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NEUROLOGIA I NEUROCHIRURGIA POLSKA

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The Official Journal of Polish Neurological Society

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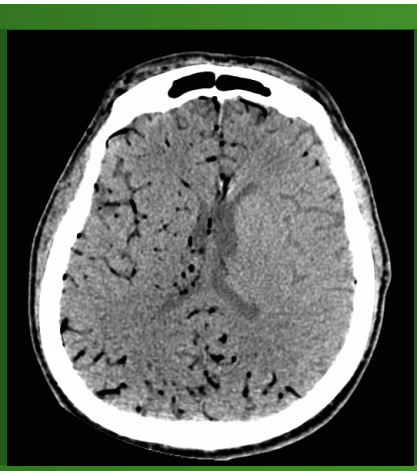

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Neurologia i Neurochirurgia Polska also known under the name of *Polish Journal of Neurology and Neurosurgery (PJNNS)* is a premier research and educational platform of the Polish Neurological Society and Polish Society of Neurosurgeons. It has a long 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: Śmiłowska K. et al., Brain vessels with low density that indicates intravascular pneumocephalus (see figure on page 254).

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Editorial Board meeting of the *Polish Journal of Neurology and Neurosurgery* — announcement of the gold open access for the journal

Zbigniew K. Wszolek[1], Łukasz Stolarczyk[2], Jarosław Sławek[3]

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The Editorial Board members met via Zoom meetings on April 20 and June 15, 2021. The status of the Journal was presented to them by Drs. Slawek and Wszolek, the editors of the Polish Journal of Neurology and Neurosurgery (PJNNS, Neurologia i Neurochirurgia Polska), and Dr. Stolarczyk and Ms. Czarnocka, the Journal administrators. In 2020, the PJNNS received a total of 334 manuscripts over all submission categories. This represents a 49.8% increase in submission numbers compared to 2019. Despite the significant increase in submitted manuscripts, the Journal's processing time continues to be very competitive (Fig. 1).

During the Editorial Board meeting, the issue of changing the format of publication of PJNNS from the current hybrid model to the gold open access model was extensively discussed. The members of the PJNNS Editorial Board expressed full support to change the publication system to the gold open access model, commencing on July 1, 2021. This change was earlier approved by the Executive Committee of the Polish Neurological Society. In 2020, 51% of published manuscripts were open access (Fig. 2). The change of publication model has also been supported by our readership. It will represent a further enhancement of availability of the

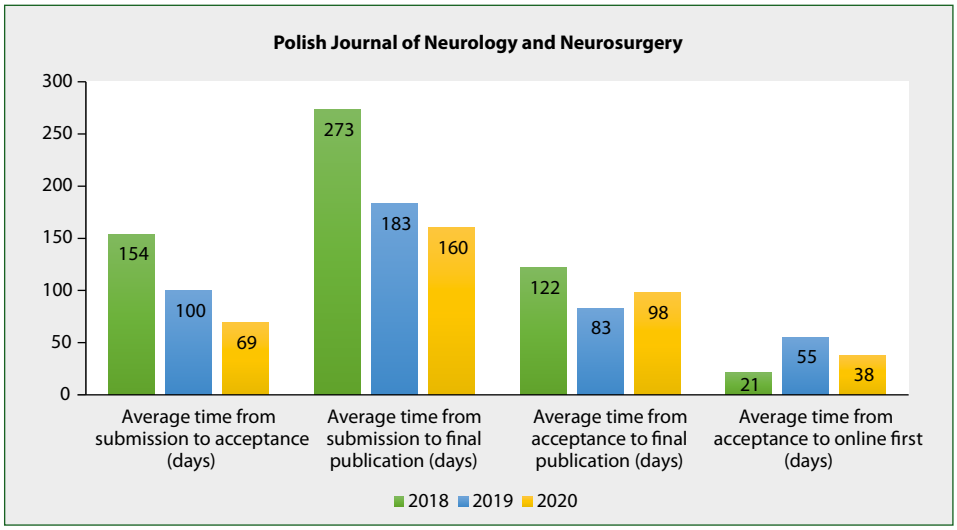

Figure 1. A major improvement in processing time of manuscripts submitted to the Polish Journal of Neurology and Neurosurgery (Neurologia i Neurochirurgia Polska) for years 2018, 2019, and 2020

