasome has also been implicated in SMA pathogenesis as SMN degradation is mediated by the ubiquitin-proteasome system (UPS)[35] and inhibiting proteasome function has been shown to increase SMN levels.[36] Furthermore, the deubiquitinase *Usp9x* associates with and stabilises the SMN complex through interaction with SMN, which it deubiquitinates.[5] *Usp9x* does not, however, deubiquitinate and stabilise the truncated SMN protein produced by *SMN2*, which is therefore rapidly degraded.[33] The identification of these novel cellular and molecular functions of SMN opens up the possibility of developing SMN-independent therapies for the treatment of SMA.

SPINAL MUSCULAR ATROPHY AS A MULTISYSTEM DISORDER

Regardless of whether therapies being developed are SMN-dependent or SMN-independent, one key element of any successful therapy is the ability to deliver it to cells, tissues, and organs affected by the disease. In the case of SMA, the main pathological target is undoubtedly alpha motor neurons;[37] ~25–30% of motor neuron cell bodies are lost from the spinal cord of late symptomatic SMA mice.[7] However, neuromuscular pathology is apparent before the overt loss of motor neuron cell bodies occurs in SMA.[14,38] Thus, the neuromuscular system appears to develop relatively normally, but early on in the disease structural and functional defects are seen in both nerve and muscle.[39,40] These include early pathological changes at the neuromuscular junction (NMJ), including nerve terminal loss, synaptic accumulation of neurofilament proteins, and defective maturation of acetylcholine receptor clusters.[39-41] Alongside these early changes in distal extremities of motor neurons, intrinsic defects have been reported in skeletal muscle, including smaller myotubes as well as reduced proliferation and fusion defects of myoblasts.[42,43] These pathological changes in muscle occur independently of neuron degeneration and correlate with SMN reduction in model systems.[38,42-44]

Aside from lower motor neurons and skeletal muscle, low SMN levels also affect other cell types throughout the nervous system in SMA. For example, a defective myelination phenotype has been observed, resulting from intrinsic defects in Schwann cells in mouse models of SMA;[45-47] altered function of astrocytes has also been implicated in SMA pathogenesis.[48,49] Furthermore, pathological changes have been reported in the thalamus, cerebral cortex, brainstem, and dorsal root ganglia in severe cases of SMA.[9] Alongside pathological changes in the nervous system and skeletal muscle, defects in peripheral tissues and organs including the heart, pancreas, blood vessels, and intestine have also been reported.[8,9,50,51] One working hypothesis to explain the presence of extra-neuronal pathology in SMA is the 'threshold hypothesis' where differential thresholds for low SMN levels exist in different cell types, with motor neurons being most vulnerable in SMA due to their exceptional sensitivity to low levels of SMN.[8,13]

Although pathological extra-neural organ system phenotypes may often manifest at the subclinical level in SMA patients, they are becoming of increasing importance as therapies prolonging a patient's survival run the risk of unmasking disorders of other organ systems. In addition, several recent studies have demonstrated that restoring SMN in motor neurons or skeletal muscle alone is insufficient to correct disease pathology in SMA mice, and that peripheral SMN restoration is likely to be essential for long-term rescue of SMA.[30,52] Taken together, these findings suggest that any successful therapy for SMA will need to target not only motor neurons and skeletal muscle, but also more widespread, systemic pathology.

DEVELOPMENT OF NOVEL THERAPIES FOR SPINAL MUSCULAR ATROPHY

At present, no curative or disease-modifying therapies are available for patients with SMA. Palliative care options can assist with management of symptoms and prevention of complications. There are, however, several clinical trials aimed at modifying the disease that are currently underway in SMA patient cohorts. Given the central role that SMN plays in the disease, it is not surprising to find that the majority of clinical trials are aimed at increasing SMN protein levels. The current clinical trials can be split into four main groups, three based around a variety of approaches to increase SMN levels and one group of complementary therapies mainly comprising neuroprotective factors or muscle strength-enhancing compounds (Table 1).

Gene therapy to replace the faulty *SMN1* gene is one of the main technological approaches entering clinical trials, based on very promising preclinical data from animal models.[80-83]

Table 1: Current overview of clinical trials for spinal muscular atrophy as of June 2016.[53-79]

Trial number	Study	Status	Type	Sponsor
Gene therapy				
NCT02122952[55]	Gene Transfer Clinical Trial for Spinal Muscular Atrophy Type 1	Ongoing - not recruiting	Phase I	Jerry R. Mendell
Molecular therapy with ASO				
NCT02386553[56]	A Study of Multiple Doses of ISIS SMNRx (ISIS 396443) Delivered to Infants With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy	Recruiting	Phase II	Biogen
NCT02462759[57]	A Study to Assess the Safety and Tolerabiity of ISIS 396443 (ISIS SMNRx) in Participants With Spinal Muscular Atrophy (SMA)	Ongoing - not recruiting	Phase II	Biogen
NCT02594124[58]	An Open-label Study (SHINE) for Patients With Spinal Muscular Atrophy (SMA) Who Participated in Studies With IONIS-SMNRx	Recruiting via invitation	Phase III	Ionis Pharmaceuticals, Inc.
NCT02292537[59]	A Study to Assess the Efficacy and Safety of IONIS-SMN Rx in Patients With Later-onset Spinal Muscular Atrophy	Ongoing - not recruiting	Phase III	Ionis Pharmaceuticals, Inc.
NCT02193074[60]	A Study to Assess the Efficacy and Safety of IONIS-SMN Rx in Infants With Spinal Muscular Atrophy	Ongoing - not recruiting	Phase III	Ionis Pharmaceuticals, Inc.
NCT02052791[61]	An Open-label Safety and Tolerability Study of Ionis SMNRx in Patients With Spinal Muscular Atrophy Who Previously Participated in IONIS SMNRx-C52 or IONIS SMNRx-CS10	Ongoing - not recruiting	Phase I	Ionis Pharmaceuticals, Inc.
NCT01839656[62]	A Study to Assess the Safety and Pharmacokinetics of IONIS SMNRx in Infants With Spinal Muscular Atrophy	Ongoing - not recruiting	Phase II	Ionis Pharmaceuticals, Inc.
NCT01780246[63]	An Open-label Safety and Tolerability Study of ISIS SMNRx in Patients With Spinal Mucular Atrophy Who Previously Participated in ISIS 396443-C51	Completed	Phase I	Ionis Pharmaceuticals, Inc.
NCT01703988[64]	An Open-label Safety, Tolerability and Dose-range Finding Study of Multiple Doses of ISIS SMNRx in Patients With Spinal Mucular Atrophy (SMNRx - C52)	Completed	Phase I/II	Ionis Pharmaceuticals, Inc.
NCT01494701[65]	An Open-label Safety, Tolerability and Dose-range Finding Study of ISIS SMNRx in Patients With Spinal Muscular Atrrophy	Completed	Phase I	Ionis Pharmaceuticals, Inc.
Small molecules enhancing SMN				
NCT02268552[66]	An Open-label Study of LM1070 in Type 1 Spinal Muscular Atrophy (SMA)	Ongoing - not recruiting	Phase I/II	Novaris Pharmaceuticals
NCT02240355[67]	A Study of RO6885247 in Adult and Pediatric Patients With Spinal Muscular Atrophy	Suspended	Phase I	Hoffman-La Roche
NCT01671384[68]	Valproate and Levocarnitine in Children With Spinal Muscular Atrophy	Recruiting	Phase III	All India Institute of Medical Sciences, New Delhi
NCT00661453[69]	CARNIVAL Type I: Valproic Acid and Carnitine in Infants With Spinal Mucular Atrophy (SMA) Type 1	Completed	Phase I/II	University of Utah
NCT00481013[70]	Valproic Acid in Ambulant Adults With Spinal Muscular Atrophy (VALIANTSMA)	Completed	Phase II	University of Utah
NCT02633709[71]	A Study to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of RO7034067 (RG7916) Given by Mouth in Healthy Volunteers	Recruiting	Phase I	Hoffman-La Roche

Table 1 continued.

Trial number	Study	Status	Type	Sponsor
Complementary/other				
NCT02644668[72]	A Study of CK-2127107 in Patients Wth Spinal Muscular Atrophy	Recruiting	Phase II	Cytokinetics
NCT02628743[73]	A Study to Evaluate Long Term Safety, Tolerability, and Effectiveness of Olesoxime in Patients With Spinal Muscular Atrophy	Recruiting	Phase II	Hoffmann-La Roche
NCT01302600[74]	Safety and Efficacy of Olesoxime (TRO19622) in 3-25 Years SMA Patients	Completed	Phase II	Trophos
NCT02227823[75]	Safety and Efficacy Study of Pyridostigmine on Patients With Spinal Muscular Atrophy Type 3 (EMOTAS)	Recruiting	Phase II	Centre Hospitalier Régional de la Citadelle
NCT01645787[76]	Short and Long Term Treatment With 4-AP in Ambulatory SMA Patients	Completed	Phase II/III	Columbia University
NCT00533221[77]	Pilot Study of Growth Hormone to Treat SMA Type II and III	Completed	Phase II	University Hospital Freiburg
NCT00528268[78]	Study to Evaluate Sodium Phenylbutyrate in Pre-symptomatic Infants With Spinal Muscular Atrophy (STOPSMA)	Completed	Phase I/II	NICHD
NCT00324454[79]	Levetiracetum for Cramps, Spasticity and Neuroprotection in Motor Neuron Disease	Completed	Phase II	Duke University

SMA: Spinal muscular atrophy; SMN: survival motor neuron; ASO: antisense oligonucleotides.

Several studies demonstrated that adeno-associated virus mediated SMN gene replacement resulted in widespread expression of SMN in the spinal cord and significantly increased survival of SMA model mice.[80-83] In the ongoing Phase I gene therapy clinical trial, SMN within a self-complementary adeno-associated virus serotype 9 (scAAV9) vector is being delivered intravenously to Type 1 SMA patients (Table 1). As the use of gene therapy approaches are still not commonplace in the clinical setting, this initial clinical trial is evaluating both the safety and efficacy of the treatment in the small group of infants enrolled in the study (Table 1).

A second therapeutic approach to increase SMN levels is to direct antisense oligonucleotides (ASOs) against sequences that inhibit the inclusion of *SMN2* exon 7. The binding of ASOs to the regulatory motif prevents the binding of repressor factors. This promotes the inclusion of exon 7, thereby increasing the amount of full-length SMN produced by *SMN2*.[84,85] The use of such ASOs in mouse models of SMA has shown promising results with survival prolonged by >100 days in some cases.[86] ASOs to treat SMA are currently in Phase II and Phase III of clinical trials (Table 1). Interestingly, trials using ASOs to treat other diseases, such as muscular dystrophy, are well advanced and have been instrumental in informing the study design and methodology used for ASOs trials in SMA.[87] The third SMN-targeted therapeutic approach entering clinical trials for SMA is based around small molecules that modify *SMN2* splicing and increase SMN levels, including the use of histone deacetylase inhibitors such as valproic acid.[88] Studies taking this more traditional pharmacological approach are currently at different stages of the clinical trial process, again after promising results from screens in rodent and cell models of SMA (Table 1).[89]

Regardless of the technological approach used to increase SMN levels, the 'therapeutic time-window' within which therapy must be delivered in order to have a maximal effect needs to be considered. Several studies have indicated that SMN is required at the early stages of development and that sufficient SMN levels are essential for NMJ maturation in the early post-natal period.[88-92] It has also been demonstrated that late stage SMN gene replacement using scAAV9 failed to ameliorate NMJ defects, while pre-symptomatic delivery of scAAV9-SMN resulted in near complete rescue of the SMA phenotype.[93] Together, these studies indicate that treatment using SMN-enhancing therapies will need to occur before overt symptoms are apparent for a full restoration of the SMA phenotype.

While the first-generation of SMN-dependent therapies progress through the clinical trial process there is a wave of second-generation SMN-independent therapies currently in preclinical and clinical development. The most clinically advanced of these are centred around administering neuroprotective factors, or enhancing muscle strength (Table 1). Olesoxime is one potential neuroprotective factor currently in Phase II clinical trials for Type 2 or 3 SMA patients. This treatment acts by binding to components of the mitochondrial permeability pore, through which it can exert neuroprotective effects.[94] Several drugs in trials to improve muscle strength have previously been approved for other disorders involving weakness of the neuromuscular system, while others are more recent developments, including CK-2127107 (Table 1). This is a skeletal muscle troponin activator currently in trials to treat neuromuscular dysfunction, muscular weakness, and muscle fatigue.[95] IGF-1 has also been used to try and improve muscular symptoms in SMA patients. Intravenous administration of AAV1-IGF-1 increased tissue levels of SMN, ameliorated muscle atrophy, and increased survival in SMA model mice.[31] However, in SMA patient trials, IGF-1 failed to reduce muscle atrophy.[96] It has recently been found that the IGF-1 receptor is increased in the spinal cord of SMA mice.[97] Hence, the beneficial effects of increasing IGF-1 might be restricted by the overexpression of IGF-1 receptor.[97] Indeed, the same group showed that reducing IGF-1 receptor expression protects motor neurons and improves motor behaviour in a mouse model of SMA.[97]

Following on from recent developments in our understanding of the cellular and molecular pathways dysregulated downstream of SMN reduction in SMA (as discussed earlier), therapeutic strategies that target these pathways are also being developed. For example, members of the ROCK pathway have become attractive therapeutic targets due to their known potential to modulate axon outgrowth and growth cone motility. Although targeting downstream effectors of ROCK did not rescue the SMA phenotype,[28] inhibiting ROCK led to positive outcomes in mouse models of SMA.[98,99] Both Fasudil and Y-27632 inhibit ROCK and lead to an increase in the survival of SMA mice and improved NMJ maturation and muscle fibre size.[98,99] Although toxicity was associated with Fasudil at high doses and motor neuron cell death was not reduced, the increase in survival points towards the importance of targeting muscle in SMA treatment strategies.[98,99]

The UPS has also recently been highlighted as a potentially attractive therapeutic target for SMA. Initial indications came from studies showing a role for the UPS in SMN degradation and stabilisation. Indeed, it has been demonstrated that inhibiting the chymotrypsin-like activity of the 26S proteasome using bortezomib increases SMN levels in peripheral tissues of SMA mice.[36] This resulted in improved motor function and increased survival. When SMA mice were treated with a combination therapy of bortezomib and trichostatin A, a histone deacetylase inhibitor that increases SMN protein levels, the improvements observed across all aspects of the SMA phenotype were greater than when mice were treated with only one treatment.[36] This study therefore provides proof-of-principal that combination therapies increasing SMN gene transcription and reducing SMN protein degradation may represent a viable therapeutic approach for SMA. However, due to toxicity issues associated with using available proteasome inhibitors such as bortezomib, targeting E3 ligases responsible for the specific ubiquitination of SMN may be a more suitable therapeutic approach to stabilise SMN.[34] E3 ligases are the enzymes responsible for conferring the specificity of the UPS, therefore targeting them would be predicted to lead to a reduction in the non-specific effects associated with targeting the proteasome.[34] Indeed, mind bomb 1 was recently identified as an E3 ligase responsible for the ubiquitination of SMN.[100] Interestingly, loss of the *Caenorhabditis elegans* orthologue of mind bomb 1 ameliorated the neuromuscular defects seen due to *SMN1* loss of function.[100]

Alongside evidence suggesting that targeting SMN protein stability via the UPS may be an attractive therapeutic approach for SMA, recent experiments have demonstrated that UBA1, a key E1 ubiquitin-activating enzyme required for UPS function, is a major downstream target of SMN whose levels are robustly depleted in SMA.[32,33] This SMN-induced reduction of UBA1 levels leads to disruption of UBA1-dependent targets, such as an accumulation of beta-catenin.[33] Experiments in SMA animal models targeting these UBA1-dependent pathways have demonstrated improvements in neuromuscular phenotype, suggesting that these pathways are amenable to therapeutic intervention.[33]

FUTURE DEVELOPMENT AND CONCLUDING REMARKS

At present, there are no curative therapies for patients with SMA; however, several clinical trials aimed at modifying the disease are underway in patient cohorts. The focus of many of these clinical trials is augmenting full-length SMN protein levels. However, there is a second wave of SMN-independent therapies in preclinical and clinical development that target cellular and molecular pathways dysregulated downstream of SMN reduction in SMA.

Several major issues and questions will need answering before we are able to design and deliver fully effective therapies. For example, debate is still ongoing regarding whether restoration of SMN levels will be more important in the central nervous system and/or in peripheral tissues to successfully treat SMA.[30,52,101] As previously mentioned, several studies in rodent models of the disease have shown that SMN restoration in extra-neural tissues and organ systems will likely be necessary for amelioration of the systemic SMA phenotype.[30,52] Were this situation to be recapitulated in human SMA patients, it could have significant consequences for the success of current clinical trials, many of which aim to increase SMN levels using delivery methods targeting the central nervous system (Table 1).

One other major issue that remains to be fully resolved concerns the presence of a therapeutic time-window after which therapy delivery can only have a minimal effect. Several studies have indicated that for maximal benefit, SMN-replacement therapies will need to be delivered before the onset of overt symptoms.[91,93] It will therefore be essential to understand how the recent mouse work relates to the temporal development of SMA pathogenesis in human patients.

It is possible that combination therapies will have the greatest potential to ameliorate the SMA disease phenotype. Combining SMN enhancement therapies with muscle strength-enhancing drugs or neuroprotective factors may help to preserve and strengthen the connections between neurons and muscles. This approach may result in effective treatment of SMA symptoms beyond the therapeutic time-window in which SMN-dependent therapies alone will be effective. An alternative combinatorial therapeutic avenue is the possibility of combining therapies to enhance SMN gene transcription with treatments to reduce SMN degradation and stabilise the protein (see previous discussion). This approach has the benefit of a possible dose reduction of the SMN-enhancing agent, which may help to reduce toxicity and increase efficacy.[36] While the future looks bright for the progress of emerging treatments for SMA, it is necessary to continue investing in research covering a range of therapeutic approaches in order to get the best chance of success for developing an effective cure for SMA.

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REFERENCES

1. Kolb SJ, Kissel JT. Spinal muscular atrophy: a timely review. Arch Neurol. 2011;68(8):979-84.

2. Lunn MR, Wang CH. Spinal muscular atrophy. Lancet. 2008;371(9630):2120-33.

3. Sugarman EA et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. Eur J Hum Genet. 2012;20(1):27-32.

4. Lefebvre S et al. Identification and characterization of a spinal muscular atrophy-determining gene. Cell. 1995;80(1): 155-65.

5. Han KJ et al. Ubiquitin-specific protease 9x deubiquitinates and stabilizes the spinal muscular atrophy protein-survival motor neuron. J Biol Chem. 2012;287(52): 43741-52.

6. Monani UR et al. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. Hum Mol Genet. 1999;8(7):1177-83.

7. Monani UR et al. The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn(-/-) mice and results in a mouse with spinal muscular atrophy. Hum Mol Genet. 2000;9(3): 333-9.

8. Hamilton G, Gillingwater TH. Spinal muscular atrophy: going beyond the motor neuron. Trends Mol Med. 2013;19(1): 40-50.

9. Harding BN et al. Spectrum of neuropathophysiology in spinal muscular atrophy type I. J Neuropathol Exp Neurol. 2015;74(1): 15-24.

10. Mercuri E et al. Patterns of disease progression in type 2 and 3 SMA: Implications for clinical trials. Neuromuscul Disord. 2016;26(2):126-31.

11. Mercuri E et al. Childhood spinal muscular atrophy: controversies and challenges. Lancet Neurol. 2012;11(5): 443-52.

12. Faravelli I et al. Spinal muscular atrophy--recent therapeutic advances for an old challenge. Nat Rev Neurol. 2015;11(6): 351-9.

13. Sleigh JN et al. The contribution of mouse models to understanding the pathogenesis of spinal muscular atrophy. Dis Model Mech. 2011;4(4):457-67.

14. McWhorter ML et al. Knockdown of the survival motor neuron (Smn) protein in zebrafish causes defects in motor axon outgrowth and pathfinding. J Cell Biol. 2003;162(5): 919-31.

15. Fischer U et al. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. Cell. 1997;90(6): 1023-9.

16. Liu Q et al. The spinal muscular atrophy disease gene product, SMN, and its associated protein SIP1 are in a complex with spliceosomal snRNP proteins. Cell. 1997; 90(6):1013-21.

17. Massenet S et al. The SMN complex is associated with snRNPs throughout their cytoplasmic assembly pathway. Mol Cell Biol. 2002;22(18):6533-41.

18. Gabanella F et al. Ribonucleoprotein assembly defects correlate with spinal muscular atrophy severity and preferentially affect a subset of spliceosomal snRNPs. PLoS One. 2007; 2(9):e921.

19. Zhang Z et al. SMN deficiency causes tissue-specific perturbations in the repertoire of snRNAs and widespread defects in splicing. Cell. 2008;133(4): 585-600.

20. Huo Q et al. Splicing changes in SMA mouse motoneurons and SMN-depleted neuroblastoma cells: evidence for involvement of splicing regulatory proteins. RNA Biol. 2014;11(11):1430-46.

21. Lotti F et al. An SMN-dependent U12 splicing event essential for motor circuit function. Cell. 2012;151(2):440-54.

22. Bäumer D et al. Alternative splicing events are a late feature of pathology in a mouse model of spinal muscular atrophy. PLoS Genet. 2009;5(12):e1000773.

23. Giavazzi A et al. Neuronal-specific roles of the survival motor neuron protein: evidence from survival motor neuron expression patterns in the developing human central nervous system. J Neuropathol Exp Neurol. 2006;65(3): 267-77.

24. Fallini C et al. Spinal muscular atrophy: the role of SMN in axonal mRNA regulation. Brain Res. 2012;1462:81-92.

25. Groen EJ et al. ALS-associated mutations in FUS disrupt the axonal distribution and function of SMN. Hum Mol Genet. 2013;22(18):3690-704.

26. Rossoll W et al. Smn, the spinal muscular atrophy-determining gene product, modulates axon growth and localization of beta-actin mRNA in growth cones of motor neurons. J Cell Biol. 2003;163(4):801-12.

27. Cheever TR et al. Axonal regeneration and neuronal function are preserved in motor neurons lacking ß-actin in vivo. PLoS One. 2011;6(3):e17768.

28. Bowerman M et al. SMN, profilin IIa and plastin 3: a link between the deregulation of actin dynamics and SMA pathogenesis. Mol Cell Neurosci. 2009;42(1):66-74.

29. Nolle A et al. The spinal muscular atrophy disease protein SMN is linked to the Rho-kinase pathway via profilin. Hum Mol Genet. 2011;20(24):4865-78.

