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Neurologia i Neurochirurgia Polska also known under the name of *Polish Journal of Neurology and Neurosurgery (PJNNS)* is a premier research and educational platform of the Polish Neurological Society and Polish Society of Neurosurgeons. It has a long and accomplished history dating back to earlier days of the XX Century. The journal publishes the results of basic and clinical research contributing to the understanding, diagnosis, and treatment of neurological and neurosurgical disorders.

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Cover photo: Wolfgang H. Jost et al., Two muscles that have gained importance through introduction of the Col-Cap concept, see figure on page 278.

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The editors of Neurologia i Neurochirurgia Polska (the Polish Journal of Neurology and Neurosurgery) announce new editorial features

To Our Readership: Last year, we introduced Invited Editorials to our Journal. This has proved to be very successful, having been met with significant interest by readers and authors alike. These editorials allow us to select articles that we as editors think are important for our readership. We also introduced a new functionality to our electronic review form that allows reviewers to suggest an Invited Editorial. Having already used reviewer suggestions many times, we ask our reviewers to continue to submit them.

In the last three issues of 2019, we published four Invited Editorials. The first, published in Issue 4/2019, was written by Dr. Deutschländer [1] from the Mayo Clinic. The second, written by Dr. Tipton [2], also from the Mayo Clinic, appeared in Issue 5/2019. The last two (both in Issue 6/2019) were written by Dr. Ambrosius [3] from the Poznan University of Medical Sciences and by Dr. Domitrz [4] from the Medical University of Warsaw. These Invited Editorials highlighted four important papers on the treatment of postural deformities by istradefylline in patients with Parkinson's Disease [5], on lifetime prevalence and clinical correlations of dystonic tics in a Polish cohort of patients with Gilles de la Tourette syndrome [6], on investigations of independent predictors of unfavourable outcomes in patients with an aneurysmal subarachnoid haemorrhage [7], and on the assessment of the prevalence and clinical characteristics of angiography headache and its relationship with primary headaches [8]. These manuscripts were submitted by authors from Fukuoka in Japan, Warsaw in Poland, Belgrade in Serbia, and Adana in Turkey.

We plan to continue with these important features, and we hope that future Invited Editorials will be met with equal interest by You, our Readers.

Now, we would like to announce another new and exciting feature for our Journal in 2020: a *Leading Topic for a Particular Issue*. We will group articles dealing with similar topics (e.g. stroke, multiple sclerosis, epilepsy) at the beginning of an issue. We will invite an expert in the particular topic to provide an overview for these articles. While this feature may not be possible for each issue, we will try to implement it as frequently as possible, depending on the number of received papers related to a particular topic. We hope that this new feature will be similarly welcomed by our readers.

Zbigniew K. Wszolek, M.D., Co-Editor-in-Chief
Jarosław Sławek, M.D., Ph.D., Co-Editor-in-Chief
Łukasz Stolarczyk, M.D., Administrative Editor

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INVITED EDITORIAL

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Botulinum toxin for cervical dystonia: addressing treatment failure and improving outcomes

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ABSTRACT

Introduction. Cervical dystonia is a form of focal dystonia characterised by tilting and turning of the head and neck. This can cause significant disability in affected patients. Botulinum toxin injections are the mainstay of therapy. However, approximately 30% of patients discontinue treatment.

Clinical reflections. Tyślerowicz et al. have provided a comprehensive review of the factors contributing to treatment failure. Such factors include appropriate identification of dystonia patterns, accurate injection of muscles, and addressing non-motor features of cervical dystonia.

Clinical implications. A systematic approach is needed to identify and address the potentially modifiable factors that contribute to treatment failure.

(*Neurol Neurochir Pol 2020; 54 (3): 218–219*)

Cervical dystonia is a common form of focal dystonia. It can cause dystonic head tremor, impaired range of motion, and musculoskeletal pain, leading to significant functional disability for the patient [1].

Effective and well tolerated treatment options are therefore vital to preserve and restore functional status. Oral medications are notoriously ineffective in the treatment of cervical dystonia, and can present significant adverse effects. Botulinum toxin therapy has therefore become the mainstay of treatment due to its proven clinical efficacy and favourable side effect profile [2, 3]. However, approximately 30% of patients discontinue treatment with botulinum toxin injections [4].

Tyślerowicz et al. have presented a review of potential pitfalls in the treatment of cervical dystonia with botulinum toxin. In addition to providing a detailed analysis of reasons for botulinum toxin treatment failure, they have discussed strategies for optimising treatment [5].

Assuring the correct diagnosis is the all-important first step in treating any neurological disorder. In the case of cervical dystonia, this includes assessing for mimics, identifying comorbid movement disorders, and fully addressing secondary causes of cervical dystonia. There are a number of movement disorders with similar phenomenology that can mimic cervical dystonia. These include tic disorders, particularly dystonic tics for which additional behavioural and pharmacological therapies may be beneficial [6, 7]. Cervical dystonia can also be seen in the context of other movement disorders including Parkinson's Disease and atypical parkinsonian disorders, which present additional symptomatology and disease progression that may affect treatment response [8].

Successful treatment with botulinum toxin requires proper identification of the pattern of dystonia. The classification system used to identify dystonic movements impacts upon which muscles are then targeted with botulinum toxin [9]. Tyślerowicz et al. emphasise the utility of the Col-Cap scheme for identifying the components of dystonic posturing [5]. Dystonic movements can include turning and tilting of the head and neck in the lateral and anterior-posterior planes. A combination of these abnormal postures is often seen [10]. Utilising techniques such as EMG guidance to assure proper placement of botulinum toxin injections may assist in this approach. The therapeutic response may also be improved by appropriately escalating the dose and the distribution of botulinum toxin injections within the targeted muscles

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[11, 12]. Proper counselling of patients is an essential complement to this optimisation process. Patients who are unaware of the potential for optimisation with repeated injections may wrongly perceive botulinum toxin treatment to be ineffective, and thus prematurely discontinue treatment [4, 13].

For patients who have had a suboptimal response to botulinum treatment over a long period, considerations may include immunity to botulinum toxin and complications of cervical dystonia such as muscle contracture and fibrosis [5].

A frequently overlooked component of treatment response is the contribution of non-motor features associated with cervical dystonia. These can include psychiatric disorders such as anxiety and depression, which can degrade quality of life and contribute to a subjective lack of improvement. Psychiatric symptoms appear to occur independently of the severity of motor symptoms, rather than as a consequence of motor symptoms [14, 15, 16]. As such, psychiatric symptoms do not demonstrate response to the treatment of motor symptoms [17, 18]. This suggests that the treatment of psychiatric comorbidities could play an important role in improving quality of life in patients with cervical dystonia.

Tyślerowicz et al. emphasise that a systematic approach is needed to examine the numerous potential contributors to botulinum toxin treatment failure [5]. Specific attention should be paid to addressing modifiable contributors.

It is important to optimise botulinum toxin treatment not only to prevent patient disability and improve quality of life, but also to maximise treatment efficacy prior to pursuing more invasive treatment options such as deep brain stimulation or selective denervation procedures.

In light of the wide array of contributors to botulinum toxin treatment failure outlined by this review, the development of a protocol-driven rehabilitative approach may be beneficial to augment the treatment of cervical dystonia.

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INVITED REVIEW ARTICLE

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Advancing gene therapies, methods, and technologies for Parkinson's Disease and other neurological disorders

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ABSTRACT

Introduction. Vector-based intracerebral gene therapies are being used to treat specific neurodegenerative conditions such as Parkinson's Disease (PD). This review presents a basis for central nervous system (CNS) gene therapy treatments of neurodegenerative diseases such as PD, as well as the need for novel skill sets and health delivery strategies within the clinical neurosciences (neurology and neurosurgery) to meet future demand for such therapies.

State of the art. Preclinical vector-based gene therapy approaches have been translated into clinical trials for PD and other neurodegenerative conditions. Unfortunately, such trials, and parallel efforts using other therapeutics, have yet to provide a breakthrough. Image-guided convection enhanced delivery (CED) optimises the parenchymal distribution of gene therapies applied within the CNS, and may ultimately provide such a breakthrough.

Clinical implications. Currently, image-guided CED and gene therapy are not part of training programmes for most neurosurgeons and neurologists. As a result, few medical centres and hospitals have sufficiently experienced teams to participate in gene transfer clinical trials for PD or other neurological conditions. If CNS gene therapies prove to be efficacious for PD and/or other conditions, the demand for such treatments will overwhelm the available number of experienced clinical neuroscience teams and treatment centres.

Future directions. Expanded indications and demand for CNS gene therapies will require a worldwide educational effort to supplement the training of clinical neuroscience practitioners. Initially, a limited number of Centres of Excellence will need to establish relevant educational training requirements and best practice for such therapeutic approaches. Advanced technologies, including robotics and artificial intelligence, are especially germane in this regard, and will expand the treatment team's capabilities while assisting in the safe and timely care of those afflicted.

Key words: gene therapy, convection-enhanced delivery, Parkinson's Disease, clinical neuroscience education, robotics, artificial intelligence

(*Neurol Neurochir Pol 2020; 54 (3): 220–231*)

Introduction

The application of novel biological and biophysical knowledge to therapeutic and technological innovations in medicine, especially since around 1990, has provided breakthrough interventions for a variety of neurological conditions previously considered to be untreatable or incapable of being completely treated. Historical indirect (systemic) attempts to provide therapeutic molecular compounds to the central nervous system (CNS) have been restricted by the presence of the blood-brain-barrier (BBB). More direct routes of drug delivery, including drug distribution via cerebrospinal fluid (by lumbar puncture, subarachnoid, drug polymer, or intraventricular delivery) is limited by diffusive properties and non-targeted distribution [1–4]. While providing drug to a targeted CNS site, direct surgical placement of

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drug-impregnated polymers for treatment of malignant CNS tumour remains limited by diffusional forces at the polymer edge (i.e. the drug only penetrates tissue millimetres from the polymer edge with a steep drop-off in concentration) and tissue injury at the site of placement.

Recently, the successful development of new molecular agents, device innovations, and improved neurosurgical techniques have helped 'unlock' the CNS to direct delivery via infusion, providing a route for the more effective treatment of specific neurological maladies. Consequently, direct intracerebral delivery strategies to the CNS, ones that circumvent the BBB through intraparenchymal CNS infusions, are being employed. For conditions requiring parenchymal penetration of the therapeutic agent, especially in treating distinct, focal targets, direct intraparenchymal delivery is preferred.

Today, more effective direct CNS intraparenchymal distribution of drugs and biologics is being achieved via convection-enhanced delivery (CED) methods, first developed in the early 1990s [5, 6]. CED uses a constant pressure gradient-dependent bulk flow within the extracellular fluid of the CNS derived from an external pump. This drives both small and large molecular species (including macromolecules and viruses [7]) within the infusate, well beyond the limits of simple diffusion from the site of injection (multiple centimetres rather than millimetres). Bulk flow associated with CED, therefore, transports the infusate homogeneously within a volume of distribution (Vd) that is dependent on the infusion volume (Vi) and the specific tissue characteristics of the target parenchymal volume. CED provides a steep concentration drop at the advancing margin of the convected infusate (Fig. 1).

Such a distribution strategy is ideal for covering a specific parenchymal volume with a homogenous concentration of a therapeutic drug, while limiting such concentrations within the surrounding tissue. Usually, intraparenchymal injection strategies without CED limit their distribution to the end of the injection needle or cannula, within a small cavity, and tend to lose the rest of their Vi to reflux outside the parenchyma, and often into the subarachnoid space. Without or with CED, distribution beyond the infusate margin continues over time, as a result of a constant physiological bulk flow induced by the 'perivascular pump' mechanism [8], as well as by diffusion.

Iterative improvements in convective delivery methodology [9–11], delivery cannula designs [12, 13], the safe use of contrast co-infusions for real-time parenchymal CED visualisations [14–17], and intraoperative magnetic resonance imaging (iMRI) [18], have catalysed the development of this unique intracerebral therapeutic direct delivery platform [17, 19], with initial applications focused on neuro-oncology [20–23] and certain inherited neurometabolic disorders [24, 25]. With advances made in gene therapies for treating specific human neurological conditions [26, 27], and specific neurodegenerative diseases [28–30], the same image-guided CED platform has been increasingly called upon in testing CNS gene therapy strategies, including two ongoing Parkinson's Disease (PD) trials [31, 32].

In this review, we will focus on the implications related to these specific PD gene therapies, and gene therapies utilising similar delivery platforms for other selected neurodegenerative conditions.

State of the art

Although the aetiopathogenesis of most idiopathic neurodegenerative disorders is likely to be due to a combination of genetic predisposition and exposome-induced epigenetic modulation [33], the pathophysiological characteristics associated with the expressed clinical phenotype are well-documented and increasingly understood. Alpha (α)-synucleinopathies are specific neurodegenerative disorders in which aggregates of α-synuclein (α-syn) protein accumulate within neurons, nerve fibres, and glia, with clear evidence of an associated parenchymal cell loss [34]. The three main types of α-synucleinopathies include PD, dementia with diffuse Lewy bodies (DLB), and multiple system atrophy (MSA). An important feature of these three neuropathologies is that differential neuronal and glial susceptibilities appear to dictate the eventual phenotypic manifestations and clinical course. PD has both idiopathic and familial/genetic forms, with the latter including mutations/alterations involving the *SNCA*, *LRRK2*, *VPS-35*, *PARKIN*, or *PINK1* genes, among others [35].

For this review related to gene therapy, we will focus primarily on idiopathic PD treatment strategies, and introduce recent considerations for MSA. Unfortunately, the primarily genetic forms of PD have yet to be tested in CNS gene therapy trials.

The idiopathic form of PD is rare before the age of 50, its incidence and prevalence progressively increasing after the age of 60 and peaking around the age of 85, with a male:female ratio of 1.4:1.0 [36]. Nearly 1 million individuals live with PD in the United States (US) in 2020, and there are at least 8-10 million worldwide. Before diagnosis, prodromal PD patients will suffer from a variety of non-motor impairments, including loss or reduction in olfaction, sleep disturbances, constipation, urinary dysfunction, orthostatic hypotension, and depression [37]. At the onset of clinical PD, findings include asymmetric motor disturbances, usually tremor or hyper-rigidity, that gradually evolve to include the cardinal signs of TRAP (Tremor, Rigidity, Akinesia, and Postural instability) with disease progression.

Approved treatments for PD have varied over the years but include both medical and surgical interventions, including but not limited to the use of levodopa (L-dopa), dopamine agonists, stereotactic brain lesioning, and deep brain stimulation (DBS). Most recently, trials testing an anti-α-syn antibody have been initiated [38]. While DBS has provided a rational and efficacious symptomatic treatment option for TRAP-related clinical features, the use of DBS has been limited due in part to associated risks and complexities of surgical implantation,

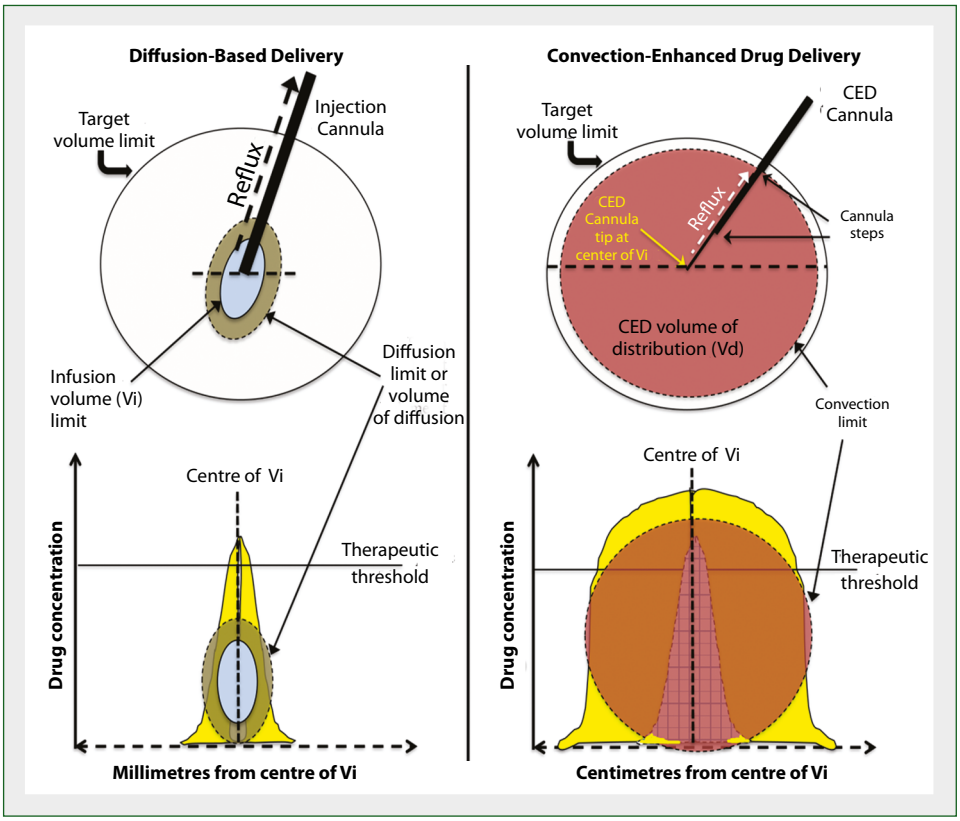

Figure 1. Schematic differences between diffusion-based and convection-enhanced delivery methods within brain parenchyma. Both methods are currently being used to provide therapeutic agents to specific target volumes within the brain.
Left: Diffusion-based drug delivery within brain parenchyma. The top left schematic depicts a typical relatively large bore injection cannula that allows rapid injection of infusate but is associated with relatively large and uncontrolled reflux along the cannula resulting from transient increase in local tissue pressure. The reflux limits the distribution (blue) of the infusion volume (Vi) within the target. Through diffusion (several millimetres), the relatively small residual Vi retained within the target (blue) is able to marginally increase the total brain volume perfused by infusate (grey). Note the depicted insufficient coverage of the target volume. The dashed black line across the diffusion volume, at the tip of the injection cannula, represents the bottom left concentration schematic for diffusion-based delivery. The bottom left schematic portrays the therapeutic concentration of infusate (yellow area under the curve) based on distance from centre of Vi, with an overlay of the limits of Vi (blue) and diffusion (grey). Note the steep concentration drop to sub-therapeutic threshold levels within short distances from the centre of Vi.
Right: Convection-enhanced delivery (CED) within brain parenchyma. The top right schematic portrays CED's smaller bore, reflux-resistant, stepped cannula. The steps on the cannula limit reflux along the cannula track and improve distribution of infusate within the extracellular fluid spaces via bulk flow. Although reflux and distribution beyond the first step, proximal to the cannula tip, allows greater coverage of the target volume, the second step typically prevents reflux along the cannula outside the target. Extracellular bulk flow induced by CED of the Vi provides a significantly larger volume of distribution (Vd) than via diffusion. The dashed black line along the Vd, at the tip of the CED cannula, represents the bottom right concentration schematic for CED-based drug delivery. As depicted in the bottom right schematic, the therapeutic concentration of infusate (yellow area under the curve) with CED extends for centimetres from the centre of Vi, providing a larger, more homogenous concentration of the therapeutic agent within the Vi, up to the convection limit. To contrast the two delivery methods, the diffusion concentration curve is also depicted

device programming, and hardware/maintenance-related complications. In general, PD has a clinical course that extends over many decades. Effective therapeutic approaches differ based on the stage of disease progression.

Within the brains of PD patients, the accumulation of aggregated α-syn protein occurs within at-risk nigrostriatal dopaminergic neurons (DANs) and is exemplified by the progressive loss of striatal (primarily putaminal) dopaminergic (DAergic) neurotransmission, dopamine (DA) levels, an imbalance in inhibitory and excitatory signalling to and from the striatum, and the eventual manifestation of the cardinal signs, with worsening disability over time [39]. Early nigrostriatal degeneration features loss of terminal DAergic dendrites and synapses, especially within the dorsolateral putamen, and progressive dysfunction and death of DAergic cell bodies (and their axons) within the substantia nigra pars compacta (SNpc), and to a lesser extent within the adjacent ventral tegmental area (VTA) [40].

Up to 25% of all DANs within the SNpc are estimated to be lost during the five years prior to the clinical onset of PD motor features, with additional exponential losses (toward ~80% DAN loss) over the subsequent 15–25 years [41]. PD features significant neurodegeneration within other important brainstem nuclei, including the dorsal motor nucleus of the vagus, the pedunculopontine nucleus, locus coeruleus, and raphe [42]. Associated noradrenergic and serotonergic losses, together with reduced DAergic expression in cerebrocortical regions (from VTA DAN losses), are likely to impact sleep, mood, and cognition in PD patients. Importantly, however, the direct and indirect striatonigral pathways, originating from medium spiny neurons of the putamen, appear to remain intact in PD [40], and play a critical role in specific gene therapy strategies, as described below.

MSA is a rare α-synucleinopathy and neurodegenerative disease characterised by clinically progressive combinations of dysautonomia, pyramidal signs, parkinsonism and/or cerebellar dysfunction. With a prevalence of 3–5 cases per 100,000 population (a total of up to 16,500), MSA meets the criteria of being an orphan disease (a condition affecting < 200,000 individuals) within the US. The clinical features at presentation are variable, but most MSA patients are categorised by predominant motor signs and segregated into either parkinsonian (MSA-P) or cerebellar (MSA-C) subtypes. Variability within, and overlap between, these two phenotypes is not uncommon, particularly in the later stages of the disease [43]. MSA-P is more prevalent in Western countries, accounting for 60–80% of MSA cases, whereas MSA-C predominates in Japan [43, 44] and the rest of Asia. The rate of disease progression, from the onset of motor signs, is relatively rapid in MSA cases, with the development of major disability within 3–5 years, death within 8–10 years, and individuals rarely surviving 15 years from diagnosis [45, 46]. Dysautonomia is an early and pervasive characteristic of both MSA subtypes, often appearing years before the onset of the motor dysfunction [47], and contributing significantly to the progressive disability.

Severe and early-onset of dysautonomia is a predictor of an aggressive disease phenotype [48] and a poorer prognosis [43]. With dysautonomia, MSA patients commonly experience genitourinary, gastrointestinal, and thermoregulatory dysfunctions, all of which have limited therapeutic options. Sleep disturbances are also prevalent in both MSA subtypes, especially rapid eye movement (REM) sleep behavioural disorder, occurring in 70–80% of MSA patients but in only 50% of idiopathic PD patients [46]. Like PD, the aetiopathogenesis of MSA remains unclear, despite disease severity being directly related to the extent and burden of α-syn accumulations within glial (oligodendroglial) cells. Glial cytoplasmic inclusions (GCIs), composed of aggregated α-syn and phosphorylated α-syn [49, 50], are found within affected oligodendroglia. Concentrations of GCIs are especially profound within the putamen and substantia nigra of MSA patients [49, 51–53]. One proposed mechanism for the associated neurodegeneration seen in MSA is the loss of neurotrophic support of central catecholaminergic neurons, as evidenced by significant loss of GDNF protein in the frontal cortex and cerebellum [54], as well as within the putamen [55]. Oligodendroglia are a key source of growth factors [56], including the production of GDNF, which is critical for the maintenance of adult catecholaminergic neurons [57]. Putaminal levels of GDNF and DA are significantly reduced in autopsied MSA patients compared to controls [55]. Such reduced GDNF levels may either result from striatal GCIs impairing the function of intrinsic neuronal populations known to produce GDNF, or from a primary loss of GDNF production within the striatum that leads to the focal accumulation of GCIs. The nigrostriatal pathway physiology is significantly altered under both proposed mechanisms, with a reduction in striatal GDNF and its trophic influence on DAN terminals. In either case, the DAergic dendrites within the putamen respond to the low intrinsic levels of GDNF by becoming 'sick but not dead' [55], reducing local DA levels, until eventually degenerating due to lack of trophic support. Under such circumstances, nigrostriatal DAergic axons undergo similar responses to reduced striatal or local oligodendroglial GDNF production.

Finally, nigral DAergic neuronal somata may be negatively influenced by lack of local oligodendroglial GDNF support and/or the negative influences provided by degenerating axon terminals within the putamen. Such nigrostriatal dysfunction is believed to result in prominent parkinsonian clinical features, as noted especially in MSA-P. The loss of oligodendroglial neurotrophic support resulting from GCIs may not only suppress nigrostriatal DAergic function, therefore, but may negatively influence other central catecholaminergic networks (noradrenergic, serotonergic), providing a basis for the evolving dysautonomia. There are currently no specific treatment options for MSA other than those directed toward temporary symptomatic relief.

Methods for replenishing the brain's neurotrophic environment in PD with direct GDNF protein delivery have been translated to the clinic, since systemic administration was unsuccessful in crossing the BBB. Initial investigations featured the use of serial, monthly intracerebroventricular infusions of recombinant GDNF protein (rGDNF) of varying doses via an implanted catheter system [58]. This randomised, double-blind, placebo-controlled trial, evaluating escalating doses of rGDNF versus placebo, in 50 subjects treated for eight months, showed no therapeutic efficacy. Probably, this lack of efficacy resulted from rGDNF not reaching the intended target structures (putamen and/or substantia nigra) in effective levels when delivered within the CSF, and as shown in other intracerebroventricular protein infusion studies [1], while activity of rGDNF was determined in subjects experiencing adverse events, especially at higher protein doses delivered. Almost concurrently, initial intraparenchymal rGDNF infusions for PD were tested [59]. Intraputaminal stereotactic delivery cannulas

delivered rGDNF continuously (0.01 μg rGDNF/μL infusate; 10.8-14.4 μg rGDNF/putamen/day) over 12 months [60], or via an intermittent delivery protocol (0.02 μg rGDNF/μL infusate; 120 μg rGDNF/putamen/4 weeks) for 40 weeks [61]. Although the safety and tolerability of the delivered rGDNF within the brain parenchyma was confirmed, and early signs of efficacy were suggested, a more recent single-centre, randomised, double-blind, placebo-controlled trial and trial extension failed to meet the primary trial endpoint [61, 62]. The authors interpreted their results as suggesting a greater likelihood of rGDNF infusion efficacy in a proposed intermittent (single dose delivered every four weeks) delivery Phase 3 trial, by extending treatment and clinical assessment from baseline to 80 weeks, and providing a higher rGDNF dose (up to 0.06 μg GDNF protein/μL infusate) [62].