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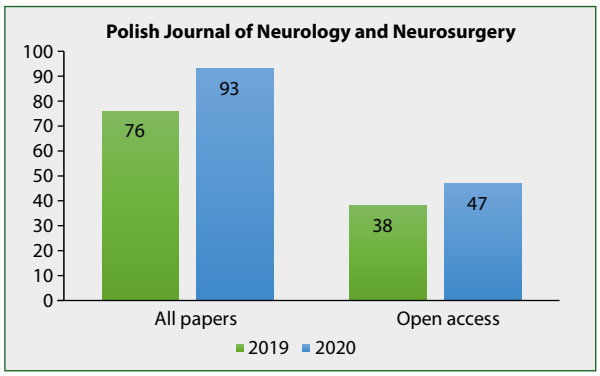

Figure 2. Open access manuscripts published by the Polish Journal of Neurology and Neurosurgery (Neurologia i Neurochirurgia Polska) compared to all published manuscripts in years 2019 and 2020

Journal's manuscripts to the public in large, and very likely will further improve the Journal's visibility, its citations and impact factor, and download of its manuscripts. The established rates are very competitive compare to other national and international neurological journals. For the members of the Polish Neurological Society and the Editorial Board members of the PJNNS, the individual cost per accepted manuscript will be USD 250; for all other authors, this will be USD 450.

The members of the Editorial Board were very pleased with the publication of the first issue (2/2021) of the PJNNS that included a Leading Topic. We will continue this feature in future issues.

INVITED EDITORIAL

Neurologia i Neurochirurgia Polska
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This Invited Editorial accompanies a Letter to the Editors, see page 322

First Polish case of *CSF1R*-related leukoencephalopathy

Zbigniew K. Wszolek

Department of Neurology, Mayo Clinic Florida, Jacksonville, Florida, United States

(*Neurol Neurochir Pol 2021; 55 (3): 239–240*)

In this issue of the *Polish Journal of Neurology and Neurosurgery*, Żur-Wyrozumska, et al. describes the very first genetically proven case of *CSF1R*-related leukoencephalopathy in Poland [1]. *CSF1R*-related leukoencephalopathy (due to mutations in *CSF1R* gene [2]) has been reported in multiple countries around the world (reviewed in Konno, et al. [3]); however, despite its worldwide occurrence, *CSF1R*-related leukoencephalopathy is still an underdiagnosed condition [4].

There are three primary reasons for this diagnostic difficulty. The disease was first recognized by Van Bogaert and Nyssen back in 1936 [5] as a subset of orthochromatic leukodystrophies. They identified it as a pigmentary orthochromatic leukodystrophy (POLD). However, until the discovery of causative *CSF1R* gene mutations in 2011 [2], only a handful of sporadic and familiar POLD cases were published (reviewed in Marotti, et al. [6]). To diagnose it with certainty, either brain biopsy or autopsy had to be done.

The second reason relates to a nomenclature confusion leading to labeling many cases of POLD as hereditary diffuse leukoencephalopathy with spheroids (HDLS). For example, in our own first publication on this subject from 2006, we erroneously named a POLD family as an HDLS family [7]. HDLS was first describe by Axelsson et al in 1984 [8] in a Swedish family with clinical and pathologic similarities to POLD families. Recently, this family was found to carry mutations in *AARS2* gene [9]. Even before this genetic discovery, we found that families mislabeled as HDLS were indeed POLD families [10]. Fortunately, these nomenclature difficulties stemming from similarities in clinical and pathologic presentations have been solved by advances in genetic technology. The nomenclature introduced by Konno et al. [3] simplifies it, and now we identify these two separate conditions as *CSF1R*-related leukoencephalopathy, formerly POLD families, and *AARS2*--related leukoencephalopathy, formerly HDLS families (Tab. 1). Unfortunately, there are also published and unpublished cases/families suspected for *CSF1R*-related leukoencephalopathy or *AARS2*-related leukoencephalopathy with negative genetic testing for both *CSF1R* and *AARS2* gene mutations [11, and personal observation]. Thus, the concept of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP), initially introduced by Marotti et al. [6] and further popularized by Wider et al. [11], is still quite useful. Konno et al. [3] make this distinction even more specific by introducing the term, *CSF1R/AARS2*-negative ALSP. It is very likely that there are other so far unidentified genes in which mutations are responsible for clinical and pathologic phenotypes currently indistinguishable from those seen in *CSF1R*-related leukoencephalopathy and *AARS2*-related leukoencephalopathy.