30. Hua Y et al. Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model. Nature. 2011;478(7367):123-6.

31. Tsai LK et al. Systemic administration of a recombinant AAV1 vector encoding IGF-1 improves disease manifestations in SMA mice. Mol Ther. 2014;22(8):1450-9.

32. Fuller HR et al. Spinal Muscular Atrophy Patient iPSC-Derived Motor Neurons Have Reduced Expression of Proteins Important in Neuronal Development. Front Cell Neurosci. 2015;9:506.

33. Wishart TM et al. Dysregulation of ubiquitin homeostasis and β-catenin signaling promote spinal muscular atrophy. J Clin Invest. 2014;124(4):1821-34.

34. Groen EJ, Gillingwater TH. UBA1: At the Crossroads of Ubiquitin Homeostasis and Neurodegeneration. Trends Mol Med. 2015; 21(10):622-32.

35. Chang HC et al. Degradation of survival motor neuron (SMN) protein is mediated via the ubiquitin/proteasome pathway. Neurochem Int. 2004;45(7):1107-12.

36. Kwon DY et al. Increasing expression and decreasing degradation of SMN ameliorate the spinal muscular atrophy phenotype in mice. Hum Mol Genet. 2011; 20(18):3667-77.

37. Powis RA, Gillingwater TH. Selective loss of alpha motor neurons with sparing of gamma motor neurons and spinal cord cholinergic neurons in a mouse model of spinal muscular atrophy. J Anat. 2015. [Epub ahead of print].

38. Murray LM et al. Selective vulnerability of motor neurons and dissociation of pre- and post-synaptic pathology at the neuromuscular junction in mouse models of spinal muscular atrophy. Hum Mol Genet. 2008;17(7):949-62.

39. McGovern VL et al. Embryonic motor axon development in the severe SMA mouse. Hum Mol Genet. 2008;17(18): 2900-9.

40. Murray LM et al. Pre-symptomatic development of lower motor neuron connectivity in a mouse model of severe spinal muscular atrophy. Hum Mol Genet. 2010;19(3): 420-33.

41. Cifuentes-Diaz C et al. Neurofilament accumulation at the motor endplate and lack of axonal sprouting in a spinal muscular atrophy mouse model. Hum Mol Genet. 2002;11(12):1439-47.

42. Martínez-Hernández R et al. The developmental pattern of myotubes in spinal muscular atrophy indicates prenatal delay of muscle maturation. J Neuropathol Exp Neurol. 2009;68(5): 474-81.

43. Shafey D et al. Hypomorphic Smn knockdown C2C12 myoblasts reveal intrinsic defects in myoblast fusion and myotube morphology. Exp Cell Res. 2005; 311(1):49-61.

44. Boyer JG et al. Early onset muscle weakness and disruption of muscle proteins in mouse models of spinal muscular atrophy. Skelet Muscle. 2013; 3(1):24.

45. Hunter G et al. SMN-dependent intrinsic defects in Schwann cells in mouse models of spinal muscular atrophy. Hum Mol Genet. 2014;23(9):2235-50.

46. Aghamaleky Sarvestany A et al. Label-free quantitative proteomic profiling identifies disruption of ubiquitin homeostasis as a key driver of Schwann cell defects in spinal muscular atrophy. J Proteome Res. 2014;13(11):4546-57.

47. Hunter G et al. Restoration of SMN in Schwann cells reverses myelination defects and improves neuromuscular function in spinal muscular atrophy. Hum Mol Genet. 2016. [Epub ahead of print].

48. Rindt H et al. Astrocytes influence the severity of spinal muscular atrophy. Hum Mol Genet. 2015;24(14):4094-102.

49. Zhou C et al. Defects in Motoneuron-Astrocyte Interactions in Spinal Muscular Atrophy. J Neurosci. 2016;36(8):2543-53.

50. Somers E et al. Vascular defects and spinal cord hypoxia in spinal muscular atrophy. Ann Neurol. 2015. [Epub ahead of print].

51. Sintusek P et al. Histopathological

Defects in Intestine in Severe Spinal Muscular Atrophy Mice Are Improved by Systemic Antisense Oligonucleotide Treatment. PLoS One. 2016;11(5):e0155032.

52. Hua Y et al. Motor neuron cell-nonautonomous rescue of spinal muscular atrophy phenotypes in mild and severe transgenic mouse models. Genes Dev. 2015;29(3):288-97.

53. TREAT-NMD: Neuromuscular Network. Available at: http://www.treat-nmd.eu/. Last accessed: 14 June 2016.

54. Cure SMA. 2014. Available at: http://www.curesma.org. Last accessed: 14 June 2016.

55. AveXis, Inc. Gene Transfer Clinical Trial for Spinal Muscular Atrophy Type 1. Clinical Trial: NCT02122952. https://clinicaltrials.gov/ct2/show/NCT02122952?term=NCT02122952&rank=1.

56. Biogen. A Study of Multiple Doses of ISIS SMNRx (ISIS 396443) Delivered to Infants With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy (NURTURE). Clinical Trial: NCT02386553. https://clinicaltrials.gov/ct2/show/NCT02386553?term=NCT02386553&rank=1.

57. Biogen. A Study to Assess the Safety and Tolerability of ISIS 396443 in Participants With Spinal Muscular Atrophy (SMA). (EMBRACE). Clinical Trial: NCT02462759. https://clinicaltrials.gov/ct2/show/NCT02462759?term=NCT02462759&rank=1.

58. Ionis Pharmaceuticals, Inc. An Open-Label Study (SHINE) for Patients With Spinal Muscular Atrophy (SMA) Who Participated in Studies With IONIS-SMNRx. Clinical Trial: NCT02594124. https://clinicaltrials.gov/ct2/show/NCT02594124?term=NCT02594124&rank=1.

59. Ionis Pharmaceuticals, Inc. A Study to Assess the Efficacy and Safety of IONIS-SMN Rx in Patients With Later-onset Spinal Muscular Atrophy. Clinical Trial: NCT02292537. https://clinicaltrials.gov/ct2/show/NCT02292537?term=NCT02292537&rank=1.

60. Ionis Pharmaceuticals, Inc. A Study to Assess the Efficacy and Safety of IONIS-SMN Rx in Infants With Spinal Muscular Atrophy. Clinical Trial: NCT02193074. https://clinicaltrials.gov/ct2/show/NCT02193074?term=NCT02193074&rank=1.

61. Ionis Pharmaceuticals, Inc. An Open-label Safety and Tolerability Study of IONIS SMNRx in Patients With Spinal Muscular Atrophy Who Previously Participated in IONIS SMNRx-CS2 or IONIS SMNRx-CS10. Clinical Trial: NCT02052791. https://clinicaltrials.gov/ct2/show/NCT02052791?term=NCT02052791&rank=1.

62. Ionis Pharmaceuticals, Inc. A Study to Assess the Efficacy, Safety and Pharmacokinetics of IONIS SMNRx in Infants With Spinal Muscular Atrophy. Clinical Trial: NCT01839656. https://clinicaltrials.gov/ct2/show/NCT01839656?term=NCT01839656&rank=1.

63. Ionis Pharmaceuticals, Inc. An Open-label Safety and Tolerability Study of ISIS SMNRx in Patients With Spinal Muscular Atrophy Who Previously Participated in ISIS 396443-CS1. Clinical Trial: NCT01780246. https://clinicaltrials.gov/ct2/show/NCT01780246?term=NCT01780246&rank=1.

64. Ionis Pharmaceuticals, Inc. An Open-label Safety, Tolerability and Dose-range Finding Study of Multiple Doses of ISIS SMNRx in Patient With Spinal Muscular Atrophy (SMNRx - CS2). Clinical Trial: NCT01703988. https://clinicaltrials.gov/ct2/show/NCT01703988?term=NCT01703988&rank=1.

65. Ionis Pharmaceuticals, Inc. An Open-label Safety, Tolerability, and Dose-range Finding Study of ISIS SMNRx in Patients With Spinal Muscular Atrophy (SMNRx). Clinical Trial: NCT01494701. https://clinicaltrials.gov/ct2/show/NCT01494701?term=NCT01494701&rank=1.

66. Novartis Pharmaceuticals. An Open Label Study of LMI070 in Type 1 Spinal Muscular Atrophy (SMA). Clinical Trial: NCT02268552. https://clinicaltrials.gov/ct2/show/NCT02268552?term=NCT02268552&rank=1.

67. Hoffman-La Roche. A Study of RO6885247 in Adult and Pediatric Patients With Spinal Muscular Atrophy (MOONFISH). Clinical Trial: NCT02240355. https://clinicaltrials.gov/ct2/show/NCT02240355?term=NCT02240355&rank=1.

68. All India Institute of Medical Sciences, New Delhi. Valproate and Levocarnitine in Children With Spinal Muscular Atrophy. Clinical Trial: NCT01671384. https://clinicaltrials.gov/ct2/show/NCT01671384?term=NCT01671384&rank=1.

69. University of Utah. CARNIVAL Type I: Valproic Acid and Carnitine in Infants With Spinal Muscular Atrophy (SMA) Type I. Clinical Trial: NCT00661453. https://clinicaltrials.gov/ct2/show/NCT00661453?term=NCT00661453&rank=1.

70. University of Utah. Valproic Acid in Ambulant Adults With Spinal Muscular Atrophy (VALIANTSMA). Clinical Trial: NCT00481013. https://clinicaltrials.gov/ct2/show/NCT00481013?term=NCT00481013&rank=1.

71. Hoffman-La Roche. A Study to Investigate the Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of RO7034067 (RG7916) Given by Mouth in Healthy Volunteers. Clinical Trial: NCT02633709. https://clinicaltrials.gov/ct2/show/NCT02633709?term=NCT02633709&rank=1.

72. Cytokinetics. A Study of CK-2127107 in Patients With Spinal Muscular Atrophy. Clinical Trial: NCT02644668. https://clinicaltrials.gov/ct2/show/NCT02644668?term=NCT02644668&rank=1.

73. Hoffman-La Roche. A Study to Evaluate Long Term Safety, Tolerability, and Effectiveness of Olesoxime in Patients With Spinal Muscular Atrophy. Clinical Trial: NCT02628743. https://clinicaltrials.gov/ct2/show/NCT02628743?term=NCT02628743&rank=1.

74. Trophos. Safety and Efficacy of Olesoxime (TRO19622) in 3-25 Years SMA Patients. Clinical Trial: NCT01302600. https://clinicaltrials.gov/ct2/show/NCT01302600?term=NCT01302600&rank=1.

75. Centre Hospitalier Régional de la Citadelle. Safety and Efficacy Study of Pyridostigmine on Patients With Spinal Muscular Atrophy Type 3 (EMOTAS). Clinical Trial: NCT02227823. https://clinicaltrials.gov/ct2/show/NCT02227823?term=NCT02227823&rank=1.

76. Columbia University. Short and Long Term Treatment With 4-AP in Ambulatory SMA Patients. Clinical Trial: NCT01645787. https://clinicaltrials.gov/ct2/show/NCT01645787?term=NCT01645787&rank=1.

77. University Hospital Freiburg. Pilot Study of Growth Hormon to Treat SMA Typ II and III. Clinical Trial: NCT00533221. https://clinicaltrials.gov/ct2/show/NCT00533221?term=NCT00533221&rank=1.

78. NICHD. Study to Evaluate Sodium Phenylbutyrate in Pre-symptomatic Infants With Spinal Muscular Atrophy (STOPSMA). Clinical Trial: NCT00528268. https://clinicaltrials.gov/ct2/show/NCT00528268?term=NCT00528268&rank=1.

79. Duke University. Levetiracetam for Cramps, Spasticity and Neuroprotection in Motor Neuron Disease. Clinical Trial: NCT00324454. https://clinicaltrials.gov/ct2/show/NCT00324454?term=NCT00324454&rank=1.

80. Dominguez E et al. Intravenous scAAV9 delivery of a codon-optimized SMN1 sequence rescues SMA mice. Hum Mol Genet. 2011;20(4):681-93.

81. Foust KD et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nat Biotechnol. 2010;28(3):271-4.

82. Passini MA et al. CNS-targeted gene therapy improves survival and motor function in a mouse model of spinal muscular atrophy. J Clin Invest. 2010;120(4):1253-64.

83. Valori CF et al. Systemic delivery of scAAV9 expressing SMN prolongs survival in a model of spinal muscular atrophy. Sci Transl Med. 2010;2(35):35ra42.

84. Keil JM et al. A short antisense oligonucleotide ameliorates symptoms of severe mouse models of spinal muscular atrophy. Mol Ther Nucleic Acids. 2014;3:e174.

85. Singh NK et al. Splicing of a critical

exon of human Survival Motor Neuron is regulated by a unique silencer element located in the last intron. Mol Cell Biol. 2006; 26(4):1333-46.

86. Nizzardo M et al. Effect of combined systemic and local morpholino treatment on the spinal muscular atrophy Δ7 mouse model phenotype. Clin Ther. 2014;36(3): 340-56.e5.

87. Guiraud S et al. The Pathogenesis and Therapy of Muscular Dystrophies. Annu Rev Genomics Hum Genet. 2015;16: 281-308.

88. Kissel JT et al. SMA valiant trial: a prospective, double-blind, placebo-controlled trial of valproic acid in ambulatory adults with spinal muscular atrophy. Muscle Nerve. 2014;49(2):187-92.

89. Naryshkin NA et al. Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. Science. 2014; 345(6197):688-93.

90. Chang WF et al. SMN is required for the maintenance of embryonic stem cells and neuronal differentiation in mice. Brain Struct Funct. 2015;220(3):1539-53.

91. Kariya S et al. Requirement of enhanced Survival Motoneuron protein imposed during neuromuscular junction maturation. J Clin Invest. 2014;124(2): 785-800.

92. Iyer CC et al. Low levels of Survival Motor Neuron protein are sufficient for normal muscle function in the SMNΔ7 mouse model of SMA. Hum Mol Genet. 2015;24(21):6160-73.

93. Robbins KL et al. Defining the therapeutic window in a severe animal model of spinal muscular atrophy. Hum Mol Genet. 2014;23(17):4559-68.

94. Sunyach C et al. Olesoxime delays muscle denervation, astrogliosis, microglial activation and motoneuron death in an ALS mouse model. Neuropharmacology. 2012;62(7):2346-52.

95. Hwee DT et al. The small-molecule fast skeletal troponin activator, CK-2127107, improves exercise tolerance in a rat model of heart failure. J Pharmacol Exp Ther. 2015; 353(1):159-68.

96. Kirschner J et al. Somatropin treatment of spinal muscular atrophy: a placebo-controlled, double-blind crossover pilot study. Neuromuscul Disord. 2014;24(2):134-42.

97. Biondi O et al. IGF-1R Reduction Triggers Neuroprotective Signaling Pathways in Spinal Muscular Atrophy Mice. J Neurosci. 2015;35(34):12063-79.

98. Bowerman M et al. Rho-kinase inactivation prolongs survival of an intermediate SMA mouse model. Hum Mol Genet. 2010;19(8):1468-78.

99. Bowerman M et al. Fasudil improves survival and promotes skeletal muscle development in a mouse model of spinal muscular atrophy. BMC Med. 2012;10:24.

100. Kwon DY et al. The E3 ubiquitin ligase mind bomb 1 ubiquitinates and promotes the degradation of survival of motor neuron protein. Mol Biol Cell. 2013;24(12): 1863-71.

101. McGovern VL et al. SMN expression is required in motor neurons to rescue electrophysiological deficits in the SMNΔ7 mouse model of SMA. Hum Mol Genet. 2015;24(19):5524-41.

WILSON'S DISEASE: AN INHERITED, SILENT, COPPER INTOXICATION DISEASE

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ABSTRACT

Wilson's disease is a rare, autosomal recessive, genetic, copper overload disease, which evokes multiple motor or neuropsychiatric symptoms and liver disease. It is the consequence of a variety of different mutations affecting the *ATP7B* gene. This gene encodes for a class IB, P-type, copper-transporting ATPase, which is located in the trans-Golgi network of the liver and brain, and mediates the excretion of excess copper into the bile. When functionally inactive, the excess copper is deposited in the liver, brain, and other tissues. Free copper induces oxidative stress, lipid peroxidation, and lowers the apoptotic threshold of the cell. The symptoms in affected persons can vary widely and usually appear between the ages of 6 years and 20 years, but there are also cases in which the disease manifests in advanced age. In this review, we discuss the considerations in diagnosis, clinical management, and treatment of Wilson's disease. In addition, we highlight experimental efforts that address the pathogenesis of Wilson's disease in *ATP7B* deficient mice, novel analytical techniques that will improve the diagnosis at an early stage of disease onset, and treatment results with copper-chelating agents.

Keywords: Inherited disease, genetics, liver, brain, metal, copper, clinical management, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) protocols, therapy, ceruloplasmin, X-linked inhibitor of apoptosis protein (XIAP), oxygen stress, trientine dihydrochloride, D-penicillamine, diagnostics.

WILSON'S DISEASE: AN INHERITED GENETIC DISORDER

Wilson's disease is a rare, autosomal recessive inherited disorder of copper metabolism with a prevalence of ~1:30,000. It is characterised by hepatic and/or neurological symptoms. However, in some populations living in socio-culturally isolated communities with a high rate of consanguinity, the frequency of the disease is higher and may increase to up to 1:1,130.[1,2] The disease was first described as a familial progressive lenticular degeneration disease associated with cirrhosis of the liver in 1912 by the British physician S. A. Kinnier Wilson.[3] This disorder was later named 'Wilson's disease'. It is caused by an overload of copper in the affected cells due to mutations within *ATP7B* (the Wilson's disease gene), which codes for a copper-transporting, eight transmembrane domain-containing P-type ATPase, primarily expressed in the liver. This gene is located on the long arm of human chromosome 13 (Figure 1A) and shares a significant sequence similarity to *ATP7A*, another copper-transporting ATPase; together these enzymes act as key regulators in copper homeostasis with a dual role in cells: to provide copper for essential cuproenzymes and to mediate the removal and excretion of excess intracellular copper.[4] Mutations affecting the functioning of the *ATP7A* gene give rise to a multisystemic disorder that is termed Menkes kinky hair disease. This rare, X-linked disease affects copper levels in the body and becomes evident in the first months after birth. It is marked by peculiar sparse white hair, doughy skin, connective tissue disturbances, and progressive neurological deterioration associated with severe mental disability and a diffuse atrophy of the cerebellar cortex that is due to a widespread degeneration of the cerebral grey and white matter.[5]

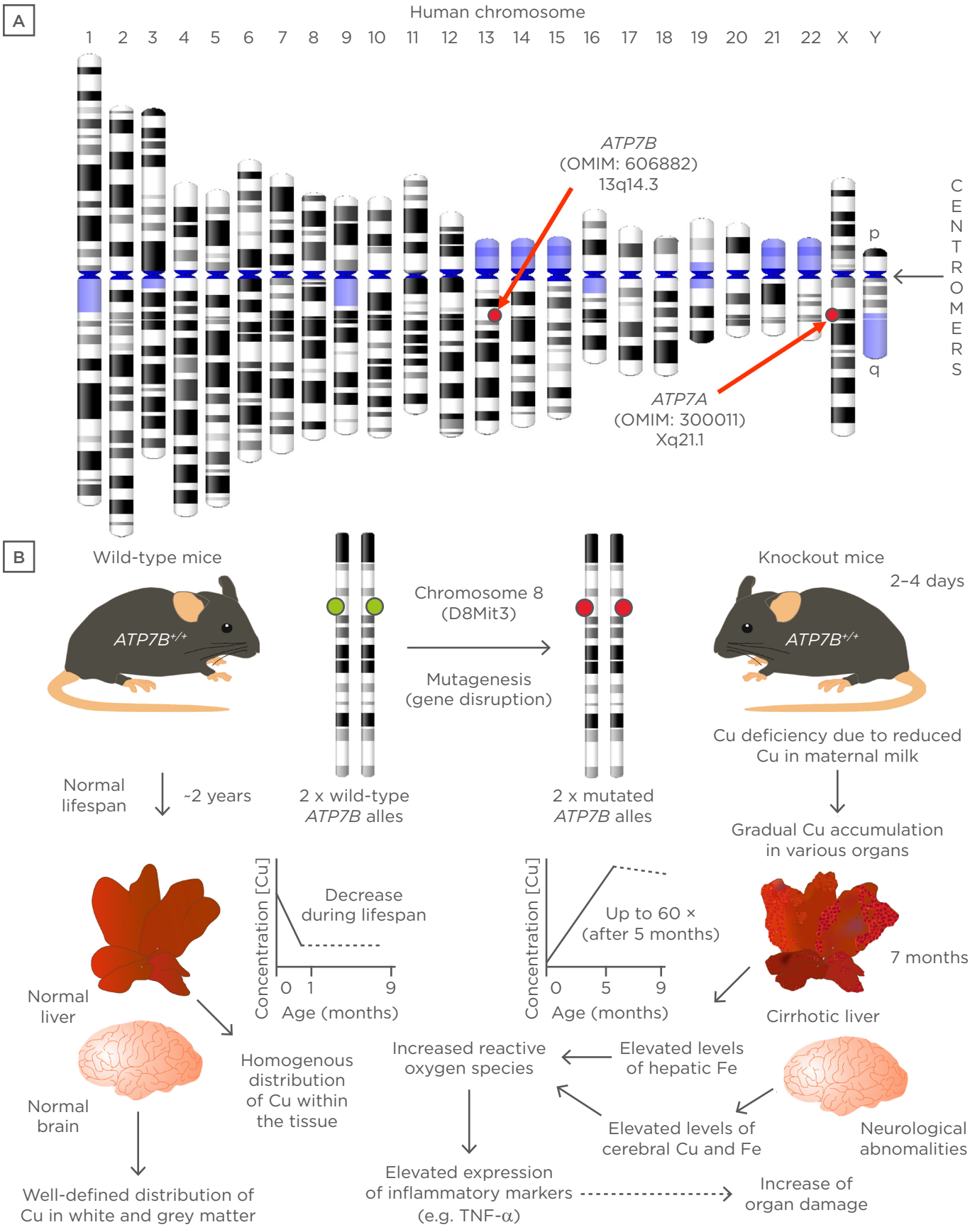

Figure 1: Clinical and experimental Wilson's disease.