The problem of demonstrating the efficacy of GNDF protein infusion in PD trials over nearly two decades has resulted in pessimism directed towards additional therapeutic investigations utilising GDNF for the treatment of PD. Despite the proposed changes to the protocol for their forthcoming rGDNF Phase 3 trial, we believe there might be other technical issues to consider, based on the described delivery protocol [61], that might ultimately affect the trial's efficacy.

First and foremost, the delivery of rGDNF within the putamen (and CNS), using either continuous or intermittent infusion methods, has yet to be confirmed using contrast co-infusion and real-time MRI. Without such confirmation, the degree of on-target distribution and cannula reflux cannot be accurately predicted. Importantly, a reflux-resistant cannula was not utilised in any of the rGDNF infusion studies to date. Although utilising 2mM gadolinium test infusions, and CED infusion rates [9], there is a lack of data confirming that the cannula systems and pumps used actually provide convective flow and distribution [60–62]. Additionally, intermittent intraparenchymal delivery of rGDNF is known to provide concentration peaks and an exponential drop-off to below baseline levels within days to a few weeks following administration [63]. A 4-week delivery strategy for rGDNF would be associated with significant periods of time with subthreshold neurotrophic levels. Finally, we and others strongly believe that effective Vd is highly dependent on the Vi delivered, especially when using CED. Based on known human putaminal volumes [64], the percentage coverage of the estimated putaminal volume, following a known Vi (in gene therapies and rGDNF infusion studies), with and without CED ([31, 32, 65–68], and clinicaltrials.gov: NCT00985517, NCT01621581, NCT01973543, NCT02418598, NCT03065192), suggest a Vi of ≥ 1,800 μL is required to achieve a ≥ 50% putaminal Vd (Fig. 2). Unless abnormal brain anatomy and physiology are present [69], such a Vi, if convected over 4–7 hours, has been well tolerated [31].

There is growing clinical evidence of viral vector-based gene therapy approaches for delivering beneficial transgenes to the brains of PD patients [70, 71]. After being effectively developed in animal models and eventually translated to the clinic, two primary viral vectors have proved most useful and safe, based on either the adeno-associated virus (AAV) [72] or lentivirus (Lenti) [73]. The AAV serotype 2 (AAV2) has a preferential affinity to neurons [15], raising its relevance when targeting putamen and other neuronal populations in the CNS, and limiting transduction of glia and other non-neuronal cells. Lenti vector tropism can be specifically engineered through pseudotyping strategies [74]. Both AAV2 and Lenti vectors have been employed to deliver relevant genetic payloads for treating PD to the human putamen. Small- and medium-sized spiny GABAergic neurons, making up 95% of putaminal neuronal populations [75], are the primary targets of transduction for both AAV2 and Lenti vectors delivered within that subcortical structure. These neuron populations are not typically associated with degeneration in PD, but are affected in MSA-P where caudal and dorsolateral populations of putaminal medium spiny neurons are severely depleted [76]. AAV2 has also been used in PD trials in attempts to influence the subthalamic nucleus, caudate, and substantia nigra [70, 71]. Currently active putaminal enzyme replacement strategies for PD utilise either AAV2 or Lenti, based on the size of the genetic payload being delivered. AAV vectors are most effective in packaging single stranded transgenes of less than 5 kilobases (kb) [77], approximating the size of the wild type viral genome, while Lenti vectors permit packaging of nearly double the payload of AAV [78]. PD trials replacing depleted aromatic amino acid decarboxylase (AADC) alone have utilised AAV2 constructs (AAV2-AADC) [31, 66, 79, 80], while those using Lenti vectors have delivered a trio of transgenes coding for AADC, tyrosine hydroxylase (TH) and GTP-cyclohydrolase 1 (GCH1) to augment dopamine production [81]. Both approaches have focused on replacing lost dopamine production capacity seen in the latter stages of PD and allowing titration of putaminal dopamine levels by altering the amount of precursor medication (L-dopa) taken. Nonhuman primate parkinsonian models have shown strong AADC expression in transduced putamen, with 10-to-20- fold improvements in behavioural responses to L-dopa medication [82, 83]. Since inception, the AAV2-AADC gene therapy for PD has primarily utilised our iMRI CED platform to gradually increase the delivered Vi to enhance putaminal transduction volume [31, 80], and is now delivering up to 1,800 μL per putamen. Using the same AAV2-AADC therapeutic delivered via a larger Vi alone (see Fig. 2G *vs* Fig. 2H), was associated with improved clinical results [31]. Similar iterations and detailed assessments of the Lenti tricistronic vector deliveries are not yet available [81]. Both strategies are progressing towards later stage investigations for efficacy, having shown safety and tolerability over many years.

A PD gene therapy abandoned in 2012, that attempted to suppress subthalamic nucleus (STN) activity with an AAV2 vector carrying the glutamic acid decarboxylase (GAD) gene (AAV2-GAD), provided clinical benefits that were no

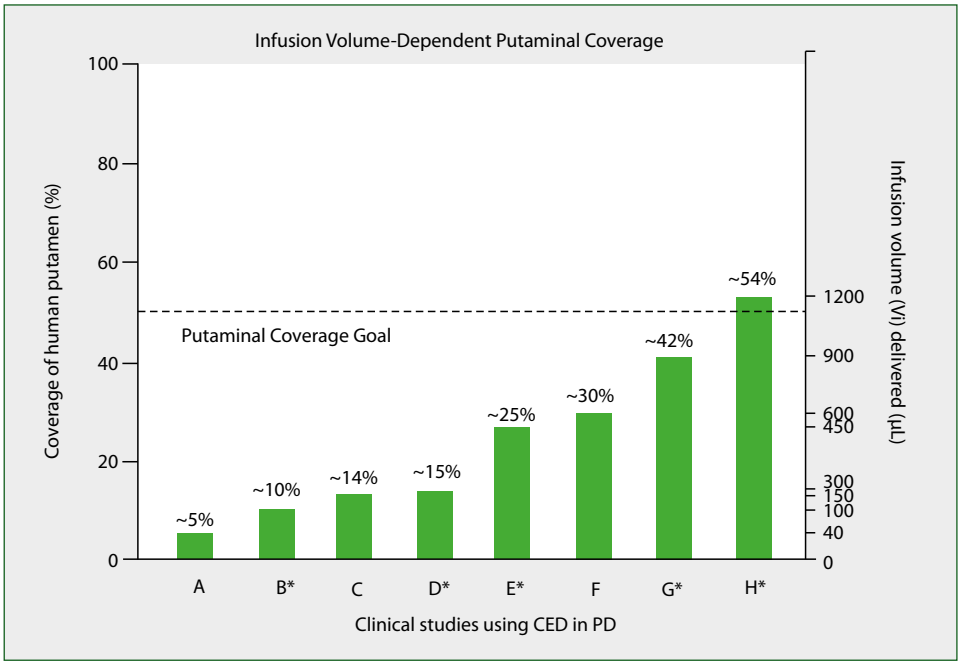

Figure 2. Infusion volume (Vi)-dependent putaminal coverage. This bar chart represents collected data from past putaminal infusion investigations, without or with CED, as described in the text. Left vertical axis presents the estimated volumetric putaminal coverage determined from a particular study, as a percentage of normal human putaminal volume (see [64]). Putaminal coverage percentage for each study is shown above the individual bar graphs. The right vertical axis presents the reported Vi in microlitres (µL). Labels A-H on the horizontal axis correspond to depicted data from a publication, clinicaltrials.gov site, or a scientific presentation. An asterisk (*) beside the letter indicates reported use of a CED platform, including contrast co-infusion and real-time iMRI monitoring. A) from [67] – 2010 AAV2-NRTN trial report; B) from [66] – 2012 AAV2-AADC trial report, and NCT02418598; C) from [67, 68] – 2013 and 2020 AAV2-NRTN trial reports, and NCT00985517; D) from AAV2-AADC infusion trial – NCT02418598; E) from [32] – 2019 AAV2-GDNF trial report, NCT01621581 and NCT01973543; F) from [61, 62] – 2019 rGDNF intermittent infusion trial reports; G) from AAV2-AADC infusion trial – NCT03065192; H) from [31] 2019 AAV2-AADC infusion trial report.

CED — convection-enhanced delivery; iMRI — intraoperative magnetic resonance imaging; AAV2 — adeno-associated virus, serotype 2; NRTN — neurturin; AAV2-NRTN — AAV2 vector carrying the NRTN gene; AADC — aromatic amino acid decarboxylase; AAV2-AADC — AAV2 vector carrying the AADC gene; NCT — national clinical trials number; GDNF — glial cell-derived neurotrophic factor; AAV2-GDNF — AAV2 vector carrying the GDNF gene; rGDNF — recombinant GDNF protein

better than DBS [84, 85]. A recent analysis of brain scan data from these trials [86], however, has encouraged a company to acquire the rights to this therapeutic approach. Unfortunately, significant limitations exist in terms of the number of subjects analysed. This approach will probably require a costly Phase 3 trial to provide evidence of efficacy.

Two neurotrophic factor gene therapies delivered via AAV2 vectors have provided either neurturin (AAV2-NRTN) or glial cell-line derived neurotrophic factor (AAV2-GDNF) to subjects with PD. Additional AAV2-NRTN trials have been abandoned due to failure to meet primary endpoints in two double-blind placebo-controlled studies, both of which targeted the bilateral putamen and one of which also infused the substantia nigra [65, 87]. Importantly, the AAV2-NRTN trials confirmed the safety of delivering growth factors to the putamen and substantia nigra [88], pathologically confirmed transduction limits using small parenchymal delivered Vi, especially without CED and optimised delivery technologies [68, 89–91], and suggested evidence that neurotrophic factor gene therapy may be more effective in earlier than later PD stages [87, 92]. The remaining neurotrophic factor gene therapy trial results in advanced PD, using AAV2-GDNF in a Phase 1 open-label study (NCT01621581), were recently published [32]. All treated subjects tolerated their iMRI CED procedure and three escalating vector doses without significant adverse events. Importantly, all 13 subjects treated are now over 36 months and some over 60 months post-op, and show a) stability of their clinical motor exams and activities of daily living, b) stability of their levodopa-equivalent daily doses, and c) significant increases in their fluorodopa positron emission tomography (PET) imaging signals at the infusion sites, when comparing baseline to the 6- and 18-month treatment intervals. The study had a Vi of 450 µL per putaminal target and was determined by iMRI to provide an average of 26% coverage. An upcoming Phase 1b trial (NCT04167540) for 12 PD subjects, due to start in 2020, will test lessons learned from previous

gene therapy investigations. The open-label Phase 1b trial will test earlier stage (n = 6) compared to moderate to advanced stages (n = 6) of PD, anticipating greater potential efficacy for GNDF effects in the former group compared to the latter, based on residual nigrostriatal DANs [41], and preclinical [93] and clinical [87, 92] opinions advanced. This trial will also test the highest ever approved dose of AAV2-GDNF vector, not tested in the Phase 1 trial due to limited putaminal coverage (26%) attained using that protocol, and distributing a similar Vi (up to 1,800 µL per putamen) with surgical methods (single occipital longitudinal trajectory spanning each putamen) [11, 94] currently preferred in the AAV2-AADC gene therapy study (NCT03733496) [31]. As mentioned earlier in this review, the specific AAV2-GDNF vector delivered to the putamen in previous and current PD trials avails itself of the persistent striatonigral projections and anterograde transport provided by that particular AAV2 vector. Such capabilities allow GDNF trophic support to the at-risk putaminal DAN terminals as well as the DAergic somata that are 'sick but not dead' within the SNpc. The more residual nigrostriatal DANs present, the more robust the GDNF-induced upregulation and sprouting [93].

Clinical implications

There are specific clinical implications related to novel treatments for neurodegenerative diseases such as PD and MSA, as well as a hereditary paediatric orphan disease with a genetic defect tied to catecholaminergic deficiencies (AADC-deficiency; AADC-d) [95]. AAV2-AADC gene therapy for PD is on track to provide therapeutic relief and mitigation of specific clinical features in subjects that would also be candidates for DBS surgery. Both AAV2-AADC and DBS, however, are not likely to influence the progressively downhill course of PD's nigrostriatal degeneration and mounting therapeutic sequelae that increase disability and shorten the lifespan of those afflicted. AADC gene therapy, however, has provided evidence of the ability to restore DA production, and some clinical benefit, when coupled to peripheral substrate (L-dopa) administration. The utility of AADC gene therapy in PD has encouraged use in AADC-d, where widespread loss of central and peripheral catecholaminergic production due to a genetic defect results in loss of intracellular AADC enzyme function and significantly impaired newborns, children and a few adults. AAV2-AADC gene therapy is currently being tested for AADC-d by two treatment teams, with one team targeting the putamen ([96]; NCT01395641 and NCT02926066) and the other focusing on the midbrain (NCT02852213). Both approaches are currently trying to restore DAergic tone in the afflicted children, and results remain preliminary at this point.

However, it is clear that additional measures will be required to replenish additional catecholaminergic centres within the CNS affected by this genetic defect. With regards to the upcoming PD trial set to test intermittent parenchymal delivery of higher doses of rGDNF over a longer time period, we anticipate limited efficacy due to: 1) a lack of documented infusate distribution and infusion reflux assessment by contrast co-infusion and real-time MRI; 2) the rapid exponential loss of infused rGDNF from the parenchyma resulting in subthreshold GDNF levels shortly after infusion and for a significant period prior to the subsequent (every four weeks) treatment; 3) the inability of rGDNF infused within the striatum to influence distal targets such as the SNpc DAergic somata; and 4) the predisposition to infection and mechanical problems associated with the required indwelling devices currently necessary for such chronic treatments. We project that using a single putaminal AAV2-GDNF gene therapy treatment for PD may obviate the need for chronic infusions of rGDNF. AAV2-GDNF gene therapy, using the verified delivery platform, has the capacity to restore constitutive, higher than baseline, levels of parenchymal GDNF within the striatum, and through striatonigral anterograde transport of the AAV2-GDNF vector, to SNpc, as defined in animal models [63, 93, 97].

We expect that a single gene therapy procedure should provide life-long transduction of target cells, and parenchymal trophic factor expression, as has been noted over at least eight years in both non-human primates [97] and humans [68]. In an alternative to antibody-based α-syn clearance for human PD [38], rodent investigations [98] suggest that AAV2-GDNF vector reduced the α-syn deposit burden in substantia nigra of aged mice, signifying that gene therapy-derived GDNF might mitigate the underlying neuropathology seen in synucleinopathies like PD and MSA. Although MSA-P has yet to be treated, therapeutic intervention using AAV2-GDNF gene therapy is currently being considered. Although the parkinsonian motor features in PD and MSA-P may benefit from AAV2-GDNF putaminal gene therapy, it remains to be seen whether the many debilitating non-motor features will also show relevant responses to treatment.

Current and future investigations will include assessment tools to determine beneficial responses in specific non-motor conditions.

Future directions

The variabilities inherent within human investigations in clinical medicine make predicting success for any novel therapy a foolhardy undertaking.

With humility, however, we anticipate that efficacious GDNF gene therapy clinical trial results, for either or both PD and MSA, will significantly increase demand for such treatments worldwide. We and others [99] believe, however, that improving gene therapy vector delivery within the CNS remains essential for proper testing of therapeutic efficacy. Optimised delivery strategies, including CED contrast co-infusions, real-time iMRI, and using increased Vi, provide safe and effective distribution methodologies, but allow the

confirmation of target coverage and off-target distribution that may impact upon clinical results.

Current neurosurgical manpower is less than 4,000 in the US [100] and approaches 50,000 worldwide [101], with markedly fewer individuals experienced in the methods and technologies described in this review. Addressing this shortfall will require the expansion of educational/training opportunities for current neurosurgeons (and neurologists), and the augmentation of tailored training programmes related to specific surgical and neurological management capabilities. Although both neurosurgeons and neurologists currently train in a variety of neuromodulation approaches, only a limited number of specialty training programmes provide trainees with the experience required to deliver safe and effective CED therapeutic interventions. Gene therapy methods to treat specific CNS disorders continue to evolve, with an emphasis on a) training and sufficient numbers of clinical providers, b) being guided by evidence-based medicine, and c) considering the development of Centres of Excellence to specifically advance such treatment opportunities. Such advances will be critical to the safe, efficacious, and standardised evolution of CNS gene therapies.

The future of effective gene therapy treatments for PD, MSA-P, and other CNS conditions, will feature CED platforms that avail themselves of growing iMRI capabilities in medical universities and hospitals. Supplements to the future neurosurgical armamentarium in meeting increased demand for such treatments will feature robotics and advanced technologies in the operating room [102–104] and artificial intelligence (AI) [105, 106] to improve the practitioner's accuracy and speed of clinical diagnoses, presurgical and surgical planning, outcome predictions, and overall management.

We await the results of forthcoming clinical trials to implement these and other strategies, focusing on safety and efficacy measures as we seek to improve the clinical trajectories in neurodegenerative and other CNS diseases with gene therapies.

Ethical permissions: *Ethical approval was not required for this invited review article.*

Conflicts of interest: *Massimo S. Fiandaca is a scientific co-founder and Vice President, Clinical Affairs for Brain Neurotherapy Bio Inc. (BNB), a biotechnology company established to advance direct brain delivery strategies, technologies, and gene therapies for neurological diseases. BNB and Dr. Fiandaca are sponsoring a Parkinson's Disease (PD) Phase 1b gene therapy clinical trial testing AAV2-GDNF at two sites: Ohio State University (OSU) and the University of California San Francisco (UCSF). Dr. Fiandaca denies other potential conflicts of interest.*

Russell R. Lonser is a scientific co-founder of BNB. He is Chairman of the Department of Neurological Surgery at OSU and oversees all neurosurgical care at that institution, including the BNB-sponsored PD Phase 1b gene therapy clinical trial testing AAV2-GDNF. Dr. Lonser is co-inventor of a patent for imaging delivery of therapeutic agents in the central nervous system (CNS). He has been a neurosurgical consultant at the Interventional Neuro Centre (INC) Bródno Mazovia Hospital, Warsaw, Poland. Dr. Lonser denies any other potential conflicts of interest.

J. Bradley Elder is an OSU Department of Neurological Surgery faculty member and principal investigator for the PD Phase 1b gene therapy clinical trial testing AAV2-GDNF. Dr. Elder has been a neurosurgical consultant at the INC Bródno Mazovia Hospital, Warsaw, Poland. Dr. Elder denies any other potential conflicts of interest.

Miroslaw Zabek is co-founder of the INC Bródno Mazovia Hospital, Warsaw, Poland and serves as Chairman of Neurosurgery at that institution. Along with Dr. Bankiewicz, he has been an active clinical investigator of a variety of neurosurgical procedures introduced to the INC Bródno, including DBS for PD and other movement disorders, and the compassionate treatment of AADC deficiency subjects using AADC gene therapy via convection-enhanced delivery (CED). Dr. Zabek has recently gained ethics committee approval at the INC Bródno for the compassionate treatment of subjects with MSA using GDNF gene therapy. Dr. Zabek denies any other potential conflicts of interest.

Krystof S. Bankiewicz is founder, Chairman, and Chief Executive Officer of BNB, in addition to co-founding the INCs at UCSF and the Bródno Hospital. Dr. Bankiewicz has also co-founded other therapeutic brain delivery companies, including MedGenesis Therapeutix (advancing rGDNF infusion) and Voyager Therapeutics (advancing AADC gene therapy for PD). He has multiple patents related to direct brain delivery to the CNS, including for gene therapy, as well as for the associated methodologies, delivery devices, and technologies to enhance such therapeutic distributions in a variety of neurological conditions. Dr. Bankiewicz has a funded clinical trial investigating treatment of AADC deficiency subjects using AADC gene therapy at UCSF and OSU. BNB and Dr. Bankiewicz are sponsors of the Parkinson's Disease Phase 1b gene therapy clinical trial testing AAV2-GDNF at OSU and UCSF. Dr. Bankiewicz denies any other potential conflicts of interest.

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Cervical dystonia — improving the effectiveness of botulinum toxin therapy

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ABSTRACT

Introduction. Cervical dystonia is the most frequent form of focal dystonia. It is characterised by involuntary muscular contractions resulting in abnormal head/neck and shoulder movements and postures, which can be associated with tremor and pain. Local intramuscular injections of botulinum toxin type A (BoNT-A) is the treatment of choice, being both effective and well-tolerated. However, a considerable number (c. 30%) of patients discontinue this treatment.

The aim of this review was to analyse the factors possibly responsible for treatment failures of cervical dystonia (CD), with special regard to the new classification known as the 'Col-Cap' concept and non-motor symptoms.

Clinical implications. Several factors analysed in this review are responsible for effective treatment: proper diagnosis of dystonia and exclusion of pseudodystonias, correct recognition of dystonia pattern and identification of new patterns according to the Col-Cap concept, muscle selection and precise injections under electromyography (EMG) and/or ultrasonography (US) guidance. Furthermore, concomitant diagnosis and treatment of non-motor symptoms such as depression, anxiety, fatigue, sleep problems, phobias and stigmatisation are crucial in obtaining the best overall effect of the treatment. Primary and secondary immunisation and non-responsiveness seem to be marginal problems nowadays due to a low potential of new BoNT-A formulations to produce neutralising antibodies.

Future directions. There is a need for new and relevant scales combining the Col-Cap concept patterns with non-motor symptoms and quality of life. There is also a lack of specific rehabilitation protocols which could enhance BoNT-A treatment results.

Key words: cervical dystonia, Col-Cap concept, botulinum toxin, treatment failures, primary non-responsiveness, secondary non-responsiveness

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Introduction

Cervical dystonia (CD) is a movement disorder characterised by involuntary, sustained or intermittent muscle contractions leading to abnormal head movements and/or positioning. It is accompanied by pain in 67–75% of patients [1]. Typical features include sensory trick and head tremors. A substantial number of patients (18–41%) demonstrate complications manifesting in premature degenerative disorder of the cervical spine, discopathy or cervical myelopathy [2]. CD significantly reduces quality of life, affects the ability to work, and socially stigmatises patients [2–3]. Spontaneous remission occurs in up to 15% of patients and is usually temporal [5, 6], but may be triggered by botulinum neurotoxin type A (BoNT-A) treatment [4]. CD is the most common focal dystonia: a meta-analysis reveals that it affects a mean 4.98 of every 100,000 people. However, a significant difference has been observed with regards to geographical location: in Japan the rate is 2.52, whereas in Europe it is 6.71 [5].

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Table 1. Summary of possible causes of non-responsiveness or unsatisfactory effect of Botulinum toxin type A treatment of cervical dystonia

1. **Primary non-responsiveness**
   - preexisting BoNT-A antibodies, chronic exposure to BoNT-A in childhood, cross-reaction of other antibodies (e.g. tetanus toxin)
2. **Secondary non-responsiveness**
   - neutralizing antibodies
   - change in the pattern of dystonia, conditioned by the central mechanism of non-specific muscle activation
3. **Misdiagnosis**
   - dystonia in neurodegenerative diseases (PSP — usually retrocollis, MSA — usually anterocollis)
   - genetic disorders: Wilson's disease, Huntington's disease, spinocerebellar ataxias, neuroacanthocytosis, NBIA
   - dystonia in mitochondrial disorders
   - secondary dystonia caused by identified factors: post-traumatic dystonia, post-stroke dystonia, post-inflammatory dystonia, tardive dystonia, toxin-related dystonia
   - functional dystonia (psychogenic)
   - pseudodystonias:
     a. related to musculoskeletal deformations: camptocormia, scoliosis, Grisel's syndrome, Arnold-Chiari syndrome, Klippel-Feil syndrome, joints deformities, arthrogryposis, Dupuytren's contracture, congenital muscular torticollis, Sandifer's syndrome,
     b. related to the compensation of improper functioning of the central nervous system or peripheral nervous system (compensatory head tilt): vestibular system disorder, oculomotor nerves palsies (6^{th}, 4^{th}), mass lesion in the posterior fossa
     c. disorders of sensory pathways: parietal lobe damage, syringomyelia, myelopathy, mono- and polyneuropathy
     d. disorders of motor pathways: Isaac's syndrome, stiff-person syndrome, tetanus, myotonic disorders, MMN
4. **Misidentification of the subtype of dystonia** (new classification –according Col-Cap concept and new CD patterns with involvement of muscles not routinely injected earlier)
5. **Improper selection of active muscles** (injections of muscles that are not responsible for specific pattern) **and missing the muscles** (no guidance, too short needles, too deep injections missing thin muscle layers, e.g. m. trapezius)
6. **Lack of monitoring techniques of injections: us, emg** (combination is the optimal method as visualization does not mean that muscle is really active)
7. **Improper adjustment of the total dose and its distribution in particular muscles**
8. **Subjective feeling of lack of improvement** (dominating non motor symptoms like depression, anxiety, phobias or sleep problems)
9. **Long-lasting dystonias causing secondary changes like myofibrosis, contractures**
10. **Improper storage and transportation of the medication**

PSP — Progressive Supranuclear Palsy, MSA — Multiple System Atrophy, NBIA — Neurodegeneration with Brain Iron Accumulation, MMN — Multifocal Motor Neuropathy

Treatment of CD with BoNT-A is the treatment of choice as recommended by the American Academy of Neurology (AAN), with high effectiveness and safety profiles [6].

Double-blind, randomised clinical, as well as open, studies have shown that 50–85% of patients demonstrate a significant improvement [7–15]. A considerable number (c. 30%, range 19–46%) of patients discontinue treatment once it appears to be ineffective [16–20]. Treatment may be considered ineffective if neither the patient nor the therapist have observed a satisfactory reduction of symptoms or if significant adverse effects occur (e.g. dysphagia or neck muscles weakness), or when clinical trials observe no significant score reduction in rating scales. Those most commonly used are the Tsui Scale and the Toronto Western Spasmodic Torticollis Rating Scale, TWSTRS.

This article aims to analyse the possible factors responsible for treatment failures, with special regard to the new classification of CD known as the Col-Cap concept and non-motor symptoms.

Making a proper diagnosis

BoNT-A can be effective both in primary and secondary dystonia. However, the latter may require specific treatment to avoid fatal progression and outcome like e.g. Wilson's disease. Therefore, a proper diagnosis is essential. Particular attention should be paid to pseudodystonias that mimic dystonia and do not respond to treatment. Revised definitions and an extended list of pseudodystonic postures was recently presented by Berlot et al. (Tab. 1) [21]. Pseudodystonic postures must be clearly differentiated from conditions related to muscles weakness which can result in a head-drop e.g. myasthenia, muscular dystrophy, and amyotrophic lateral sclerosis. Moreover, pathological anteflexion can be confusing (e.g. anteflexion in multiple system atrophy) because there is no consensus as to whether it is related to dystonia of flexors or myositis and weakness of neck extensors [22]. It has been speculated that neck extensor myopathy might follow mechanical over-stretching from flexor dystonia. There is evidence that even muscles which are not under mechanical stress can develop myopathic changes. Pathologic changes seen in inflammatory myopathies (IBM) have been found in clinically affected paraspinal muscles. This shows the neurodegenerative nature of both IBM and parkinsonism.

Additionally, functional CD may be refractory to BoNT-A treatment. It usually presents as fixed dystonia or is multidirectional, variable with different patterns at the same time, accompanied by enormous effort put into head positioning, with improvement after distraction, and no sensory twitches.

Proper identification of dystonia pattern

One of the most common reasons behind therapy failures seems to be incorrect identification of the clinical pattern of CD. To properly identify the subtype of dystonia, the patient should be examined carefully, not only in a relaxed seated position when their upper body is in a resting state, but also with open and closed eyes (because closing eyes can worsen dystonic posture), standing and/or lying positions, while walking or performing activities such as writing (these can enhance dystonic movements).

By observing the patient from the side, the front, and also from the back, we can assess the change in head and neck position in each of the three planes. Sometimes an objective assessment may be difficult due to the activity of compensating muscles as well as coexistence of tremors and/or myoclonus. We should pay attention to the so-called 'sensory twitches' (as they may change the pattern temporarily), shoulder and scapula positions, and potential muscle hypertrophy and pain. If the arm is elevated, we should assess the patient by stabilising their arms by pushing down the elevated arm. This may reveal its compensating character to maintain the head erect position due to a severe head tilt.

The Col-Cap concept was first set out by Reichel et al. in 2009 [23]. Their careful examination identified new patterns not previously recognised. Initially, CD had been classified into four types. These related to: turning the head (torticollis), tilting the head to one side (laterocollis), backwards (retrocollis), or forwards (anterocollis). More than 50% of cases were diagnosed as torticollis, 10–15% as laterocollis or retrocollis, and less frequently anterocollis [24]. The 'Col-Cap' concept (*collum-caput* = neck-head) was invented on the basis of imaging examinations (CT/MRI of the head, cervical spine and also soft tissues) and functional anatomy. According to this approach, based on the various movements of the head (muscles insertions between skull and C2 spine level) and neck (muscles insertions between C2 and C7 level), 10 major subtypes of CD were identified (Fig. 1): transverse (torticaput/collis), frontal (laterocaput/collis), sagittal with tilting forwards (anterocaput/collis) or backwards (retrocaput/collis) [25, 26]. These distinctive patterns are 'realised' by the activity of different (i.e. different from those in the 'classic four') muscles involved in the particular type. So an inaccurate diagnosis may result in a lack of effect. According to the multicentre study recently published by Jost et al. analysing 306 consecutive patients with CD, pure forms are rare (16.3%), whereas combinations of 2–6 of the subtypes are common (83.7%). Among all the subtypes, the most common primary form is torticaput (49%), and the second most common is laterocaput (16.7%) [27]. One can also distinguish combinations of incorrect positions such as a mixture of laterocollis to one side and laterocaput in the opposite direction; this is known as lateral shift. In addition, we see a combination of anterocollis and retrocaput called anterior sagittal shift, and consequently posterior sagittal shift as a combination of retrocollis and anterocaput.

To identify these new patterns, characteristic points/lines should be identified, which enables levels of movement (collis, caput) to be distinguished. In the case of torsion, the main anatomical structures are: superior thyroid incisure of the larynx and manubrium of the sternum above the jugular incisure. Rotational torticollis is diagnosed if, during rotation, the larynx shifts in relation to the sternum. If these points remain in the same line, but the chin shifts in relation to the larynx, rotational head position is diagnosed (torticaput). In the sagittal plane, the meatus acusticus externus and the clavicula are useful anatomical landmarks. If only the 'head' is concerned, the meatus acusticus stays in line with the clavicula (anterocaput, retrocaput). If the projection is in front or behind the clavicula, the 'neck' level is involved (anerocollis/anterocaput) (Fig. 1) [28]. A treatment protocol differentiating the head and the neck level may result in better outcomes. However, to date this has only been shown in one retrospective study [29].

Based on the Col-Cap concept, 'main' muscles should be injected as the first choice, then 'secondary' in each subtype. In complex patterns, the leading (or primary) one should be first injected and in refractory cases previous injection patterns should be assessed and modified in the subsequent cycle [29].

Correct selection of active muscles and guided injections (US/EMG)

Before the Col-Cap concept, muscles were selected on the basis of functional anatomy, a physical examination accompanied by assessment of muscle hypertrophy, location of pain, or arm elevation. Several published studies used electromyography (EMG) as an injection technique for the identification and localisation of muscles [30–34]. There are studies which strongly support the role of EMG showing that injections performed only according to anatomical landmarks can be imprecise (83% reached the sternocleidomastoid, but only 47% the levator scapule muscle) [35]. Moreover, EMG guidance increases the sensitivity and specificity of the muscle selection, even when performed by BoNT-A specialists. Clinical predictions of individual muscle involvement are only 59% sensitive and 75% specific without EMG use. It has been pointed out that muscular hypertrophy or shoulder elevation indicates 'dystonic' muscle activity in only 70% of patients, showing how a classical physical examination can be confusing [36].

The role of EMG has been supported by randomised, blinded studies which have demonstrated that EMG-guided injections vs. anatomically-based bring significantly better results, measured by rating scales and subjective assessment: 82% (TWSTRS) and 61% (patients assessment) vs. 8% (TWSTRS) and 25% (patient report) respectively [34]. The same was proved in another study, which showed a significant difference in Jankovic scale ($p = 0.05$) between guided and blinded injections [37]. Retrospective analysis confirmed the benefits of

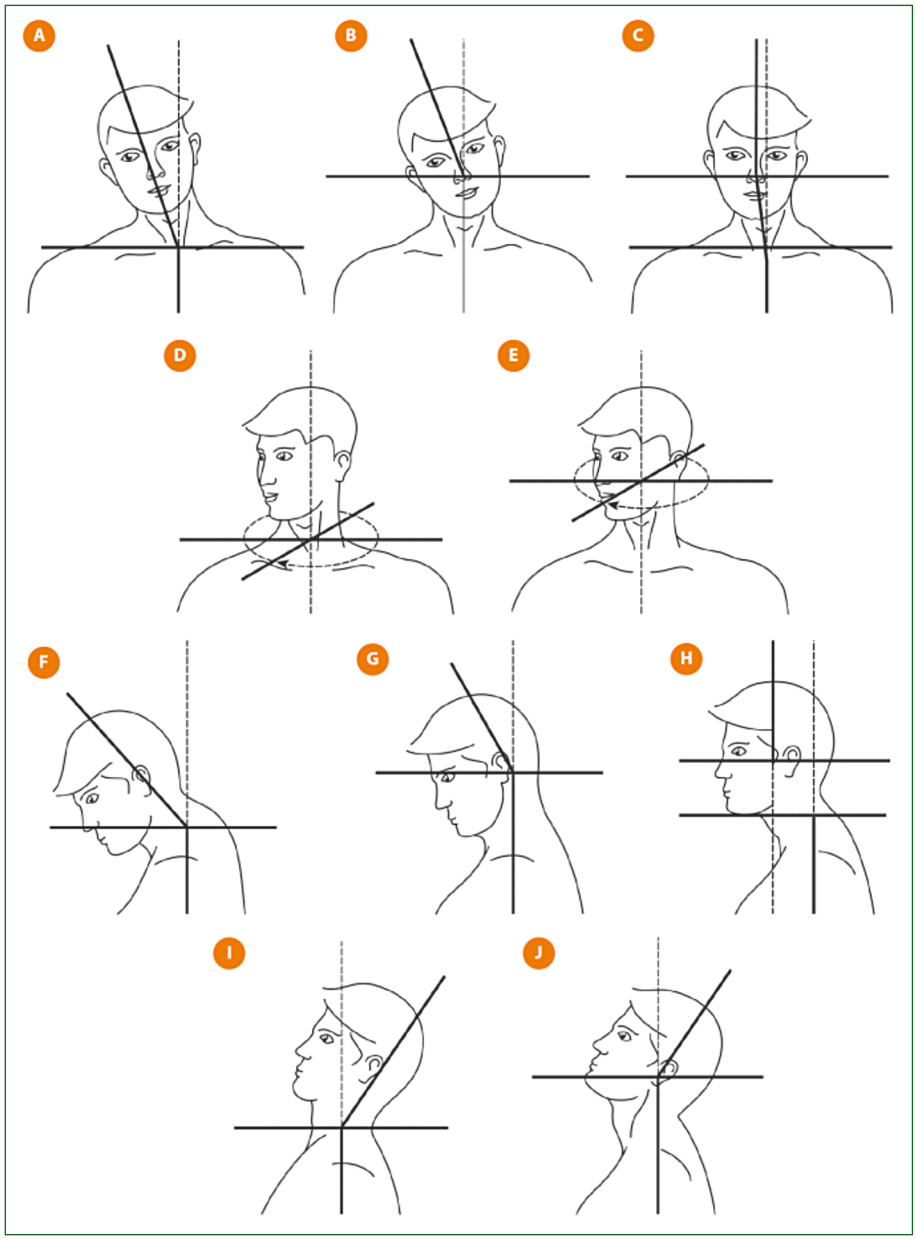

Figure 1. Ten basic clinical patterns of cervical dystonia according to col-cap concept. **A.** Laterocollis, **B.** Laterocaput, **C.** Lateral shift (combination of laterocillis to one and laterocaput to opposite side), **D.** Torticollis, **E.** Torticaput, **F.** Anterocollis, **G.** Anterocaput, **H.** Sagittal-anterior shift (combination of anterocollis and retrocaput), **I.** Retrocollis, **J.** Retrocaput. Additional rare variant may be present (no picture) presenting as Sagittal posterior shift (combination of retrocollis and anterocaput). Courtesy of Via Medica and Jarosław Sławek, Monika Rudzińska eds, In: Toksyna botulinowa w praktyce neurologicznej, Via Medica, Gdańsk 2015, vol. 1, Fig. 2

using polymyography in groups of patients previously treated with BoNT with an unsatisfactory response. After one year of treatment, reasonable or good final results were obtained in 60% of patients measured on both the Tsui Scale ($p < 0.01$) and the subjective assessment of patients ($p < 0.001$) [38]. Subsequently, one small open trial showed that EMG improved the treatment outcome in 9/10 patients, demonstrating a significant improvement in TWSTRS (mean improvement of 64%) [39]. In contrast, there are studies which do not confirm the utility of EMG guided injections. In a systematic review by Nijmeijer et al., the average improvement on the Tsui Scale was greater (31.9 vs. 43.7%) in studies that used only clinical evaluation [40]. But because of profound differences in methodology, dosage, patient characteristics, and primary and secondary endpoints, no statistical analysis could be performed, and no firm conclusions could be drawn.

To sum up, a consensus of experts recommends a combination of clinical assessment and EMG examination as well

as EMG-guided injections in patients treated for the first time (Level A) [34, 41]. But for those who have undergone unsuccessful treatment, EMG usefulness is less proven (Class III studies and Level C) [42, 43].

We must remember that EMG use does not allow us to distinguish between 'dystonic' and compensatory muscle activities or to visualise the tip of the needle (the injector is not sure if the needle is positioned in the intended muscle). Such precise targeting of muscles can be achieved using other methods such as ultrasonography (US) or computed tomography (CT).

Ultrasound is an easily available, non-invasive method enabling the visualisation of muscles and surrounding structures (nerve bundles and large vessels) in real time, which may increase the accuracy of injections of not only deep, but also superficial (sometimes as superficial as for example very flat trapezius) muscles. Studies regarding spasticity treatment with BoNT under US guidance have shown its efficacy in localising especially deep muscles. Recent publications are open studies conducted in small groups of patients or case studies on injections of deeply located muscles where the approach is challenging, such as the longus colli muscle or obliquus capitis inferior [41–44]. One of these studies assessed the impact of monitoring techniques such as US and/or CT in a group of eight patients requiring injections in deep cervical muscles (the obliquus capitis inferior, the longus colli muscle, obliquus capitis superior, scalenus anterior and scalenus posterior). The Tsui Scale confirmed a significant improvement occurring within four weeks (11.75 vs. 1.50) and on the TWSTRS scale in each of the subscales (20.0 vs. 5.25, 20.0 vs. 7.00, and 13.10 vs. 6.50) [48]. A study conducted in a group of five patients to assess the incidence of swallowing problems after injections revealed that an ultrasound examination, carried out in order to locate the EMG needle during injections in the sternocleidomastoid, significantly reduced such adverse effects (0% vs. 34.7%) [45]. Nevertheless, no randomised, controlled studies have proved the greater effectiveness of US-guided versus blinded injections.

Muscles which seem to 'benefit' mostly from US monitoring include: suprahyoid muscles, scalenus muscles, the longissimus capitis and cervicis, semispinalis capitis and cervicis, obliquus capitis inferior (crucial muscles in some col-cap patterns), but also the sternocleidomastoid, levator scapulae, and trapezius (frequently injected too deeply) [46].

It seems that in some cases, particularly with accompanying tremors or after several non-effective treatment attempts, EMG and US methods, applied simultaneously, should be considered [47]. The needle size should also be adjusted because one that is too short will not reach deep muscle layers, although these can be easily detected thanks to US guidance.

Optimal dosage and its distribution in particular muscles

The optimal dose of BoNT-A was obtained in pivotal studies. The recommendations according to SPC (summary product characteristics) are based on these clinical trials. However, dosage should be adjusted in subsequent treatment cycles both in terms of effectiveness and safety. There are studies which indicate that efficacy and the incidence of side effects depend on the BoNT-A dose. A study assessing abobotulinumtoxin A showed that the largest and longest improvements were obtained in the 1,000U group [12]. Nevertheless, the highest number of adverse events also occurred in this group. All groups (placebo, 250U, 500U and 1,000U) demonstrated improvements > 20% on the Tsui Scale after two weeks. However, in week 4 such improvements were still observed only in the 500 U and the 1,000 U groups. In these groups, also compared to the 250U and placebo groups, patients reported > 50% improvement on CGI scale statistically more frequently. Therefore, the experts' recommendations, based on those studies [12, 48], suggest starting treatment by administering 500 U of abobotulinumtoxin. However, lower (200–400 U) doses might be equally effective and safe if precisely administered e.g. under EMG guidance [49]. In clinical practice, the dose should be adjusted to muscle bulk and body mass, although formal studies have not been performed.

There are no randomised, controlled studies comparing the effectiveness of the number of injections per muscle. A comparative study on 49 patients showed that multi-point injections increased treatment effectiveness: they reduced pain ($p < 0.002$), increased the range of motion ($p < 0.001$), and lengthened the duration of effect ($p < 0.001$) [50]. Experts recommend distributing the dose to 1–4 points, depending on the area of the muscle [51].

Subjective feelings of lack of improvement and non-motor symptoms

Patients suffering from cervical dystonia, like those with other movement disorders, present a wide spectrum of non-motor symptoms which have not been considered in clinical trials assessing BoNT-A effectiveness, but may influence the overall result of therapy.

Patients with CD appear to be more aware of having abnormal dyskinetic movements than do patients with Huntington's or Parkinson's Diseases [52]. Non-motor symptoms have been noted in several studies: 61.8% of patients with CD presented lack of self-confidence due to stigmatisation, 59.8% had sleep problems, and 51% fatigue [53]. Depression was prevalent in as many as 47.5% of patients and this was the major determinant of poor quality of life [3]. In another study, poor quality of life was more common in CD than in blepharospasm and writer's cramp and also depression and anxiety were the major correlates [54].

Depression, along with other emotional-psychological disorders, may constitute a clinical spectrum of CD independently of motor symptoms. Mood disturbances coupled with anxiety, also adjustment disorders or obsessive-compulsive behaviours, occur significantly more often in patients with focal dystonias

(57.3% of patients with dystonia, compared to 24.1% of healthy subjects and 34.6% of patients with hemifacial spasm) [55]. Another study showed that patients with CD are much more frequently affected by depression (15–53.4%) and anxiety disorders (26.4– 83.3%), and 4.5 times more often by agoraphobia or panic attacks than the general population. No correlation was found between age, duration of dystonia or its severity, as well as duration of BoNT-A treatment, which may indicate that mental disorders are primary, but not secondary, to dystonia.

A study by Berardelli et al. showed that during a five-year follow up of treatment with BoNT injections, it significantly improved dystonic movements (TWSTRS 33.4 ± 11.1 at baseline, 26.9 ± 10.9 after five years). However, the incidence of neuropsychiatric disorders did not improve at all (65% at baseline, 64% after five years), which suggests an independent mechanism and, possibly, the need for additional treatment [56]. A similar effect was observed in Sławek et al.'s study, showing in a group of 101 patients with CD treated with BoNT-A that size effect for TWSTRS (motor presentation) was significant after treatment: 1.1 (SD ± 0.6), but for depression (Montgomery Åsberg Depression Rating Scale) it was only 0.5 (SD ± 0.7) [4].

Stamelou et al. presented evidence indicating an important non-motor component to primary dystonia, including abnormalities in sensory and perceptual functions, as well as the neuropsychiatric, cognitive and sleep domains [57]. Widespread loss of inhibition and pathologically increased plasticity appear to play important roles in the pathophysiology of primary dystonia [58]. The hypothesis is that non-motor features of dystonia could be explained by a common pathophysiological deficit that also underlies the motor symptoms [59–61]. Genetic susceptibility is the key to the pathophysiology of dystonia, indicated by the numerous non-motor abnormalities that are found in unaffected first-degree relatives of patients with adult-onset focal dystonia and non-manifesting gene mutations carriers. This genetic background may predispose patients to develop dystonia in the presence of other factors that may have important non-motor components, such as repetitive activity, trauma, or emotional distress [61].

Considering the accompanying non-motor disorders, such as pain or a broad spectrum of psychiatric diseases, it seems that analgesics or antidepressants would be beneficial. There are, however no controlled studies supporting such practice. The only randomised, controlled trial conducted recently looked at the efficacy of escitalopram in the treatment of CD with concomitant tremor. It did not reveal any beneficial effects of the drug on either motor or non-motor symptoms; the authors underline however that this should not be a reason for resigning from such therapy [62].

Primary and secondary immunoresistance

Patients who do not respond to the therapy can be classified as those who did not respond at all from the beginning (so-called primary non-responders, PNR), or those who stopped responding to the treatment after a good initial effect (so-called secondary non-responders, SNR).

It is suspected that primary resistance may be associated with preexisting BoNT-A antibodies (AB), chronic exposure to BoNT-A in childhood, or cross-reaction of other AB (tetanus toxin AB) [63, 64]. However, this is only speculation unsupported by studies. Secondary non-responsiveness is defined differently by different authors. Some authors claim that two consecutive ineffective treatment cycles (i.e. no subjective improvement or exacerbation by at least 2 points on the Tsui Scale as well as absence of side effects typical for BoNT-A) which occur after at least two effective cycles in the past (defined as improvement on the Tsui Scale by at least 3 points and/ /or atrophy in injected muscles and/or an occurrence of side effects typical for BoNT-A) are enough to diagnose secondary resistance [18]. Others believe that secondary resistance can be diagnosed only after three consecutive ineffective treatment cycles [65]. One should remember that it may be a pseudoimmunoresistance due to other reasons mentioned earlier, and that the real one should be confirmed in laboratory or clinical tests (see below).

It is estimated that secondary resistance affects approximately 3–5% of patients [66]. Previously the production of AB to BoNT-A was considered the main cause of secondary treatment failure (STF). Some publications indicate that higher BoNT-A doses, administered at shorter intervals and frequent injections (within six weeks of the previous injection, so called booster injections), increase this risk of development of AB [67, 68]. Most of the previous studies reporting AB in groups of CD patients were unbiased regarding STF and had a short duration. Therefore, AB rates reported in these studies more or less represent the incidence of AB induction during 1-2 years of therapy and the range is between 0.5% and 2.0% [69–71]. In long-term treated patients with CD, the prevalence of AB is higher, indicating 14% in the group of patients still responding to therapy who underwent BoNT-A injections over 10 years [72]. Another study showed that duration of treatment of ~15 years carries a risk of up to 40% of becoming AB positive, not influencing the treatment result. In addition, the study showed that single dose per session and BoNT-A formulation were the most significant factors influencing AB formation [73].

Furthermore, the amount of complexing proteins, which differs in the three most commonly used preparations, plays an essential role. Antibodies were found in 9.5% of patients treated only with original onabotulinumtoxinA with a large amount of complexing proteins (100 U/25 ng protein), whereas AB were not detected in any patients treated exclusively with the new preparation of onabotulinumtoxinA (100U/5 ng protein) marketed more than 20 years ago [74]. Antibodies were not found in patients treated with incobotulinumtoxinA, which does not contain complexing proteins [75, 76].

Neutralising antibody titre decreases after discontinuation of therapy. The rate of decline differs for each individual, and

the decline can last for up to four years. Minimum 12-week intervals between injections are still advisable. However, some studies on incobotulinumtoxinA reveal that shorter intervals are also safe and do not produce antibodies [19].

In summary, studies show that AB and their titre do not necessarily contribute to secondary resistance; subsequent studies revealed that antibodies are found in approximately 50% of patients with secondary resistance [77, 78] and the reasons for this situation possibly differ.

According to some studies, secondary resistance could be associated with a higher dose of BoNT-A, administration of other therapies (rehabilitation, pharmacological treatment), a significant number of side effects, and more frequent interruptions in BoNT-A treatment [79].

The lack of response due to the neutralising antibodies formation may be diagnosed with specific tests. MPA (mouse protection assay), HDA (hemidiaphragm assay (HDA), and new enzyme-linked immunosorbent assay (ELISA) are currently available. According to the latest reports, a combination of ELISA and HDA is a quick method characterised by the highest sensitivity and its price is reasonable. For 100% sensitivity its specificity is 90%, and for 100% specificity its sensitivity is 55%. Sensitivity of previously used tests, such as MPA or WBA (western blot assay) was lower and ranged from 33% to 53% [80].

Instead of laboratory tests, in clinical practice we can use simple, objective tests involving a unilateral application of BoNT-A in the frontal muscle [74] or in the extensor digitorum brevis muscle. The frontal test is performed by administering 30 units of aboBotulinumtoxinA or 10 units of ona/incobotulinumtoxinA [71] in the frontalis muscle on one side. Clinical assessment (ability to raise eyebrows) is recommended after 2–4 weeks. Asymmetry indicates that BoNT-A is effective [43]. For an extensor digitorum brevis muscle test we administer 100 U of abo- or 20 units of onabotulinumtoxinA in this muscle and assess the compound muscle action potential response (CMAP) by stimulating the peroneal nerve at baseline and two weeks after injection (CMAP should more than halve compared to its original value). For a decline of up to 20%, immunoresistance is quite probable; for values of 20–50%, the result is doubtful [81].

In patients with immunoresistance, we can discontinue injections for 12–18 months and observe the patient at regular intervals. If dystonia significantly reduces quality of life, an alternative is re-administration of BoNT-A. Or one may consider the use of botulinum toxin B, which is safe and effective in the treatment of cervical dystonia, but presents high immunogenicity potential, in particular in patients already resistant to BoNT-A treatment [82, 83].

Intrinsic muscle changes and change of dystonia pattern

Muscle fibrosis and contractures, being the result of long-lasting disease as well as a change in the pattern of dystonia, play an important role in secondary resistance apart from immunisation. The muscular dystonia pattern may change in some patients over time. BoNT-A injections may 'activate' other previously inactive muscles (contributing to the similar clinical pattern of dystonia), which implies the activity of a central mechanism, conditioning the position of the head or neck through non-specific muscle activation. The clinical pattern of dystonia in the course of the disease can also change. This probably results from activation of other muscles, which in turn results from a peripheral block of initially active dystonic muscles, or a change of the activation centre at the level of the central nervous system [84–86].

Evaluating the effectiveness of treatment

A lack of improvement in a patient's assessment may contradict the positive change in rating scales. The most commonly used are Tsui, CIDP-58 and TWSTRS.

The TWSTRS is most commonly used in clinical trials and serves as the primary endpoint for assessing the effectiveness of BoNT-A in treating CD [14, 17, 87–90]. In most studies, an improvement by 25–30% measured with this scale is considered significant. It shows a strong correlation with the Tsui Scale [91]. Despite many advantages however, it does not take into account the evaluation of dystonic tremor; there is no clear definition of the midline and the full range of motion [92]. This scale also cannot assess properly the complex patterns of CD demonstrated in the Col-Cap concept.