A) Chromosomal localisation of human *ATP7A* and *ATP7B* genes. The genes *ATP7A* and *ATP7B* are both crucial copper-transporting ATPases, which are located on the long arms of chromosomes 13 and X, respectively. The schematic overview of the human ideogram was created with the Genome Decoration Page.[40] B) *ATP7B* knockout mouse, a model of experimental Wilson's disease. By use of mutagenesis both alleles of the murine *ATP7B* gene that are located on chromosome 8, region D8Mit[3,41] were

Figure 1 continued.

functionally disrupted.[22] During weaning, maternal mice produce milk with low copper content, and progeny of these mice thus show a significant deficiency in copper during the first days of life. Thereafter, the progeny show a gradual accumulation of copper in various organs. This intoxication induces cirrhotic liver and neuronal abnormalities. In addition, the accumulation of copper is associated with increased concentrations of iron, which induces reactive oxygen species and inflammatory responses. In contrast, wild-type mice with intact *ATP7B* genes show a decrease in copper content during the first month that remains stable for the rest of their lifespan.
Cu: copper; Fe: iron; TNF-α: tumour necrosis factor alpha.

In humans, approximately 50% of dietary copper (~0.8–2 mg/day) is absorbed in the proximal small intestine. After uptake by hepatocytes, biliary excretion is the main pathway for the elimination of excess copper. In Wilson's disease, the export of copper into bile is impaired, leading to a pathological copper accumulation, primarily within the liver and subsequently in the brain (particularly in the basal ganglia) and other tissues (e.g. kidneys and cornea). In addition to reduced biliary copper excretion impaired *ATP7B* function leads to abnormalities in the incorporation of copper into ceruloplasmin, which under normal conditions is the major copper-carrying protein in the blood. Failure to incorporate copper during ceruloplasmin biosynthesis results in the secretion of an apoprotein (i.e. apoceruloplasmin) that is devoid of enzymatic activity and degrades rapidly. The resulting decrease in serum ceruloplasmin concentration, which was first described in 1952, is a diagnostic hallmark of Wilson's disease.[6] Ceruloplasmin accounts for most of the copper in serum, thus the total serum copper is abnormally low in 80–95% of all Wilson's disease cases.[7]

The toxicity of copper in Wilson's disease is evident in a number of different mechanisms. Free copper has the capacity to generate free radicals and direct oxidative stress, resulting in lipid peroxidation of membranes, DNA, and mitochondria.[8] Several independent experimental studies indicate that mitochondrial dysfunction might be the key factor precipitating liver failure due to impairment of mitochondrial energy production.[9,10] Recently it has been suggested that dysregulation of nuclear receptors such as the liver X receptor/retinoid X receptor (LXR/RXR) heterodimer, which critically impact lipid metabolism and the inflammatory response, is one of the major events that triggers the onset and progression of liver pathology in Wilson's disease.[11] In addition, elevated traces of copper induce a conformational change in the X-linked inhibitor of apoptosis protein (XIAP), decreasing its ability to inhibit caspase-3, thereby lowering the apoptotic threshold and sensitising the cell to apoptosis. This leads to a further reduction in the concentration of XIAP levels.[12]

Typically, hepatic symptoms in affected patients develop in children or young adults. A wide range of clinical presentations may be observed with respect to hepatic symptoms, including clinically asymptomatic states with only biochemical abnormalities, steatosis, splenomegaly, and hepatomegaly.[13] Symptomatic liver disease may manifest itself as fulminant hepatic failure associated with haemolysis, compensated or decompensated cirrhosis, or as acute or chronic hepatitis. Non-genetic diseases that cause repeated episodes of liver failure or chronic liver cirrhosis may cause an acquired 'non-Wilsonian' form of hepatocerebral degeneration. These similarities often result in patients being mistakenly diagnosed as Wilson's disease-affected patients. However, this disorder is most likely the consequence of toxic deposition of manganese, rather than copper.[14]

The neurological symptoms of Wilson's disease usually develop later than the liver disease, and occur most often in the third decade of life. Common findings are tremor, lack of motoric co-ordination, dysarthria, dystonia, and spasticity. Many of the individuals with neurological symptoms may have concomitant liver disease, which is frequently asymptomatic. The wide range of the disease pattern is not explained by genetic mutations alone. Several small studies have attempted to correlate a particular *ATP7B* mutation with the phenotypic presentations, however no clear phenotype-genotype correlation in Wilson's disease has been established.[15] Environmental, epigenetic, and genetic modulators probably play a role in the observed phenotypic variability. This assumption was recently

supported by a trial in which clinical and genetic genotype-phenotype correlations were studied in two large families living in a socio-culturally isolated community. This study revealed an equal influence of presumed other genetic modifiers and environmental factors on clinical presentation and age of onset of Wilson's disease in patients with a particular genotype.[2]

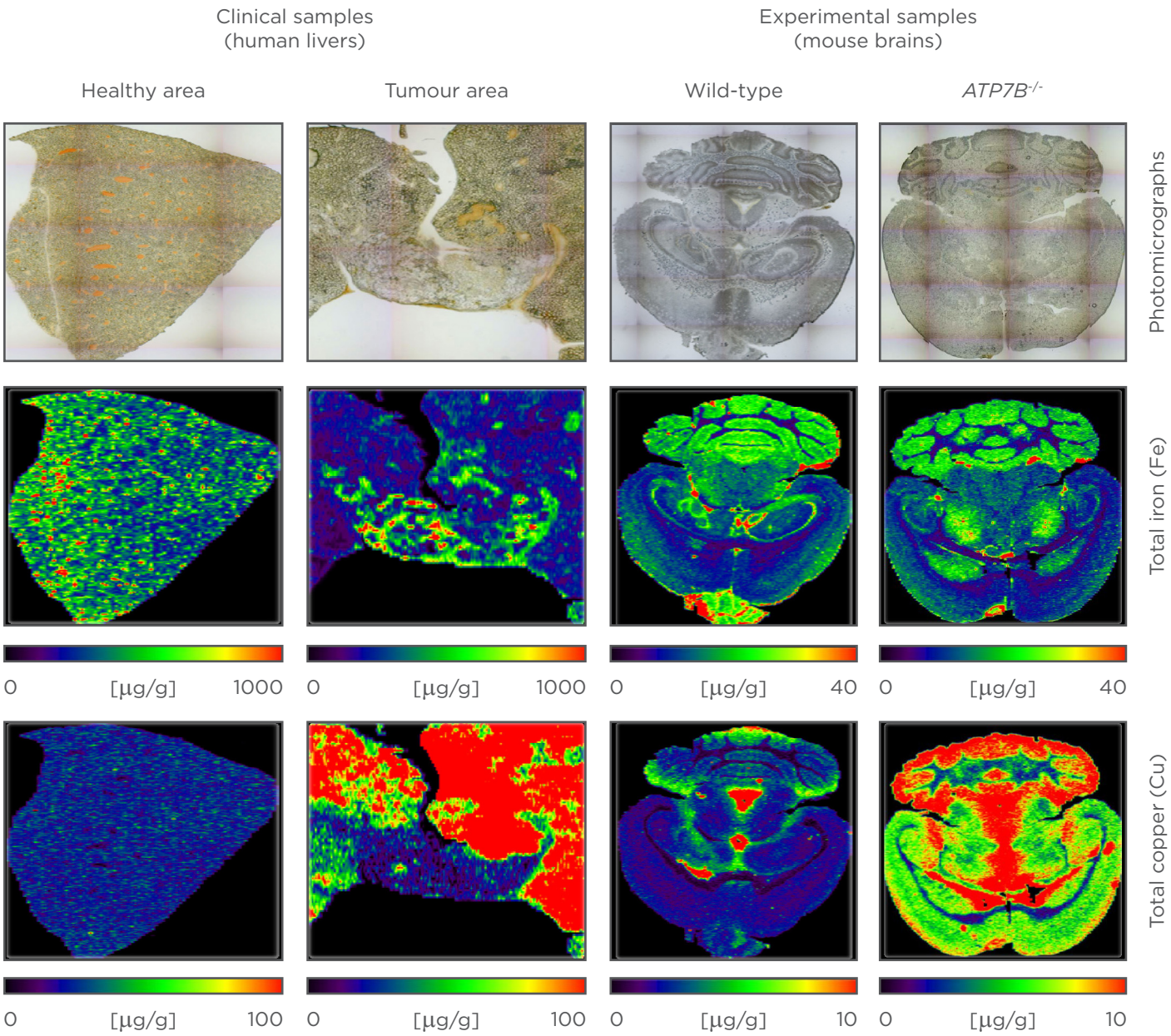

Figure 2: Element measurement by laser ablation inductively coupled plasma mass spectrometry protocols in clinical and experimental Wilson's disease.

Human liver specimen of a Wilson's disease patient from normal and tumourigenic areas (left panels) and murine brain specimens (right panels) isolated from wild-type and *ATP7B* deficient mice were prepared.[22,10] The prepared sections were subjected to LA-ICP-MS measurements and the spatial distribution of iron and copper were measured. The determined element concentrations in µg/g tissue are visualised in a colour scale using the open software ELAI that allows reconstruction of metal distribution maps using Microsoft Excel with the aid of Visual Basic for Applications user-defined functions.[42] Note that (i) the cerebral concentration and disease-associated accumulation of copper is not uniform and preferentially affects specialised brain areas, and (ii) the selective copper increase in the liver is associated with alterations in spatial concentration and distribution of iron. More details about LA-ICP-MS measurements in experimental and clinical samples of Wilson's disease patients are given elsewhere.[25,26]

LA-ICP-MS: laser ablation inductively coupled plasma mass spectrometry.

In most Wilson's disease patients presenting with neurological signs, copper overload is associated with corneal deposition of copper within the Descemet's membrane. Typically, these Kayser–Fleischer rings have a golden-brown appearance. Although this discolouration is not considered pathognomonic of Wilson's disease, it is important in the diagnosis of this copper overload disease. Kayser–Fleischer rings, are also found in cases of cryptogenic cirrhosis, without other clinical features of Wilson's disease.[16,17] This exemplifies some of the variations within clinical presentation and demonstrates that the correct clinical diagnosis may be difficult, and can be delayed. On the other hand, the characteristic clinical features associated with Wilson's disease are only a benchmark; alone they are insufficient to make a reliable diagnosis. Moreover, the clinical consequences of Wilson's disease are variable, from an asymptomatic state to fulminant hepatic failure, chronic liver disease with or without cirrhosis, neurological, and psychiatric manifestations.[18]

In summary the pathophysiologic reason why Wilson's disease exhibits such a broad spectrum of manifestations is unclear. The diverse array of symptoms often makes diagnosis hard to establish, and commonly used diagnostic markers, such as elevated urinary copper excretion, can be found in non-Wilsonian conditions, for example cholestasis syndromes or acute non-Wilsonian liver failure. Low ceruloplasmin is also found in aceruloplasminaemia, an inherited disorder caused by mutations in the ceruloplasmin gene. This extremely rare disease is associated with neurological symptoms and especially with iron overload due to the role of ceruloplasmin in the absorption, storage, and excretion of iron.

EXPERIMENTAL MODELS FOR STUDYING WILSON'S DISEASE

The mechanisms underlying the pathophysiology of Wilson's disease are not well understood. Established animal models include the Long Evans Cinnamon rat,[19,20] the toxic milk mouse,[21] and the *ATP7B*$^{-/-}$ mouse,[22-24] all of which show hepatic copper accumulation. The inbred Long Evans Cinnamon rat model develops hereditary hepatitis and shows spontaneous hepatic copper accumulation, resulting in concentrations 40-times higher than those observed in normal Long Evans Agouti rats, while the serum ceruloplasmin and copper concentrations in these rats is significantly reduced.[19] These alterations are induced by a deletion that removes the ATP binding domains and induces a phenotype that has clinical features similar to those observed in human Wilson's disease patients.[20] The toxic milk mouse carries a single nucleotide mutation that results in the substitution of an evolutionarily conserved methionine to valine in the eighth transmembrane domain.[21]

In our laboratory, we use *ATP7B* null mice that were engineered by homologous recombination of a gene disruption that affects chromosome 8, which prevents the normal translation of the *ATP7B* protein.[22] This genetically modified mouse strain shows an increase in copper concentration in many organs and is associated with a high propensity for the development of morphological abnormalities, which resemble cirrhosis, in animals aged >7 months.[23] Similarly to Wilson's disease patients, the genetic abnormalities in these mice gradually induce liver pathologies, which develop in defined stages (Figure 1B) progressing from mild necrosis and inflammation to extreme hepatocellular injury, nodular regeneration, and bile duct proliferation.[23] Interestingly, these mice not only displayed copper accumulation but also tend to acquire an elevated hepatic iron content combined with significantly lower concentrations of serum iron, serum transferrin saturation, and blood haemoglobin levels.[24] We have recently demonstrated by use of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) protocols that the disease-associated accumulation of hepatic copper in patients suffering from Wilson's disease also results in an increase in intrahepatic iron concentrations (Figure 2, left panels).[25] This study also revealed that hepatic zinc homeostasis is critically affected in these patients.[25] The LA-ICP-MS technique is a robust method, which allows the simultaneous quantitative imaging of various metals in tissue samples with high sensitivity, spatial resolution, specificity, and quantification ability. We have previously used this innovative methodology to confirm the time-dependent increase of copper in the brain parenchyma (Figure 2, right panels) within the *ATP7B*$^{-/-}$ mice model,[26] and to demonstrate that this method is highly appropriate for discovering and diagnosing metal imbalances in fibrotic and cirrhotic liver disorders,[27] and for documenting the drastic time-dependent metal alterations in the livers of *ATP7B*-deficient mice.[28]

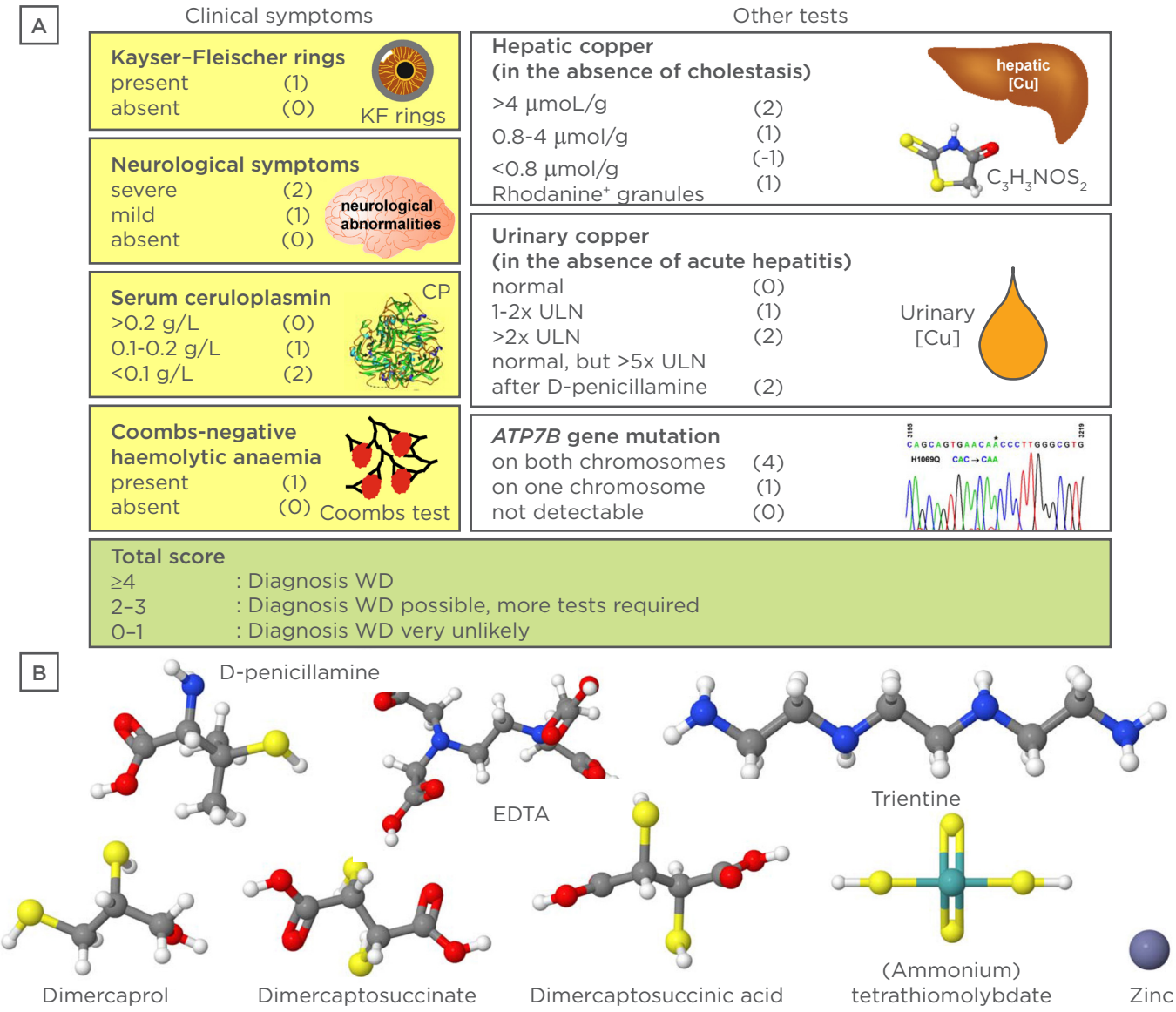

Figure 3: Diagnosis, phenotypic classification, and therapy of Wilson's disease.

A) Diagnosis of Wilson's disease. A diagnostic score that is based on clinical symptoms (occurrence of Kayser–Fleischer rings, existence of neurological symptoms, concentration of serum ceruloplasmin, result of Coombs test) and other tests (hepatic copper content, outcome of rhodanine stain, urinary copper content, genetic mutations) was proposed in 2003 and adapted in 2010.[30,31] Based on the composite of these key parameters, the diagnosis 'Wilson's disease', 'possible Wilson's disease', or 'Wilson's Disease is very unlikely' is made. Further details of scoring and treatment guidelines were established by different international organisations.[35,36] The structure of human ceruloplasmin was prepared using X-ray co-ordinates with access. no. 4ENZ[43] that are deposited in the RCSB Protein Data Bank[44] and the software Ribbons XP (version 3.0), and the formula of rhodanine with CAS identity no. 141-84-4 was displayed with Jmol, version 14.2.15.[45]

B) Metal chelators in the treatment of Wilson's disease. D-penicillamine (CAS 52-67-5), dimercaprol (CAS 59-52-9), EDTA (CAS 60-00-4), ammonium tetrathiomolybdate (CAS 15060-55-6), triethylenetetramine (trientine dihydrochloride, CAS 112-24-3), dimercaptosuccinate (CAS 2418-14-6), and dimercaptosuccinic acid (CAS 304-55-2) act as potent copper chelators. They primarily develop their therapeutic efficacy by enhancing urinary copper excretion. Zinc salts on the other side act as inducers of metallothioneins, thereby favouring reduced uptake, a negative cooper balance, and a reduction of free plasmatic copper. The different images of the compounds were prepared using the open source software Jmol, version 14.2.15.[45]

ATP7B: gene encoding the *ATP7B* protein; CP: ceruloplasmin; EDTA: ethylenediaminetetraacetic acid; KF: Kayser–Fleischer; ULN; upper limit of normal; WD: Wilson's disease; Cu: copper.

However, LA-ICP-MS imaging studies have previously demonstrated that copper was not increased proportionally in the periventricular regions, and that the cerebral copper overload was associated with alterations in cerebral content of other metals (e.g. manganese and zinc); the concentration of other metals (iron) showed only marginal changes. This high cerebral copper accumulation in specific brain areas, and the resulting cognitive alterations during Wilson's disease, might be the consequence of differential regional susceptibility to copper within the brain.[25] In contrast, the age-dependent accumulation of hepatic copper in respective mice and also in patients suffering from Wilson's disease is strictly associated with a simultaneous increase in iron and zinc in the liver.[25] All these findings established by LA-ICP-MS show that Wilson's disease is more than just a simple 'copper imbalance' and that treatment strategies using chelators such as D-penicillamine and trientine dihydrochloride (discussed forthwith) must be carefully executed and clinically monitored. The development of an assay allowing the analysis of the regional distribution of copper and other metals in the murine brain allows analysis of currently available and novel treatments for their experimental effect on tissue metal content. This is of great interest as neurological symptoms are often especially hard to treat and do not respond to therapy as reliably as hepatic symptoms.

Recent work that was performed in the *ATP7B*$^{-/-}$ mouse model showed that an adeno-associated vector-based transfer of a functional *ATP7B* complementary DNA that was placed under the control of the liver-specific α1-antitrypsin promoter is sufficient to induce a dose-dependent therapeutic effect, which was visible in reduced serum transaminases and urinary copper excretion, normalisation of holoceruloplasmin, and restoration of physiological biliary copper excretion in response to copper overload.[29] This study impressively demonstrates that the mentioned experimental models are definitely helpful in addressing special aspects of the pathogenesis of Wilson's disease.

DIAGNOSTICS IN WILSON'S DISEASE

Diagnosis is currently established based on clinical findings and laboratory abnormalities. Sternlieb's criteria makes diagnosis straightforward if two or more of the following symptoms are present: Kayser–Fleischer rings, typical neurologic symptoms, low serum ceruloplasmin levels (<20 mg/dL), and increased hepatic copper content (>250 μg/g dry weight). Diagnosis is far more complex in patients presenting with liver disease. In most patients, a combination of various parameters is required to establish a diagnosis as no single finding is adequate for diagnosis of Wilson's disease. A diagnostic score based on all available tests was proposed by the Working Party at the 8th International Meeting on Wilson's disease that was held in Leipzig in 2001.[30] The Wilson's disease scoring system provides a good level of diagnostic accuracy[23] and the diagnostic algorithm that is applied for scoring is shown in Figure 3A.

Kayser–Fleischer rings are seen in most neurological Wilson's disease patients and in ~70–80% of hepatic patients. Serum ceruloplasmin is typically decreased below 20 mg/dL in patients with Wilson's disease,[31] and total serum copper concentration below 70 μg/dL. Urinary copper excretion is commonly increased in patients with Wilson's disease and conventionally, the level required for diagnosis is 100 μg/24 hours (or 1.6 μmol/24 hours). Liver biopsy with determination of hepatic copper content remains the gold standard for diagnosis, and though the normal hepatic copper content in healthy subjects is <40 μg/g dry weight, this typically exceeds 250 μg/g dry weight in Wilson's disease patients.[32] Based on a recently published prospective study,[33] the optimal cut-off value of liver copper content indicative of Wilson's disease can potentially be reduced to >210 μg/g dry weight. This concentration achieves the highest sensitivity and specificity results (i.e. >95%) to date.