In addition, due to its complex nature, the scale can hardly ever be applied in everyday practice. The authors of one recent study determined the number of points in the TWSTRS scale which contributes to a minimally clinically perceptible change in the Patient Global Impression of Change (PGIC) scale: a change by 3 points in milder cases (≤ 28.5) and 18 points in severe cases (> 52) [93]. A linear relationship was also found between the TWSTRS total scores and the PGIC: an improvement by 2.9 points in the TWSTRS scale corresponded to a change by one category in the PGIC scale.

The lack of a specified rating scale including both motor (with respect to the new Col-Cap patterns) and wide spectrum of non-motor CD features seems to be an unmet need. Moreover, it may create difficulties when planning new studies [94].

The next unmet need is the lack of consensus on specific rehabilitation programmes dedicated to CD patients and aimed at enhancing the effect of BoNT-A therapy.

In conclusion, CD treatment with BoNT-A remains a challenge for the physician. It is rare to obtain satisfactory effects at the first session. If unsuccessful, the long list of possible reasons, which have been the subject of this paper, should be considered.

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REVIEW ARTICLE

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Smoking as a risk factor of onset and relapse of Multiple Sclerosis — a review

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ABSTRACT

Introduction and objective. Multiple Sclerosis (MS) is a chronic demyelinating disease caused by damage to myelin in the brain and spinal cord. The cause of the disease is unclear, but it is probably correlated with dysregulation of the immunological system, as well as non-modifiable and modifiable risk factors.

Unfortunately, there is no cure for MS. However, the course of the condition has been shown to be modifiable by treatment and various environmental factors. Cigarette smoking is one of the most common addictions around the world, and may be a key modifiable risk factor in MS. Here, we review data available on Pubmed and Scopus from the last 10 years. The following consecutive key words were used in our search: "multiple sclerosis", "smoking", "cigarette", "impact", "progression", and "tobacco". This search yielded 248 initial articles, 43 of which were included in our review.

Current state of knowledge. In our review, we have examined the impact of smoking on the immunology, course, treatment, relapse, recurrence, quality of life, and changes visualised on MRI among patients with MS in general. We have also explored these patterns in MS subtypes. In general, smoking is reported to have negative effects on MS, including a decrease in quality of life, as well as cognitive and mental state, and an increase in disability, as well as in the frequency of relapses and recurrences.

Clinical implications. Smoking has a widespread negative impact on patients with MS. Thus, it is important to educate patients and to help them to give up smoking to improve their health and quality of life.

Future directions. Further research about the impact of smoking and nicotine on MS and other neurodegenerative diseases is needed; in particular, research on e-cigarettes.

Key words: multiple sclerosis, smoking, cigarettes, tobacco, impact, progression

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Introduction and objectives

Multiple Sclerosis (MS) is a neurological disease caused by a chronic, inflammatory demyelinating process of nervous tissue in the brain and spinal cord. It is estimated that the number of cases of MS in the population is 30.1 per 100,000 people [1]. MS can have a wide range of physical and mental symptoms, including double vision, blindness, ataxia, urination disorders, and cognitive impairment. The pathogenesis of MS is still unclear; however, MS onset is correlated with impaired functioning of the immune system. Thus, it is possible that the onset of MS depends on an occurrence of genetic and environmental risk factors and may be triggered by a viral infection. Environmental and genetic factors have been shown to influence MS onset.

Identifying the factors that have an impact on the development of the disease is critical for preventing the progression of MS. The literature suggests that key factors are likely to include diet, vitamin D deficiency, overweight status, viral infection, stress, and smoking. Importantly, many of these environmental and lifestyle factors can be modified. A diagnosis of MS requires the occurrence of certain symptoms and signs in combination with medical imaging (i.e. magnetic resonance imaging, MRI) and laboratory testing. The McDonald criteria, updated in 2017, are commonly used in the diagnosis of MS [2, 3]. MS remains an incurable condition. However, new

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drugs are available that have shown promise in stunting the progression of the disease for years. The severity of MS can be assessed using the Expanded Disability Status Scale (EDSS) [4]. One key modifiable risk factor for poor outcomes is smoking.

Smoking results in a broad range of harmful effects from the risk of lung cancer and other cancers to autoimmune diseases. Tobacco smoke contains several substances that have been shown to negatively influence biological processes in the body through various mechanisms. In recent years, it has been suggested that smoking may be implicated in MS pathogenesis, and that it may affect the progression of the disease including its severity [5, 6]. Our study comprises a review of the data available on smoking and MS published in Pubmed and Scopus over the past 10 years. The following consecutive key words were used in our search: "multiple sclerosis", "smoking", "cigarette", "impact", "progression", and "tobacco". This search yielded 248 initial articles, 43 of which were included in our review.

Current state of knowledge

Impact of smoking on experimental model of MS in mice

The harmful effects of smoking may be due to the different components of smoke rather than solely related to nicotine itself.

Gao et al. examined the impact of nicotine and the non-nicotine components in cigarette smoke on MS using an experimental autoimmune encephalomyelitis (EAE) model in mice. They found that nicotine moderated the severity of EAE, as evidenced by reduced demyelination, increased body weight, and attenuated microglial activation. After the development of EAE symptoms, nicotine administration prevented further disease exacerbation, suggesting that it may have therapeutic utility for EAE/MS. Importantly, the other (non-nicotine) components of cigarette smoke, delivered as cigarette smoke condensate (CSC), were shown to accelerate and increase the adverse clinical symptoms during the early stages of EAE. Among the non-nicotine compounds, acrolein was identified as the key potential mediator. The protective role of nicotine may be explained by its immunomodulatory functions. Within the nervous system, nicotinic receptors are primarily expressed on microglia, which relate to their immune-regulatory functions. CSC infusion into the spinal cord has been shown to correlate with microglial activation. Moreover, therapeutic nicotine administration has been shown to attenuate EAE symptoms. Nicotine has also been shown to demonstrate anti-inflammatory properties [7].

For example, Enzmann et al. examined the impact of specific genetic factors on the effects of smoking using the EAE model. Using Swiss Jim Lambert (SJL/J) mice, a transgenic model of relapsing-remitting MS (RRMS), the authors observed a very low incidence of EAE in both the smoke-exposed and control groups. In a model of optico-spinal encephalomyelitis (OSE) in C57 Black 6 (C57BL/6) mice, a double transgenic model, the early onset of EAE precluded a meaningful evaluation of the effects of cigarette smoke. In EAE models induced by immunisation, daily exposure to cigarette smoke caused a delayed onset of EAE followed by a protracted disease course in SJL/J mice. In contrast, cigarette smoke exposure was shown to ameliorate the EAE clinical score in C57BL/6J mice. Taken together, the influence of cigarette smoke on MS has been shown to depend on the type of transgenic mouse [8].

To date, the literature remains unclear about which components of cigarettes are harmful. Research suggests that nicotine administered alone may actually have a protective role in the course of MS. The other components of cigarette smoke may contribute to the detrimental effects of smoking in MS.

Correlation between smoking and immunology, biochemistry

Smoking has been shown to impact upon various features of the immune system in MS patients. According to Paknejad et al., Calcium binding protein B (S100B) plays a role in the pathogenesis of the disease via detection of specific T cells against S100B in the peripheral blood of MS patients. Based on these findings, levels of S100B in serum is considered to be a sensitive biomarker of disease activity. In one study, the level of S100B was significantly higher among smokers with RRMS compared to non-smokers [9]. Socha et al. examined the impact of smoking on selenium concentration (Se), glutathione peroxidase (GSH-Px) activity, and total antioxidant status (TAS) in serum drawn from patients with RRMS. Patients with MS had lower Se, GSH-Px, and TAS compared to individuals in the control group. Moreover, in that study, a significant decrease in TAS was observed in the serum of smokers compared to non-smokers. Thus, the immune system may be affected by low levels of antioxidants. Oxidative stress is an important factor involved in the pathogenesis of MS, including speeding up the production of reactive oxygen species. The generation of reactive oxygen species has been linked with both demyelination and inflammation [10].

A study by Tao et al. demonstrated a link between a history of tobacco smoking and symptom onset 3.05 years later. This result is consistent with the recognised notion that smoking is a risk factor of MS. The anti-inflammatory effects of nicotine on T-cells, B-cells, and dendritic cells might provide some answers. Tobacco smoking may have a greater influence on neurodegeneration rather than inflammation itself. A greater effect on neurodegeneration than inflammation may play a role in the onset of a subset of cases [11]. Another line of research has examined the role of perfluorinated alkylated substances (PFASs). PFASs are synthetic chemical compounds that have both immunosuppressive and immunotoxic effects. However, after running the experiment, the authors concluded that PFAS exposure is not an important risk factor for MS. Nevertheless, they observed cellular immune activation among smokers, evidenced by: 1) a lower frequency of CD8+ T cells characterised

by the expression of CD26 and; 2) CD161, which presumably defines mucosal associated invariant T (MAIT) cells; and 3) an increased percentage of inducible T cell costimulatory (ICOSL+) plasmacytoid dendritic cells (pDC). MAIT cells express proinflammatory and protective functions in MS.

In this context, lower levels of MAIT cells observed in smokers may be associated with their migration to the lungs. The impact of this phenomenon on the relapse of MS is still unclear. Furthermore, levels of ICOS+ Tfh cells in patients with RRMS have been shown to be higher than levels reported in healthy individuals. Smokers have also been shown to have increased levels of the co-stimulatory molecules ICOSL and CD86 on antigen presenting cells, including pDCs and B cells. ICOSL interacts with ICOS and plays a crucial role in the development of Tfh cells. Moreover, smoking induces APC with higher T-cell activation [12].

Ammitzbøll et al. examined immune cells from three groups: healthy smokers, healthy non-smokers, and non-smokers with MS. There was a significant increase in the number of granulocytes, monocytes, B cells, CD4+ and CD8+ T cells among smokers. Smokers also showed lower levels of MAIT cells. Based on these results, the authors suggest that smoking exerts proinflammatory effects rather than specific immunological ones [13]. Ammitzbøll et al. examined the expression of the class A orphan G-protein coupled receptor (GPR15) gene. Expression of GPR15 was increased among healthy smokers as well as non-smokers and smokers with RRMS. Expression of GPR15 was normal among patients with progressive MS. GPR15 may function as a chemoattractant receptor and has been associated with effector T cells in inflammatory processes. Smokers show higher expression of GPR15 on their CD4+ T-cells. Based on these results, the authors put forward the hypothesis that higher GPR15 expression on CD4+ T-cells is observable in RRMS upon activation. Moreover, GPR15+ CD4+ T cells were shown to produce higher levels of IL-17, which defines Th17 cells, suggesting a crucial role in the pathogenesis of MS. RRMS smokers had increased level of GPR15+ cells detected in their cerebrospinal fluid (CSF), which might explain the observed harmful effects of smoking in MS [14].

It is thus possible that smoking could have a comprehensive influence on immune system functions by changing levels of certain proteins or enzymes. Smoking may also affect the distribution of immune cells and growth factors.

Correlation between smoking and different forms of MS

MS is divided into a few types based on its course, severity, occurrence of relapse, recurrence, and remission. Smoking can have different effects on different types of MS.

In general, there are three clinical forms of MS: RRMS, primary progressive MS (PPMS), and secondary progressive MS (SPMS) [15]. Lublin et al. described a distinct, fourth subtype of MS, which is described as a clinically isolated syndrome (CIS). Patients with CIS have been found to frequently transition to other types of MS [16]. Indeed, Van der Vuurst de Vries et al. examined CIS patients during a five year follow-up and examined the risk of clinically definite MS (CDMS) in smoking and non-smoking patients at the time of the first demyelinating event. They found that smoking at the time of CIS was an independent predictor for CDMS diagnosis [hazard ratio (HR) 2.3; $p = 0.002$]. Interestingly, CIS patients who formerly smoked did not have a higher risk for CDMS compared to those who had never smoked. The researchers also found that the number of cigarette packs smoked per year was higher in the group that was diagnosed with CDMS (CIS–CDMS) during follow-up than in the group that remained in the CIS category. Patients who smoked at the time of CIS had a shorter time to CDMS diagnosis than patients who were not active smokers (HR 2.1 $p < 0.001$) [17]. Similar results have been reported in other studies, wherein patients with CIS had significantly higher risks of secondary progressive disease in males (HR 1.83, 95% CI: 1.3–2.7) and in those with a history of smoking (HR 1.4, 95% CI: 1.0–2.0). Progressive disease was found to occur four years earlier in patients who had a history of smoking relative to non-smoking patients [18].

In contrast to the aforementioned studies, Horakova et al. found that active smoking status was not associated with the number of relapses (all p-values > 0.26), progression to CDMS (all p-values > 0.44), or time to first relapse (all p-values > 0.41). However, smoking was associated with observable changes in MRI scans in this study and in other published studies [32, 33]. Arikanoglu et al. also demonstrated that there was no difference between smokers and non-smokers with CIS in relation to rate and time of conversion to CDMS. Nevertheless, smokers presented more changes in white matter [19].

Progression of RRMS to SPMS may be associated with exposure to smoking. According to O'Gorman et al., there were significantly higher risks of secondary progressive disease in males and in patients with a history of smoking. Again, SPMS was found to occur approximately four years earlier in patients with a history of smoking relative to non-smokers. However, smoking was not found to affect the age of onset of primary progressive disease [18]. In other research, a higher proportion of patients with RRMS were found to be smokers compared to non-smokers ($p = 0.001$). Moreover, a greater frequency of SPMS was significantly associated with an increase in the number of cigarettes to more than 10 ($p = 0.001$).

Over and beyond this, smokers have been shown to be at increased risk for progression of RRMS to SPMS compared to non-smokers (HR 2.25, $p = 0.004$). Also, the risk of SPMS was 2.43 ($p = 0.007$) times higher for an increase in the number cigarettes smoked per day, compared to the risk of SPMS among non-smokers [20]. On the other hand, Kvistad et al. demonstrated that, in the case of RRMS, smoking was not associated with the occurrence of new changes in MRI, relapses, or progression in EDSS [21]. Javizian et al. examined the impact of smoking on disease activity in PPMS. The median

time to EDSS 4 was four years in ever-smokers and five years in never-smokers (p = 0.27). The median time to EDSS 6 was nine years in both ever-smokers and never-smokers (p = 0.48). Smoking did not increase the risk of faster progression to EDSS 4 or EDSS 6, or the progression from EDSS 4 to 6.

Hence, cigarette smoking does not appear to influence disability accumulation in PPMS [22]. Taken together, smoking might have an impact on the earlier occurrence of CDMS in patients with CIS. However, the findings are mixed. Smoking can also increase the odds of progression of RRMS to SPMS.

Correlation between smoking and occurrence of relapses in MS

The age at onset of MS can vary, and depends on genetic profile, type of disease, and environmental factors. In MS, relapses and the progression of symptoms and disability are inevitable. However, there are factors that can promote or stifle these processes.

According to Briggs et al., MS patients who had been smokers had an 8.2% younger age-at-onset than non-smokers, which equated to an approximate 2.6-year difference (p = 5.7 × 10−10). Another study suggested that smoking can increase the risk of early relapse (p = 0.053) [23]. Smokers may also be more liable to develop a more severe type of MS, and MS patients with RRMS more likely to develop SPMS. According to Roudbari et al., compared to non-smokers, current and former smokers showed a relative risk of 2.43 and 3.55 respectively for the progression of MS after one year. When age at disease onset, number of relapses per year, and gender were taken into account, the hazard ratio for smokers compared to non-smokers was 2.25 (p = 0.004) [20].

The impact of smoking on the occurrence of relapses and disease activity is unclear. A study by Weiland et al. found no significant association between smoking and relapse rate or disease activity controlling for age and gender. No significant differences in 12-month self-reported physician-diagnosed relapse rates or disease activity were found according to smoking status, amount currently smoked, or time since smoking cessation. However, disease activity was reduced among patients who gave up smoking more than 10 years ago (p = 0.046) and 1–10 years ago (p = 0.047) [24].

On the other hand, a separate line of research showed no link between smoking and severity of MS. According to Kvistad et al., there was no association between cotinine (the main metabolite of nicotine) levels and MRI activity among smokers. In that same study, smokers did not display more relapses or EDSS progression [21]. In another article, active smoking was not associated with the number of relapses, progression to CDMS, or time of first relapse. However, smoking was associated with an increased number, and volume, of contrast-enhancing lesions (CEL) during a two year study period [25]. Kinga et al. examined the impact of smoking on the EDSS annualised relapse rate (ARR). They found no significant differences in EDSS ARR among smokers vs. non-smokers [26].

Thus, the findings regarding the impact of smoking on occurrences of relapses are contradictory. Some studies have reported a higher risk of relapse among smokers with MS, whereas other studies have shown no increased risk.

Smoking and range of disability in MS

The natural course of MS inevitably correlates with gradually progressing disability. However, environmental factors can alter this course by either stifling or promoting the progression of disability. A study by Briggs et al. examined disability by using the Timed 25-Foot Walk, a marker of lower limb disability, and the Performance Scales Sum, a measure of global disability. Compared to non-smoking MS patients, smoking MS patients showed a slower walking speed as well as higher global disability. Smoking status and insurance payer had the largest impacts on global disability as measured by the Performance Scales Sum [27]. Marck et al. implemented an educational intervention to MS patients that focused on healthy lifestyles, including giving up smoking. The researchers examined adherence to healthy behaviours after three years of education. They found that patients who followed the new healthy lifestyle reported improved physical and mental health [28]. Tanasescu et al. examined the effects of smoking cessation over time on reaching Expanded Disability Status Scale (EDSS) scores 4 and 6 among smokers with MS. They found that participants who gave up smoking had a 0.96 times lower risk of reaching EDSS 4 each year, and a 0.97 times lower risk of reaching EDSS 6 each year. Furthermore, they found that non-smokers had a significantly lower level of disability in all of the self-reported measures compared to current smokers. Relative to a patient who had continued smoking, a patient who gave up smoking 10 years earlier had a 33% and a 26% lower risk of reaching EDSS scores 4 and 6, respectively [29]. Similar results were described by Manouchehrinia et al. In that study, almost 1,000 patients were examined. MS patients who had a lifetime history of smoking were 1.34 and 1.25 times more likely than never-smokers to reach EDSS scores 4 and 6, respectively. A higher risk of reaching EDSS scores 4 and 6 was found for current smokers compared to non-smokers. Former (but not current) smokers had a significantly lower risk of reaching EDSS scores 4 (0.50–0.83) and 6 (0.53–0.90) than current smokers. There were also no significant differences in the time to EDSS scores 4 and 6 between patients who stopped smoking before MS onset, and those who stopped after developing MS.

Other studies have examined the effects of daily smoking on the Multiple Sclerosis Severity Score (MSSS). Average MSSS was increased by 0.04 for each additional cigarette smoked per day [30]. According to Ivashynka et al., the median MSSS was higher (3.2 vs. 2.3, p = 0.002) in patients with a lifetime history of smoking vs. patients without a lifetime history. Patients with a lifetime history of smoking were almost twice as likely to fall into the upper MSSS tertile compared to smoking-naïve patients. Similar to the effects of age and

sex, smoking habit increased the risk of falling into the worst MSSS tertile by 10.81 ($p < 0.01$) [31]. Ektan et al. examined the impact of smoking on respiratory problems and level of functioning. Among MS patients, smoking was associated with decreased functioning of the respiratory system compared to non-smokers. Smokers with MS reduced their daily walking distances. Respiratory failure was also shown to decrease the level of functioning among patients with MS [32].

Briggs et al. evaluated global disability among MS patients using the Patient Determined Disease Steps (PDSS) and Item Response Theory (IRT) summed score. They found that active smokers had significantly higher disability scores as measured with the IRT than non-smokers had. Global disability assessed by the PDDS did not differ between active smokers and non-smokers, although there was evidence of female smokers reporting higher disability than female non-smokers [33]. Ballesteros et al. found an increased risk of disability progression in daily smokers compared to non-smokers and ex-smokers (3.56 times and 2.32 times, respectively) [34]. According to Weiland et al., current smokers showed an almost doubled odds of requiring major mobility support (e.g. bilateral support, wheelchair, becoming bedridden) compared to never-smokers. In that same study, former smokers showed a 1.24 times increased odds of requiring major mobility support compared to patients who had never smoked. No association was observed between smoking and relapse rate, or between smoking and disease activity, after controlling for age and gender. Nevertheless, among former smokers, a longer duration of smoking cessation was associated with reduced disease severity. In another study, smokers had significantly lower HRQOL than never smokers and former smokers, and heavier smoking was associated with greater decreases in HRQOL [24]. On the other hand, according to the available data, the impact of smoking on disability can differ according to the type of MS. For example, in progressive onset MS, the consumption of alcohol, coffee, tea, fish, and cigarettes did not have a significant effect on the time to reach EDSS 6. However, in relapsing onset MS, smoking has been associated with an increased risk of reaching EDSS 6 [35]. Those results are in agreement with other research. Indeed, Javizian et al. examined more than 400 patients with PPMS and demonstrated that patients who had a history of smoking had a median time of about four years to progression to EDSS 4. The median time to progression to EDSS 4 was five years in MS patients without a history of smoking ($p = 0.27$). Median time to progression to EDSS 6 was the same (nine years) in patients with and without histories of smoking ($p = 0.48$). In that study, smokers were not at increased risk of faster progression to EDSS 4 or 6, or progression from EDSS 4 to 6. Age at disease onset, however, was the strongest risk factor for progression to EDSS 4 and 6, and from EDSS 4 to 6 [22].

Kvistad et al. examined a group of 87 patients with RRMS every six months over a two-year period. EDSS and MRI changes were analysed. Tobacco users did not have more relapses or EDSS progression. This could suggest that RRMS progression might be mediated by other factors [21].

Smoking and level of disability are strongly correlated. The research projects cited above have demonstrated a link between smoking and an increase in disability, as well as a decrease of motor functioning, in patients with MS.

Impact of smoking on cognitive functions and mental state in MS

MS is correlated with an impairment in cognitive functioning. Smoking and body mass index (BMI) display consistent and significant deleterious associations with perceived cognitive impairment (PCI) [36].

Özcan et al. used the Brief Repeatable Battery of Neuropsychological Tests (BRB-N) to assess cognitive functioning in patients with MS. Patients who smoked at least 10 pack-years were considered to be heavy smokers. The researchers found a greater degree of impairment in cognitive functioning among patients who were heavy smokers than non-smoking patients ($p = 0.04$) [37]. In other research, current smoking was associated with severe cognitive symptoms, and smoking increased the risk of occurrence of cognitive symptoms by almost three times. Former smokers were more than twice as likely to experience symptoms compared to non-smokers [38]. Due to chronicity and cumbersome symptoms, patients with MS can have mental health problems such as depression. Briggs et al. examined the impact of prognostic factors among patients with RRMS, using the Patient Health Questionnaire 9 (PHQ-9), a nine-item scale that assesses the presence and severity of depression. Briggs et al. [27] found that patients who smoked had higher depressive scores compared to non-smokers. Taylor et al. assessed the impact of lifestyle factors on the occurrence and onset of depression 2.5 years later, using the Patient Health Questionnaire-2 (PHQ-2) and the PHQ-9. The PHQ-2 is a scale that is commonly used to screen for depression at onset, and examines the frequency of depressed mood and anhedonia over the past two weeks. Taylor et al. found that smokers, in general, had more often depression at the beginning of their disease compared to non-smokers, and that depression was more severe at the follow-up in smokers relative to non-smokers [39].

Another study used the Multiple Sclerosis Impact Scale 29 (MSIS-29). Compared to ex-smokers, smokers showed higher MSIS-29 scores, reflecting a poorer mental state. Smoking has also been associated with increased risk of anxiety and depression among patients with MS [29]. Aetiological pathways in depression among people with MS may be related to the neurotoxic effects of smoking [40]. Jelinek et al. proved that smoking was significantly associated with mental state. In that study, the Mental Health Composite score (MHC) was used, where higher scores correlate with better mental health status. Jelinek et al. [41] found that, relative to smokers, non-smoking MS patients scored 6–7 points better on the MHC.

Anxiety and fatigue have also been associated with restless legs syndrome (RLS) among patients with MS. Contentti et al. found that smoking cigarettes significantly increased ($p = 0.03$) the risk of RLS among patients with MS [42]. Patients with MS who are former and current smokers are more likely to achieve worse results on specific tests, which correlated with a decline in cognitive functioning.

In sum, current and former smokers have been found to experience mental health problems more frequently than non-smokers.

Impact of smoking on lesions found in MRI scans among patients with MS

Smoking among patients with MS has also been shown to influence changes visualised in MRI. Horakova et al. examined the impact of smoking on changes in patients with CIS over a two-year period. Smoking was associated with an increased number of contrast enhancing lesions (CEL) ($p = 0.002$), and a trend towards an increased volume of lesions between baseline and the two-year follow up ($p = 0.014$). The mean number of CEL at two years in the group of smoking MS patients was 0.51, compared to 0.19 lesions in the group of MS patients who were not actively smoking. However, smoking status was not associated with the number of new and newly enlarging T2 lesions ($p = 0.86$) or brain atrophy, as assessed by percentage brain volume change ($p = 0.64$) [25]. According to Durhan et al., a tendency towards greater lesion load in MRI was found in smoking patients. Indeed, T1 hypointense lesions and perilesional white matter had reduced fractional anisotropy and increased mean diffusivity to a similar degree in CIS patients who smoked vs. those did not smoke. Compared to non-MS patients who smoke, CIS patients who smoke had more extensive normal-appearing white matter changes revealed by increased mean diffusivity. Moreover, among CIS patients, the mean diffusivity value in the left superior longitudinal fasciculus was significantly higher in smokers than non-smokers [43].