In regards to liver histology, there is no single pathognomonic feature for the diagnosis of Wilson's disease. While the pathology can be similar to an ethanol-induced steatohepatitis in some patients, other patients may show histological signs resembling autoimmune hepatitis. Although histological findings are not often helpful for the diagnosis of Wilson's disease, the exclusion of other aetiologies by liver biopsy may be equally important. *De novo* diagnosis by molecular studies remains difficult due to the large number of disease-specific mutations and variants (>500) that are scattered across the *ATP7B* gene and have a different impact on the disease, hampering the interpretation of specific changes.[34] However, depending on the population tested, a few mutations can be prevalent and can

facilitate the otherwise cumbersome diagnostic mutation analysis.

THERAPY FOR WILSON'S DISEASE

Copper accumulation is progressive and ultimately fatal without specific therapy; therefore, once a diagnosis of Wilson's disease is established, lifelong treatment with an appropriate metal chelator is recommended.[35] In addition, patients should avoid the intake of foods and water with high concentrations of copper in most cases, especially during the first year of treatment.[36] An effective treatment offers patients an excellent long-term survival for this otherwise fatal illness.

Different metal chelating agents and zinc salts are commonly used (Figure 3B). Under treatment, liver function is improved within 6–12 months in most Wilson's disease patients. D-penicillamine and trientine dihydrochloride are chelating agents that enhance urinary excretion of copper to remove excess. A pioneering study that enrolled 53 Wilson's disease patients revealed that the medical therapy with D-penicillamine was sufficient to reduce the size and intensity of Kayser–Fleischer rings in 18 out of 20 patients, suggesting that the concentration of serum copper directly correlates to the phenotypic manifestation of this ophthalmic symptom.[37]

In contrast to chelating agents, zinc blocks copper absorption in the gastrointestinal tract by inducing metallothionein synthesis in enterocytes. Metallothionein has a high affinity for copper, and the copper is discarded during normal cell turnover. The dose of elemental zinc is 150 mg per day, given in three doses.

According to the practice guidelines from the American Association for the Study of Liver Diseases (AASLD) on Wilson's disease,[36] the initial treatment for symptomatic patients should include a chelating agent. Although a larger body of published evidence exists for D-penicillamine, trientine dihydrochloride seems to have a favourable safety profile, especially in patients with neurological symptoms. Treatment of pre-symptomatic patients and lifelong maintenance therapy of successfully treated symptomatic patients can be accomplished with zinc salts or chelating agents in a reduced dosage. Treatment has to be monitored to ensure both efficacy of the drug and patient compliance, and to identify any adverse events.

While Wilson's disease is a very treatable condition, in about 5–10% of all patients a liver transplantation is needed. In particular, this procedure is necessary in patients who have developed considerable liver damage by the time of diagnosis. However, liver transplant is rarely indicated in patients presenting with acute liver failure, which is often due to the presence of concurrent liver diseases. As well as current medical therapies, liver transplantation is a promising therapeutic option with satisfactory long-term outcomes in the subset of Wilson's disease patients with acute liver failure or acute-on-chronic liver failure presenting severe complications. This is well documented in a report that summarised the long-term outcome of 121 Wilson's disease patients following liver transplantation in France in which the patient survival rates were 87% at 5, 10, and 15 years.[38] Similar outcomes after orthotopic liver transplantation combined with improved neuropsychiatric symptoms were also observed in Germany.[39]

FUTURE ASPECTS AND PERSPECTIVE IN MANAGEMENT OF WILSON'S DISEASE

Wilson's disease is an inherited copper overload disease related to the *ATP7B* gene. In recent years, rodent models have helped us to understand the mechanisms by which the effects of excess copper concentrations are exerted. The outcome of this disease can be significantly improved by proper treatment with chelating agents and zinc. However, it is essential that the disease is diagnosed as early as possible to prevent hepatic damage and neurological symptoms developing before the onset of any clinical signs. Nowadays, the diagnosis is based on scoring systems that use clinical symptoms (e.g. Kayser–Fleischer rings, neurological symptoms), genetic testing, measurement of serum ceruloplasmin, hepatic/urinary copper, and other diagnostic tests (e.g. Coombs test). However, there are still challenges in diagnosis.

Based on the high number of different mutations in the *ATP7B* gene, genetic testing is difficult. In addition, the diagnosis of Wilson's disease is challenging because the test parameters overlap with those of other diseases such as cholestasis, and the development of the Kayser–Fleischer rings may also be absent in patients with hepatic Wilson's disease. The present guidelines for treatment of Wilson's disease aim to remove excess body copper, reduce the intake of copper-rich

foods, and to treat any damage of the liver and the central nervous system.

However, novel analytical techniques such as LA-ICP-MS that have the capacity to simultaneously measure and image various metals with high sensitivity, spatial resolution, specificity, and quantification ability in experimental and clinical samples have shown that Wilson's disease is not simply a copper overload disease. The elevated levels of copper are associated with alterations in iron and zinc homeostasis. It will therefore be mandatory to unravel which of the clinical symptoms are associated with which metal. Another important objective in therapy will be the development of drug systems that allow chelators to pass through the blood–brain barrier and deliver chelators to more inaccessible body regions. Such devices might be more useful than the application of undirected chelators, in particular in those patients in whom neurological symptoms are worse than the hepatic disease. These delivery systems might also be favourable to overcome the initial worsening of the neurological conditions, which have often been reported after application of conventional drugs such as D-penicillamine; such therapeutics are experimentally tested and will be evaluated in clinical trials worldwide. Recent encouraging experimental studies have also shown that the administration of functional *ATP7B* transgenes is sufficient to cure key parameters in models of Wilson's disease. It will be interesting to see how these findings will translate clinically and how such procedures might be suitable to engender long-term metabolic correction of human Wilson's disease.

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REFERENCES

1. Reilly M et al. An epidemiological study of Wilson's disease in the Republic of Ireland. J Neurol Neurosurg Psychiatry. 1993;56(3):298-300.

2. Cocoş R et al. Genotype-phenotype correlations in a mountain population community with high prevalence of Wilson's disease: genetic and clinical homogeneity. PLoS One 2014; 9(6):e98520.

3. Wilson SAK. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain 1912;34(1):295-309.

4. La Fontaine S, Mercer JF. Trafficking of the copper-ATPases, ATP7A and ATP7B: role in copper homeostasis. Arch Biochem Biophys. 2007;463(2):149-67.

5. Menkes JH et al. A sex-linked recessive disorder with retardation of growth, peculiar hair, and focal cerebral and cerebellar degeneration. Pediatrics. 1962;29:764-79.

6. Scheinberg IH, Gitlin D. Deficiency of ceruloplasmin in patients with hepatolenticular degeneration (Wilson's disease). Science 1952;116(3018):484-5.

7. Ala A et al. Wilson's disease. Lancet 2007;369(9559):397-408.

8. Nagasaka H et al. Relationship between oxidative stress and antioxidant systems in the liver of patients with Wilson disease: hepatic manifestation in Wilson disease as a consequence of augmented oxidative stress. Pediatr Res. 2006;60(4):472-7.

9. Zischka H et al. Liver mitochondrial membrane crosslinking and destruction in a rat model of Wilson disease. J Clin Invest. 2011;121(4):1508-18.

10. Sauer SW et al. Severe dysfunction of respiratory chain and cholesterol metabolism in Atp7b-/- mice as a model for Wilson disease. Biochim Biophys Acta 2011;1812(12):1607-15.

11. Hamilton JP et al. Activation of liver X receptor/retinoid X receptor pathway ameliorates liver disease in Atp7B-/- (Wilson disease) mice. Hepatology. 2016; 63(6):1828-41.

12. Mufti AR et al. XIAP Is a copper binding protein deregulated in Wilson's disease and other copper toxicosis disorders. Mol Cell. 2006;21(6):775-85.

13. Stremmel W et al. Wilson disease: clinical presentation, treatment, and survival. Ann Intern Med. 1991;115(9): 720-6.

14. Nagappa M et al. Non-Wilsonian hepatolenticular degeneration: Clinical and MRI observations in four families from south India. J Clin Neurosci. 2016;27:91-4.

15. Ferenci P. Phenotype-genotype correlations in patients with Wilson's disease. Ann N Y Acad Sci. 2014;1315:1-5.

16. Frommer D et al. Kayser-Fleischer-like rings in patients without Wilson's disease. Gastroenterology. 1977;72(6):1331-5.

17. Jawairia M et al. Unexplained findings of kayser-fleischer-like rings in a patient with cryptogenic cirrhosis. Case Rep Gastrointest Med. 2012;2012:438525.

18. Rodriguez-Castro KI et al. Wilson's disease: A review of what we have learned. World J Hepatol. 2015;7(29):2859-70.

19. Li Y et al. Spontaneous hepatic copper accumulation in Long-Evans Cinnamon rats with hereditary hepatitis. A model of Wilson's disease. J Clin Invest. 1991;87(5):1858-61.

20. Wu J et al. The LEC rat has a deletion in the copper transporting ATPase gene homologous to the Wilson disease gene. Nat Genet. 1994;7(4):541-5.

21. Theophilos MB et al. The toxic milk mouse is a murine model of Wilson disease. Hum Mol Genet. 1996;5(10): 1619-24.

22. Buiakova OI et al. Null mutation of the murine ATP7B (Wilson disease) gene results in intracellular copper

accumulation and late-onset hepatic nodular transformation. Hum Mol Genet. 1999;8(9):1665-71.

23. Huster D et al. Consequences of copper accumulation in the livers of the Atp7b-/- (Wilson disease gene) knockout mice. Am J Pathol. 2006;168(2):423-34.

24. Merle U et al. Evidence for a critical role of ceruloplasmin oxidase activity in iron metabolism of Wilson disease gene knockout mice. J Gastroenterol Hepatol. 2010;25(6):1144-50.

25. Boaru SG et al. Laser ablation inductively coupled plasma mass spectrometry imaging of metals in experimental and clinical Wilson's disease. J Cell Mol Med. 2015;19(4):806-14.

26. Boaru SG et al. Simultaneous monitoring of cerebral metal accumulation in an experimental model of Wilson's disease by laser ablation inductively coupled plasma mass spectrometry. BMC Neurosci. 2014;15:98.

27. M-M P et al. Novel bioimaging techniques of metals by laser ablation inductively coupled plasma mass spectrometry for diagnosis of fibrotic and cirrhotic liver disorders. PLoS One 2013;8(3):e58702.

28. M-M P et al. Bioimaging of copper deposition in Wilson's disease mouse liver by laser ablation inductively coupled plasma mass spectrometry imaging (LA-ICP-MS). Int J Mass Spectrom 2013;354-355:281-7.

29. Murillo O et al. Long-term metabolic correction of Wilson's disease in a murine model by gene therapy. J Hepatol. 2016; 64(2):219-26.

30. Ferenci P et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int. 2003;23(3):139-42.

31. Nicastro E et al. Re-evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. Hepatology. 2010;52(6):1948-56.

32. Ferenci P et al. Diagnostic value of quantitative hepatic copper determination in patients with Wilson's Disease. Clin Gastroenterol Hepatol. 2005;3(8):811-8.

33. Yang X et al. Prospective evaluation of the diagnostic accuracy of hepatic copper content, as determined using the entire core of a liver biopsy sample. Hepatology. 2015;62(6):1731-41.

34. Kenney SM, Cox DW. Sequence variation database for the Wilson disease copper transporter, ATP7B. Hum Mutat. 2007;28(12):1171-7.

35. European Association for Study of Liver. EASL Clinical Practice Guidelines: Wilson's disease. J Hepatol. 2012;56(3):671-85.

36. Roberts EA, Schilsky ML. American Association for Study of Liver Diseases (AASLD). Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008;47(6):2089-111.

37. Wiebers DO et al. The ophthalmologic manifestations of Wilson's disease. Mayo Clin Proc. 1977;52(7):409-16.

38. Guillaud O et al. Long term results of liver transplantation for Wilson's disease: experience in France. J Hepatol. 2014;60(3):579-89.

39. Weiss KH et al. Outcome and development of symptoms after orthotopic liver transplantation for Wilson disease. Clin Transplant. 2013;27(6): 914-22.

40. National Center for Biotechnology Information. Genome Decoration Page. Available at: http://www.ncbi.nlm.nih.gov/genome/tools/gdp. Last accessed: 24 April 2016.

41. Reed V et al. Mapping of the mouse homologue of the Wilson disease gene to mouse chromosome 8. Genomics 1995;28(3):573-5.

42. Uerlings R et al. Reconstruction of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) spatial distribution images in Microsoft Excel 2007. Int J Mass Spectrom. 2016;395: 27-35.

43. Samygina VR et al. Ceruloplasmin: macromolecular assemblies with iron-containing acute phase proteins. PLoS One. 2013;8(7):e67145.

44. Research Collaboratory for Structural Bioinformatics. RCSB Protein Data Bank. Available at: http://www.rcsb.org. Last accessed: 24 April 2016.

45. Jmol: an open-source Java viewer for chemical structures in 3D. Available at: http://jmol.sourceforge.net. Last accessed: 24 April 2016.

MULTIPLE SCLEROSIS: WHERE DO WE GO FROM HERE?

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ABSTRACT

Multiple sclerosis (MS) is the most common cause of neurological disability in young populations after trauma and represents a significant personal, social, and economic public health burden. The clinical course and response of MS to therapy is highly heterogeneous, but most patients progress from a relapsing-remitting disease course, in which patients may respond to immunomodulatory drugs, to a steady progression and neurodegeneration that is unresponsive to any currently available treatment. In the last few years, novel disease-modifying therapies for MS have become available but the aetiology of the disease remains an enigma. The search for clinical biomarkers that are able to stratify MS patients and allow the personalisation of treatment strategies, has developed greatly in recent years though only a few have been integrated into routine clinical practice.

Keywords: Multiple sclerosis (MS), biomarkers, clinical activity.

HISTORICAL PERSPECTIVE OF MULTIPLE SCLEROSIS

The first description of multiple sclerosis (MS) dates back to the 14th Century, but it was not until the 19th Century that the first anatomopathological descriptions were made. In 1838, the first report associating the presence of demyelinating lesions with clinical features was published. This discovery was published following an 1835 report of clinical findings, which would subsequently become associated with MS, in a patient who later developed demyelination.[1] Some decades later, in 1868, the French pathologist and founder of modern neurology Jean-Martin Charcot formally described the disease entity as 'sclérose en plaques' and first detailed the correlation between clinical and *post mortem* findings.[1] By 1955, descriptions of the disease had expanded, due to the discovery of the 'disseminated' expansion of lesions in the central nervous system (CNS), and due to the 'multiple' lesions and episodes of neurological dysfunction.[1] While the French still conserve the original name given by Charcot, the most commonly used label for the disease is multiple sclerosis. Thomas Rivers was the first to induce experimental autoimmune encephalomyelitis (EAE), the animal model of MS; in 1933, Rivers repeatedly injected brain emulsions and extracts from rabbits into primates, inducing the development of CNS demyelinating lesions. This finding, an immune response in the CNS myelin of mammals, suggested an autoimmune aetiology, with a mechanism of injury relating to chronic inflammation as a result of the presence of self-antigens. In 1948, Elvin Kabat described 'increments' in the oligoclonal immunoglobulins (Ig) within the cerebrospinal fluid (CSF) of patients with MS, sustaining a local inflammatory nature of the disease.[2] The aetiology of MS remains elusive, though several immunomodulatory disease-modifying therapies (DMTs) have shown efficacy in altering the course of the relapsing form of MS, and delaying neurological deterioration, although the mechanisms of action of these drugs are not fully understood. Unfortunately, there is still no effective therapy for the progressive forms of MS.

EPIDEMIOLOGY OF MULTIPLE SCLEROSIS

MS can be defined as a chronic inflammatory demyelinating disease of the CNS in which repeated episodes result in the formation of persistently demyelinated plaques of glial scar tissue, associated with varying degrees of axonal loss.[3] Anatomopathologically, it is characterised by the loss and disruption of the myelin sheath that surrounds the axons in the brain and spinal cord, producing multifocal lesions in the CNS white matter,[4] which can lead to axonal degeneration and progressive neurological dysfunction. Common symptoms include visual disturbances, loss of balance and co-ordination, spasticity, sensory disturbances, bladder and bowel incontinence, pain, weakness, fatigue, and paralysis. MS therefore severely compromises the quality of life of the patient and their family and has a large adverse socioeconomic impact on MS patients, their families, and society as a whole.

Incidence and Prevalence

MS is the most common neurological disease that causes disability in young adults.[5] The disease has an increasing prevalence worldwide, which may be attributable to environmental factors or to increasing awareness and more accurate diagnosis.

According to the Atlas of MS, updated in 2013 by the Multiple Sclerosis International Federation, the number of people with MS has increased from 2.1 million in 2008 to 2.3 million in 2013. The median estimated prevalence worldwide is 33 per 100,000 inhabitants,[6] and median estimated incidence is 2.5 per 100,000 inhabitants.[7] Prevalence varies greatly; North America and Europe have the highest prevalence with 140 and 108 per 100,000, respectively, whereas in Sub-Saharan Africa and East Asia the figures are 2.1 and 2.2 per 100,000, respectively.[6] Data from this atlas generally confirm the observation by John Kurtzke in 1975, stating that MS prevalence increases the further a country is from the equator. A North-to-South gradient of declining prevalence of MS seems to be present in Europe, but there are several exceptions to this rule. For instance, Southern European countries like Spain and Italy have recently seen an increase in MS prevalence (>100 per 100,000).[6]

Age of Onset and Sexual Dimorphism in Multiple Sclerosis

Although the age of onset varies widely within the disease, clinical manifestations normally start at childbearing age, ~30 years of age.[6] Children can also suffer from MS; ~3% of MS patients experience their first symptom prior to age 18 years.[8]

As observed in other autoimmune diseases,[9] MS more frequently affects young women than men (ratio 2:1).[6,7] This female predominance is thought to be due to environmental rather than genetic factors. Potential factors underlying the sex-bias in MS are the effects of sex hormones on immune responses[10,11] and the differential distribution of sex hormone receptors in immune cell subsets.[12,13] Interestingly, the disease course of MS is modified by pregnancy and decreases after menopause.[14] During pregnancy the frequency of MS relapses clearly decreases, with a subsequent surge *postpartum*.[15]

NATURAL HISTORY OF MULTIPLE SCLEROSIS

Clinical Course

MS is a clinically heterogeneous disease, which varies according to the location of plaques in the CNS. Eighty percent of MS patients present with an acute attack, known as clinically isolated syndrome (CIS), which can affect one or several CNS sites.[16] The most commonly affected sites in CIS include the optic nerve, spinal cord, brainstem, and cerebellum; in some rare cases the cerebral hemisphere may be affected.[17] Thus, the most common symptoms include unilateral optic neuritis with visual disturbances; with paraesthesias in the extremities, and weakness in the feet or hands reflecting sensory and motor dysfunction of the spinal cord, respectively. When white matter lesions are detected by magnetic resonance imaging (MRI), the risk of suffering a second relapse increases.[18] New attacks occur with different frequencies, but on average rarely exceed 1.5 episodes per year.[19] Most patients with CIS develop relapsing–remitting multiple sclerosis (RRMS) within 5 years of onset, and a majority of patients with RRMS (~65%) develop secondary progressive multiple sclerosis (SPMS) after a median of 10–15 years from disease onset.[16,20] RRMS is characterised by recurrent relapses with total or partial recovery and an inflammatory course that can be modified with therapy. Around 20% of patients have a progressive onset

without relapses, known as primary progressive multiple sclerosis (PPMS).[19] Some MS patients present a milder form of the disease and are defined as 'benign MS' patients. Due to the difficulty in predicting disease progression, it takes decades after the initial diagnosis to know if a course is benign. The term 'benign MS' is somewhat controversial since it has classically been based mainly on changes in motor functions.[21] These patients may have normal employment and domestic activities for some decades, however studies have shown that over a number of years, their cognitive function deteriorates and they suffer fatigue, pain, and depression that negatively impacts their quality of life.[22-24]

RRMS and PPMS show different clinical courses. RRMS patients traditionally display a 'two-stage' disease: a first stage in which there is a predominance of inflammation (relapses and remissions) compared with SPMS, and a second stage with predominant neurodegeneration and progression (demyelination and axonal loss). However, this classical timeline view is no longer so clearly demarcated, as neuroimaging studies have shown the coexistence of inflammation and neurodegeneration from the onset of the disease.[25] In addition, recent studies indicate that inflammation is abundant in PPMS and correlates with axonal damage and disease progression,[26] involving follicular T helper (Th) cells, Th17, and activated B cells.[27] The presence of meningeal inflammation is associated with an increased rate of clinical progression in PPMS.[28,29]

Therapeutic strategies differ depending on the target phase of the disease: immunomodulatory therapies combat inflammation in the inflammatory phase and neuroprotective agents fight against myelin/neural degeneration in the progressive phase. By contrast, PPMS patients present with a steady progression and degeneration from the onset of disease.

Acute inflammation occurs during relapses with partial or complete remyelination during remissions, but progressive neurodegeneration leads to a higher brain volume loss and clinical disability. For the SPMS and PPMS clinical forms, treatments available to date are unable to stop the progression of the disease.

Prognosis

A review of large long-term studies in MS has identified different prognostic factors associated with MS disability and progression.[30] For RRMS patients, negative prognostic factors identified were a higher initial relapse rate, a shorter interval to the second relapse, a higher level of disability in the first 5 years, and the involvement of more systems. A shorter time-to-progression is typical in SPMS, while PPMS demonstrates a faster rate to disability in the first 2 and 5 years and the involvement of more than three systems. The presence of these prognostic factors does not necessarily imply disability as there is a large variability in patient outcomes.[30] A recent study by Tintore et al.[31] that included 1,015 CIS patients with a mean follow-up of 6.8 years, identified that the number of lesions (≥10) detected by MRI and the presence of oligoclonal bands (OCB) are prognostic factors for the development of MS and early disability.

The life expectancy and prognosis for MS patients is highly variable. Recently a large study[32] identified that life expectancy is reduced by 7 years in MS patients compared with matched healthy controls, and that mortality from both infectious diseases and diseases of the respiratory system is higher in MS patients. The symptoms of MS are lifelong, painful, and debilitating; the treatment and prevention of comorbidities in MS should therefore be considered in the management of these patients to improve their condition, survival, and quality of life.