In other research in patients with CIS, white matter lesions were detected in MRI scans of all of the smoking patients. In non-smoking CIS patients, white matter lesions were identified in a smaller number — only 63.5% (54 of 64) ($p = 0.02$) [19]. Graetz et al. found that patients who smoked showed reduced grey matter fraction, lower brain parenchymal fraction, and increased cerebrospinal fluid fraction compared to non-smoking patients. Nevertheless, no effect was observed on white matter fraction [44].

In contrast to the aforementioned studies, Kvistad et al. reported no association between tobacco use and the occurrence of new gadolinium-enhancing T1 lesions, new or enlarging T2 lesions, or their aggregate. Furthermore, in that study there was no association between cotinine levels and MRI activity among smokers [21]. In fact, smoking may display a neuroprotective effect. In other research, smoking has been associated with less cortical and deep grey matter damage occurrence and with increased grey matter volumes in several regions of the brain [45].

MS, smoking, quality of life, and prognosis

In general, quality of life is lower among patients with MS compared to individuals without MS. This may be due to chronicity and severity of the disease. Briggs et al. analysed survey data from 950 patients. Health-related quality of life (HRQOL) was assessed via the SF-12v2, which is divided into subscales. HRQOL in smokers was significantly lower than in non-smokers, and current smokers' HRQOL was appreciably lower than HRQOL of former smokers. It is worth noting that the relationship between smoking and HRQOL only reached statistical significance among women, although the non-significant effects in the smaller male sample were in the same direction [33].

Similar results were achieved by Weiland et al. Compared to MS patients who smoked 1–15 or 16+ cigarettes per day, non-smokers had significantly better quality of life across all subscales examined. No significant difference in quality of life tests between patients smoking 1–15 or 16+ cigarettes per day were observed. While time since giving up smoking had no significant impact on the overall quality of life and physical health composite, the number of years since cessation was significantly associated with QOL on the mental health composite and emotional wellbeing subscales [24]. Jelinek et al. used the Multiple Sclerosis Quality of Life-54 (MSQOL-54), which is used to assess physical health-related QOL and mental health-related QOL. Compared to smokers, non-smoking MS patients scored 4–5 points higher in physical health (i.e. PHC) and 6–7 points higher in mental health (i.e. MHC) scales [41].

The mortality rate among patients with MS is generally high; however, some environmental factors can substantially increase this ratio. According to Manouchehrinia et al., the mortality rate was similar between MS patients who had never smoked or formerly smoked and individuals without MS who had never smoked or formerly smoked. However, current smokers with MS had an 84% higher rate of death compared to current smokers without MS. Current smokers with MS had about a ten-year reduction in life expectancy compared to non-smokers with MS [46].

According to Hedström et al., MS risk is correlated with smoking in a dose-dependent manner, and this risk is similar for smoking and exposure to second-hand smoke [47]. On the other hand, Mandia et al. found no significant difference in MS severity between patients who currently smoke and patients who formerly smoked or have no history of smoking. No significant relationship between second-hand smoke and MSSS was found [48]. In general, smokers with MS have been shown to have lower quality of life compared to non-smokers, as measured by various scales. Moreover, smoking MS patients may have a higher mortality rate than non-smoking MS patients. Quality of life and mortality rates are both likely to be associated with the number of cigarettes smoked per day.

Clinical implications

The broadly negative effects of smoking on the metabolism and the proper functioning of the human body are well understood. According to the research data reviewed in this report, cigarettes may have an impact on the onset, course, and effectiveness of treatments for some conditions, including MS.

Most of the reviewed research indicates that smoking has a negative role in MS, by triggering the onset of symptoms, the occurrence of relapse, and activity of drugs. Moreover, exposure to cigarette smoke can impair mental state, quality of life, and cognitive functioning, and increase the severity of disability among MS patients. Smoking might also impact upon changes in the brain, as measured using MRI.

On the other hand, the harmful effect of cigarettes may be moderated by the subtype of MS. Nonetheless, these findings are important points to consider to encourage patients with MS to give up smoking. In particular, healthcare systems should make efforts to provide patients with thorough information on the modifiable factors in MS, particularly smoking, and the impact of these factors on the course of the disease.

The provision of this information could lead to positive changes in both quality of life and efficiency of treatment. Such education may also reduce the social costs of eventual treatment.

Future directions

The role of nicotine and smoking in the pathogenesis of MS and other neurodegenerative disorders remains unclear. However, the potential positive impact of nicotine is promising. Our review found a lack of research about the effects of e-cigarettes on MS.

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Biomarkers in Multiple Sclerosis: a review of diagnostic and prognostic factors

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ABSTRACT

Introduction. Multiple Sclerosis (MS) is a chronic, demyelinating disease of the central nervous system which affects mostly young people. Because it leads to disability and cognitive impairment, it is crucial to recognise MS at an early stage.

State of the art. Magnetic resonance imaging is the golden standard in MS diagnosis. However, it is not an infallible diagnostic tool, especially at the stage of clinically isolated syndrome. The incorporation of oligoclonal bands in the diagnostic process of MS is a step towards the extension of diagnostic methods. Recently, a lot of research has been carried out on potential biomarkers in blood serum and cerebrospinal fluid that may be useful in the diagnosis of MS.

Clinical implications. This article summarises current knowledge on the use of new prognostic factors such as neurofilament light chain, chitinase 3-like 1 and 2, heat shock proteins, and tubulins in MS.

Future directions. Despite numerous studies on the use of biomarkers in the diagnosis of MS, more extensive research is needed to determine the clinical usefulness of these molecules and to develop diagnostic tests applicable in everyday practice. This in turn may result in earlier MS detection, faster implementation of treatment, and better therapeutic effects.

Key words: multiple sclerosis, biomarker, risk factor, cerebrospinal fluid, serum

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Introduction

Multiple Sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS) with a wide spectrum of clinical and imaging changes [1, 2]. The first episode of neurological symptoms with the features of inflammatory demyelination is referred to as clinically isolated syndrome (CIS) [3]. This develops acutely or subacutely, lasts at least 24 hours, and runs a course similar to that of a typical MS relapse but in a patient not yet diagnosed with MS [3, 4]. Research has shown that up to 60–70% of patients with a first clinical episode do not meet the criteria for MS. However, up to 85% of these patients will develop full-blown MS in the future [5, 6].

According to the new, revised McDonald criteria from 2017, magnetic resonance imaging (MRI) is the golden standard in MS diagnosis [7]. The risk of conversion from CIS to clinically definite MS (CDMS) in patients with characteristic lesions in MRI is up to 60–80 %, while in patients without abnormalities specific for MS this risk is 20% at most [3, 8, 9]. Unfortunately, MRI is not effective enough to detect all active demyelinating changes in the CNS. MS affects approximately 2.3 million people, mostly between the ages of 20 and 40, leading to their disability and cognitive impairment. This makes it a significant problem for society as a whole [10]. Currently available treatment can delay the progression of MS only if it is used at the beginning of the disease. This is why early diagnosis of MS has become a burning clinical challenge. It is essential to properly recognise it from the very earliest stages of the disease. The above-mentioned McDonald criteria put emphasis on the early detection of the CIS. In addition to magnetic resonance, oligoclonal bands (OCBs) play a key role in the diagnostic process. Moreover, recent promising studies have been carried out to determine

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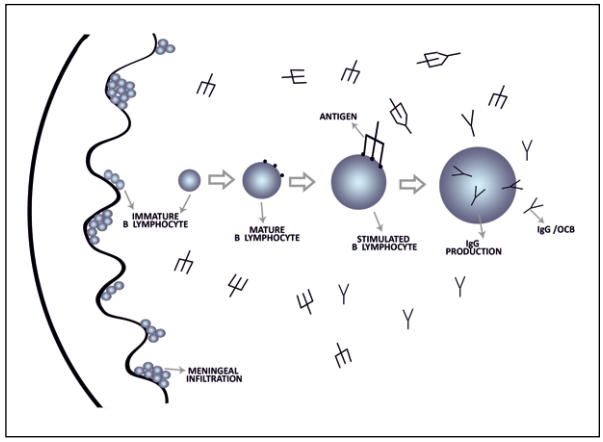

Figure 1. Intrathecal synthesis of immunoglobulins G forming oligoclonal bands. Immature B lymphocytes form infiltrates meninges, where they go through maturation process and acquire affinity to antigen. Mature B lymphocytes become stimulated B lymphocytes after contact with antigen. Stimulated B lymphocytes start producing immunoglobulins G, which form oligoclonal bands; IgG — immunoglobulin G; OCB — oligoclonal band

the presence and concentration of new biochemical indicators in the cerebrospinal fluid (CSF) and blood serum that may be useful in MS diagnosis.

State of the art

Oligoclonal bands as predictive factors of MS

OCBs are mostly immunoglobulins G (IgG), which are produced intrathecally by stimulated clones of B lymphocytes and plasma cells. It is believed that the final maturation of B lymphocytes, and their affinity for a closer unknown antigen, occurs in the peritoneum and meninges (Fig. 1) [11, 12]. CSF and the serum are tested by isoelectric focusing (IEF) gel electrophoresis for the presence of so-called bands. There are six types of OCBs: types 2 and 3 indicate intrathecal synthesis; the other four are negative for MS (Tab. 1) [13–15].

The importance of oligoclonal bands in the diagnosis of MS has been the subject of several studies. A large study conducted by 33 centres included 1,047 patients with CIS observed for a minimum of two years. With a median follow-up of 4.31 years, 623 patients with CIS converted to MS. Oligoclonal bands were detected in 95% of patients [6].

Another multicentre retrospective study, conducted by German-Austrian scientists, included 406 patients. The diagnostic criteria for MS were met by 11% of patients (44 people), and the remaining 89% (362 people) were diagnosed with CIS. OCBs were positive in 86% of patients with CIS; 74% of these patients developed MS. OCBs were not detected in 14% of patients; 44% of these patients converted to MS. The median conversion time for CIS patients with positive OCBs was 25 months, compared to 47 months in patients with negative OCBs [16].

Table 1. Types of OCBs depending on their pattern in IEF and occurrence in body fluids [13]

IEF pattern	Occurrence in body fluids
1	No OCBs in CSF and serum
2	CSF-restricted IgG OCBs
3	CSF-restricted OCBs and additional identical bands in CSF and serum (combination of patterns 2 and 4)
4	Identical OCBs in CSF and serum ('mirror pattern')
5	Monoclonal bands in CSF and serum
6	Presence of a single band limited to CSF

OCBs — oligoclonal bands; IEF — isoelectric focusing; CSF — cerebrospinal fluid; IgG — immunoglobulin G

Another study included 120 patients with CIS. Conversion to MS was observed in 42% of cases, and 58% of patients was defined as stable CIS. Positive OCBs were detected in 61% of patients with CIS at the beginning of the study. During follow-up, 55% of patients with positive OCBs converted to MS, and 21% of patients with negative OCBs developed MS. The median time needed for conversion was similar in both groups [17]. These two studies show that people with CIS and positive test results for OCBs are twice as likely to develop MS than people with negative OCBs. However, in the German-Austrian study, the conversion time to MS was almost twice as long in patients with negative OCBs than it was in patients with positive OCBs.

Recent research has also focused on the value of intrathecal immunoglobulin M (IgM) synthesis in the diagnosis of MS. A study involving 126 patients with CIS showed that IgM levels have a higher reliability index for the conversion of patients with CIS to CDMS compared to IgG [18].

Generally, oligoclonal bands are a strong independent predictor of the risk of conversion in patients with CIS to CDMS: this has been proven by numerous studies. Based on this, an oligoclonal bands test was included in the latest (2017) McDonald criteria, not only as a criterion of MS but also as a predictor for a second relapse occurrence in patients diagnosed with CIS [7]. Moreover, OCBs are not specific indicators for MS, because an increased level of OCBs can occur in other infectious and inflammatory diseases of the central nervous system [19, 20].

New potential biochemical indicators of MS

Neurofilament light chain

Neurofilaments (NFs) are larger molecules of the neuronal cytoskeleton. They are divided into three subgroups: light, medium, and heavy [21]. Neurofilaments are neuron-specific proteins that are released during neuronal damage. This process is the main element of pathology in MS. Therefore it may result in the occurrence of neurofilament light chain (NFL) in the cerebrospinal fluid and then in the blood serum [22–24]. It is believed that axonal damage occurs in the early stages of MS [25, 26].

The role of the neurofilament light chain as a diagnostic factor in MS has been the subject of much research. A prospective longitudinal cohort study included 41 patients with CIS or relapsing-remitting multiple sclerosis (RRMS) and 22 healthy patients. Patients' cerebrospinal fluid was analysed for many biomarkers, including neurofilament light chain. In addition, during the two-year follow-up, the activity of the disease was evaluated by assessing relapses, worsening of disability, or magnetic resonance imaging activity. The study showed that NFL turned out to be the best predictor of MS development at the baseline. Within two years of follow-up, based on the NFL level, 93% of patients who showed signs of disease activity, as well as 67% of patients who did not, were correctly classified. The overall percentage of correct classifications was 85% (33/39 patients) [27].

Another study included 85 RRMS patients whose serum was collected and tested for NFL and its potential role as a predictor of disease activity. Patients were followed for two years. They did not receive disease-modifying treatment for the first six months, then for the next 18 months they received interferon-beta 1a (IFNB-1a). Baseline assessment included the collection of serum samples, MRI and the Expanded Disability Status Scale (EDSS). Serum samples were additionally collected after three, six, 12 and 24 months, MRI was performed after nine, 12 and 24 months and EDSS was evaluated every six months. Patients with new T1 gadolinium-enhancing lesions and new T2 lesions had significantly higher serum NFL levels compared to patients without new changes in MRI. The presence of T1 gadolinium-enhancing lesions was correlated with serum NFL levels two months before and one month after biochemical measurements. The level of NFL decreased after inclusion of IFNB-1a treatment [28].

A third study was carried out on 86 patients with optic neuritis (ON) as the first manifestation of demyelination. The level of neurofilament light chain was examined in the cerebrospinal fluid. Patients were followed on average for 13.6 years, and 81.4% of patients were evaluated using MRI and EDSS scores. The remaining 18.6% of patients were questioned by telephone. During follow-up, 53.5% (46 patients) developed CDMS, and NFL predicted long-term disability by the multiple sclerosis severity scale [29].

The last study was performed on 75 patients with radiologically isolated syndrome (RIS). The level of NFL was measured in cerebrospinal fluid. Neurofilament light chain was an independent risk factor for conversion to a CIS and MS. A high level of NFL was associated with a tendency to shorter conversion time to CIS, and much shorter to CDMS, which was more evident in RIS patients aged at least 37 years compared to younger patients [30].

In summary, the NFL level in the cerebrospinal fluid in patients with CIS and RRMS appears to be a strong potential prognostic factor in the assessment of disease activity [27, 31]. Serum NFL is a promising biochemical indicator for the effects of treatment in RRMS and an alternative to MRI in the assessment of subclinical disease activity [28, 32, 33]. Moreover, NFL turned out to be a predictor of long-term physical and cognitive disability after optic neuritis as the first demyelinating manifestation, and an independent risk factor for conversion to CIS and CDMS in patients with RIS [29, 30].

Chitinase 3-like 1(Human YKL-40, CHI3L1) and chitinase 3-like 2 (Human CHI3L2)

Chitinase 3-like 1 (Human YKL-40, CHI3L1) and chitinase 3-like 2 (Human CHI3L2) are produced by neutrophils, astrocytes and macrophages as enzymatically inactive proteins which are involved in tissue remodelling and inflammation [34]. The levels of these substances increase in the cerebrospinal fluid during various acute and chronic neuroinflammatory states, including MS [35].

One of the first studies to determine the usefulness of chitinase 3-like 1 in the diagnosis of MS was a study performed on a group of 60 CIS patients, 30 of whom underwent conversion to CDMS, while the remaining 30 patients were stable CIS.

Analysis of patient cerebrospinal fluid revealed a significant correlation between the high level of CHI3L1 and the number of gadolinium-enhancing lesions as well as the number of T2 lesions in MRI at the beginning of the study, and was associated with a faster progression of disability and shorter conversion time to CDMS. The level of chitinase 3-like 1 was also higher among patients who underwent conversion to MS compared to those who remained stable CIS [36].

The above results have been confirmed in another longitudinal cohort study carried out in 15 European MS centres. A total of 813 cerebrospinal fluid samples were collected from patients with a CIS. The CHI3L1 level was higher in patients who converted to CDMS compared to those who continued treatment as a CIS. A high level of CHI3L1 was also associated with a shorter conversion time to MS and faster development of disability [37].

Another study investigated the relationship between CHI3L1 levels and response to interferon-beta (IFNβ) and glatiramer acetate (GA) in patients with MS. The level of CHI3L1 in the cerebrospinal fluid of 117 patients with RRMS was measured, including 76 patients treated with IFNβ and 41 treated with GA. The level of CHI3L1 was associated with the response to INFβ treatment, and was higher in the group of non-responders. Similar effects were not found among patients treated with GA. CHI3L1 may thus act as a response biomarker to INFβ in patients with RRMS [38].

Chitinase 3-like 1 can be used as a biomarker of disability development and as an effective marker to distinguish patients who will convert to CDMS from those who will remain as stable CIS in the future [36, 37]. CHI3L1 is also a potential biomarker for response to INFβ treatment in patients with RRMS [38].

On the other hand, the potential of chitinase 3-like 2 in the diagnosis of MS was examined in a prospective cohort study which included 73 patients with optic neuritis as the first demyelinating event, plus 26 age-matched healthy subjects. The

level of CHI3L2 was determined in the cerebrospinal fluid. The predictive capacity of CHI3L2 was compared to that of CHI3L1. The level of CHI3L2 was significantly elevated in patients with ON and was associated with the risk of developing MS. In addition, CHI3L2 was correlated with the risk of cognitive impairment and MS development in patients after ON.

CHI3L2 is a promising risk factor in patients with the first episode of demyelination. In multifactorial risk analysis of MS, CHI3L2 has been shown to be more effective than CHI3L1 [39].

Heat shock protein 70 and heat shock protein 90

Heat shock proteins (HSPs) are molecular chaperones that play vital homeostatic roles in the central nervous system and whose distribution between different species is conservative. They can be divided into different groups depending on the molecular weight [40]. Heat shock protein 70 (HSP70) is localised in the cytosol, where it supports and protects cells against lethal stress-induced damage, as well as in the cell membrane and the intracellular space, where it plays an important role in the immune response [41, 42]. Elevated levels of HSP70 can have a beneficial effect on MS, protecting neurons and oligodendrocytes during an inflammatory process from death through apoptosis. However, the extracellular HSP70 may be responsible for induction of immunologic reaction [43, 44].

The first study was based on analysis of the HSPA1L gene polymorphism encoding the HSP70-hom protein among 191 MS patients and 365 healthy subjects. There was a strong correlation between the polymorphism of the studied gene and the risk of MS development, as well as a significant relationship between the expression of the HSP70-hom protein and the severity of MS [45].

The purpose of further research was to determine the role of HSP70 as a potential biomarker in the differentiation of neurodegenerative and inflammatory processes in MS. The serum of 94 patients with MS was examined, including 26 with CIS, 40 with RRMS, 19 with secondary progressive MS (SPMS), and nine with primary progressive MS (PPMS). The control group consisted of 41 patients with non-inflammatory neurological diseases (NIND), 28 with other inflammatory neurological diseases (OIND), and 114 healthy donors (HD). The level of HSP70 in the serum of patients with MS was significantly higher than in HD, and significantly lower than in OINDs. Moreover, the analysis showed that the HSP70 level in patients with CIS or RRMS was significantly higher than in patients with PPMS or SPMS, which may be correlated with the strongly expressed inflammatory process in the first group [46].

HSP70 is considered to be a useful biomarker to monitor inflammatory processes in MS in the future [45, 46]. Nevertheless, there is still no consensus as to whether HSP70 mediates the beneficial or negative effects of MS.

Heat shock protein 90 (HSP90) has similar properties to HSP70, differing only in molecular weight. It modulates inflammatory processes by producing anti-inflammatory cytokines and modulating the response with toll-like receptor 2 and 4 (TLR2 and TLR4) [47].

One study evaluated the effect of HSP90 on steroid response in the treatment of relapses in MS patients. It was shown that the amount of HSP90 in the glucocorticoid receptor (GR) complex was significantly higher in patients with steroid-resistant MS than in patients with steroid-sensitive MS. The mechanism of non-response to glucocorticoids may be associated with an increased presence of HSP90 in the cytoplasmic GR complex, which causes inhibition of GR translocation to the nucleus and reduction of its transcription [48].

Tubulin beta (TUBβ)

Tubulins (TUBs) are heterodimeric proteins consisting of an alpha and a beta subunit and are major components of microtubules. The synthesis of class II tubulin isotype increases in development and regeneration of neurons.

In one study, the level of cytoskeletal proteins, including tubulin beta, in the cerebrospinal fluid of patients with MS and their correlation with clinical indicators of MS was assessed. The study was performed in 51 patients, including 33 with MS and 18 with other neurological diseases (OND). Tubulin beta (TUB**β**) level was significantly higher in MS patients than in OND patients [49]. Preliminary results show that TUB**β** is a promising diagnostic factor in MS, but further analyses are needed.

Combined measurements of biochemical indicators and their impact on the diagnosis of MS

Recently, studies have been conducted to determine the effect of combined measurements of some of the above-discussed biomarkers levels in CSF and their role in the diagnosis of MS.

In a cross-sectional cohort study by Spanish researchers, the correlation between NFL and CHI3L1 levels in CSF in 157 MS patients, including 99 patients with RRMS, 35 with SPMS, and 23 with PPMS, was investigated. After 50 months of observation, it was found that NFL and CHI3L1 levels in CSF were higher in patients with MS compared to patients in the disease-free control group. Increased levels of NFL in RRMS and SPMS patients were characteristic of clinical relapse, while high CHI3L1 levels were associated with progressive disease. NFL and CHI3L1 levels correlated with each other and with IgM-oligoclonal bands in RRMS patients. A formula of combined measurements of biomarkers was useful in determining MS phenotypes and in predicting clinical progression. High NFL and low CHI3L1 levels occur more frequently in RRMS compared to SPMS and PPM. In turn, elevated levels of both biomarkers were ahead of diagnosis of clinical progression in patients with RRMS [50].

A second study tracked the diagnostic value of NFL and CHI3L1 levels in CSF in 177 newly diagnosed patients with CIS or RRMS. Patients were clinically followed for an average

Table 2. Usefulness of prognostic biomarkers in diagnosis of different types and stages of MS

	CIS	RIS	ON	RRMS	CDMS	S–R MS
NFL	+	+	+	+	+	–
CHI3L1	+	–	–	+	+	–
CHI3L2	–	–	+	–	–	–
HSP70	+	–	–	+	–	–
HSP90	–	–	–	–	–	+
TUBβ	–	–	–	–	+	–

NFL — neurofilament light chain; CHI3L1 — chitinase 3-like 1; CHI3L2 — chitinase 3-like 2; HSP70 — heat shock protein 70; HSP90 — heat shock protein 90; TUBβ — tubulin beta; CIS — clinically isolated syndrome; RIS — radiologically isolated syndrome; ON — optic neuritis; RRMS — relapsing-remitting multiple sclerosis; CDMS — clinically defined multiple sclerosis; S-R MS — steroid-resistant multiple sclerosis; (+) useful in diagnostics; (–) not useful in diagnostics

of 5.7 years. Both NFL and CHI3L1 concentrations in CSF were associated with a higher risk of relapse during the first two years in one dimensional analyses, in contrast to multivariable analysis where only the NFL level was associated with relapse risk. No relationship was found between NFL or CHI3L1 concentrations and risk of conversion to SPMS or disability progression [51].

The research we have described shows that combined measurements of new biochemical indicators such as NFL and CHI3L1 levels in CSF may bring benefits in the diagnosis and prognosis of MS and may set a new direction for research using other biomarkers.

Clinical implications

MS is a disease that affects mainly young people and leads to their disability, which is why it is so important to diagnose it as early as possible. The insufficient accuracy of MRI in early MS diagnosis has led to a search for new predictors. Determining the OCBs level in the cerebrospinal fluid is included in the latest McDonald's criteria for the diagnosis of MS. Recently, promising studies have been carried out on potential new biochemical markers in the blood serum and cerebrospinal fluid that may be useful in the diagnosis of different forms and stages of MS (Tab. 2). The first results of research on compounds such as neurofilament light chain, chitinase 3-like 1 and 2, heat shock proteins, tubulins, or combined measurements of some of these biomarkers, bring fresh hope for patients and for doctors seeking to diagnose MS.

Future directions

Despite numerous studies on the use of biomarkers in the diagnosis of MS, more extensive research is needed to determine the clinical usefulness of these molecules. Identification of sensitive and specific biomarkers in CSF and blood serum of patients with CIS, the development of standardised diagnostic tests detecting these markers, and their use in everyday clinical practice, may result in earlier MS detection, faster implementation of treatment, and better therapeutic effects.

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Fibrinogen concentrations in ischaemic stroke patients with metabolic disorders

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ABSTRACT

Background. Hyperfibrinogenemia plays a crucial role in the coagulation cascade leading to the formation of clots. It is involved in the process of platelet aggregation, primary haemostasis, and leukocyte-endothelial cell interactions. The aim of our study was to assess the correlations between fibrinogen concentration and particular risk factors for vascular diseases and atherosclerotic changes in stroke patients.

Methods. The study group consisted with 94 patients with acute ischaemic stroke with normo- or hyperglycaemia and normo- or hyperlipidemia. 21 healthy subjects served as a control group. Fibrinogen level, HbA_{1c} and lipid profile were measured in all patients. Using a flow cytometer, we assessed CD61-positive microparticles which were defined as platelet-derived microparticles (PDMPs). The level of sP-selectin in serum was measured using the ELISA method.

Results. A significant positive correlation was observed between fibrinogen concentration and sP-selectin ($p = 0.001$), HbA1c ($p < 0.05$) level, and percentage of PDMPs ($p < 0.05$) in the study patients. Furthermore, we noticed a significant negative correlation between fibrinogen concentration and the level of HDL ($p < 0.05$). No correlation was observed between fibrinogen and TC, LDL and TG levels.

Conclusions and clinical implications. Our findings suggest that an elevated fibrinogen level may represent a marker of prothrombotic condition exacerbated in the state of hyperglycaemia and activation of platelets and endothelial cells. This suggests an important role played by fibrinogen in the process of thrombogenesis.

Key words: fibrinogen, sP-selectin, PDMPs, hyperglycaemia, hyperlipidemia

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Introduction

A significant role in the pathogenesis of ischaemic stroke is played by changes in the vascular endothelium and platelets activation. This process leads to atherothrombotic complications, and as a result contributes to brain infarct.

Metabolic abnormalities such as hyperglycaemia and hyperlipidemia are very common concomitant diseases observed in patients with ischaemic stroke. They are significant risk factors for vascular diseases and they play a key role in platelets activation and the development of atherosclerosis. It is also known that hyperfibrinogenemia plays a crucial role in the coagulation cascade leading to the formation of clots.

Studies have shown that a high plasma concentration of fibrinogen — an acute phase protein involved in the process of platelet aggregation, primary haemostasis, and leukocyte-endothelial cell interactions — is associated with an increased risk of total stroke [1, 2]. Hyperfibrinogenemia has been identified as an independent risk factor for both venous and arterial thrombosis [3] and might contribute to the formation and progression of atherosclerotic plaques [4]. However, is also associated with early signs of atherosclerosis, even in asymptomatic individuals [5].

It must be emphasised that an increased level of fibrinogen after acute ischaemic stroke is also associated with a worse neurological outcome [6] and reduced efficacy for thrombolysis after stroke [7–9], caused by increased thrombus resistance to thrombolysis [10]. The adhesion molecule, P-selectin, also plays an important role in atherogenesis and plaque formation. Increased concentration of the soluble form of P-selectin

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(sP-selectin) has been observed in the plasma of patients with vascular diseases and this also reflects the activation of endothelial cells and platelets [11]. Furthermore, sP-selectin is also associated with the formation of platelet-derived microparticles (PDMPs) which participate in the haemostatic response to vascular injury [12]. This can exert procoagulant activity and might play an important role in thrombotic disorders.

Clinical rationale for study

The aim of our study was to assess the correlation between fibrinogen concentration and other risk factors for vascular diseases and atherosclerotic changes such as hyperlipidemia, hyperglycaemia, sP-selectin and PDMPs concentration. Better understanding of the mechanisms of interaction between the factors involved in the coagulation processes may play an important role in the prevention of ischaemic diseases of the central nervous system. An elevated fibrinogen level may indicate a prothrombotic condition of stroke patients, supporting the important role of this factor in the process of thrombogenesis. Indeed, it could be a simple marker of an increased risk of ischaemic events.

Material and methods

The study group consisted of 94 patients who were admitted to the Department of Neurology and Stroke at the Medical University of Lodz, Poland with a diagnosis of acute non-lacunar ischaemic stroke. The diagnosis of stroke was established by a combination of medical history, clinical examination, and cerebral CT or MRI scans.

The stroke patients presented different concentrations of lipids and glycaemia ranges (presented as HbA_{1c} percentages) from low to high. The control group consisted of 21 normolipidemic and normoglycaemic patients with no history of cerebrovascular diseases, who were hospitalised in the Department of Neurology and Stroke due to discopathy or tension-type headache. The exclusion criteria were a history of infection shortly before stroke, severe liver disease, renal failure, evidence of malignant, chronic inflammatory diseases, and haemorrhagic diathesis. The risk factors for ischaemic stroke (arterial hypertension, ischaemic heart disease) were similar in the study and the control groups.

Plasma samples were taken no more than seven days after the onset of symptoms. Fibrinogen, $HbA_{1c,}$ total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoproteins cholesterol (LDL-C) levels were measured in a fasting state in all patients. Serum LDL-C levels were calculated using Friedewald's formula. Biochemical determinations were performed with an Olympus AU640 Analyser (Olympus Optical Co Ltd, Shizuoka, Japan). Blood plasma was obtained from EDTA-anticoagulated samples after 10 min of centrifugation and stored at –80°C until measurement. Blood concentration of sP-selectin was measured, according to the manufacturers' instructions, with commercially available ELISA kits (R&D Systems, Abingdon, UK).

Flow cytometry (FACScan, Becton Dickinson, San Jose, CA, USA) was used to measure the PDMPs. To avoid platelet activation, blood was withdrawn without stasis. The sample contained 0.1 mL of blood and 1 mL of a 0.5% solution of paraformaldehyde in PBS. All platelet measurements were performed within 90 min of blood withdrawal. The antibody anti-CD61-FITC (Dako) — a fluorescein-isothiocyanate-conjugated antibody to glycoprotein IIIa — was used as an activation-independent marker of platelets. To assess the extent of the nonspecific association of protein with platelets, a control tube containing antiCD61-FITC and nonfractionated PE conjugated IgG (Becton Dickinson) was used for each blood sample. The reaction mixture was incubated in a dark room, at room temperature, for 30 min. Then, the antibody-bound platelets were fixed with 200 µl of FACS flow liquid and analysed. Platelets were subtracted from other blood cells and identified by flow cytometry based on the size and platelet-specific CD61 surface expression. CD61-positive microparticles were defined as platelet-derived microparticles (PDMPs). They were distinguished from other platelets on forward scatter histograms based on their size < 0.2 µm. WinMDI 2.8 was used to analyse the data collected by flow cytometry.

Since all study variables did not pass the D'Agostino normality test, differences between groups were analysed using a Kruskal-Wallis test followed by a post-hoc Duna test for multiple comparisons adjustment. A Spearman's correlation was run to assess the relationship between continuous variables. All statistical analyses were performed using Statistica for Windows v. 8.0. The null hypothesis was rejected if $p < 0.05$.

The study was approved by the Ethics Committee of the Medical University of Lodz, Poland (No. RNN/465/11/KB).

Results

The stroke patients presented different concentrations of lipids: total cholesterol, LDL and HDL cholesterol and glycaemia ranged from normal to high values (patients with normo- or hyperlipidemia and normo- or hyperglycaemia). Mean concentrations of fibrinogen in patients were within the normal range (Tab. 1).

The results of our study showed a significantly higher sP-selectin concentration and a significantly higher percentage of PDMPs in stroke patients compared to control subjects ($p < 0.0001$; Tab. 2). Moreover, we observed a significant positive correlation between fibrinogen concentration and sP-selectin level in the group of stroke patients ($p = 0.001$; Fig. 1). A positive correlation was also noted between the concentration of fibrinogen and PDMPs ($p < 0.05$; Fig. 2). The results of our study also indicated a positive correlation between fibrinogen concentration and the level of HbA_{1c} ($p < 0.05$; Fig. 3).

Table 1. Clinical characteristics of study groups

	Stroke patients (n = 94) mean ± SD	CS (n = 21) mean ± SD
Sex, male/female	44/50	10/11
Age, years	71.43 ± 10.3	60.2 ± 14.6
TC [mmol/L]	4.68 ± 1.2	4.06 ± 0.5
LDL [mmol/L]	3.44 ± 1.35	2.45 ± 0.13
HDL [mmol/L]	1.15 ± 0.3	0.95 ± 0.17
TG [mmol/L]	1.56 ± 0.72	1.31 ± 0.48
HbA_{1c} [%]	6.5 ± 1.25	5.22 ± 0.37
FBG [mg/dL]	349 ± 87	322 ± 84
CRP	10.3 ± 14	5.8 ± 6.8

CS — control subjects; SD — standard deviation; TC — total cholesterol; LDL — low-density lipoprotein cholesterol; HDL — high-density lipoprotein cholesterol; TG — triglyceride; HbA_{1c} — glycosylated haemoglobin A_{1c}; FBG — fibrinogen; CRP — C Reactive Protein

Table 2. Percentages of platelet derived microparticles (PDMPs) and soluble P-selectin concentration in study group

Study parameters	Median	
	Stroke patients	CS
PDMPs [%]	12.78*	8.3
sP-selectin [ng/mL]	115.45*	83.32

*$p < 0.0001$ *vs* control

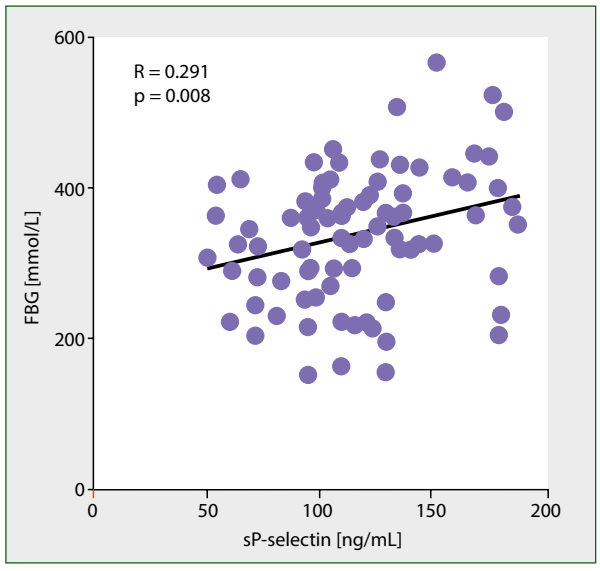

Figure 1. Correlation between fibrinogen concentration and sP-selectin level

Based on our previous studies, we expected that we would observe a positive correlation of atherogenic lipid fractions with fibrinogen. In fact, we did not observe any relation between fibrinogen concentration and TC, LDL, and TG levels. However, we found a significant negative correlation between fibrinogen concentration and the level of HDL in our study patients ($p < 0.05$; Fig. 3).

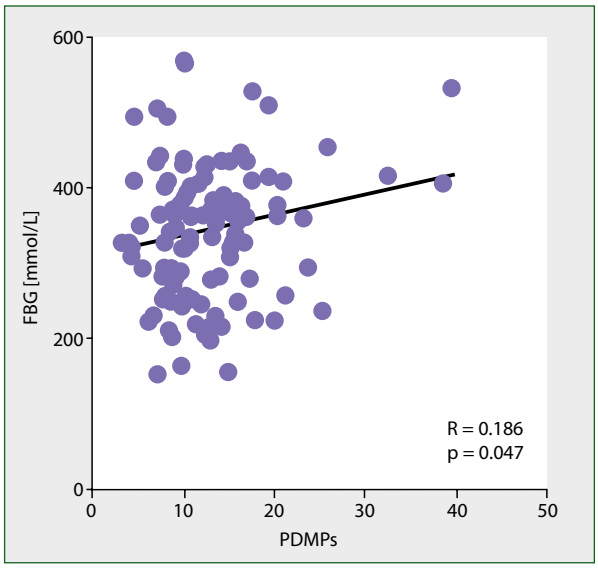

Figure 2. Correlation between fibrinogen concentration and % of PDMPs

Discussion

The principal finding of our study is a positive association between plasma fibrinogen concentration and the risk factors for atherosclerotic changes in patients with acute ischaemic stroke.

It is known that metabolic disturbances like hyperlipidemia and hyperglycaemia are factors with strong atherogenic properties, and that hyperfibrinogenemia plays a crucial role in the coagulation cascade leading to the formation of clots. Atherogenesis and atherothrombotic complications are also initiated in part by fibrin deposition [13, 14]. Fibrinogen also accelerates platelet aggregation, and increases its reactivity [15].

Our study found a positive correlation between fibrinogen level and serum concentration of sP-selectin. This suggests the important role they play in inflammation and haemostasis disorders. It has been proposed that soluble cell adhesion molecules, such as sP-selectin, could be a marker of the endothelial damage preceding atherosclerosis [16]. However, the increased plasma concentration of soluble P-selectin reflects also platelet activation related to the release of this adhesion molecule from activated platelets [17] and exerts procoagulant activity resulting from their high levels in the blood [18]. A previous study has demonstrated that fibrinogen increases platelet intracellular P-selectin level and affects P-selectin expression on the surface of platelets [19], leading to their

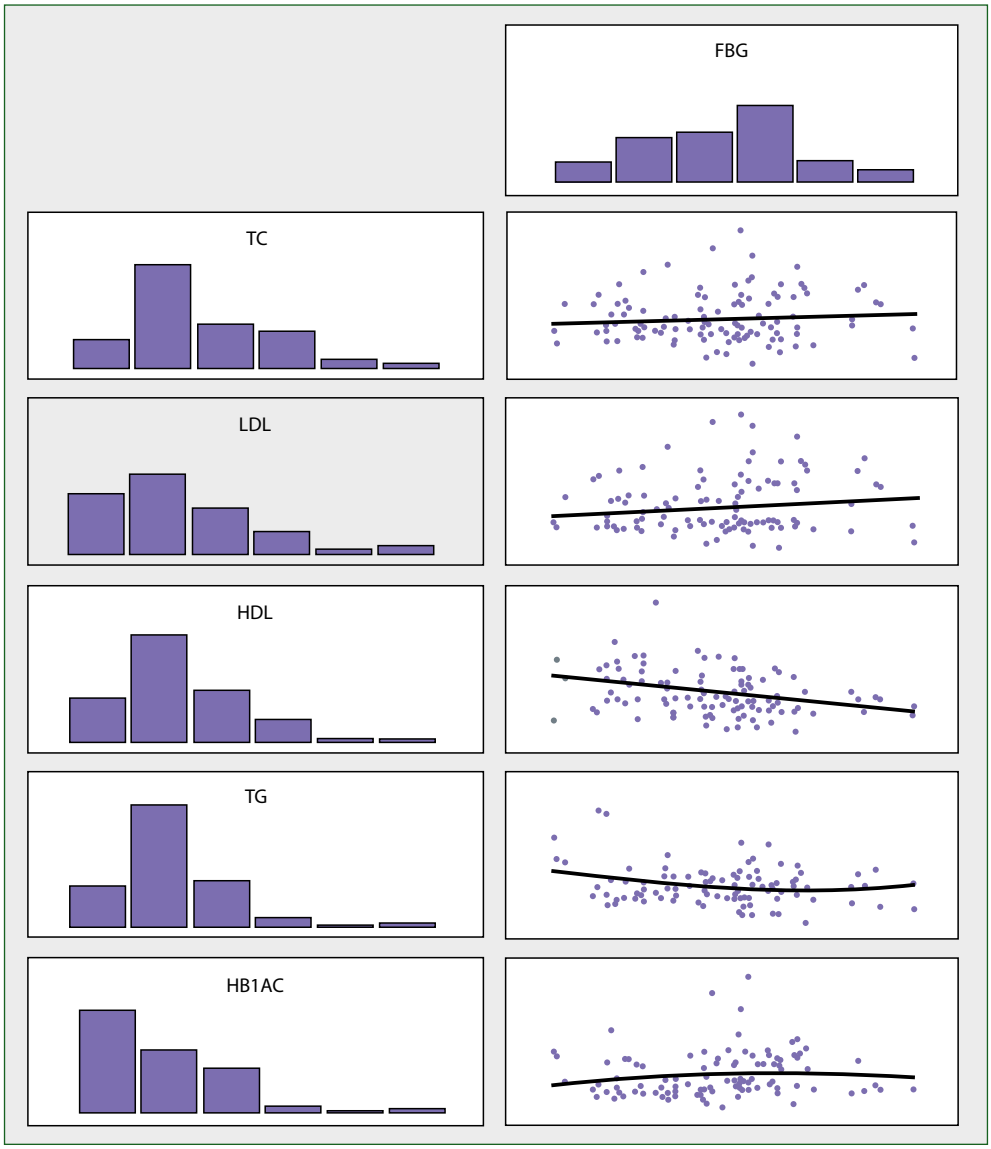

Figure 3. Correlations between fibrinogen concentration and TC, LDL, HDL, TG, HBA1c levels

activation. Because P-selectin expression on the platelet surface is short lasting, circulating degranulated platelets rapidly lose the surface P-selectin, and its level rises in the plasma pool [20]. It is important also to note that activated platelets cause Weibel-Palade body release, leading to P-selectin–mediated leukocyte rolling [21].

These findings taken together suggest that platelet P-selectin plays a crucial role in the process of inflammation and atherogenesis. In our study, we could not be sure of the origin of s-P selectin level. It could reflect platelets activation as well as endothelial cells damage. However, in both cases, fibrinogen plays an important role as the activator of the thrombogenic process. The role of blood platelets in the development of atherosclerotic lesions and in the enhancement of the prothrombotic state is also significant and mostly results from their interactions with damaged endothelial cells [22].

PDMPs play an important role in coagulation. So, an increased PDMPs level can lead to the state of hypercoagulability [23]. It has been reported that PDMP blood concentrations are significantly higher in hyperlipidemic patients with diabetes mellitus (DM), suggesting that PDMPs may participate in atherosclerosis development [24]. An elevated level of PDMPs observed in patients with ischaemic stroke may suggest their thrombogenic potential [24, 26].

The results of our study confirmed these observations, because we noticed in ischaemic stroke patients a positive correlation between fibrinogen and PDMPs level, the two potential athero- and thrombogenic factors. Another study also showed that in the acute phase of cerebral infarction, an increased fibrinogen level was associated with elevated levels of platelet-derived microparticles [27]. Thus, this correlation may reflect the influence of fibrinogen on platelet activation

and the role of these factors in the process of clot formation. It is also possible that the local generation of PDMPs in atherosclerotic arteries may promote arterial occlusion.

The next most important finding of our study was the significant positive correlation between fibrinogen concentration and the level of HbA_{1c} in patients with acute ischaemic stroke. A similar observation indicating a correlation between HbA_{1c} and fibrinogen levels was found in a study of diabetic patients with cardiovascular diseases [28]. Diabetes mellitus and hyperglycaemia lead to a hypercoagulable state, and several factors contribute to the prothrombotic condition which characterises patients with DM. The most important of these are increased coagulation, impaired fibrinolysis, endothelial dysfunction, and platelet hyperreactivity [29].

Our results indicate that a concomitance of hyperglycaemia and hyperfibrinogenemia may accelerate vascular complications. This conclusion is confirmed by the study of Lee et al. [30] which suggested that hyperfibrinogenemia in patients with acute stroke and diabetes mellitus was associated with early neurological deterioration. In DM patients, prolonged glycation related to insulin resistance increases the risk of thrombosis [31]. It has been shown that in patients under diabetic conditions fibrinogen is glycated. That leads to changes in the fibrin clot structure that reduce permeability and decrease fibrinolysis [32, 33]. These findings may explain the worse neurological outcome in patients with acute ischaemic stroke and DM.

The findings of our study do not indicate an influence of hyperlipidemia on fibrinogen concentration, although we found a negative correlation between fibrinogen and HDL levels in our study patients. A similar observation was found in the study by Pacilli et al. [29]. They noted a negative correlation between HDL level and fibrinogen concentration in diabetic patients with coronary artery disease. These results suggest an influence of poor glycaemic control and low HDL level on atherosclerotic processes.

Clinical implications

To sum up, hyperfibrinogenemia plays an important role in thrombotic disorders. In patients with acute ischaemic stroke the fibrinogen concentration is strongly correlated with atherogenic factors like hyperglycaemia, increased level of sP-selectin, and PDMPs, which reflect both atherosclerosis progression and platelet activation. Our findings suggest that an elevated fibrinogen level may represent a marker of prothrombotic condition exacerbated in the state of hyperglycaemia. Our findings indicate an important role played by fibrinogen in the process of thrombogenesis.

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Late onset multiple sclerosis — multiparametric MRI characteristics

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ABSTRACT

Introduction. Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) with heterogenic character. Typical age of onset is between 20 and 35 years. Clinically definite multiple sclerosis (CDMS) can occur also in patients older than 50 years. This type of MS is called Late Onset Multiple Sclerosis (LOMS). Until now, the differences in clinical course, type of first symptoms, and prognosis of LOMS have not been well established. Also the MRI characteristics of patients with LOMS have not been determined. Neither conventional nor nonconventional MRI features are known to be typical for LOMS.

Clinical rationale for the study. To investigate the MRI characteristics of LOMS patients based on conventional and non-conventional techniques.

Materials and methods. Twenty patients with LOMS were included in the study and 17 patients with typical onset of MS (TOMS) served as a comparative group. The two groups were matched in terms of disease duration and EDSS score. Conventional (T1- and T2-weighted images) and non-conventional (magnetization transfer images, proton magnetic resonance spectroscopy) MRI techniques were performed in all participants. Parameters from both techniques were compared between LOMS and TOMS groups.

Results. Patients with late onset of MS had lower Brain Parenchyma Fraction (BPF) ($p < 0.001$) and Grey Matter Fraction (GMF) values ($p = 0.008$) than the TOMS group. There was no statistical differences in White Matter Fraction (WMF) values between the groups ($p = 0.572$). Patients with LOMS and TOMS statistically differed in the peak height ($p = 0.018$), peak location ($p < 0.001$), and MTR mean value ($p < 0.001$). Patients with LOMS manifested lower concentrations of NAA+NAAG and NAA+NAAG/Cr than patients with TOMS ($p = 0.009$ and $p < 0.001$ respectively). No statistical difference was found between the groups in terms of mean mIn ($p = 0.346$) and mean GPC+PCh ($p = 0.563$). We did not find a statistical difference in T1- and T2- lesion load ($p = 0.1$, $p = 0.3$ respectively) although T1/T2 lesion ratio was higher in the LOMS group.

Conclusion and clinical implications. MRI parameters in patients with LOMS differed significantly from those obtained from the TOMS group. Our results, which indicate that in LOMS patients brain tissue damage is more advanced than in TOMS patients, may contribute to a better understanding of the heterogeneity of MS.

Key words: Late onset multiple sclerosis, multiple sclerosis, magnetic resonance imaging, brain atrophy, proton magnetic resonance spectroscopy, magnetization transfer ratio

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Introduction

In the typical course of multiple sclerosis (MS), the first symptoms occur between the ages of 20 and 35 years. However, according to epidemiological findings, in 4.6–9.6% of MS cases the first symptoms are observed in patients aged over 50 [1, 2, 3]. This form of the disease is referred to as Late Onset Multiple Sclerosis (LOMS) [4–8]. Very recently published results from Denmark have shown that the incidence of LOMS has increased since the 1950s, particularly in women [9]. The

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population of these MS patients clinically differs from the population of patients who are affected by MS at the typical age (Typical Age of Onset Multiple Sclerosis, TOMS). In LOMS patients, the primary progressive form of the disease (PPMS) is more common than in TOMS (50–80% in LOMS vs 10–20% in TOMS) [5–7, 10–13]. The first symptoms of MS in LOMS and TOMS are also different. In LOMS, cerebellar and sensory symptoms have been reported to be much more common than in TOMS [4, 5]. Gait disturbances with spastic paraparesis are the most common motor symptoms of LOMS [6]. The prognosis in the LOMS form of the disease is less favourable than for patients with the TOMS form [5, 6, 14]. LOMS patients seem to progress more rapidly than TOMS, especially with the primary progressive course of disease [13, 15, 16]. LOMS has also been associated with a severe disease course and has been found to be a strong predictor of conversion from RR to secondary progressive MS [14]. Additionally, there has been shown to be no impact of interferon beta treatment on disability progression in RR- LOMS patients [13]. Although magnetic resonance imaging is an established and very important paraclinical tool in the diagnosis of MS, there is very limited data concerning MRI in LOMS patients. In one study [11], the sensitivity and specificity of radiological MS criteria in patients aged over 50 years were evaluated. The study found that MRI Barkhof criteria provided the best compromise for the diagnosis of MS patients with late onset. Other studies have reported different brain and spinal cord localisations in LOMS and TOMS patients, and more brain MRI inflammatory activity in TOMS than in LOMS patients [17, 18]. A recently published study showed severe grey matter and brainstem atrophy in LOMS patients, with primary cognitive dysfunction [19]. The purpose of this study was to describe radiological characteristics based on conventional and non-conventional MRI techniques in LOMS patients.

Patients and methods

Patients

Twenty LOMS patients (15 women, 5 men) consecutively admitted to our neurological department were included in the study. Late onset MS patients were defined as patients who fulfilled McDonald's criteria of 2010 [21, 22], with first symptoms having appeared after their 50th birthday. Additionally, 17 (11 women, 6 men) typical age of onset MS (TOMS) patients, defined as patients who fulfilled the 2010 McDonald criteria [20, 21] with first symptoms appearing between the ages of 20 and 45, were included in the study as a comparative group. All patients were assessed by an experienced neurologist and neurological status was measured by the Expanded Disability Status Scale (EDSS) [22]. The two groups were matched in terms of gender, disease duration, and EDSS score.

Methods

Each patient underwent magnetic resonance imaging on a 1.5 Tesla scanner (Avanto , Erlangen, Germany). In the first step, conventional MRI included dual-echo (TR = 5,000 msec, TE = 20/80 msec; 50 slices, thickness = 3mm gap = 0.0 mm, matrix 154x256, and FOV = 250 mm), T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE TR = 9.7 msec, TE = 4 msec; eff thick 1.5 mm, no partitions 164, matrix 192 × 256), T1 weighted imaging with and without contrast administration (TR = 30 ms, TE = 11 msec, thickness = 3 mm gap = 0.0 mm , FOV 250 mm, matrix 256 × 256), fl 2D MT ON (TR = 800 msec, TE = 10 msec, thickness = 3 mm, FOV = 250 mm, matrix = 159x256); fl 2D MT OFF (TR = 800 msec, TE = 10 msec, thickness = 3mm, FOV = 250 mm, matrix = 159 × 256) were acquired. In the second step, water suppressed proton magnetic resonance spectroscopy (H-MRS) was performed using a stimulated echo acquisition mode sequence STEAM (TE = 20 ms, TR = 6,000 ms, 64 averages). Volume of interest (VOI) = 8 ml was located in NAWM in left and right centrum semiovale far from white matter lesions. To avoid the inclusion of lesions, cerebrospinal fluid, or grey matter in VOI, the borders of the VOI at upper and lower slides were checked. Before positioning the voxel, global shimming of the whole brain was performed. After location, the VOI local magnetic field was homogenised by localised shimming on the water peak. Water suppression was achieved by means of chemically selective saturation pulse.