AETIOPATHOLOGY

The aetiology of MS is still unresolved. A number of theories have been proposed as to the nature of the disease, including origins of autoimmune, infectious, genetic, metabolic, dietary, or neurodegenerative nature. None of these hypotheses alone can explain the clinical heterogeneity of the disease, therefore it is more probable that all of these factors contribute to the generation and maintenance of the disease to some extent. The major aetiopathogenic factors known to be involved in MS disease are summarised in Figure 1 and Table 1. MS is currently considered a complex disorder, which is triggered in genetically susceptible individuals by different environmental and stochastic factors.[33] Environmental factors, such as vitamin D levels, excessive hygiene during childhood, and neurotropic viruses have all previously been widely associated with MS aetiology. Different pieces of evidence suggest that the disease might be

triggered by an infectious agent and evolve into an immune-mediated chronic disease, however to date no virus has been isolated or directly linked with MS.

The immune system also plays an important role in MS pathophysiology. This is supported by diverse facts:

- Susceptibility to MS is linked to important genes of the immune response
- MS lesions are crowded with inflammatory lymphocytes and macrophages
- OCB of Ig are present in the CSF of most MS patients
- Available DMTs target inflammation in the CNS, reducing the number of relapses and lesions detected by MRI, although they are not effective in attenuating the neural damage observed in disease progression

The most accepted theory for MS is that autoreactive T lymphocytes directed against myelin peptides reach the CNS by crossing the blood brain barrier (BBB), and triggering the pathological events that lead to demyelination and axonal damage. This insult to axons can be mild and reversible or severe and irreversible, with transection and likely loss of neuronal function.

The contribution of the target organ, the CNS, has been almost completely ignored in the literature.[33] Pathological and imaging studies,[34] as well as research on the molecular aspects of the disease, in EAE and MS patients, now provide further evidence that CNS-specific factors are important.[35] In summary, MS is a complex autoimmune disease with multiple intrinsic and extrinsic factors that may trigger autoreactivity to self-antigens in the CNS.[33]

RISK FACTORS ASSOCIATED WITH MULTIPLE SCLEROSIS

Environmental risk factors include, among others, infections, smoking, and vitamin D status (Table 1). Smoking or exposure to cigarette smoke contributes to both increased disease susceptibility and more rapid disease advancement. The relative risk for MS development is approximately 1.5-times higher for smokers compared with nonsmokers.[36] Epstein–Barr virus (EBV) infection is considered a risk factor for MS; higher rates of EBV infection have been observed in children with MS compared with age-matched controls.[37] Individuals who acquired EBV in adulthood had a 2 to 3-fold higher risk of MS.[38] The geographical distribution of MS also correlates with the duration and intensity of sun exposure;[39] some large longitudinal studies support an inverse association between vitamin D and risk of MS.[40,41]

DIAGNOSIS OF MULTIPLE SCLEROSIS

The cornerstone of MS diagnosis is based on clinical evidence from a detailed neurological history and physical examination. Symptoms and signs of disorder in the motor, sensory, visual, and autonomic systems, as well as many others may be observed. The diagnosis of MS relies on the demonstration of disease dissemination in space (at least two independent CNS lesions) and time (two or more episodes of neurological dysfunction separated by at least 30 days).[19]

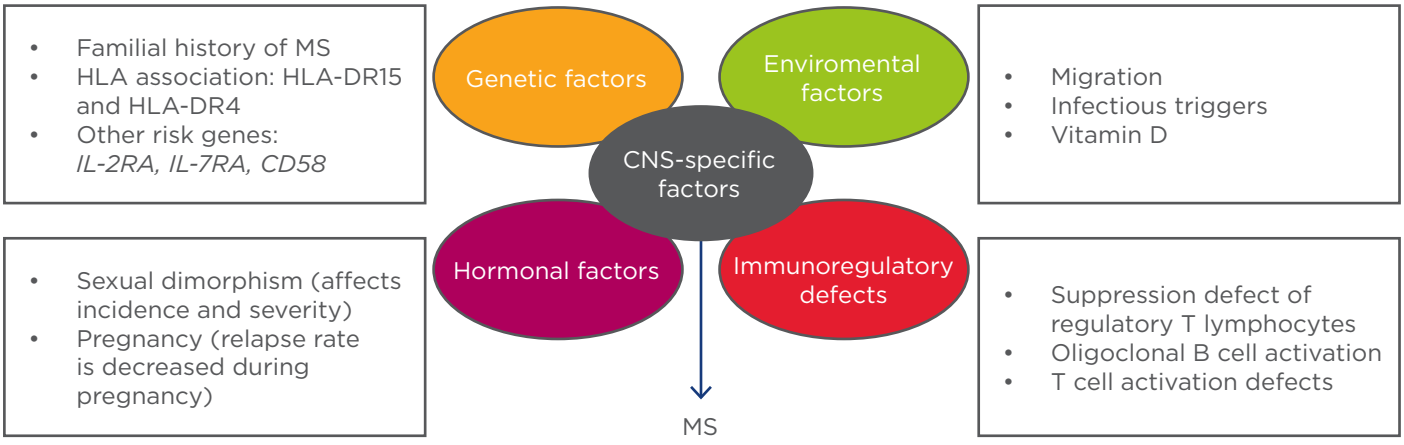

Figure 1: Factors affecting the development of multiple sclerosis.
HLA: human leukocyte antigen; IL: interleukin; CNS: central nervous system; MS: multiple sclerosis.

Table 1: Aetiopathology of multiple sclerosis.

Environmental factors	
Migration	MS development risk is associated with the place of residence in childhood[42]
Hygiene hypothesis	Sanitation level of the surrounding environment during childhood may affect the risk of later developing MS[43] An increase in MS incidence is associated with a reduction in intestinal parasitic infections[44]
Neurotropic viruses	HHV-6 is only expressed in the oligodendrocytes from MS plaques[45] HHV-6 DNA seems to correlate with exacerbations in the RRMS phase[46] Children with MS with EBV seem to be infected at higher rates than their age-matched controls[47] Molecular mimicry between myelin basic protein and an EBV peptide may be a pathophysiological mechanism to induce demyelination[48] IgG against other neurotropic viruses as measles, rubella, and varicella zoster have been reported in the CSF of MS patients[49]
Vitamin D	The geographical distribution of MS correlates with the duration and intensity to sun exposure[39] Large studies have found that taking vitamin D supplements and having high serum levels of 25(OH)D protects against MS[40,41]
Genetics	
Familial studies	Twin studies have reported concordance rates of MS of ~25% for monozygotic twins and ~5% for dizygotic twins.[50] Non-twin-siblings have a 20 to 40-fold increased risk
MHC II risk alleles	DRB1*1501 allele: strongest and most replicated genetic association with MS[51] Being heterozygous for the DRB1*1501 allele increases the risk of MS by 3-fold and homozygosity by over 6-fold[52] DR3 and DR4 haplotypes: present in Sardinian and other Mediterranean MS patients[53,54]
Non-MHC II risk alleles	*IL-2RA*,[55] *CD58*,[56] *STAT3*,[57] *IL-7RA*[58]
Immunoregulatory defects	
Treg cells	Defective suppressive function of Treg cells[59] Natural Treg cell thymic output is diminished compared to that of healthy controls[59,60] Decreased expression of FoxP3 levels in Treg cells from MS patients, with decreased function[61]
Oligoclonal B cell activation	B cells abnormally activated in meningeal follicles differentiate to plasma cells that produce intrathecal immunoglobulins detected as OCB in most MS patients[62]

MS: multiple sclerosis; HHV-6: human herpes virus 6; RRMS: relapsing–remitting multiple sclerosis; EBV: Epstein–Barr virus; MBP: maltose-binding protein; IgG: immunoglobulin G; CSF: cerebrospinal fluid; 25(OH)D: 25-hydroxy vitamin D; MHCII: major histocompatibility complex class II; STAT3: signal transducer and activator of transcription 3; IL: interleukin; OCB: oligoclonal bands; Treg: regulatory T cells.

The introduction of Poser criteria[63] for diagnosis divided MS patients in two major groups: 'definite' and 'probable'. These groups are defined by either a clinical or laboratory-supported diagnosis. The criteria also allowed the classification of MS patients according to the number of attacks, clinical and paraclinical evidence, and CSF OCB or increased IgG index. The Poser criteria were developed before MRI scans and were superseded by the McDonald criteria,[64] which underline the importance of MRI in the diagnosis of MS and allow earlier diagnosis of patients with CIS. The McDonald criteria were published in 2001 and were revised in 2005 and 2010.[65,66] The revision from 2005 included changes focussing on demonstrating dissemination of lesions in time, clarification of the use of spinal cord lesions, and simplification of PPMS diagnosis.[65] The 2010 revision was written with the objective of simplifying and accelerating diagnosis; the criteria relating to use of imaging techniques for demonstrating the dissemination of CNS lesions in space and time was simplified, and the applicability allowed an earlier diagnosis and more uniform use in populations other than Western Caucasian adults.[66]

Clinical

The cornerstone of the MS diagnosis continues to be based on clinical evidence from a detailed neurological history and physical examination, but paraclinical tests are useful in establishing an accurate MS diagnosis. Some symptoms and signs of dysfunction to the motor, sensory, visual, and autonomic systems can be observed, but many others can occur.

There are two characteristic clinical symptoms of MS: Lhermitte's sign (electrical sensation that runs down the spine or limbs on neck flexion) and Uhthoff's phenomenon (transient worsening of symptoms when the body temperature increases, for instance following exercise or a hot bath).[19] Although these symptoms are considered MS-specific they may be present in other diseases. Because MS shares clinical manifestations with other conditions, the differential diagnosis and exclusion of other diseases is an integral part of MS diagnosis.[67] Paraclinical features can help establish diagnosis.

Magnetic Resonance Imaging

MRI is one of the main tools for supporting and accelerating MS diagnosis due to its availability and sensitivity. White matter abnormalities are characteristic and demonstrated in >95% of MS patients. MRI shows the anatomical dissemination of lesions and if used serially over time, can highlight newly developed plaques in the absence of clinical episodes.[19]

MRI provides information about the histopathology of MS lesions. The pathological hallmark of MS is focal demyelination in the lesions, with variable degrees of inflammation, demyelination, gliosis, and axonal injury.[68] The site of the lesion is very important for MS diagnosis, as MS lesions are commonly located in the brainstem, spinal cord, cerebellum, and periventricular white matter.[69] Typical MRI protocols include T1-weighted (T1) imaging with and without gadolinium (Gd) administration, T2-weighted (T2 imaging), proton-density (PD), diffusion-weighted imaging and calculation of the apparent diffusion coefficient, in which active plaques may demonstrate restricted diffusion,[70] and fluid-attenuated inversion recovery techniques (FLAIR).[71] In T2 scans lesions are highlighted by hyperintense regions whereas in T1 scans lesions are highlighted by hypointense regions. T2, PD, and FLAIR demonstrate the most severely demyelinated lesions well.[71] In the acute phase, T1 hypointensity reflects oedema and demyelination, which disappears when inflammation attenuates. On the contrary, chronic foci of T1-hypointensity (known as black holes) reflect persistent axonal loss.[72]

Typically, several T2-hyperintense lesions are commonly observed in MS patients; characteristic abnormalities on T2 images occur in >95% of patients with clinically definite MS, and in 50–70% with a CIS.[73] MRI positivity alone cannot provide a correct diagnosis because lesions are not exclusively characteristic of MS disease, and also appear in people without clinical signs of disease and in people >50 years old. However, lesions detected in the spinal cord are abnormal at any age.[19] Gd enhancement in T1 imaging indicates active lesions, inflammation, and evidence of breakdown of the BBB.[68] Cortical atrophy may also be prominent and correlates with cognitive impairment. Active white matter lesions are classified according to the four observable distinct patterns that originate from different pathophysiological mechanisms.[74] Pattern IV lesions, for instance, are found in ~5% of PPMS patients.[74] Some authors have recently stated that patients with one pattern of lesion conserve it throughout their disease course, while others have described a progression from heterogeneity in lesions to homogeneity over the disease course.[74] Despite controversies concerning lesion heterogeneity, it is clear that as the disease progresses, active lesions become fully demyelinated and convert to an inactive morphology.[74] Recent guidelines recommend the use of MRI for prognostic and therapy monitoring tasks.[75]

New MRI techniques such as volumetric MRI and magnetisation transfer ratio (MTR) are now available. Volumetric MRI allows measurement of brain and spinal cord atrophy in MS and progressive atrophy may have potential as a marker of progression in the monitoring of MS patients.[76] MRI cannot effectively detect cortical demyelination in MS patients but recent studies have shown that MTR imaging is sensitive to cortical lesions in these patients.[77,78]

Cerebrospinal Fluid

A lumbar puncture can be performed to better elucidate the aetiology of a clinical episode. The detection of two or more OCB of IgG in the CSF (and not in the serum of MS patients) is the

most important CSF test finding, and is a red flag in MS diagnosis, as OCB are seen in most patients (>90%).[19] OCB reflect intrathecal Ig synthesis, however the detection of OCB is not exclusive to MS and has diagnostic value only once other causes of CNS inflammation have been excluded.[19] Complementary tests are available, such as the IgG index (IgG index = [IgG (CSF) / IgG (serum)] / [Albumin (CSF) / Albumin (serum)]), which is increased in 80% of patients (ratio >0.7), and measures of cell count (50% with >4 white cells/µL, but only 1% with cell counts >35/µL);[19] these complementary tests are useful in the differential diagnosis of MS.

BIOMARKERS IN MULTIPLE SCLEROSIS DISEASE

MS is not only heterogeneous in its clinical manifestations and forms, but also in disease course and response to therapy. The search for reliable biomarkers that help in the diagnosis, stratification, treatment response, and prediction of MS clinical disability has developed greatly in recent times. The number of available DMTs for MS has increased sharply in recent years, but biomarkers monitoring treatment response, adverse effect risk, and disease progression, and classifying clinical forms of MS, are still lacking. These biomarkers would enable more personalised treatment, something that is urgently needed.

Table 2: Actual and promising novel body fluid biomarkers for diagnosis, prognosis, subtyping, and therapeutic response evaluation of multiple sclerosis.

Biomarkers	Source	MS findings
Diagnostic		
IgG OCB	CSF	Increased in MS but with low specificity
IgG index	CSF/Blood	Increased ratio (>0.7) in MS
MBP-MOG antibodies	Blood	Increased levels in MS patients
Vitamin D	Blood	Decreased levels in MS
Neurofilament light chain	CSF	Increased levels in MS patients
Anti-aquaporin-4	Blood	Differential diagnosis: Present in patients affected by NMO, absent in MS
CSF/serum albumin ratio	CSF/blood	Differential diagnosis: Increased in NMO
N-acetylaspartate	Blood/CSF	Differential diagnosis: Increased in MS in respect to NMO
Prognostic		
MBP and MOG antibodies	Blood	Increased levels in patients developing MS after the first CIS episode
Chitinase-3-like protein 1	CSF	Increased levels in patients developing MS after the first CIS episode
Kappa-free light chains	CSF	Increased levels in patients developing MS after the first CIS episode
IgM OCB	CSF	Increased levels in patients developing MS after the first CIS episode Correlation with disability progression: aggressive course
MRZ-specific IgG antiviral antibodies	CSF	Increased levels in patients developing MS after the first CIS episode (higher specificity than IgG OCBs)
Chemokine ligand 13	CSF	Increased levels in patients developing MS after the first CIS episode, but not specific
Epstein–Barr virus antibodies	Blood/CSF	Increased specific IgG antibodies in MS patients with an early disease onset
VEGF-A	Blood monocytes	Diminished mRNA expression in SPMS compared with RRMS
NO metabolites	CSF	Increments correlate with high disability progression
Neurofilament heavy chain	CSF	Increased in progressive forms of MS
Tubulin/actin	CSF	Increased in progressive disease forms
Glial fibrillary acidic protein	CSF	Increased in SPMS patients with respect to RRMS
Brain-derived neurotrophic factor	CSF	Decreased in SPMS patients with respect to RRMS

Table 2 continued.

Biomarkers	Source	MS findings
Subtype specific		
miR-223, miR-23a, miR-15b	Blood	Decreased in PPMS
HGF, CCL11	Blood	Increased in progressive forms of MS
EGF, CCL4	Blood	Decreased in progressive forms of MS
bFGF	Blood	Decreased in PPMS patients
VEGF	Blood	Increased in SPMS patients
Therapeutic response		
MBP-MOG antibodies	Blood	Good responders to B cell target therapy
Vitamin D	Blood	Increased in IFN-β responders
Neurofilament light chain	CSF	Levels raise to normal in natalizumab responders
Brain-derived neurotrophic factor	Cell culture	Increased levels in glatiramer acetate treated patients
Neutralising antibodies against IFN-β	Blood	Present in IFN-β non-responders
Neutralising antibodies against natalizumab	Blood	Present in natalizumab non-responders
VZV antibodies	Blood	Recurrence risk of infection in previously VZV-infected MS patients receiving fingolimod treatment
Anti-JCV	Blood	Risk of developing progressive multifocal leukoencephalopathy in patients infected by JCV receiving natalizumab treatment

Ig: immunoglobulin; MRZ: measles, rubella, and varicella zoster viruses; CSF: cerebrospinal fluid; MS: multiple sclerosis; MBP: myelin basic protein; MOG: myelin oligodendrocyte glycoprotein; NMO: neuromyelitis optica; CIS: clinically isolated syndrome; MR2: mouse monoclonal receptor; VEGF-A: vascular endothelial growth factor A; mRNA: messenger RNA; SPMS: secondary progressive multiple sclerosis; RRMS: relapsing–remitting multiple sclerosis; PPMS: primary progressive multiple sclerosis; NO: nitric oxide; HGF: hepatocyte growth factor; EGF: epidermal growth factor; bFGF: basic fibroblast growth factor; IFN: interferon; VZV: varicella zoster virus; JCV: John Cunningham virus; OCB: oligoclonal band.

The multiplicity of putative biomarkers, limited information on their independent diagnostic/prognostic value, and the lack of validation in independent patient cohorts are major hurdles for their application in routine clinical practice.

The ideal biomarker for MS should have the following characteristics:[79]

- Measures clinically relevant MS outcomes
- Preferably reflects a causal association
- Detected in an easily accessible biological sample with minimal pre-analytical perturbations
- The assay for its identification is simple, affordable, and stable, and can be validated independently

Promising novel body fluid biomarkers for diagnosis, prognosis, MS subtyping, and therapeutic response evaluation have been detailed elsewhere[80] and are summarised in Table 2.

Multiple Sclerosis Biomarkers Used in Clinical Practice

Currently there is a scarcity of biomarkers that can be used in clinical practice; these are limited to CSF IgG OCB,[81] IgG index,[81] neutralising antibodies against interferon (IFN)-β[82] and natalizumab,[83] varicella zoster virus (VZV) antibodies,[84] anti-John Cunningham virus (JCV) antibodies,[85] and anti-aquaporin-4 (AQP4) antibodies.

Diagnostic Biomarkers

Detection of two or more CSF IgG OCB in a patient with clinical signs of MS provide very useful diagnostic value.[81] OCB can be detected from disease onset and persist during disease course

regardless of disease activity. An increased IgG index (ratio >0.7, reflecting intrathecal IgG production) supports MS diagnosis but has no effect on clinical decision making.[81]

AQP4-IgG are highly specific autoantibodies that target the astrocytic water channel AQP4 and are present in the serum of patients with neuromyelitis optica. These antibodies have become the first clinically useful diagnostic biomarker that allows the classification of a subgroup of patients with inflammatory demyelinating disorders that selectively affect the spinal cord and the optic nerves. The prognosis and treatment is different between these diseases,[86] making differential diagnosis all the more necessary.

Treatment Response Biomarkers

The presence of IFN-β neutralising antibodies has been found in patients with relapse, and therefore physicians might consider stopping treatment with IFN-β in these cases as it may be ineffective.[82] Recent DMTs such as natalizumab and fingolimod (sphingosine 1-phosphate receptor modulator) have shown unexpected fatal adverse reactions. Around 5% of MS patients treated with natalizumab (anti-α4-integrin monoclonal antibody) will develop anti-natalizumab antibodies, which are associated with reduced therapeutic efficacy of natalizumab and infusion-related adverse events.[87] Patients on natalizumab are at increased risk for progressive multifocal leukoencephalopathy (PML) caused by reactivation of the JCV.[88] This risk for PML can be calculated with an algorithm that includes three risk factors: anti-JCV antibody status, previous use of immunosuppressants, and duration of natalizumab treatment.[89] Thus, anti-JCV antibody measurement is a useful biomarker in the stratification of patient risk.[85] Patients on oral fingolimod are at increased risk for developing herpetic infections,[90] and it is advisable that physicians test for VZV antibodies and consider vaccinating seronegative patients at least 1 month before starting treatment with fingolimod.[84]

The DMTs available for MS include: injectable treatments with immunomodulatory properties such as IFN-β formulations, glatiramer acetate and mitoxantrone; two monoclonal antibodies: natalizumab (anti-α4-integrin, which inhibits lymphocyte migration through the BBB)[91] and alemtuzumab (anti-CD52 with immunosuppressive properties);[92] and three oral drugs: fingolimod (an immunosuppressive metabolite that recruits lymphocytes within the lymph organs, inhibiting their migration to the CNS),[93] teriflunomide (inhibits *de novo* synthesis of pyrimidine, preventing clonal expansion of activated lymphocytes),[94] and dimethyl fumarate (with immunomodulatory and antioxidative properties).[95]

Potential Cerebral Spinal Fluid Biomarkers to Support Early Multiple Sclerosis Diagnosis

CSF is the body fluid in direct contact with the CNS, the target organ of MS, and the measurement of biomarkers in the CSF may shed light on the pathological processes occurring. CSF is obtained through an invasive procedure and therefore its collection can only be justified for initial diagnosis, and exceptionally for monitoring the disease. Some CSF biomarkers identifying CIS patients likely to covert to MS have been validated in independent patient cohorts and are closer to clinical implementation. Increased chitinase-3-like protein 1, secreted by activated macrophages, may define those patients with CIS that later convert to clinically definite MS.[96] Increased CSF kappa-free light chains, secreted by the plasma cells, might further support MS diagnosis.[97] CIS patients with IgM CSF OCB have an increased risk of converting to clinically definite MS and show a more aggressive disease course.[98] An intrathecal polyspecific reaction to neurotropic viruses such as measles, rubella, and VZV (MRZ-specific IgG) is associated with an increased risk of conversion to MS.[49] Chemokine ligand 13(CXCL13), involved in B cell recruitment to the CNS during inflammation, has a relevant role in B cell activation. CXCL13 levels in MS are increased in CIS 'converters' compared with 'non-converters',[99] although CXCL13 is not specific to MS and appears in other inflammatory or infectious diseases of the CNS.[99]

Potential Peripheral Blood Biomarkers of Multiple Sclerosis

Peripheral blood biomarkers represent a much less invasive procedure (compared with CSF testing) and their integration, from bench to bedside, would be better for patients and more practical, as these samples can be more easily collected. The number of MS biomarkers in the diagnosis phase is large[49,80,89] and beyond the scope of this review. Biomarkers for the differentiation between the progressive (SPMS and PPMS) forms and the RRMS form are still lacking. Recently, it has been shown that the levels of non-coding RNAs, such as serum microRNAs (miRNAs) miR-223,

miR-23a, and miR-15b are decreased in PPMS and are strongly correlated with the Expanded Disability Status Scale. Therefore, they can be considered candidate biomarkers for differentiating PPMS from RRMS.[100] Our group recently published a study with two independent observational cohorts of different biomarkers for the classification of MS clinical subtypes. We found that a combination of four plasma proteins: hepatocyte growth factor (HGF), eotaxin, epidermal growth factor (EGF), and macrophage inflammatory protein (MIP)-1β, serve as an effective tool in the clinical subtyping of MS patients. HGF and eotaxin were risk factors for developing a progressive form of MS (SPMS or PPMS), while EGF and MIP-1β were protective factors of progression.[101] The combination of these four plasma levels by multivariate logistic regression provided a higher sensitivity and specificity than when the proteins were considered independently. This approach of combining analytes might be clinically useful and its practical application should be replicated and validated in larger cohorts. We also found that plasma fibroblast growth factor levels were decreased in PPMS patients, and that vascular endothelial growth factor was increased in SPMS patients.

CONCLUSIONS

MS remains a heterogeneous and complex disease. Despite recent advances in DMTs, the progressive and neurodegenerative forms of the disease remain incurable. The aetiology of MS remains an enigma and there is an urgent need to recognise and predict outcomes in individual MS patients that could enable more personalised treatment strategies. Therefore, the identification and development of targeted therapies and biomarkers has moved to the forefront of MS translational research.

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REFERENCES

1. Pearce JM. Historical descriptions of multiple sclerosis. Eur Neurol. 2005;54(1):49-53.

2. Kabat EA et al. A study of the crystalline albumin, gamma globulin and total protein in the cerebrospinal fluid of 100 cases of multiple sclerosis and in other diseases. Am J Med Sci. 1950;219(1):55-64.

3. Steinman L. Multiple sclerosis: a two-stage disease. Nat Immunol. 2001;2(9):762-4.

4. Trapp BD et al. Axonal transection in the lesions of multiple sclerosis. N Engl J Med. 1998;338(5):278-85.

5. Noseworthy JH et al. Multiple sclerosis. N Engl J Med. 2000;343(13):938-52.

6. Federation MSI. Atlas of MS. 2013. Available at: www.msif.org/wp-content/uploads/2014/09/Atlas-of-MS.pdf. Last accessed: 13 January 2016.

7. World Health Organization. Atlas multiple sclerosis resources in the world. 2008. Available at: http://www.who.int/mental_health/neurology/Atlas_MS_WEB.pdf. Last accessed: 13 January 2016.

8. Chitnis T et al. Demographics of pediatric-onset multiple sclerosis in an MS center population from the Northeastern United States. Mult Scler. 2009;15(5):627-31.

9. McCombe PA et al. Sexual dimorphism in autoimmune disease. Curr Mol Med. 2009;9(9):1058-79.

10. Sanchez-Ramon S et al. Pregnancy-induced expansion of regulatory T-lymphocytes may mediate protection to multiple sclerosis activity. Immunol Lett. 2005;96(2):195-201.

11. de Andres C et al. Short-term sequential analysis of sex hormones and helper T cells type 1 (Th1) and helper T cells type 2 (Th2) cytokines during and after multiple sclerosis relapse. Eur Cytokine Netw. 2004;15(3):197-202.

12. Aristimuno C et al. Sex-hormone receptors pattern on regulatory T-cells: clinical implications for multiple sclerosis. Clin Exp Med. 2012;12(4):247-55.

13.Valor L et al. Estradiol-dependent perforin expression by human regulatory T-cells. Eur J Clin Invest. 2011;41(4):357-64.

14. Whitaker JN. Effects of pregnancy and delivery on disease activity in multiple sclerosis. N Engl J Med. 1998;339(5):339-40.

15. Salemi G et al. The relapse rate of multiple sclerosis changes during pregnancy: a cohort study. Acta Neurol Scand. 2004;110(1):23-6.

16. Scalfari A et al. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. Brain. 2010;133(7):1914-29.

17. Miller DH et al. Clinically isolated syndromes. Lancet Neurol. 2012;11(2):157-69.

18. Fisniku LK et al. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. Brain. 2008;131(3):808-17.

19. Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372(9648):1502-17.

20. Ciccarelli O, Thompson A. Multiple sclerosis in 2015: Managing the complexity of multiple sclerosis. Nat Rev Neurol. 2016;12(2):70-2.

21. Correale J et al. Benign multiple sclerosis: does it exist? Curr Neurol Neurosci Rep. 2012;12(5):601-9.

22. Correale J et al. Benign multiple sclerosis: a new definition of this entity is needed. Mult Scler. 2012;8(2):210-8.

23. Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. Lancet Neurol. 2008;7(12):1139-51.

24. Amato MP et al. Benign multiple sclerosis: Cognitive, psychological and

social aspects in a clinical cohort. J Neurol. 2006;253(8):1054-9.

25. Charil A, Filippi M. Inflammatory demyelination and neurodegeneration in early multiple sclerosis. J Neurol Sci. 2007;259(1-2):7-15.

26. Frischer JM et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain. 2009; 132(Pt 5):1175-89.

27. Romme Christensen J et al. Systemic inflammation in progressive multiple sclerosis involves follicular T-helper, Th17- and activated B-cells and correlates with progression. PLoS One. 2013;8(3):e57820.

28. Choi SR et al. Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. Brain. 2012;135(Pt 10):2925-37.

29. Howell OW et al. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain. 2011;134(Pt 9):2755-71.

30. Degenhardt A et al. Clinical prognostic factors in multiple sclerosis: a natural history review. Nat Rev Neurol. 2009;5(12):672-82.

31. Tintore M et al. Defining high, medium and low impact prognostic factors for developing multiple sclerosis. Brain. 2015;138(Pt 7):1863-74.

32. Marrie RA et al. A systematic review of the incidence and prevalence of comorbidity in multiple sclerosis: overview. Mult Scler. 2015;21(3):263-81.

33. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol. 2005;23:683-747.

34. McFarland HF. Correlation between MR and clinical findings of disease activity in multiple sclerosis. AJNR Am J Neuroradiol. 1999;20(10):1777-8.

35. John GR et al. Multiple sclerosis: re-expression of a developmental pathway that restricts oligodendrocyte maturation. Nat Med. 2002;8(10):1115-21.

36. Wingerchuk DM. Smoking: effects on multiple sclerosis susceptibility and disease progression. Ther Adv Neurol Disord. 2012;5(1):13-22.

37. Pohl D et al. High seroprevalence of Epstein-Barr virus in children with multiple sclerosis. Neurology. 2006;67(11):2063-5.

38. Ramagopalan SV et al. Association of infectious mononucleosis with multiple sclerosis. A population-based study. Neuroepidemiology. 2009;32(4):257-62.

39. Handel AE et al. Environmental factors and their timing in adult-onset multiple sclerosis. Nat Rev Neurol. 2010;6(3): 156-66.

40. Munger KL et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004;62(1):60-5.

41. Munger KL et al. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006; 296(23):2832-8.

42. Elian M et al. Multiple sclerosis among United Kingdom-born children of immigrants from the Indian subcontinent, Africa and the West Indies. J Neurol Neurosurg Psychiatry. 1990;53(10):906-11.

43. Leibowitz U et al. Epidemiological study of multiple sclerosis in Israel. II. Multiple sclerosis and level of sanitation. J Neurol Neurosurg Psychiatry. 1966; 29(1):60-8.

44. Fleming JO, Cook TD. Multiple sclerosis and the hygiene hypothesis. Neurology. 2006;67(11):2085-6.

45. Challoner PB et al. Plaque-associated expression of human herpesvirus 6 in multiple sclerosis. Proc Natl Acad Sci U S A. 1995;92(16):7440-4.

46. Berti R et al. Increased detection of serum HHV-6 DNA sequences during multiple sclerosis (MS) exacerbations and correlation with parameters of MS disease progression. J Neurovirol. 2002;8(3): 250-6.

47. Banwell B et al. Clinical features and viral serologies in children with multiple sclerosis: a multinational observational study. Lancet Neurol. 2007;6(9):773-81.

48. Lang HL et al. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat Immunol. 2002;3(10):940-3.

49. Teunissen CE et al. Body fluid biomarkers for multiple sclerosis-the long road to clinical application. Nat Rev Neurol. 2015;11(10):585-96.

50. Hansen T et al. Concordance for multiple sclerosis in Danish twins: an update of a nationwide study. Mult Scler. 2005;11(5):504-10.

51. Schmidt H et al. HLA-DR15 haplotype and multiple sclerosis: a HuGE review. Am J Epidemiol. 2007;165(10):1097-109.

52. Sadovnick AD. Genetic background of multiple sclerosis. Autoimmun Rev. 2012;11(3):163-6.

53. Marrosu MG et al. Dissection of the HLA association with multiple sclerosis in the founder isolated population of Sardinia. Hum Mol Genet. 2001;10(25):2907-16.

54. Marrosu MG et al. HLA-DQB1 genotype in Sardinian multiple sclerosis: evidence for a key role of DQB1 *0201 and *0302 alleles. Neurology. 1992;42(4):883-6.

55. Maier LM et al. IL2RA genetic heterogeneity in multiple sclerosis and type 1 diabetes susceptibility and soluble interleukin-2 receptor production. PLoS Genet. 2009;5(1):e1000322.

56. De Jager PL et al. The role of the CD58 locus in multiple sclerosis. Proc Natl Acad Sci U S A. 2009;106(13):5264-9.

57. Jakkula E et al. Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. Am J Hum Genet. 2010;86(2):285-91.

58. Hafler DA et al. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med. 2007;357(9):851-62.

59. Viglietta V et al. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J Exp Med. 2004;199(7):971-9.

60. Venken K et al. Natural naive CD4+CD25+CD127 low regulatory T cell (Treg) development and function are disturbed in multiple sclerosis patients: recovery of memory Treg homeostasis during disease progression. J Immunol. 2008;180(9):6411-20.

61. Huan J et al. Decreased FOXP3 levels in multiple sclerosis patients. J Neurosci Res. 2005;81(1):45-52.

62. Obermeier B et al. Related B cell clones that populate the CSF and CNS of patients with multiple sclerosis produce CSF immunoglobulin. J Neuroimmunol. 2011;233(1-2):245-8.

63. Poser CM et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol. 1983; 13(3):227-31.

64. McDonald WI et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Ann Neurol. 2001;50(1):121-7.

65. Polman CH et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Ann Neurol. 2005;58(6):840-6.

66. Polman CH et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011; 69(2):292-302.

67. Miller DH al. Differential diagnosis of suspected multiple sclerosis: a consensus approach. Mult Scler. 2008;14(9):1157-74.

68. Filippi M et al. Association between pathological and MRI findings in multiple sclerosis. Lancet Neurol. 2012;11(4): 349-60.

69. Fazekas F et al. The contribution of magnetic resonance imaging to the diagnosis of multiple sclerosis. Neurology. 1999;53(3):448-56.

70. Nusbaum AO et al. Quantitative diffusion measurements in focal multiple sclerosis lesions: correlations with appearance on TI-weighted MR images. AJR Am J Roentgenol. 2000;175(3):821-5.

71. Keegan BM, Noseworthy JH. Multiple sclerosis. Annu Rev Med. 2002;53: 285-302.

72. van Waesberghe JH et al. Axonal loss in multiple sclerosis lesions: magnetic resonance imaging insights into substrates of disability. Ann Neurol. 1999;46(5):747-54.

73. Miller DH et al. Role of magnetic resonance imaging within diagnostic criteria for multiple sclerosis. Ann Neurol. 2004;56(2):273-8.

74. Mallucci G et al. The role of immune cells, glia and neurons in white and gray matter pathology in multiple sclerosis. Prog Neurobiol. 2015;127-128:1-22.

75. Wattjes MP et al. Evidence-based guidelines: MAGNIMS consensus guidelines on the use of MRI in multiple sclerosis--establishing disease prognosis and monitoring patients. Nat Rev Neurol. 2015;11(10):597-606.

76. Fox NC et al. Progressive cerebral atrophy in MS: a serial study using registered, volumetric MRI. Neurology. 2000;54(4):807-12.

77. Chen JT et al. Clinically feasible MTR is sensitive to cortical demyelination in MS. Neurology. 2013;80(3):246-52.

78. Abdel-Fahim R et al. Improved detection of focal cortical lesions using 7T magnetisation transfer imaging in patients with multiple sclerosis. Mult Scler Relat Disord. 2014;3(2):258-65.

79. Rajasekharan S, Bar-Or A. From bench to MS bedside: challenges translating biomarker discovery to clinical practice. J Neuroimmunol. 2012;248(1-2):66-72.

80. Katsavos S, Anagnostouli M. Biomarkers in Multiple Sclerosis: An Up-to-Date Overview. Mult Scler Int. 2013;2013:340508.

81. Link H, Huang YM. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. J Neuroimmunol. 2006;180(1-2):17-28.

82. Polman CH et al. Recommendations for clinical use of data on neutralising antibodies to interferon-beta therapy in multiple sclerosis. Lancet Neurol. 2010;9(7):740-50.

83. Lundkvist M et al. Characterization of anti-natalizumab antibodies in multiple sclerosis patients. Mult Scler. 2013;19(6):757-64.

84. Loebermann M et al. Vaccination against infection in patients with multiple sclerosis. Nat Rev Neurol. 2011;8(3):143-51.

85. Bloomgren G et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med. 2012;366(20):1870-80.

86. Wingerchuk DM, Weinshenker BG. Neuromyelitis optica: clinical predictors of a relapsing course and survival. Neurology. 2003;60(5):848-53.

87. Calabresi PA et al. The incidence and significance of anti-natalizumab antibodies: results from AFFIRM and SENTINEL. Neurology. 2007;69(14): 1391-403.

88. Clifford DB et al. Natalizumab-associated progressive multifocal leukoencephalopathy in patients with multiple sclerosis: lessons from 28 cases. Lancet Neurol. 2010;9(4):438-46.

89. Comabella M, Montalban X. Body fluid biomarkers in multiple sclerosis. Lancet Neurol. 2014;13(1):113-26.

90. Uccelli A et al. Primary varicella zoster infection associated with fingolimod treatment. Neurology. 2011;76(11):1023-4.

91. Stüve O et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. Ann Neurol. 2006;59(5): 743-7.

92. Minagar A et al. Alemtuzumab and multiple sclerosis: therapeutic application. Expert Opin Biol Ther. 2010;10(3):421-9.

93. Ingwersen J et al. Fingolimod in multiple sclerosis: mechanisms of action and clinical efficacy. Clin Immunol. 2012;142(1):15-24.

94. Claussen MC, Korn T. Immune mechanisms of new therapeutic strategies in MS: teriflunomide. Clin Immunol. 2012; 142(1):49-56.

95. Bomprezzi R. Dimethyl fumarate in the treatment of relapsing-remitting multiple sclerosis: an overview. Ther Adv Neurol Disord. 2015;8(1):20-30.

96. Hinsinger G et al. Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis. Mult Scler. 2015;21(10):1251-61.

97. Senel M et al. Cerebrospinal fluid immunoglobulin kappa light chain in clinically isolated syndrome and multiple sclerosis. PLoS One. 2014;9(4):e88680.

98. Ferraro D et al. Cerebrospinal fluid oligoclonal IgM bands predict early conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome. J Neuroimmunol. 2013;257(1-2):76-81.

99. Khademi M et al. Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course. Mult Scler. 2011;17(3):335-43.

100. Fenoglio C et al. Decreased circulating miRNA levels in patients with primary progressive multiple sclerosis. Mult Scler. 2013;19(14):1938-42.

101. Tejera-Alhambra M et al. Plasma biomarkers discriminate clinical forms of multiple sclerosis. PLoS One. 2015;10(6):e0128952.

WHOLE SPECTRUM OF NATURAL PROGRESSION OF HAEMANGIOBLASTOMA SEEN WITHIN A SINGLE PATIENT: A VERY RARE CASE REPORT AND LITERATURE REVIEW

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ABSTRACT

This paper reports a rare case of sporadic variation of haemangioblastoma (HB) presenting as multiple lesions within the posterior fossa. A whole spectrum of radiological variants of HB were seen during its natural progression in one patient. A discussion of the management algorithm taken while managing this case is provided, and there is also a literature review to outline current insights on such a rare epiphenomenon.

Keywords: Haemangioblastoma (HB), progression, management.

INTRODUCTION

Sporadic variants of haemangioblastoma (HB) presenting as multifocal central nervous system (CNS) lesions within the posterior fossa are a rare phenomenon.[1] Herein, there is a case report of such an extreme rarity. There is a paradigm shift in the approaches used to manage such multiple lesions compared with focal variants. Multiple surgeries and radiation therapy are required to manage as well as halt the proliferation, relapse, and dissemination of multifocal CNS HB.[1]

In this report there is a discussion of the management algorithm taken for the case and a review that adds to knowledge regarding the behaviour and progression of such a rare entity.

CASE REPORT

A 38-year-old female presented to our neurosurgical clinic with a history of persistent dizziness. The patient reported no history of trauma, falls, weakness of limbs, changes in bladder and bowel habits, or similar episodes in the past; nor did she have a history of ear discharge, hearing impairment, nasal regurgitations, choking episodes, or change in the pitch of her voice. The patient stated that she had no significant past medical or surgical illnesses and there was no significant family history of such conditions. Her vital parameters were within normal range, and upon neurological examination the patient was found to be well-oriented to time, place, and person. Examination also revealed normal function of the cranial nerves. The patient demonstrated unusual cerebellar signs more on the left compared to the right, exhibiting nystagmus, and the presence of an abnormal finger to nose test. Power and tone of the muscles were normal.

Radioimaging identified the presence of multiple lesions within the posterior fossa, showing high enhancement (Figure 1). It revealed a whole spectra of imaging variants of the lesion, ranging between solid, solid with microcysts, and cystic lesions with an enhancing nodule. Cystic lesions with a mural nodule were present within the cerebellomedullary junction on the left, whereas a solid lesion with microcysts was present in the cerebellopontine angle (CPA) on the right (Figure 2). There were additional small solid lesions scattered predominantly in the tentorium along with multiple flow voids highly suggestive of vascular lesions. Magnetic resonance (MR) spectroscopy showed characteristic lipid peaks,

which were highly suggestive of HB. A screening ultrasound and computed tomography of the abdomen and pelvis, as well as a fundoscopy examination (to rule out retinal angiomas), presented normal results, ruling out von Hippel–Lindau (VHL) syndrome. Blood metanephrines and urinary vanillylmandelic acid levels were also within normal range (screening for phaeochromocytoma [PCC]). A systemic work-up was performed to rule out the differentials of the lesions being metastatic, due to the multiple lesions in the posterior fossa.

The disease was explained to the patient and she was advised to have surgery. The predominant presence of a large cystic lesion on her left side resulted in the patient undergoing a left lateral suboccipital approach with aspiration of the cystic content, followed by *en bloc* excision of the nodule (Figure 3).

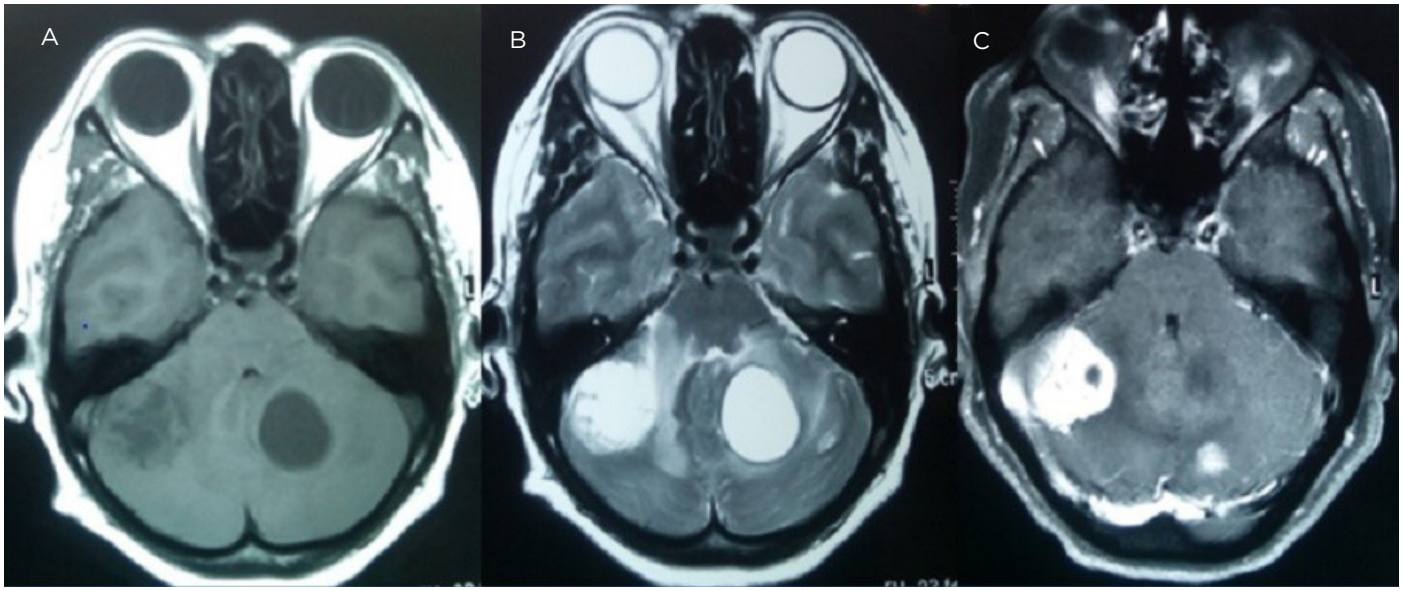

Figure 1: Characteristic magnetic resonance imaging findings of the lesions with enhancement.
A) T1; B) T2; C) T2 contrast.