MRI data analysis

The volumes of focal lesions on T2-weighted images and T1-weighted images were measured with the application of the semi-automated technique (JavaImage, Xinapse version 5.0, UK) [21]. Brain atrophy was evaluated based on the MP-RAGE sequence. Brain Parenchyma Fraction (BPF), Grey Matter Fraction (GMF), and White Matter Fraction (WMF) were calculated using JavaImage software. Evaluation of normal appearing brain tissue (NABT) on the basis of magnetization transfer was conducted with the use of two gradient sequences: flash 2D without magnetization transfer and flash 2D with magnetisation transfer. With the use of adequate algorithms, a computer program calculated the magnetisation transfer ratio map (MTR) for particular analysed points [24]. Evaluation of the NABT was made with the use of MTR histograms. In the first stage, the brain tissue was extracted semiautomatically from cranial bones and the cerebrospinal fluid. Next, the MTR map was masked with focal lesions. After isolating them from the map, the remaining part of the image which showed only the NABT was used to make a MTR histogram. Mean value of MTR, peak position and peak height of the MTR histogram were analysed.

Results of H-MRS were estimated using the linear combination model (LCModel, Provencher, 1993) [25]. Concentrations of the following metabolites were estimated using a basis set of 15 metabolites: creatine (Cr), total N-acetyl-aspartate compounds (tNAA = N-acetyl-aspartate (NAA) + N-acetyl--glutamate (NAAG)), choline-containing compounds (tCho) — including glycerophosphocholine and phosphocholine (GPC+PCh), and myoinositol (mIn). All subjects gave written, informed consent before entering the study. The study was approved by the Local Ethics Committee.

Statistical analysis

Statistical analysis was performed using Statistica 10th CSS. The results of the quantitative variables are presented as a mean ± SD (standard deviation), and median ± SD, as required.

The data was verified for normality (Shapiro–Wilk test) of distribution and equality of variances. To compare the means, the Student's t-test was used when the distribution was normal and in other cases U Mann-Whitney test was used to compare received average values. To exclude the impact of age on the brain atrophy measures (BPF, GMF, WMF) ANCOVA analysis with age as a covariate was conducted. However, for all three parameters (BPF, WMF, GMF) assumptions of ANCOVA was not met (linearity of relationship between outcome variables i.e. brain atrophy parameters and covariate i.e. age as well as lack of normal distribution for outcome variables and presence of outliers).

Therefore, we could not conduct ANCOVA analysis and we have checked the presence of relationship (linear regression) and correlation (Spearman's correlation coefficient) between age and each of the brain atrophy parameters for both groups (TOMS and LOMS). The limit of statistical significance was set at $p < 0.05$ for all the analyses.

Results

Demographic characteristics of the LOMS and TOMS patients

The mean age of LOMS patients was 57.8 years (SD ± 4.7) and the mean age of TOMS patients was 34.3 years (SD ± 6.7). None of the patients had comorbidities such as hypertension, hyperlipidemia or heart disease. None of the patients were undergoing immunomodulatory or immunosuppression treatment.

The demographic and clinical characteristics of the MS patients are set out in Table 1.

MRI analysis

Conventional MRI results

The T2 and T1-lesion volume was similar in both groups ($p = 0.3$, $p = 0.1$ respectively). T1 /T2 volume ratio was higher in the LOMS group but not statistically significantly (Tab. 2).

Nonconventional MRI results

Analysis of the BPF in the LOMS and TOMS groups showed that in LOMS BPF was significantly lower than in TOMS ($p < 0.01$). Similarly, the LOMS group was characterised by lower GMF values than the TOMS group ($p = 0.008$). There was no statistical difference between the groups in WMF ($p = 0.527$) (Tab. 2). There was no correlation between age and any of the three brain atrophy parameters either in the TOMS, nor in the LOMS group (LOMS: BPF $r = 0.13$, $p = 0.598$; GMF $r = -0.25$, $p = 0.287$; WMF $r = 0.32$, $p = 0.163$, TOMS: BPF $r = -0.06$, $p = 0.815$, GMF $r = -0{,}14$. $p = 0.585$; WMF $r = 0.07$, $p = 0.775$ respectively).

Analysis of MTR histogram data revealed that in the LOMS group mean MTR value was significantly lower than in the TOMS group ($p \leq 0.001$). We also found that the peak height and peak position were significantly lower in LOMS patients compared to TOMS patients ($p = 0.018$, $p < 0.001$ respectively) (Tab. 2). Analysis of H-MRS parameters between the LOMS and TOMS groups showed that in LOMS patients concentrations of NAA+NAAG was significantly lower than in TOMS patients ($p = 0.009$). Concentrations of the remaining H-MRS metabolites from the NAWM were comparable in both groups ($p > 0.05$) (Tab. 2).

Discussion

In this study, we assessed differences in MRI characteristics between late and normal age of onset multiple sclerosis patients. We found that LOMS patients differ from TOMS patients in non-conventional MRI characteristics.

Table 1. Demographic and clinical characteristics of LOMS and TOMS groups. Table presents mean values; brackets contain standard deviation values and size of group (n)

Variables	LOMS n = 20	TOMS n = 17	p
Sex M/F	5/15 (25%)	6/11 (35%)	< 0.001
Age at occurrence of first symptoms (years)	53.7 (3.6)	29.8 (7.7)	< 0.001
Duration of MS (years)	5.0 (3.15)	5.12 (3.77)	0.92
EDSS (median)	3.5 (1.56)	3.5 (1.53)	0.89

LOMS — Late Onset Multiple Sclerosis; TOMS — Typical Onset Multiple Sclerosis; EDSS — Expanded Disability Status Scale

Table 2. Conventional and non-conventional MRI results in LOMS and TOMS groups

Variables		LOMS n = 20	TOMS n = 17	P
Volume of lesions on T2-weighted images [mm^3] ± SD		11,197.2 ± 10,018.3	10,157.5 ± 12,489.8	0.3
Volume of lesions on T1-weighted images [mm^3] ± SD		5,357.7 ± 5,213.1	4,044.34 ± 7,094.6	0.1
T1 volume/T2 volume ratio ± SD		0.72 ± 1.23	0.41 ± 0.32	0.88
Brain Fractions				
	BPF	0.752 ± 0.03	0.82 ± 0.07	< 0.001
	GMF	0.4 ± 0.03	0.43 ± 0.13	0.008
	WMF	0.36 ± 0.05	0.37 ± 0.16	0.572
MTR Histogram				
	Peak location	34.94 ± 1.25	42.19 ± 1.94	< 0.001
	Peak height	56.79 ± 6.13	67.2 ± 12.19	0.018
	MTR mean value	28.82 ± 1.58	36.72 ± 2.71	< 0.001
H-MRS				
	mean NAA+NAAG	7.1 ± 1.56	8.81 ± 1.42	0.009
	mean In	4.88 ± 1.40	4.49 ± 1.13	0.346
	mean GPC+PCh	1.59 ± 0.41	1.65 ± 0.31	0.563

LOMS — Late Onset Multiple Sclerosis; TOMS — Typical Onset Multiple Sclerosis; SD — standard deviation; BPF — Brain Parenchymal Fraction; GMF — Grey Matter Fraction; WMF — White Matter Fraction; MTR — Magnetic Transfer Ratio; H-MRS — Water Suppressed Proton Magnetic Resonance Spectroscopy; NAA+NAAG — N-acetyl-aspartate (NAA) + N-acetyl-glutamate (NAAG); GPC+PCh — glycerophosphocholine and phosphocholine (GPC+PCh); In — myoinositol (mIn)

To the best of our knowledge, there are no published results concerning a multiparametric MRI comparison of a LOMS and TOMS study. Most of the published results concerning differences between LOMS and TOMS patients have referred to the clinical presentation [5–8, 11–13] However, it seems that an MRI study could add more data that would explain the observed differences.

We did not find a difference of T1 and T2-lesion volume between the LOMS and TOMS groups. However, T1/T2 lesion volume ratio was higher in the LOMS than in the TOMS group. Results of published studies indicate that around one third of the lesions visible on T2-weighted images correspond to hypointensities ('black holes') on T1-weighted sequences [26]. Additionally, a comparative analysis of the histopathology and MRI examination revealed that focal hypointense lesions on T1-weighted images are associated with a decrease in axonal density and axonal loss [27]. Higher T1/T2 lesion volume ratio in LOMS might indicate that there is a greater contribution of axonopathy on pathology in these types of MS. We speculate that the process which takes place in focal changes in LOMS is more destructive than in TOMS patients, with more dominant axonal damage.

Another interesting finding comes from our brain atrophy analysis. Brain Parenchyma Fraction was significantly lower in our LOMS group compared to our TOMS group. The findings of a longitudinal MRI analysis throughout the lifespan in healthy subjects revealed that brain volume change is an ongoing process [28]. Brain volume increases in childhood and adolescence until the age of 13 years, while between 18 and 35 years of age there is a second period of brain volume growth, or at least stability. After the age of 35, there is a steady brain volume loss (0.2% per year) accelerating to 0.5% per year at age 60 [28].

Additionally, recently published results showed that percentage of brain volume change was also associated with magnetic field strength [29]. In multiple sclerosis patients, the rate of brain atrophy is faster than in healthy age-matched controls (0.5%/- 1.35%/year vs 0.1–0.3%/year) respectively [30]. Brain volume loss in MS patients depends on different factors such as disease stage, neurological disability, type of pharmacological treatment, and other factors unrelated to the disease [31]. Although the LOMS group was significantly older than the TOMS group, we exclude the impact of age as a covariant on differences of brain atrophy parameters between the LOMS and TOMS groups.

A lack of correlation between age and brain atrophy parameters confirms that the significant difference in brain atrophy parameters between TOMS and LOMS is not related to differences in age between the groups. All participants were scanned on the same scanner (1.5 T) with no up-grade during the study period and with the same MRI protocol. Therefore, we can exclude the impact of technical factors on brain volume differences between LOMS and TOMS patients.

Because cardiovascular risk factors such as hypertension, hyperlipidemia and heart disease are associated with an increased number of white matter focal abnormalities and decreased whole-brain and grey matter volume, we excluded from our final analysis MS patients with these comorbidities [32–34].

Finally, our groups were also adequately matched in terms of gender, disease duration and disability level (EDSS). Because we can exclude the impact of these factors of the final results on our findings, we can speculate that the pathological processes contributing to the pathology of LOMS and TOMS are different. Lower BPF in LOMS patients indicates that in this type of MS the neurodegenerative process is much more advanced than in TOMS. We can also assume that the process of brain plasticity is less effective in LOMS than in TOMS.

Another interesting result came from tissue brain fraction analysis. Grey matter fraction in LOMS patients was significantly lower than in the TOMS group. We did not find such differences when comparing white matter fraction. This may indicate that in the LOMS group grey matter atrophy is much more advanced than in TOMS patients.

Based on published results [35–37], we can also speculate that in the LOMS group grey matter atrophy precedes white matter damage. These findings, and the lack of WMF atrophy, may also suggest that in LOMS patients a neurodegenerative process in grey matter makes a much greater contribution to MS pathology and global brain atrophy than in TOMS patients. Grey matter atrophy in our LOMS group is in line with previously published results which showed that in LOMS patients with cognitive dysfunction grey matter damage is a very characteristic MRI finding [19]

The results of the magnetization transfer imaging support another argument for differences between LOMS and TOMS patients. In the LOMS group, the peak location, the peak height, and the mean MTR value were all lower than in the TOMS group. Lower MTR values signify greater diminution of structure integrity and higher intensity of pathological processes [38]. Especially in MS, it shows not only demyelination but also axonal loss [39]. Because patients were adequately matched with regard to disease duration and neurological deficits, we assume that damage of NAWM in LOMS patients is much more advanced than in TOMS patients. We can also speculate that damage of NAWM has a greater impact on MS pathology in LOMS than in TOMS. We could also speculate that subclinical NAWM changes may occur earlier in LOMS than in TOMS. This might also imply a different type of mechanism responsible for the pathology in the central nervous system in those two types of MS.

Our results from H-MRS spectroscopy added more information concerning LOMS and TOMS differences. We found that the tNAA concentration was significantly lower in the LOMS group than in the TOMS group. Based on previously published results which detected that decreased tNAA concentration correlates with axonal damage and loss, lower tNAA in the LOMS group with normal concentration of other metabolites in our study seems to be further evidence of more widespread axonal pathology in LOMS than in TOMS patients.

The very interesting question arises as to why the first symptoms in LOMS occur later than in TOMS. It seems possible that LOMS patients, earlier than TOMS patients, are affected by pathological processes but on a subclinical level. The dynamic of this process seems to be slower in LOMS than in TOMS. We can also assume that natural brain damage that occurs in older patients may contribute to the first presentation of MS. We can also speculate that for a long period, the reparatory processes are more effective in LOMS than in TOMS patients, which prevents an earlier occurrence of neurological symptoms. Along with the course of the disease and with advancing age, the mechanism of remyelination declines, prompting the presentation of MS clinical symptoms [3].

Our study has some limitations. It is a description of a small population of LOMS and TOMS patients; due to this fact, the presence of multiple sclerosis phenotypes (RRMS vs. PPMS) in both groups of patients was not included. We are aware that some differences in MRI of these two forms of MS may affect the obtained results in conventional MRI techniques. Nonetheless, to the best of our knowledge, this is the first description of conventional and non-conventional MRI in a LOMS population.

Conclusions

Differences in MRI presentation between LOMS and TOMS patients confirm the heterogenic character of MS, with probably more advanced axonal pathology in LOMS than in TOMS patients. Our results may contribute to a better understanding of the differences in the pathogenesis of various types of MS, and we hope may support improved therapeutic decision-making.

Conflict of interests: *None*

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Sex-related differences among ischaemic stroke patients treated with intravenous thrombolysis in Poland

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ABSTRACT

Aim of study. We investigated sex differences in ischaemic stroke patients treated with intravenous alteplase. **Clinical rationale for study.** We suggest that it is necessary to improve care for women with atrial fibrillation. Our data suggests that closer evaluation of treatment for ischaemic stroke in men and women is needed, preferably in the form of a prospective study.

Materials and methods. This was a multicentre analysis of 1,830 ischaemic stroke patients treated with alteplase from 2004 to 2012. Data was prospectively collected in the Safe Implementation of Treatments in Stroke (SITS) registry. The main outcome measures were symptomatic intracerebral haemorrhage (sICH) within 36 hours of treatment, three months of functional independence, and mortality.

Results. Women were significantly older (mean age 71.3 *vs* 66.2 years; $p < 0.01$), more often suffered from hypertension (78.3% *vs* 70.1%; $p < 0.01$) and cardio-embolic strokes (34.7% *vs* 27.1%; $p < 0.01$), and presented heavier baseline deficits. There were no differences in sICH, but after three months fewer women were functionally independent (46.5% *vs* 53.3%; $p < 0.01$) and women had higher mortality (26.0% *vs* 19.7%; $p < 0.01$).

Conclusions: Of the ischaemic stroke patients treated with intravenous thrombolysis, women had worse long-term outcomes than men. This discrepancy may be explained by the older age and higher proportion of cardio-embolic strokes with more severe baseline deficits. However, multiple logistic analysis did not show that sex itself had an impact on the greater mortality in women after a stroke, or on the poorer prognosis.

Key words: alteplase, ischaemic stroke, sex differences, risk factors, outcome

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Introduction

In recent years, a lot of research has been published looking into ischaemic stroke's epidemiology, prognosis, risk factors, pathogenesis, clinical picture and course, as well as its treatment and sex-dependent outcomes [1]. Many studies have shown that women suffer from more severe strokes than men, and have less favourable prognoses, something which is additionally modified by their home country's level of development [2–9].

Women also appear to be less often treated with intravenous thrombolysis [9–13]. It is uncertain if they equally benefit from intravenous and intra-arterial thrombolysis treatment [12–17]. One should also take into account specific national or regional differences in patients' profiles [18].

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Clinical rationale for the study

The aim of this study was to investigate sex-related differences in patient profiles and outcomes among Caucasian ischaemic stroke patients from Polish centres treated with intravenous thrombolysis.

Materials and methods

This is a retrospective multicentre analysis of 1,830 consecutive ischaemic stroke patients treated with intravenous alteplase (tPA) in Polish centres from 1 January 2004 to 31 December 2012. The data was prospectively recorded in the Safe Implementation of Treatments in Stroke – International Stroke Thrombolysis Registry [19]. The main outcome measures were: functional independence (0–2 in modified Rankin Scale score-mRS) at three months; sICH within 36 hours of treatment; and three-month mortality [19]. Dependency was defined as an mRS score of 3–5 [20].

Stroke events were defined according to the World Health Organisation's criteria, and stroke was confirmed in all patients by neuroimaging [21].

Stroke subtypes were classified on admission according to the Trial of Org10172 in Acute Stroke Treatment (TOAST) criteria [22]. Stroke severity was measured using the National Institutes of Health Stroke Scale score (NIHSS) on admission and at discharge. Conventional stroke risk factors, including hypertension, diabetes mellitus, atrial fibrillation (AF), hyperlipidemia and tobacco smoking, were defined as self-reported in previous medical records or newly diagnosed. Obesity was defined as a body mass index ≥ 30kg/m^2. Pre-stroke mRS, at discharge, and three months after the stroke, were assessed during an interview with the patient or his or her proxy.

Statistical analysis

Continuous variables are presented as mean with standard deviation or median and quartiles, depending on the normality of data distribution. Categorical variables are presented as a number of observations with percentage. For comparisons between men and women, Mann-Whitney U tests and Fisher's exact tests were used, as appropriate [23]. A p value = 0.05 was considered significant. All analyses were performed in R v. 3.4.0. [24]. A multiple logistic regression model, which was adjusted for age, onset-to-door time, door-to-treatment time, presence of hypertension, diabetes, AF, smoking (currently), and baseline mRS score before stroke (0 and 1 only), was applied to identify factors contributed considerably to the main endpoints (death, mRS after three months, sICH according to SITS).

Results

The studied population included 1,830 consecutive ischaemic stroke patients treated with alteplase between January 2004 and December 2012 in many centres in Poland. The analysed group included 819 women (44.8%). Women were significantly older (mean age 71.3 *vs* 66.2 years), were more frequently dependent before a stroke (9.3% *vs* 3.1%), were more often burdened with AF (35.8% *vs* 26.3%) and hypertension, but were less often smokers (13.3% *vs* 33.9%) (Tab. 1). Women suffered more severe strokes (median baseline NIHSS score 13 *vs* 11). Stroke aetiology in women was more often cardioembolic (34.7% *vs* 27.1%) (Tab. 1), although they used anticoagulants less often (3.8% *vs* 4.2%).

Women had significantly longer onset-to-door time (ODT) (median 70 *vs* 62 min) and longer onset-to-treatment time (OTT) (median 160 *vs* 154 min), with a borderline difference in door-to-treatment time (DTT) (median 75 *vs* 80 min, p = 0.05). Their stroke unit stay was significantly shorter (median 8.7 *vs* 9.1 days). There were no significant differences in the prescription rates of hypotensive medications, aspirins, vitamin K antagonists or new oral anticoagulants, but women were less likely than men to be prescribed statins on discharge from hospital (83.4% *vs* 90.1%).

Women had significantly higher mortality, both at day 7 (15.5% *vs* 9.6%) and three months after the onset of symptoms (26.0% *vs* 19.7%) (Tab. 2). This discrepancy was not modified by the presence of AF, hypertension or diabetes. There were no significant differences in the occurrence of sICH according to the ECASS (European Cooperative Acute Stroke Study) or the SITS definition (Tab. 2) [19]. However, a tendency towards a higher occurrence of sICH according to ECASS in women was particularly marked in subgroups of patients with diabetes, with heart failure, with aspirin use before stroke, and with disability before stroke (Tab. 3).

Multiple logistic analysis did not confirm that sex itself had an impact on greater mortality in women after a stroke or a worse prognosis (Tab. 4).

Discussion

In line with data from other cohorts, we found that women with acute ischaemic stroke treated with tPA are older than men and more often suffer from strokes of cardioembolic aetiology [7, 9, 17, 25–29]. However, the mean age at stroke onset among Polish women was seven years lower than in Sweden (mean age 71.3 *vs* 78.4 years). In other words, female Polish patients experienced a stroke seven years earlier than female Swedish patients [30]. Female Polish patients, despite a higher proportion of pre-stroke AF, used oral anticoagulants as frequently as men (3.8% *vs* 4.2%). Similarly to Swedish patients [30], no differences were found in the prescription rate of anticoagulants after an ischaemic stroke. Higher proportions of AF and hypertension in women have also been reported in other cohorts [9, 28, 29, 31].

Probably because of all the abovementioned reasons, the neurological condition on admission of women was significantly worse than men [30, 32, 33]. We observed that the ODT,

Table 1. Sex differences in risk factors and clinical characteristics among patients with ischaemic stroke treated with intravenous thrombolysis

Characteristics	Men	Women	p-value
Cases, n (%)	1.011 (55.2)	819 (44.8)	< 0.01
Age, years, mean (SD)	66.2 (11.0)	71.3 (11.6)	< 0.01
Atrial fibrillation, n (%)	262 (26.3)	289 (35.8)	< 0.01
Congestive heart failure, n (%)	214 (21.5)	151 (18.8)	0.16
Hyperlipidemia, n (%)	317 (33.4)	254 (32.6)	0.76
Hypertension, n (%)	700 (70.1)	637 (78.3)	< 0.01
Diabetes mellitus, n (%)	184 (18.4)	171 (21.1)	0.15
Previous stroke, n (%)	138 (13.8)	107 (13.2)	0.78
Smoking – previous, n (%)	189 (28)	47 (6.8)	< 0.01
Smoking – current, n (%)	324 (33.9)	106 (13.3)	< 0.01
Baseline NIHSS score, median (Q1-Q3)	11 (7–16)	13 (7.5-18)	< 0.01
SBP mean, mm Hg (SD)	151.4 (20.8)	152.4 (20.4)	0.18
DBP mean, mm Hg (SD)	85.7 (13.1)	83.7 (12.9)	< 0.01
Glucose, mg/dl, (SD)	131.1 (47.3)	135.5 (49.3)	0.01
Large-vessel disease, CAS, n (%)	155 (16.6)	69 (9.3)	< 0.01
Large vessel disease, other, n (%)	301 (32.2)	249 (33.4)	0.64
Cardio-embolic, n (%)	253 (27.1)	259 (34.7)	< 0.01
Lacunar stroke, n (%)	57 (6.1)	57 (7.6)	0.24
Other/unusual n (%)	35 (6.0)	56 (4.7)	0.28
Unknown, n (%)	111 (11.9)	76 (10.2)	0.31

SBP — systolic blood pressure; DBP — diastolic blood pressure

Table 2. Sex differences in outcomes at three months after ischaemic stroke treated with alteplase

Outcome	Men	Women	p-value
Death, n (%)	199 (19.7)	213 (26.0)	< 0.01
mRS 0-1, n (%)	358 (35.4)	245 (29.9)	< 0.01
mRS 0-2, n (%)	539 (53.3)	381 (46.5)	< 0.01
Intracerebral haemorrhage according to ECASS def., n (%)	45 (4.7)	50 (6.5)	0.11
Intracerebral haemorrhage according to SITS def., n (%)	14 (1.5)	16 (2.0)	0.36

ECASS — European Cooperative Acute Stroke Study; SITS — Safe Implementation of Treatments in Stroke; mRS — modified Rankin Scale score

Table 3. Frequency of symptomatic intracerebral haemorrhage (sICH) according to ECASS definition in women and men depending on presence of additional factor

Additional factor	Men sICH		Women sICH		p-value
	with factor	without factor	with factor	without factor	
Diabetes, n (%)	9 (5.3)	36 (4.6)	13 (8.0)	36 (6.0)	1.36 (CI 0.52–3.08)
Atrial fibrillation, n (%)	20 (8.2)	23 (3.3)	27 (9.9)	22 (4.5)	1.31 (CI 0.74–2.56)
Hypertension, n (%)	8 (4.8)	12 (4.2)	42 (7.0)	32 (4.8)	1.4 (CI 0.86–2.27)
Heart failure, n (%)	11 (5.5)	32 (4.3)	15 (10.9)	34 (5.5)	1.47 (CI 0.66–3.38)
Aspirin use before stroke, n (%)	12 (4.0)	28 (4.4)	21 (8.4)	28 (5.6)	1.56 (CI 0.75–3.28)
Disability before stroke, (mRS > 1) n (%)	3 (4.5)	39 (4.5)	14 (11.3)	32 (5.1)	1.28 (CI 0.78–2.05)

Table 4. Multiple regression logistic model adjusted for age, onset-to-door time (OTD), door-to-treatment time (DTT), presence of hypertension, diabetes mellitus, AF, smoking (current), score 0–1 in mRS before stroke, initial stroke severity on NIHSS scale

Variable	OR ± 97.5 CI	2.5% CI	97.5% CI
Death	1.2104	0.8752	1.6736
Intracerebral haemorrhage according to SITS def.	1.2493	0.5059	3.12222
mRS 0–1 after 3 months	1.0037	0.7803	1.2909
mRS 0–2 after 3 months	1.0137	0.7851	1.3098

OR — odds ratio; CI — 95% confidence interval; SITS — Safe Implementation of Treatments in Stroke; mRS — modified Rankin Scale score

as well as the OTT, in women was on average several minutes longer than in men. This might be caused by their older age, more frequent functional dependence, and more frequent living alone [32]. The difference may not be of major clinical importance overall, but it still deserves to be addressed. Attempts should be made to equip older women who live alone with the knowledge of how to recognise stroke and how to react. It might also be profitable to consider providing them with a special device to facilitate communication with ambulances in case of emergency. As there were no differences in DTT, we can assume that the processing of patients qualified for tPA is equal for both sexes while in hospital.