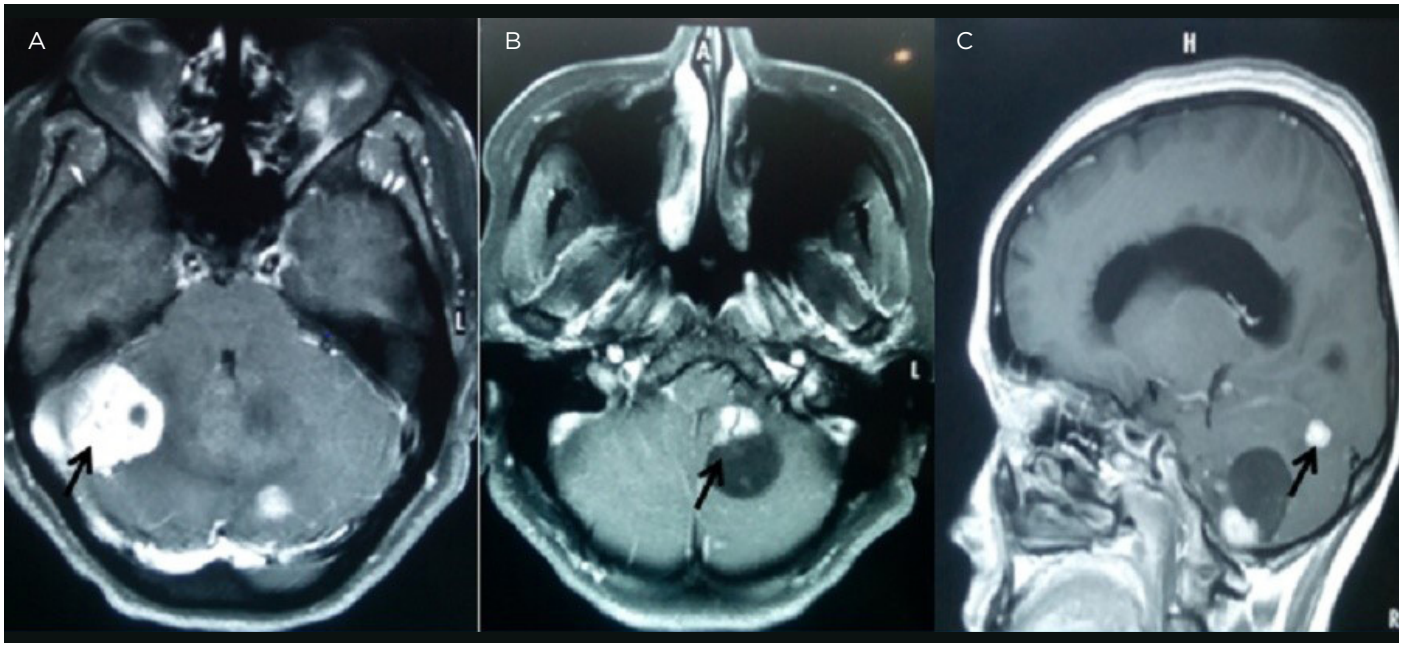

Figure 2: Spectra of radioimaging variants of one lesion.
A) Solid with microcyst; B) cyst with nodule; C) solid.

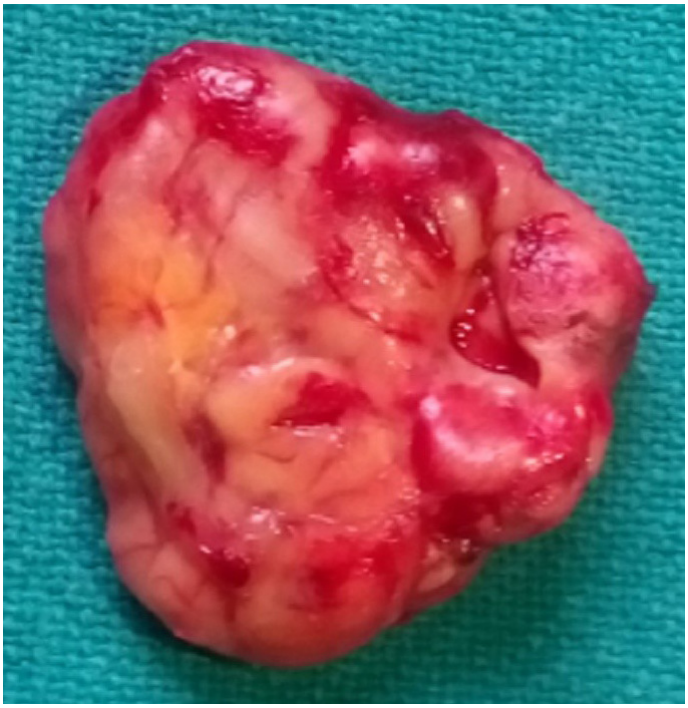

Figure 3: Gross specimen of the solid haemagioblastoma excised *en bloc*.

The histological examination confirmed the diagnosis of HB. Two weeks later, the patient underwent a right retrosigmoid approach with complete excision of the lesion adhered to the pia of the cerebellar hemisphere and projecting into the CPA. The postoperative imaging revealed bilateral cerebellar oedema with narrowing of the forth ventricle. Since the patient was asymptomatic with regards to signs and symptoms of raised intracranial pressure, she was started on oral acetazolamide therapy 250 mg every 8 hours (as an anti-oedema measure) and was strictly monitored for features of acute hydrocephalus. The protocol of starting with oral acetazolamide before commencing diuretic therapy (intravenous furosemide, 20 mg every 8 hours) was followed. This strategy was chosen as diuretics may abruptly decrease the mean arterial pressure thereby reducing the cerebral perfusion pressure and increasing the risk of vasospasm in the posterior circulation during the postoperative period. If a patient is still symptomatic with evidence of progressive hydrocephalus in the repeat imaging, despite these measures, then we opt for external ventricular drainage placement for cerebrospinal fluid diversion. Steroids are not routinely used in such scenarios as it increases the risk of infection and adversely affects wound healing. The patient, however, showed gradual clinical improvement with repeat imaging, revealing no progression of the hydrocephalus; she made an uneventful recovery.

Follow-up was recommended every 6 months with routine imaging to assess the progression of other small solid lesions, and the patient was also counselled for periodic screening to rule out dissemination or other systemic involvement as a result of the HB.

DISCUSSION

Posterior fossa HB is a rare entity.[2] These tumours have been broadly divided into sporadic form and those associated with VHL syndrome.[3] Classically, patients with VHL syndrome present with multiple craniospinal as well as systemic vascular lesions with a high propensity for recurrence in the future.[4]

The *VHL* gene checks transcriptional elongation along with its interplay with other transcriptional factors like elongin B and C. Mutation in the tumour suppressor gene *VHL* leads to upregulation of vascular endothelial growth factor (VEGF) proteins, thereby leading to unopposed angiogenesis in targeted organs following its action on VEGF1 and VEGF2 receptors in stromal cells.[5] This leads to systemic tumours such as craniospinal HB, retinal angiomas, renal cell carcinoma, PCC, endolymphatic sac tumour, and others. Radiologically and with regard to its natural progression, HB has been classified into: purely cystic, cystic lesions with mural nodules, solid lesions, and solid lesions with microcysts.[6]

Judicious surgery for symptomatic lesions along with stringent follow-up for non-progressive variants is the cornerstone of management.[7] Typically, lesions within the posterior fossa present with cerebellar or brain stem compression. However, they have the propensity for spontaneous rupture as well.[8] These lesions are pial vascular lesions showing characteristic homogenous enhancement. They show characteristic vascular blush on angiography, however systemic screening needs to be performed to rule out metastasis in patients harbouring such multiple lesions. Advancements in radioimaging with newer modalities like positron emission tomography, dynamic contrast enhanced MR imaging, and MR spectroscopies have added new dimensions in diagnosis of these lesions.[9-11]

The correct therapeutic approach needs to be undertaken while managing HB. Cystic lesions classically tend to grow and cause compressive symptoms. Surgical excision is

therefore justified with *en bloc* removal of the nodule. Aspiration of the cystic content is not justified due to the fact that the mural nodules are foci for secretion into the cyst, and therefore, there remains a high chance of recurrence.[12] Compared with the cystic lesions, solid lesions should be managed similarly to those with cerebral arteriovenous malformations.[13] Selective preoperative embolisation of major feeders[14] and wide exposure with a tailored skull base approach with circumferential dissection for *en bloc* resection[15] are the key aspects to be followed during their management. The excision can be aided by the use of intraoperative fluorescence and indocyanine green.[16,17] There is also risk of dissemination of the lesions following tumour cell spillage during improper tumour handling.[18]

Preoperative embolisation helps reduce intraoperative bleeding, thereby facilitating excision. N-butyl cyanoacrylate (NBCA) and onynx have been used for preoperative embolisation.[19] NBCA, however, has the risk of gluing the microcatheter following its polymerisation after its contact with blood. Onynx, due to its diffusive properties, allows rapid embolisation of the feeders.[20] The major limitation of its use is the associated lengthy procedure: it can approach 1 hour in duration. A severe complication to be aware of during the embolisation of cerebellar HB is the risk of intratumoural bleeding and death, due to venous congestion in small capillaries and subsequent rupture.[21]

Studies have proven the efficacy of stereotactic radio surgery (SRS) for the management of small, recurrent, deep seated, and disseminated lesions.[22-24] Radiotherapy provides good tumour control. The 1, 2, and 6-year local control rates were 98%, 88%, and 73%, respectively, in one study.[24] Another study validated VHL disease-associated HB, solid tumour, lower tumour volume, and greater marginal dose with improved progression-free survival.[25] There are advantages of SRS and therapy compared with external beam radiation therapy, such as accurate and conformal dose to the target, minimal radiation effects to surrounding neurovascular structures, and provision for providing fractionated doses.[24] Complications related to radiotherapy include perilesional oedema, hydrocephalus, malignant transformation, *de novo* malignancy, and radiation necrosis.[26,27] Some authors therefore do not advocate SRS for prophylactic treatment of asymptomatic tumours.[26]

Our case was unique in that it was a sporadic form of HB presenting with multiple lesions within the posterior fossa. Furthermore, it presented with a whole spectrum of radiological variants seen during its natural progression. We opted for surgical management as both the cystic and solid lesions were large, symptomatic, and were leading to effacement of the forth ventricle.

Patients are advised for a lifelong follow-up with periodic screening for early diagnosis of recurrence, progression, and dissemination of the lesion. Patients with sporadic HB are to be followed up at 6, 12, and 24 months, respectively. For VHL-associated HB, yearly craniospinal MR imaging (for HB), annual ophthalmoscopy (for retinal angiomas), abdominal computed tomography (for renal cell carcinoma, pancreatic cysts, PCC), audiometry (for endolymphatic sac tumour), and blood and urinary screening for PCC is advocated.[28] Gene-targeted therapy in familial CNS HB, though still not proven, may provide new insights in the future.[1] Anti-angiogenic treatments have been tried, but results have shown them to be futile.[29] Timely gene testing and genetic counselling is prudent in early diagnosis and management of HB associated with VHL syndrome.[30,31]

CONCLUSION

A case of a sporadic variant of HB presenting with multifocal lesions in the posterior fossa was diagnosed. In addition to this, there were multiple radiological variants seen. Sites of involvement were also variable, concerning the cerebellomedullary junction, tentorium, as well as the CPA. Therefore, such presentation should be kept in mind during the differential diagnosis of posterior fossa lesions, as it may create uncertainty regarding the correct therapeutic approach to managing the condition. Due to the paucity of such cases, it is prudent to advise the patients to undertake regular screening for timely diagnosis of any systemic involvement, as well as for early management of recurrence of such lesions.

REFERENCES

1. Chu LZ et al. Multifocal central nervous system hemangioblastoma: A case report and review of the literature. Genet Mol Res. 2014;13(3):7904-11.

2. Friedmann DR et al. Nonschwannoma tumors of the cerebellopontine angle. Otolaryngol Clin North Am. 2015;48(3): 461-75.

3. Conway JE et al. Hemangioblastomas of the central nervous system in von Hippel-Lindau syndrome and sporadic disease. Neurosurgery. 2001;48(1):55-63.

4. Abd Hamid D et al. Cerebellar hemangioblastoma in a patient with von Hippel-Lindau disease: A case report. Malays J Med Sci. 2000;7(2):43-8.

5. Wizigmann-Voos S, Plate KH. Pathology, genetics and cell biology of hemangioblastomas. Histol Histopathol. 1996;11(4):1049-61.

6. Choyke PL et al. von Hippel-Lindau disease: Genetic, clinical and imaging features. Radiology. 1995;194(3):629-42.

7. Lonser RR et al. Prospective natural history study of central nervous system hemangioblastomas in von Hippel-Lindau disease. J Neurosurg. 2014;120(5): 1055-62.

8. Ene CI et al. Spontaneous hemorrhage from central nervous system hemangioblastomas. World Neurosurg. 2015;83(6):1180.

9. Sharma P et al. Von Hippel-Lindau syndrome: Demonstration of entire disease spectrum with 68Ga-DOTANOC PET-CT. Korean J Radiol. 2014;15(1): 169-72.

10. Cha J et al. Differentiation of hemangioblastoma from metastatic brain tumor using dynamic contrast-enhanced MR imaging. Clin Neuroradiol. 2016. [Epub ahead of print].

11. Isobe T et al. Proton magnetic resonance spectroscopy findings of hemangioblastoma. Jpn J Radiol. 2010; 28(4):318-21.

12. Miyagami M et al. Long-term prognosis of hemangioblastomas of the central nervous system: Clinical and immunohistochemical study in relation to recurrence. Brain Tumor Pathol. 2004;21(2): 75-82.

13. Igari Y et al. Sudden death due to a cystic lesion in the cerebellum. Forensic Sci Int. 2014;245C:e25-8.

14. Sakamoto N et al. Preoperative endovascular embolization for hemangioblastoma in the posterior fossa. Neurol Med Chir (Tokyo). 2012;52(12): 878-84.

15. Moon BH et al. Large solid hemangioblastoma in the cerebellopontine angle: Complete resection using the transcondylar fossa approach. Brain Tumor Res Treat. 2014;2(2):128-31.

16. Rey-Dios R et al. Intraoperative fluorescence for resection of hemangioblastomas. Acta Neurochir (Wien). 2013;155(7):1287-92.

17. Hojo M et al. Usefulness of tumor blood flow imaging by intraoperative indocyanine green videoangiography in hemangioblastoma surgery. World Neurosurg. 2014;82(3-4):e495-501.

18. Akimoto J et al. Disseminated cerebellar hemangioblastoma in two patients without von Hippel–Lindau disease. Surg Neurol Int. 2014;5:145.

19. Shin GW et al. Preoperative embolization of cerebellar hemangioblastoma with onyx: Report of three cases. Neurointervention. 2014;9(1): 45-9.

20. Seong Eom K et al. Preoperative embolization of a cerebellar haemangioblastoma using onyx: A case report and literature review. Neurol Neurochir Pol. 2011;45(3):292-6.

21. Cornelius JF et al. Hemorrhage after particle embolization of hemangioblastomas: Comparison of outcomes in spinal and cerebellar lesions. J Neurosurg. 2007;106(6):994-8.

22. Hanakita S et al. The long-term outcomes of radiosurgery for intracranial hemangioblastomas. Neuro Oncol. 2014; 16(3):429-33.

23. Lee GJ et al. The clinical experience of recurrent central nervous system hemangioblastomas. Clin Neurol Neurosurg. 2014;123:90-5.

24. Puataweepong P et al. The clinical outcome of intracranial hemangioblastomas treated with linac-based stereotactic radiosurgery and radiotherapy. J Radiat Res. 2014;55(4): 761-8.

25. Kano H et al. The role of stereotactic radiosurgery for intracranial hemangioblastomas. Neurosurgery. 2008; 63(3):443-50; discussion 450-1.

26. Asthagiri AR et al. Prospective evaluation of radiosurgery for hemangioblastomas in von Hippel–Lindau disease. Neuro Oncol. 2010;12(1):80-6.

27. Moss JM et al. Stereotactic radiosurgical treatment of cranial and spinal hemangioblastomas. Neurosurgery. 2009;65(1):79-85; discussion 85.

28. Bamps S et al. What the neurosurgeon should know about hemangioblastoma, both sporadic and in Von Hippel-Lindau disease: A literature review. Surg Neurol Int. 2013;4:145.

29. Seystahl K et al. Neuropathological characteristics of progression after prolonged response to bevacizumab in multifocal hemangioblastoma. Oncol Res Treat. 2014;37(4):209-12.

30. Frantzen C et al, "Von Hippel-Lindau Syndrome," Pagon RA et al. (eds.), GeneReviews® (2000), Seattle (WA): University of Washington, Seattle; 1993–2016.

31. Nielsen SM et al. Von Hippel-Lindau disease: Genetics and role of genetic counseling in a multiple neoplasia syndrome. J Clin Oncol. 2016. [Epub ahead of print].

ADVANCES IN THE MANAGEMENT OF TRANSIENT ISCHAEMIC ATTACK AND STROKE

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ABSTRACT

Among many important advances in the management of transient ischaemic attack (TIA) and stroke are: the updated definition of TIA; risk stratification scores for TIA; the urgent diagnostic and treatment process for TIA; thrombectomy treatment for large vessel occlusive ischaemic stroke; cryptogenic stroke evaluation and treatment, including long-term monitoring for paroxysmal atrial fibrillation; and strategies to improve outcomes for patients including mirror therapy for neglect and timing of mobilisation after stroke. Future research will focus on: antiplatelet strategies after TIA; selecting patients for treatment with recanalisation therapies in an extended time window; uncovering the cause of strokes previously defined as cryptogenic; and better defining the optimal timing and dose of mobilisation after stroke.

Keywords: Transient ischaemic attack (TIA), acute stroke treatment, cryptogenic stroke, stroke rehabilitation.

INTRODUCTION

Owing to the results of important research in transient ischaemic attack (TIA) and stroke, secondary stroke rates and stroke-related deaths have dropped over the last two decades. This review will focus on advancements in the areas of TIA, acute stroke, cryptogenic stroke, and rehabilitation management.

TRANSIENT ISCHAEMIC ATTACK

About 20% of ischaemic strokes are closely preceded by a TIA.[1] The classic definition of TIA, i.e. 'focal cerebral dysfunction of an ischaemic nature lasting ≤24 hours with a tendency to recur', has been largely replaced by one that emphasises tissue rather than time.[2] The American Heart Association (AHA)/American Stroke Association (ASA) definition is "a transient episode of neurologic dysfunction caused by focal brain, spinal cord, or retinal ischemia."[3] Inherent in this definition is the early acquisition of neuroimaging; patients with evidence of new ischaemic injury are identified as having had a stroke, while those without a new injury are defined as having a TIA. The concept of an acute ischaemic cerebrovascular event, similar to the concept of an acute coronary syndrome, has also been proposed.

The risk of stroke following TIA is highest in the first 48 hours after occurrence.[4] Risk stratification has been a topic of major interest in order to better guide the timeliness and extent of healthcare resource utilisation for suspected TIA patients. The most widely used prediction rule is the ABCD2 score;[5] however, it has been shown to have only modest predictive ability.[6,7] Additionally, the ABCD2 score has been shown to have low sensitivity when used by non-neurologists.[8] Although data do not generally support the use of the ABCD2 score until after the condition has been confirmed as

TIA by a stroke specialist, its use is supported by current AHA/ASA and National Institute for Health and Care Excellence (NICE) guidelines to aid early management decisions.[9] While some report utility of the ABCD2 score to be useful in discriminating TIAs from mimics,[10] several studies have shown that the ABCD2 score is not reliable at discriminating between high and low-risk TIAs. Dual TIA (i.e. a TIA event within 7 days before another TIA) contributes to short and long-term prediction of stroke, and also improves ABCD2 performance.[7] ABCD2 is not accurate in predicting atrial fibrillation (AF) or large vessel stenosis, both of which require prompt intervention.[11] Incorporation of brain imaging improves prediction of stroke.[7,12-14] ABCD3 and ABCD3-I are both superior in the prediction of short and long-term risk of stroke.[7] Carotid stenosis and intracranial stenosis are associated with recurrent strokes,[7] therefore the addition of vasculature imaging techniques to risk scores provides better accuracy for predicting the recurrence of stroke. Intracranial large vessel occlusion is also a predictor of decline in functional status in patients with TIA.[15]

Whether patients with a TIA should be admitted to the hospital or not is a point of spirited discussion. Current guidelines recommend urgent management for patients presenting with TIA, but it is unclear if these patients should be hospitalised.[16] The primary reason for hospitalisation is completion of TIA evaluation and secondary prevention.[3,17] Specialised TIA clinics and observation units in the emergency department may provide a safe approach to TIA while avoiding hospital admissions.[18] Reductions in hospital admissions of up to 80% can be achieved with this approach and may improve patient satisfaction. Admissions would be targeted towards patients requiring an urgent procedure, transition to anticoagulation, or for medical management of vessel-narrowing producing clinical worsening due to haemodynamic compromise. Short-term dual antiplatelet treatment of TIA and minor stroke was shown to be beneficial in the CHANCE trial. However, uncertainty about the generalisability of the results given the high event rates in both arms of the trial along with the relatively low use of statin and anti-hypertensive agents did not lead to the sudden discontinuation of the POINT trial, which is ongoing. Importantly, the event rates of 10% at 90 days seen over a decade ago now appear to be in the range of 3–4% with the urgent evaluation and management strategy.[19]

ACUTE REVASCULARISATION THERAPY IN ISCHAEMIC STROKE

Intravenous alteplase is the only US Food and Drug Administration (FDA)-approved lytic therapy for the treatment of acute ischaemic stroke.[20] The NINDS trial showed improved outcomes in acute ischaemic stroke patients treated with tissue plasminogen activator (tPA) <3 hours from symptom onset and several subsequent trials have corroborated these findings.[21-24] Although not approved by the FDA in the 3–4.5 hour window, ECASS III showed benefit of tPA administration during this period and its use during this time has been endorsed by the AHA/ASA.[25,26] The ENCHANTED study did not show non-inferiority of alteplase 0.6 mg/kg compared with 0.9 mg/kg with respect to death and disability at 90 days; however, ordinal analysis of the modified Rankin scale score did show non-inferiority. Additionally, major symptomatic haemorrhage occurred less frequently in the 0.6 mg/kg group (1.0% versus 2.1%, p=0.01).[27]

The guidelines for the use of tPA were developed according to the population enrolled in the trials and as such there were initially many contraindications to tPA, which were not based on any specific safety issues observed. Given that patients had to meet very specific criteria, tPA, although effective, was only given to a small percentage of stroke patients.[26,28] There is little evidence of how some of these contraindications affect safety in patients that receive tPA. Multiple studies show that patients may benefit from an individualised decision to give tPA.[28]

Historically, the computed tomography (CT) scan was the most widely used method for the acute evaluation of stroke since it can accurately identify hyperacute haemorrhage (0–6 hours). Gradient echo magnetic resonance imaging (MRI) has been shown to be as good in detecting acute haemorrhage as the CT scan.[29-31] Diffusion weighted imaging (DWI) is more sensitive than the CT scan for the detection of acute ischaemia.[31-33] Multimodal CT and MRI (parenchymal, perfusion, and vascular imaging) have the potential to identify patients with an ischaemic penumbra that may benefit from reperfusion therapy. MRI to identify chronic vascular injury can be useful after acute reperfusion therapies have taken place.[31] There are several ongoing trials for treating patients who wake up with stroke symptoms using thrombolytic therapies (alteplase

or tenecteplase) and/or endovascular procedures.[34] The imaging parameters are an estimation of a DWI/fluid-attenuated inversion recovery mismatch or evaluation of penumbral tissue on MRI or CT perfusion.

tPA has been shown to be better than conservative care in patients with large vessel occlusion, however it only has limited efficacy in this situation.[35] Initial endovascular therapies for stroke included intra-arterial infusion of thrombolytic agents, as well as first-generation mechanical devices, such as the Merci retriever. In 2013, however, the largest randomised trial at that time, the Interventional Management of Stroke (IMS) III trial showed no benefit to endovascular therapy above and beyond best medical therapy alone. However, there had been advances in both endovascular technique as well as in imaging which were not incorporated into the trial. The major advance in endovascular therapy was the use of stent-retrievers, which resulted in dramatically increased rates of rapid recanalisation as compared with previous-generation devices. In late 2014 and early 2015, five randomised trials, MR CLEAN, ESCAPE, EXTEND-IA, SWIFT PRIME, and REVASCAT showed the benefit of endovascular therapy compared with the best available medical therapy.[36-40] Collectively, endovascular therapy improved functional independence on the modified Rankin scale (≥2) at 90 days by absolute difference of 20% (46% versus 26%).[41]

In spite of the dramatic changes in stroke management during the last two decades, only a small percentage (5%) of patients with acute ischaemic stroke receive tPA.[42] Endovascular therapy is safe and effective when there is a small amount of tissue that is already infarcted prior to treatment. However, many patients are ineligible because there has been too much damage prior to treatment. In these cases, neuroprotective agents may be useful in combination with reperfusion strategies.[26,43] Although hundreds of putative neuroprotective agents showed benefit in animal models, none resulted in a positive human trial.[44-46] Future studies may combine a neuroprotective strategy with recanalisation rather than neuroprotection alone.

On the endovascular side, an important question is how wide is the timeframe within which the procedure can be performed, and for whom. Treatment is relatively safe and effective within 6 hours from symptom onset. Additional imaging using CT angiography collateral score assessment, DWI, or perfusion imaging with either CT or MRI may allow better selection of patients within that time window. In addition, some patients present with large vessel occlusion and a low National Institutes of Health Stroke Score (NIHSS).

Contentious issues which should be noted include whether or not to treat stroke patients with thrombectomy, and in patients with tandem occlusion of cervical internal carotid artery and intracranial occlusion whether acute stenting or angioplasty alone should be performed.

CRYPTOGENIC STROKE

Stroke of unexplained cause or 'cryptogenic' stroke accounts for approximately 30% of all ischaemic strokes.[47,48] According to the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria, cryptogenic stroke is defined when no stroke mechanism can be identified after routine, exhaustive, or incomplete evaluation.[49] One of the neuroimaging features in cryptogenic stroke patients is the presence of superficial infarcts occurring in up to 60% of cases,[50] which led investigators to use the term 'embolic stroke of undetermined source' in reference to a non-lacunar infarction occurring in the absence of a specific identifiable embolic source, such as AF, valvular heart disease, or large artery stenosis.[51] An important mechanism in cryptogenic stroke that, when identified, leads to anticoagulation therapy is paroxysmal AF. It is detected in 20–30% of patients with cryptogenic stroke by outpatient telemetry or implantable loop recorders. Less documented embolic sources include patent foramen ovale (PFO), atheroma of the aortic arch, and artery-to-artery embolism from sub-stenotic atherosclerotic plaque.[49]

Paradoxical embolism through a PFO is hypothesised to be one of the possible mechanisms leading to cryptogenic stroke. There is a weak association, however, between PFO and cryptogenic stroke.[52] Investigators designed the Risk Of Paradoxical Embolism (ROPE) score to identify patients with cryptogenic stroke and a PFO in whom the PFO is the likely stroke mechanism. In those patients, the risk of recurrent stroke was low. In addition, several clinical trials showed no significant benefit of PFO closure over medical treatment in reducing the risk of recurrent stroke in patients with cryptogenic stroke.[53] Antiplatelet therapy remains the mainstay

of treatment in most patients with cryptogenic stroke and evidence of a PFO.[54] Several studies have demonstrated increased prevalence of aortic arch atheromas >4 mm in size[55-57] and ipsilateral non-stenosing complex internal carotid plaques in patients with cryptogenic stroke. Antiplatelet therapy agents remain the mainstay of treatment in patients with cryptogenic stroke and evidence of thick aortic arch plaque.[54,58]

Atrial dysfunction or 'cardiopathy' has been introduced as a possible stroke mechanism in patients with cryptogenic stroke. Serum biomarkers of atrial dysfunction such as N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) have been shown to be associated with an increased risk of cardioembolism independent of AF.[59] Atrial arrhythmias and electrocardiogram findings such as supraventricular tachycardia[60] and increased p-wave terminal force in lead V1[61] have also been associated with ischaemic stroke risk in the absence of AF, particularly those related to embolism (cardioembolic and cryptogenic stroke subtypes).[62] Left atrial enlargement is also associated with the risk of incident ischaemic stroke in the absence of AF[63] and recurrent ischaemic stroke particularly related to embolism (cryptogenic or cardioembolic), an association independent of AF.[64]

AF, with its implied left atrial stasis in the setting of an irregular contractile function, has been used to provide a direct mechanistic explanation for embolism.[65] A *post hoc* analysis of the Asymptomatic AF and Stroke Evaluation in Pacemaker Patients and the AF Reduction Atrial Pacing Trial (ASSERT), however, showed a lack of a temporal relationship between subclinical AF detected on pacemaker interrogation and stroke, suggesting that AF signifies underlying atrial cardiopathy, which is possibly the direct cause of stroke in most of these patients. Recent randomised trials proved the efficacy of outpatient cardiac monitoring in detecting AF after cryptogenic stroke with a detecting rate of 16% at 30 days with non-invasive monitoring[66] and 30% at 3 years with implantable monitors[67] leading to anticoagulation therapy for secondary stroke prevention.

Unless AF is detected after a cryptogenic stroke, we agree with the AHA/ASA guidelines guidelines which which suggest that antiplatelet therapy be the treatment for secondary stroke prevention in this population.[54] Despite this therapeutic approach, the risk of recurrent stroke after cryptogenic stroke remains substantial, reaching up to 20% at 2 years.[68] A *post hoc* analysis of the Warfarin-Aspirin Recurrent Stroke trial showed that warfarin was superior to aspirin in reducing recurrent stroke risk in patients with NT-proBNP ≥750 ng/dL (hazard ratio: 0.30, 95% confidence interval: 0.12–0.84; p=0.021), a marker of atrial cardiopathy, in the absence of AF. This suggests the need for clinical trials comparing anticoagulation versus antiplatelet therapy in patients with cryptogenic stroke and atrial cardiopathy.

ADVANCES IN STROKE REHABILITATION

Stroke rehabilitation is an important part of the continuum of care and important studies have come out in the last few years highlighting potential methods to improve outcomes. The focus of this section will be on the results of studies focussed on mirror therapy, intensive early mobilisation, and non-invasive brain stimulation.

Spatial neglect is a disabling sequela of stroke, which can lead to difficulties with participation in rehabilitation and long-term functional outcomes. Mirror therapy has been used in an attempt to overcome these deficits. The Mirror Therapy in Unilateral Neglect After Stroke (MUST) trial was an open-label, blinded endpoint, randomised trial of patients with unilateral neglect 48 hours after stroke.[69] Patients assigned to mirror therapy looked into a mirror as they worked with the limb on the neglected side. Patients assigned to sham treatment looked at the non-reflecting side of the mirror during therapy sessions. Treatment regimen was 1–2 hours per day for 5 days per week, over 4 weeks. Twenty-six patients were treated with mirror therapy and 20 with sham. At 1, 3, and 6 months, patients assigned to mirror therapy had significantly improved spatial attention on the affected side, as measured by the star cancellation test, line bisection test, and picture identification task. In addition, the percentage of patients with a good outcome at 6 months, as defined by a modified Rankin scale score of 0–2, was 10% in the sham treatment group and 46% in the mirror therapy group (p=0.01). Further trials with the modified Rankin scale as the primary outcome measure would be very meaningful, for a treatment that is not very expensive.

The timing and amount of mobilisation following ischaemic stroke has been a subject of significant interest to those caring for patients in the post-acute setting. The efficacy and safety of very early mobilisation within 24 hours of stroke onset (AVERT) parallel group, single-blind, randomised controlled trial at 56 acute stroke units in five countries comparing usual stroke-unit care alone or very early mobilisation in addition to usual care.[70] Patients were eligible for participation if they had haemorrhagic or ischaemic stroke, including those who underwent thrombolysis. The very early mobilisation intervention included: 1) mobilisation within 24 hours of stroke onset; 2) focussing on sitting, standing, and walking (i.e. out-of-bed activity); 3) at least three additional out-of-bed sessions compared to usual care. The intensity of intervention was prescribed according to functional ability, with four levels specified, and titrated according to recovery. There were 1,054 patients who received very early mobilisation and 1,050 received usual care. The groups were well matched on baseline characteristics. Time to first mobilisation was at a mean of 18.5 hours after stroke in the very early mobilisation group and at a mean of 22.4 hours in the usual care group. The daily amount of treatment averaged 31 minutes in the very early mobilisation group and 10 minutes in the usual care group. A favourable outcome (modified Rankin scale score of 0–2) was more likely to occur in the usual care group (46% versus 50%, p=0.004). On secondary analysis, there was no significant shift in the modified Rankin scale score. Time to walking 50 metres unassisted by 3 months was virtually identical in the two groups. The rates of late and serious complications (such as pneumonia and venous thromboembolism) were not significantly different between the two groups. The researchers plan to perform a further dose–response analysis to establish the effect of dose of rehabilitation on efficacy and safety outcomes. Future research questions will include the best time to initiate rehabilitation after stroke, the type of training to be initiated, and the type of patient who might benefit most.

CONCLUSION

Among many important advances in the management of TIA and stroke are the updated definition of TIA, risk stratification scores for TIA; the urgent diagnostic and treatment process for TIA; thrombectomy treatment for large vessel occlusive ischaemic stroke, cryptogenic stroke evaluation and treatment including long-term monitoring for paroxysmal AF; and strategies to improve outcomes of patients including mirror therapy for neglect and timing of mobilisation after stroke.

Future research will focus on antiplatelet strategies after TIA; selecting patients for treatment with recanalisation therapies in an extended time window; uncovering the cause of strokes previously defined as cryptogenic; and better defining the optimal timing and dose of mobilisation after stroke.

REFERENCES

1. Rothwell PM, Warlow CP. Timing of TIAs preceding stroke Time window for prevention is very short. Neurology. 2005;64(5):817-20.

2. Toole JF. The Willis lecture: transient ischemic attacks, scientific method, and new realities. Stroke. 1991;22(1):99-104.

3. Easton JD et al. Definition and evaluation of transient ischemic attack: a scientific statement for healthcare professionals from the American Heart Association/American Stroke Association Stroke Council; Council on Cardiovascular Surgery and Anesthesia; Council on Cardiovascular Radiology and Intervention; Council on Cardiovascular Nursing; and the Interdisciplinary Council on Peripheral Vascular Disease: The American Academy of Neurology affirms the value of this statement as an educational tool for neurologists. Stroke. 2009;40(6):2276-93.

4. Johnston SC et al. Short-term prognosis after emergency department diagnosis of TIA. JAMA. 2000;284(22):2901-6.

5. Johnston SC et al. Validation and refinement of scores to predict very early stroke risk after transient ischaemic attack. Lancet. 2007;369(9558):283-92.

6. Perry JJ et al. Prospective validation of the ABCD2 score for patients in the emergency department with transient ischemic attack. CMA. 2011; 183(10):1137-45.

7. Kiyohara T et al. ABCD3 and ABCD3-I scores are superior to ABCD2 score in the prediction of short- and long-term risks of stroke after transient ischemic attack. Stroke. 2014; 45(2):418-25.

8. Bradley D et al. Frequent inaccuracies in ABCD^2 scoring in non-stroke specialists' referrals to a daily Rapid Access Stroke Prevention service. J Neurol Sci. 2013;332(1-2):30-4.

9. Royal College of Physicians (UK). Stroke: National Clinical Guideline for Diagnosis and Initial Management of Acute Stroke and Transient Ischaemic Attack (TIA). NICE Clinical Guidelines, No. 68. 2008. Available at: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0009998/. Last accessed: 8 February 2016.

10. Sheehan OC et al. Diagnostic usefulness of the ABCD2 score to distinguish transient ischemic attack

and minor ischemic stroke From noncerebrovascular events The North Dublin TIA Study. Stroke. 2009; 40(11):3449-54.

11. Wardlaw JM et al. ABCD2 score and secondary stroke prevention Meta-analysis and effect per 1,000 patients triaged. Neurology. 2015; 85(4):373-80.

12. Giles MF et al. Addition of brain infarction to the ABCD2 score (ABCD2I) a collaborative analysis of unpublished data on 4574 patients. Stroke. 2010; 41(9):1907-13.

13. Merwick Á et al. Addition of brain and carotid imaging to the ABCD^2 score to identify patients at early risk of stroke after transient ischaemic attack: a multicentre observational study. Lancet Neurology. 2010;9(11): 1060-9.

14. Ay H et al. A score to predict early risk of recurrence after ischemic stroke. Neurology. 2010;74(2):128-35.

15. Poisson SN et al. Intracranial large vessel occlusion as a predictor of decline in functional status after transient ischemic attack. Stroke. 2011;42(1):44-7.

16. Stamplecoski M et al. Predictors of Hospitalization in Patients with Minor Stroke and TIA. Stroke. 2015; 46:AWP280.

17. Blacquiere D et al. Delays in Presentation to Stroke Prevention Clinic after TIA or Minor Stroke are Associated with Increased Recurrent Events, Hospitalization and Mortality. Stroke. 2015;46:AWP282.

18. Siket MS, Edlow J. Transient ischemic attack: an evidence-based update. Emerg Med Pract. 2013;15(1):1-26.

19. Amarenco P et al. One-Year Risk of Stroke after Transient Ischemic Attack or Minor Stroke. N Engl J Med. 2016;374(16):1533-42.

20. Marier J. Tissue plasminogen activator for acute ischemic stroke. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. N Engl J Med. 1995;333(24): 1581-7.

21. Albers GW et al. ATLANTIS trial: results for patients treated within 3 hours of stroke onset. Alteplase Thrombolysis for Acute Noninterventional Therapy in Ischemic Stroke. Stroke. 2002;33(2):493-6.

22. Hacke W et al. Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke: the European Cooperative Acute Stroke Study (ECASS). JAMA. 1995;274(13):1017-25.

23. Hacke W et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Second European-Australasian AcuteStroke Study Invesigators. Lancet. 1998; 352(9136):1245-51.

24. Sandercock P et al. The IST-3 collaborative group.The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. Lancet. 2012;379(9834):2352-63.

25. Hacke W et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med. 2008;359(13):1317-29.

26. Culp WC et al. Window for stroke therapy effectiveness extended by dodecafluoropentane emulsion in rabbits. J Vasc Interv Radiol. 2015; 26(2)Suppl:S113.

27. Anderson CS et al. Low-Dose versus Standard-Dose Intravenous Alteplase in Acute Ischemic Stroke. N Engl J Med. 2016. [Epub ahead of print].

28. Parker S et al. TPA Contraindications have Little Impact on Thrombolysis Outcomes. Stroke. 2015;46:ATP58.

29. Kidwell CS et al. Comparison of MRI and CT for detection of acute intracerebral hemorrhage. JAMA. 2004; 292(15):1823-30.

30. Fiebach JB et al. Stroke magnetic resonance imaging is accurate in hyperacute intracerebral hemorrhage: a multicenter study on the validity of stroke imaging. Stroke. 2004;35(2): 502-6.

31. Merino JG, Warach S. Imaging of acute stroke. Nat Rev Neurol. 2010; 6(10):560-71.

32. Sobesky J et al. Does the mismatch match the penumbra? Magnetic resonance imaging and positron emission tomography in early ischemic stroke. Stroke. 2005;36(5):980-5.

33. Lee LJ et al. Impact on stroke subtype diagnosis of early diffusion-weighted magnetic resonance imaging and magnetic resonance angiography. Stroke. 2000;31(5):1081-9.

34. Thomalla G, Gerloff C. Treatment Concepts for Wake-Up Stroke and Stroke With Unknown Time of Symptom Onset. Stroke. 2015; 46(9):2707-13.

35. Bhatia R et al. Low rates of acute recanalization with intravenous recombinant tissue plasminogen activator in ischemic stroke real-world experience and a call for action. Stroke. 2010;41(10):2254-8.

36. Berkhemer OA et al. Stent-Retriever Thrombectomy for Stroke. N Engl J Med. 2015;373(11):1076.

37. Campbell BC et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. N Engl J Med. 2015;372(11):1009-18.

38. Goyal M et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med. 2015;372(11):1019-30.

39. Jovin TG et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. N Engl J Med. 2015; 372(24):2296-306.

40. Saver JL et al. Stent-retriever thrombectomy after intravenous t-PA vs. t-PA alone in stroke. N Engl J Med. 2015;372(24):2285-95.

41. Grech R et al. Stent-based thrombectomy versus intravenous tissue plasminogen activator in acute ischaemic stroke: A systematic review and meta-analysis. Interv Neuroradiol. 2015;21(6):684-90.

42. Adeoye O et al. Recombinant tissue-type plasminogen activator use for ischemic stroke in the United States: A doubling of treatment rates over the course of 5 years. Stroke. 2011; 42(7):1952-5.

43. Saver JL et al. Methodology of the Field Administration of Stroke Therapy - Magnesium (FAST-MAG) phase 3 trial: Part 1 - rationale and general methods. Int J Stroke. 2014;9(2):215-9.

44. Saver JL et al. Methodology of the Field Administration of Stroke Therapy - Magnesium (FAST-MAG) phase 3 trial: Part 2 - prehospital study methods. Int J Stroke. 2014;9(2):220-5.

45. Dávalos A et al. Citicoline in the treatment of acute ischaemic stroke: an international, randomised, multicentre, placebo-controlled study (ICTUS trial). Lancet. 2012;380(9839):349-57.

46. Heiss WD et al. Cerebrolysin in patients with acute ischemic stroke in Asia: results of a double-blind, placebo-controlled randomized trial. Stroke. 2012;43(3):630-6.

47. Petty GW et al. Ischemic stroke subtypes: a population-based study of incidence and risk factors. Stroke. 1999;30(12):2513-6.

48. Sacco RL et al. Infarcts of undetermined cause: the NINCDS Stroke Data Bank. Ann Neurol. 1989; 25(4):382-90.

49. Yaghi S, Elkind MS. Cryptogenic stroke: A diagnostic challenge. Neurol Clin Pract. 2014;4(5):386-93.

50. Lamy C et al. Clinical and imaging findings in cryptogenic stroke patients with and without patent foramen ovale: the PFO-ASA Study. Atrial Septal Aneurysm. Stroke. 2002;33(3):706-11.

51. Hart RG et al. Embolic strokes of undetermined source: the case for a

new clinical construct. Lancet Neurol. 2014;13(4):429-38.

52. Alsheikh-Ali AA et al. Patent foramen ovale in cryptogenic stroke: incidental or pathogenic? Stroke. 2009;40(7):2349-55.

53. Spencer FA et al. Systematic review of percutaneous closure versus medical therapy in patients with cryptogenic stroke and patent foramen ovale. BMJ Open. 2014;4(3):e004282.

54. Kernan WN et al. Guidelines for the prevention of stroke in patients with stroke and transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke. 2014;45(7):2160-236.

55. Di Tullio MR et al. Aortic atheromas and acute ischemic stroke: a transesophageal echocardiographic study in an ethnically mixed population. Neurology.1996;46(6):1560-6.

56. Freilinger TM et al. Prevalence of nonstenosing, complicated atherosclerotic plaques in cryptogenic stroke. JACC Cardiovasc Imaging. 2012; 5(4):397-405.

57. Gupta A et al. Magnetic resonance angiography detection of abnormal carotid artery plaque in patients With cryptogenic stroke. J Am Heart Assoc. 2015;4(6):e002012.

58. Amarenco P et al. Clopidogrel plus aspirin versus warfarin in patients with stroke and aortic arch plaques. Stroke. 2014;45(5):1248-57.

59. Montaner J et al. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. Stroke. 2008;39(8):2280-7.

60. Kamel H et al. Paroxysmal supraventricular tachycardia and the risk of ischemic stroke. Stroke. 2013;44(6):1550-4.

61. Kamel H et al. P-wave morphology and the risk of incident ischemic stroke in the Multi-Ethnic Study of Atherosclerosis. Stroke. 2014;45(9): 2786-8.

62. Kamel H et al. Electrocardiographic Left Atrial Abnormality and Risk of Stroke: Northern Manhattan Study. Stroke. 2015;46(11):3208-12.

63. Di Tullio MR et al. Left atrial size and the risk of ischemic stroke in an ethnically mixed population. Stroke. 1999;30(10):2019-24.

64. Yaghi S et al. Left atrial enlargement and stroke recurrence: the northern Manhattan stroke study. Stroke. 2015; 46(6):1488-93.

65. Goldstein LB et al. Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke. 2011; 42(2):517-84.

66. Gladstone DJ et al. Atrial fibrillation in patients with cryptogenic stroke. N Engl J Med. 2014;370(26):2467-77.

67. Sanna T et al. Cryptogenic stroke and underlying atrial fibrillation. N Engl J Med. 2014;370(26):2478-86.

68. Ntaios G et al. Embolic Strokes of Undetermined Source in the Athens Stroke Registry: An Outcome Analysis. Stroke. 2015;46(8):2087-93.

69. Pandian JD et al. Mirror therapy in unilateral neglect after stroke (MUST trial): a randomized controlled trial. Neurology. 2014;83(11):1012-7.

70. Bernhardt J et al. AVERT Trial Collaboration Group. Efficacy and safety of very early mobilisation within 24 h of stroke onset (AVERT): a randomised controlled trial. Lancet. 2015;386(9988):46-55.

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