Yeo et al. [15] observed, in a group of 2,460 ischaemic stroke patients treated with intravenous alteplase, that if the outcome of women improved significantly (a 10 or more points reduction in NIHSS scale) within 2–24 hours, there was a doubled chance of regaining full independence at three months after an ischaemic stroke. This illustrates the necessity of women receiving particularly good care within the first 24 hours from the onset of an ischaemic stroke. If such care were to become standard, then the number of women with greater independence at three months would increase.

Our study has some limitations. It used data from a voluntary multicentre registry. The evaluation of control CT scan was performed on site, and the diagnosis of sICH was made at the discretion of an attending physician. It is impossible to determine how many patients were not reported, and why. Because only a fraction of Polish stroke centres participate in the SITS, one may expect that our results are generalisable to dedicated stroke units, but probably not fully to all Polish stroke units. Nonetheless, the registry provides the best available multicentre real life data.

Clinical implications/future directions

Our findings confirm that there are several important sex-related differences in ischaemic stroke patients treated with intravenous alteplase. Although women do not seem to be at a clearly increased risk of intracerebral haemorrhage, they more often have a poor long-term outcome and higher mortality. This discrepancy may be to some extent explained by the older age and higher proportion of cardio-embolic strokes with more severe baseline deficit.

One may assume that the optimisation of primary prevention, improved stroke awareness, and improved communication with ambulance dispatchers could reduce the gap between men and women. Therefore, specific central health policies should be encouraged and properly implemented.

Our data suggests that closer evaluation of the treatment of ischaemic stroke in men and women in Poland is needed, preferably in the form of a prospective study. It is also necessary to improve care for women with AF.

Conflict of interests: *None*

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Preferred muscles in cervical dystonia

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ABSTRACT

The classification of abnormal posture and the assessment of the affected muscles in cervical dystonia (CD) have changed in recent years. To determine the frequency of injected muscles, we studied 306 patients with CD. The mean age was 55.5 ± 13.1 years (range 21–90), 67% were female. Splenius capitis was the most commonly injected muscle (83%), followed by sternocleidomastoid (79.1%), and trapezius muscles (58.5%). The three next most common were the levator scapulae, semispinalis capitis, and obliquus capitis inferior muscles. The study shows that the most commonly injected muscles have remained unchanged over the past few decades, although the concept has changed. However, several new muscles have been added that were previously never, or hardly ever, considered.

Key words: cervical dystonia, torticollis, sternocleidomastoid muscle

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Introduction

For a long time, the injection scheme in so-called spasmodic torticollis was relatively standardised, with only a few muscles routinely injected. This was because only four basic patterns of CD were recognised: torticollis, laterocollis, anterocollis and retrocollis. Furthermore, in most countries, approval was only valid for rotary torticollis. Sternocleidomastoid, splenius capitis and trapezius muscles were recommended and injected most frequently [1]. With increasing experience, the injection patterns became more complex and, at least since the introduction of the Col-Cap concept, other muscles such as the obliquus capitis inferior and levator scapulae have also been taken into account [2, 3].

In our study, we examined which muscles are injected most frequently in daily clinical practice, regarding the new Col-Cap concept which has distinguished 11 new patterns. These new patterns include the movements of the head (caput) and neck (collis) and/or their combinations [2]. The most common patterns according to this new approach are: torticaput (49%) and laterocaput (16.7%). All other subtypes were less than 10% of the study population [5].

Patients and methods

Between January and June 2019, we examined prospectively, in seven centres specialized in movement disorders, 306 patients with CD. Patients were included if they had idiopathic CD with pronounced symptoms that interfered with their daily activities, and had been admitted at least three months after their previous BoNT treatment, the effect of which had worn off. The centres were Besançon (France),

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Table 1. Frequency of injections (all patients)

SCM	SM	LS	SsCap	SsCer	SCap	SCer	OCI	Trap	Long
79.1%	11.1%	48.7%	38.2%	22.9%	83.0%	6.9%	35.3%	58.5%	16.7%

SCM — Sternocleidomastoideus; SM — Scalene muscles; LS — Levator scapulae; SsCap — Semispinalis capitis; SsCer — Semispinalis cervicis; SCap — Splenius capitis; SCer — Splenius cervicis; OCI — Obliquus capitis inferior; Trap — Trapezius; Long — Longissimus. All others: 20.1%

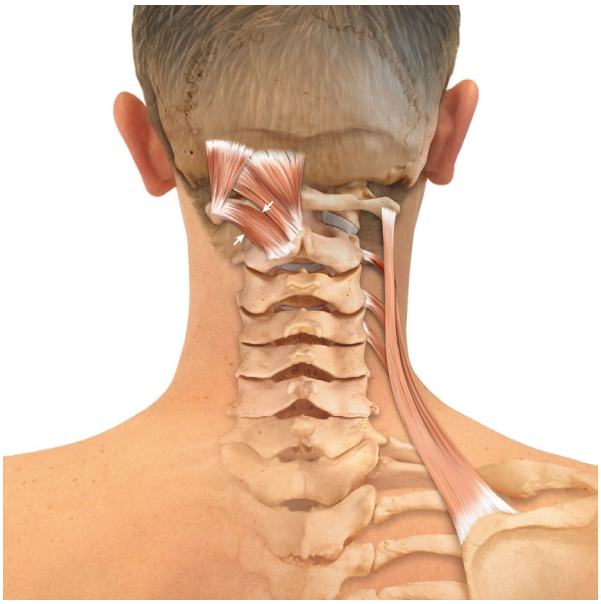

Figure 1. Two muscles that have gained importance through introduction of the Col-Cap concept: obliquus capitis, pictured on the left between the two arrows, and levator scapulae, pictured on the right of the neck

Copenhagen (Denmark), Gdańsk (Poland), Lille (France), New Delhi (India), Poznań (Poland), and Wolfach (Germany). All investigators (WHJ, LT, SP, JS, AD, BBS, AK) are specialists in movement disorders and have long-term experience with BoNT and CD treatment. All injections were performed with the use of ultrasonography guidance. Our study focused on which muscles were most commonly injected.

Results

306 patients with CD (mean age 55.5 ± 13.1 years, range 21–90, 67% female) were injected and assessed. Splenius capitis was the most common choice in 83%, followed by sternocleidomastoid in 79.1% and trapezius muscles in 58.5% (Tab. 1). This was followed by levator scapulae, semispinalis capitis, and obliquus capitis inferior in 38.2%, 48.7% and 35.3% respectively. The most common primary form was torticaput (49%), and the second most common was laterocaput (16.7%) [5].

Discussion

For a long time, the sternocleidomastoid (SCM), splenius capitis and trapezius muscles were mainly selected to treat cervical dystonia [1]. After the introduction of the Col-Cap concept, it became apparent that the SCM had been overused because it was not involved in torticollis, the supposedly most common form of CD [2–4].

In the past we treated several of the muscles involved, but without distinguishing between caput and collis forms. For example, we treated the sternocleidomastoid muscle because we assumed that it was an important muscle in torticollis. Meanwhile we know that torticaput (but not torticollis) was the most common subtype and that the muscles mentioned, i.e. sternocleidomastoid, splenius capitis and trapezius, were chosen correctly [5]. That explains why the injected muscles may have changed little over the course of the process, although the concept has changed.

It is evident from our study that the muscles mentioned above were injected most frequently. But our study also demonstrates that other muscles previously not recommended should be considered, such as the levator scapulae and obliquus capitis inferior muscles (Fig. 1). The semispinalis cervicis (22.9%) and the longissimus (16.7%) are also muscles which have only recently started to be targeted. For some deep muscles (such as the obliquus capitis inferior) or muscles with relatively thin layers (such as the trapezius), the fact that they can only be injected specifically and safely with ultrasound guidance should play a role [6]. In the future, muscles such as the longus colli may also be injected as improved technology combines ultrasonography and electromyography guidance [7, 8].

In summary, our study demonstrates that in CD the same muscles are the ones that are most commonly injected as before, but additional ones, which were rarely taken into account in the past, are now being injected in order to increase the effect of treatment. The Col-Cap concept should be used in everyday clinical practice, and not only in complicated cases and treatment failures.

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Radiation therapy in patients with implanted deep brain stimulation

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ABSTRACT

Background. As deep brain stimulation (DBS) and radiation therapy (RT) have become established treatments for movement disorders and malignancies respectively, patients being treated with both simultaneously are becoming more frequent.

Objectives. Literature regarding the safety of RT in patients with implanted DBS is scarce, and there are no clear guidelines on how to manage them.

Methods. We present a follow-up of two Parkinson's Disease (PD) patients with DBS undergoing RT in the context of previous literature.

Results. No adverse events nor malfunctioning of the DBS system were observed. This was in line with previous reports.

Conclusions. Since there are no clear safety guidelines for RT in DBS patients, it is important to document experience in this field. A combined approach involving multidisciplinary discussions between neurosurgeons, radiotherapists, clinical oncologists and neurologists is recommended.

Key words: deep brain stimulation, radiation therapy, Parkinson's Disease, safety guidelines

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Introduction

Since DBS became an effective treatment of PD and other movement disorders, more than 150,000 devices have been implanted worldwide [1]. On the other hand, 14 million people are diagnosed with malignancies every year, and half of them will require RT in the course of their disease [2, 3]. Therefore, to consult a patient with DBS who requires RT is becoming more frequent. We looked for biomarkers of good outcome after surgery. The question of neoplastic disease in remission as an indication for potential DBS therapy in advanced PD remains unclear [4]. Unfortunately, literature on the safety of radiation therapy in patients with implanted DBS is scarce, and there are no clear guidelines on how best to manage them [5].

Methods

We followed up two PD patients with implanted DBS who required RT due to various malignancies in the context of previous reports of such a coincidence.

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Results

Patient 1 was a 67-year-old male with a diagnosis of PD 27 years earlier. He underwent left-sided pallidotomy in 2002, with a significant improvement which lasted for about eight years, and again in 2012 at the age of 65. Due to disabling peak L-dopa dose dyskinesias and motor fluctuations, he was qualified to bilateral subthalamic nucleus deep brain stimulation (STN-DBS). Two-stage neurosurgical implantation of a St Jude Libra DBS system was done without any complications. Improvement after STN-DBS was measured with UPDRS. Improvement in UPDRS was 27% one year after surgery. Reduction of LEDD at 43% throughout a two-year observation resulted in significant improvement of dyskinesias. At the time of qualification for DBS, he was diagnosed with prostate cancer during hormonotherapy, and was stable on urological examination and biochemical markers (PSA). Nevertheless, in November 2013 local progression of the disease was diagnosed and pelvic RT with 30 Gy in 10 fractions without any complications was performed. Three months later, he was admitted to the Neurology Department due to a first-in-life incident of generalised seizures. CT brain scan showed two lesions in the left hemisphere, one of them near the DBS electrode (Fig. 1), with further local progression of cancer with metastases to retroperitoneal lymph nodes. The patient was qualified for palliative RT of the brain metastatic tumours with 6 MV photons and a dose of 20 Gy in five fractions. Estimated maximal dose for brain DBS electrodes was 21 Gy. Neurostimulation was ON during the whole RT procedure. After treatment, regression of tumours was observed in MRI. No complications for patient or the DBS system were seen for the next six months. Unfortunately, due to disease progression and urosepsis, the patient died in May 2015. An autopsy was not performed.

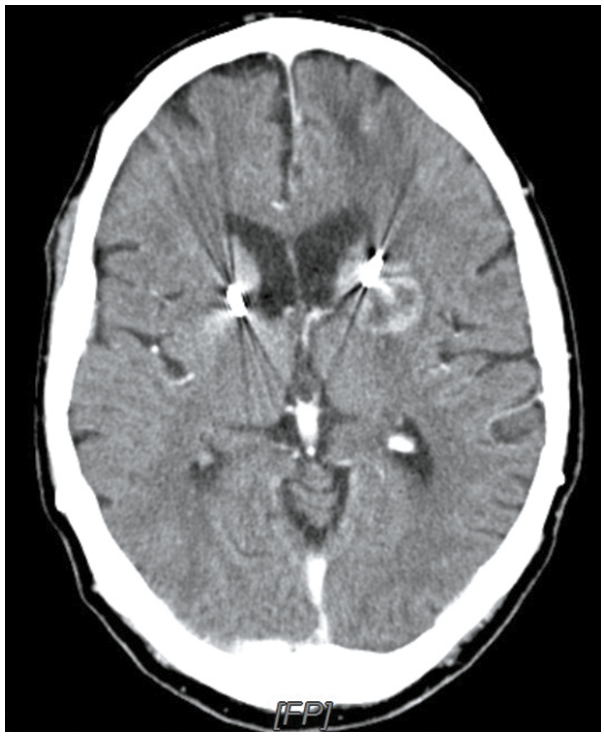

Figure 1. Two metastatic brain tumours, one near DBS electrode, in left hemisphere of Patient 1 (CT scan)

Patient 2 was a 68-year-old female who had suffered from PD for 29 years. In 2010, due to motor fluctuations and very severe peak levodopa dose dyskinesias, she was qualified for bilateral DBS-STN (Medtronic Soletra). At the time of qualification, she had a history of left-sided mastectomy due to breast cancer. At the 7 years follow up, a motor improvement in UPDRS of 40% and a reduction of LEDD of 51%, with a significant decrease of dyskinesias, were observed. In 2015, left IPG was replaced with Medtronic Activa SC. In 2016, local recurrence of breast cancer close to the IPG was diagnosed and she was qualified to RT. In September 2016, radiation therapy of the left supraclavicular and subclavian area with 15 MV photons and dose of 20 Gy in five fractions was carried out. Maximal estimated dose for left IPG was 1.7 Gy. Neurostimulation was ON during the whole RT procedure. Immediately after RT and during the last control (February 2017), no dysfunction of the DBS system was observed, with oncological remission.

Discussion

Experience of the use of RT in patients with DBS is scarce. Nutt et al. published an example of very serious consequences of diathermy for DBS [6]. Similarly, full body coil MRI might be harmful for DBS patients [7].

These arguments prompted us to seek to determine the safety guidelines for other procedures such as RT in conjunction with DBS. The leading DBS manufacturer has stated that "the DBS system may be affected by, or adversely affect,… radiation therapy." [8]. There are only two previous case reports detailing the safety of irradiation of a pulse generator device, and two reports on the safety of cranial RT in a patient with an implanted DBS. In the report by Mazdai et al. [9], a patient being treated with DBS for severe PD underwent radiation therapy to the head and neck. In this case, the estimated dose to the device was 7.5 Gy. In a similar report by Borkenhagen et al. [10], a patient with bilateral DBS devices implanted for the treatment of PD underwent radiation therapy to a left upper lung tumour directly underneath the location of the IPG. The mean dose to the device was 5.53 Gy, and the maximum dose was 48.12 Gy. Follow-up interrogation of IPG revealed no changes in its settings or evidence of malfunctioning. In both cases, the IPGs were found to be in good working order, despite receiving a radiation dose exceeding typical pacemaker tolerances (3–5 Gy) [11]. In the third case, a patient who had a DBS implanted for the treatment of severe PD underwent a course of hypofractionated radiation therapy (21 Gy in three fractions of 7 Gy per fraction delivered over seven days) for the treatment of two brain metastases using stereotactic dynamic intensity modulated arc therapy [12]. In this case, the

Table 1. Data on radiation therapy in previously reported cases and our two patients

Author and year of publication (number of pts)	Indication for DBS	DBS system	Tumour localisation (radiation dose)	Beam energy	Radiation dose for IPG	Radiation dose for electrodes	Clinical consequences
Mazdai et al. 2006 (n = 1)	PD	Medtronic	Head and neck (66 Gy — 33 frac.)	4 MV photons	7.5 Gy (total)	–	None for DBS system
Brokenhagen et al. 2014 (n = 1)	PD	Medtronic	Lung close to IPG	6MV photons	Mean 5.53 Gy Max. 48.12 Gy	NA	Three years follow-up (tumour cured). None for DBS system
Guy et al. 2014 (n = 1)	PD	–	Lung (brain metastases –21 Gy — 3 frac.)	–	< 0.01 Gy	< 1 Gy	None for DBS system
Kotecha et al. 2016 (n = 1)	Tremor	Medtronic, lead model 3389	Brain metastases (WB-RT, 30 Gy — 10 frac.)	6 MV photons	0.61 Gy (total)	Mean 28 Gy Max. 33 Gy	None for DBS system
Patient 1	PD	St. Jude Medical Libra	Brain metastases (WB-RT, 20 Gy — 5 frac.)	6 MV photons	–	Mean 9.9 Gy Max. 21 Gy	None for DBS system
Patient 2	PD	Medtronic Activa	Breast cancer (20 Gy — 5 frac.)	15 MV photons	Mean 0.6 Gy Max. 1.7 Gy	–	None for DBS system

electrodes received less than 1 Gy and the pulse generator received less than 0.01 Gy. Regarding the fourth patient, the IPG was well outside the field of radiation therapy and received a nominal dose of only 6.1 cGy/fraction (61 cGy total), but the electrodes received a maximum of 33 Gy [13]. Data from these previous four case reports and from our two patients is set out in Table 1. Much larger experience with pacemakers and implantable cardioverter defibrillators (PM/ICD) shows that patients undergoing RT with electrons or kV photons do not need supplementary device evaluations in the PM/ICD clinic. Because the impact of RT on a device depends on the beam energy rather than the total dose of radiation, it is recommended to limit photon beam energy to ≤ 10 MV when possible. The frequency of pacemaker malfunction is about 3%, and mainly consists of device resets and, exceptionally, replacements [14].

To minimise IPG exposure to RT, especially when the device is located very close to a tumour, surgical relocation of IPG using a longer extension should be considered [15]. Maintaining cardiostimulation during radiation therapy, especially for patients who also have an implanted ICD, is crucial. Similarly, in patients with movement disorders, turning off the neurostimulation makes the RT procedure impossible to perform due to involuntary movements. Thus, turning off the stimulation during RT is in fact not recommended because in the majority of reported cases IPGs were turned on, and procedures were safe. Radiotherapy is an established therapy method in oncology, so it is important to suggest that manufacturers consider a built-in 'safe RT' approach in the devices. Furthermore, we believe it would be sensible to report all cases of DBS patients undergoing RT and to create a web-based registry of such coincidences.

Conclusions

As the number of patients with DBS continues to rise, the influence of RT on those patients should be analysed. It is important to document the experience of DBS patients simultaneously receiving RT. We believe that all previously reported cases add to the argument for adopting a combined approach for patients, with multidisciplinary discussions between neurosurgeons, radiotherapists, clinical oncologists and neurologists. Drawing on the analagous experience of cardiologists in the field of implantable pulse generators, safety guidelines will be established in the future.

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Does amantadine have a protective effect against COVID-19?

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The SARS-CoV-2 pandemic is causing the scientific community to look for the best treatment to fight the virus. Studies are being carried out worldwide with this purpose looking into antiviral drugs, antibiotics, antimalarials, and anticoagulants. All these investigations are in their early stages due to the nature of the pandemic, which has spread very rapidly since the first case was reported on 17 November, 2019 [1]. This means that there are no studies available that could give evidence of the most appropriate treatment for SARS-CoV-2.

Although authors such as Rodón et al. have indicated a cytotoxic effect on Vero E6 cells exposed to a fixed concentration of SARS-CoV-2 in the presence of decreasing concentrations of amantadine [2], Rejdak et al. described a series of 22 neurological patients treated with amantadine: all the patients were tested after reported person-to-person contact with SARS-CoV-2 infected subjects, and had viral infection confirmed with a real-time reverse transcription polymerase chain reaction test for the qualitative detection of nucleic acid from SARS-CoV-2 in upper and lower respiratory specimens. All of them had spent two weeks in quarantine since their documented exposure, and none developed clinical manifestations of infectious disease [3], or Aranda that exposes the hypothesis that amantadine blocks the viroporine channel of COVID-19, preventing the release of the viral nucleus into the cell cytoplasm [4] and Tipton repurposing other medications, especially those with known antiviral properties such as amantadine and memantine.

These medications are inexpensive, widely used, and have well-known side effect profiles that are relatively mild compared to other potential COVID-19 treatments such as hydroxychloroquine [5].

The reason for this letter to the editors is to present the case of a 75-year-old woman, with Parkinson's Disease of 16 years' duration, treated with opicapone, 50 mg/day (Ongentys •), pramipexol, 2.1 mg/day (Mirapexin •), levodopa, 1,000 mg/day, benserazide, 250 mg/day (Madopar •) and amantadine, 100 mg/day, with a history of hyperthyroidism treated with levothyroxine, 25 mg/day (Euthyrox •), and stomach cancer five years ago treated with surgery (Billroth II gastrectomy) and pre- and post-surgical chemotherapy, now well controlled with no cancer recurrence.

The husband of this patient, after seven days with fever with an unusual sporadic cough, was diagnosed by a positive COVID-19 PCR test with bilateral pneumonia that was the reason for hospital admission and resulted in his death.

The patient, 45 days after her husband's death, had not had any symptoms related to COVID-19, without fever, cough or anosmia, and was being looked after at home by her family. Remaining isolated without going outdoors and maintaining only contact with their direct caregivers, both had negative COVID-19 PCR tests.

Having seen the previously cited articles which hypothesise that amantadine may have a protective effect against the coronavirus [6, 7], this patient has been taking amantadine for seven months.

Is it possible that she was not infected with the coronavirus despite having lived with her husband with COVID-19 symptoms for seven days, even sharing a bed, with the exposure to coughing and aerosols that this produces? May amantadine work by inhibiting coronavirus infection?

For this reason, it is advisable, as the authors recommend [4, 5], that more studies be carried out with patients with Parkinson's Disease who are on amantadine treatment and have been directly exposed to SARS-CoV-2, in order to demonstrate the hypothesis that they put forward, and which I second.

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Response to "Does amantadine have a protective effect against COVID-19?"

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Information about SARS-CoV-2 is accumulating at an unprecedented rate. Any hope of returning to a pre-COVID way of life seems to rest on the shoulders of researchers tasked with developing effective treatments and a vaccine.

This has generated an expectation for science to move faster than ever before. Evidence of acute infection can be assessed in a matter of days; however, the long- term effects may require years before reaching the threshold for clinical detection. Long-term effects are also subject to more confounders. Therefore, while acute care investigators are illuminating immediate viral effects, those studying neurodegeneration are left to ponder and to speculate about the downstream effects of viral exposure. There is now a reemerging focus on a possible link between SARS-CoV-2 and neurodegenerative diseases, specifically Parkinson's Disease (PD) [1].

Many studies across the globe have published COVID-19 patient characteristics and comorbidities to help us understand which people are more susceptible to infection and who is likely to have a more severe disease course. One of the largest studies looked at the characteristics of 5,700 patients hospitalised with COVID-19 [2]. Older individuals trended towards poorer outcomes, which is consistent with current thinking. Comorbidities with the largest representation included cancer, cardiovascular disease, chronic respiratory disease, immunosuppression, and others.

PD affects over 1% of the population over the age of 60, and 5% of those older than 85 [3]. Moreover, PD is the second most common neurodegenerative disease behind Alzheimer's Disease (AD), yet neither disease was represented in this cohort, which had a median age of 63. Perhaps there are protective factors in these otherwise vulnerable populations that seem to make them disproportionately less affected. It is also possible that medications used to treat the symptoms of PD and AD are effective against SARS-CoV-2.

In our Letter to the Editors of *Neurologia i Neurochirurgia Polska*, we hypothesised that some adamantane derivatives used in neurodegenerative populations may play a protective role against SARS-CoV-2 [4]. These include amantadine, commonly used to treat patients with PD, and memantine, which is commonly used in dementing illnesses such as AD.

Our hypothesis was largely based on evidence that similar medications are efficacious against other coronaviruses [5]. Given the newness of SARS-CoV-2, this hypothesis is un--tested and there were no randomised control trials at the time. To date, there still have been no direct trials; however, supportive evidence is emerging. In this issue, Cortés Borra responds to our Letter to the Editors, and describes a 75-year--old woman with a longstanding history of PD treated with amantadine, among other medications [6]. Unfortunately, the woman's husband died from COVID-19 pneumonia. Despite an almost certain SARS-CoV-2 exposure from direct contact with her husband, COVID-19 PCR testing was negative, and she remained symptom-free.

Even though anecdotal evidence like this must always be interpreted with extreme caution, Cortés Borra's Commentary appears to support our hypothesis. Adding to this is a recently published study that aimed at determining whether patients with PD were at greater risk of COVID-19 [7]. While the study found no significant difference from the general population, the authors showed that vitamin D supplementation was associated with lower rates of infection in patients with PD. We find it interesting that there was a relative risk reduction of 100% among PD patients on amantadine. This was not statistically significant, and absolute risk reduction was only 1%; however, only 2% of PD patients were taking amantadine. Rejdak and Grieb found that 5/5 PD patients on amantadine tested positive for SARS-CoV-2 but experienced no symptoms after > 14 days [8]. Similarly, 7/7 patients on memantine had asymptomatic SARS-CoV-2 infections.

Reports vary, but this asymptomatic rate is much higher than a recent report of 56% among nursing home residents where only 3% remained asymptomatic one week after testing [9].

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We find this difference in symptomatic rates to be remarkable given that elderly individuals are generally considered to be 'high risk'.

It could be that the high risk of the PD population goes beyond that of older age, and may be tied to viral neurotropism. Validating this will require well-designed studies with years of follow up. However, an adequately powered retrospective study would be sufficient to disprove our hypothesis. We feel this study should be conducted and, if our hypothesis stands, be followed by prospective validation.

We congratulate Cortés Borra on his contribution and hope that others will make further progress in this field and thus protect our patients.

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Glossary

AD — Alzheimer's Disease
COVID-19 — coronavirus 19
PD — Parkinson's Disease
SARS-CoV-2 — Severe Acute Respiratory Syndrome Coronavirus 2

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