The third and most important reason is that clinical features of *CSF1R*-related leukoencephalopathy are very broad, encompassing headaches, seizures, spasticity, rigidity, tremors, psychiatric features, dementia, among others, thus leading to misdiagnosis or delayed diagnosis. Fortunately, a much wider availability of clinical genetic testing at this juncture makes

Table 1. Current and previously used nomenclature

Current nomenclature	Previously used nomenclature
CSFIR-related leukoencephalopathy	POLD cases/families
	Previously mislabeled HDLS cases/families
AARS2-related leukoencephalopathy	HDLS cases/families
CSF1R/ANRS2-negative ALSP	Genetically negative for *CSF1R/AARS2* mutation cases/families

CSF1R-related leukoencephalopathy — colony stimulating factor 1 receptor-related leukoencephalopathy; POLD — pigmented orthochromatic leukodystrophy; HDLS — hereditary diffuse leukoencephalopathy with axonal spheroids; *AARS2*-related leukoencephalopathy — alanyl tRNA synthetase-related leukoencephalopathy; ALSP — adult-onset leukoencephalopathy with axonal spheroids and pigmented glia

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the diagnosis easier and faster as demonstrated in the case presented by Żur-Wyrozumska et al. [1]. I congratulate Żur-Wyrozumska et al. for their diagnostic success and for bringing this case to the attention of the readership of the *Polish Journal of Neurology and Neurosurgery*. It is very likely that more cases of this disease will be identified in Poland.

At the present time, *CSF1R*-related leukoencephalopathy is an incurable disease. However, a better understanding of the pathophysiology and molecular biology of this illness makes development of a halting progression therapy a possibility [4]. In fact, hematopoietic stem cell transplantation has already been used to treat several patients (briefly discussed in Tipton, et al. [12]).

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REVIEW ARTICLE

Neurologia i Neurochirurgia Polska
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Genetics of Parkinson's disease in the Polish population

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ABSTRACT

Introduction. Genetic forms of Parkinson's disease (PD) often cluster in different ethnic groups and may present with recognisable unique clinical manifestations. Our aim was to summarise the current state of knowledge regarding the genetic causes of PD and describe the first Polish patient with *SNCA* duplication.

Methodology. We searched the electronic database, PubMed, for studies between January 1995 and June 2020 that evaluated genetics in Polish patients with PD, using the search terms 'Parkinson's disease, 'Polish', 'genetics', 'mutations', and 'variants'.

Results. In total, 73 publications were included in the review; 11 genes responsible for monogenic forms and 19 risk factor genes have been analysed in the Polish population. Pathogenic variants were reported in four monogenic genes (*LRRK2*, *PRKN*, *PINK1*, and *SNCA*). Eight genes were associated with PD risk in the Polish population (*GBA*, *TFAM*, *NFE2L2*, *MMP12*, *HLA-DRA*, *COMT*, *MAOB*, and *DBH*). Multiplex ligation-dependent probe amplification and Sanger sequencing in *PRKN*, *PINK1*, *DJ1*, *LRRK2*, and *SNCA* revealed *SNCA* duplication in a 43-year-old Polish patient with PD examined by movement disorder specialists.

Conclusion. Only a limited number of positive results have been reported in genes previously associated with PD in the Polish population. In the era of personalised medicine, it is important to report on genetic findings in specific populations.

Key words: genetics, Parkinson's disease, Polish population, *SNCA* duplication

(*Neurol Neurochir Pol 2021; 55 (3): 241–252*)

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative movement disorders worldwide, affecting people of all ethnic groups [1]. The cardinal motor features include tremor, rigidity, bradykinesia or akinesia, and postural instability [2–4]. The pathophysiology of this disease is based on degeneration of dopaminergic neurons in the substantia nigra [1]. The characteristic neuropathological feature is the presence of Lewy bodies composed of aggregated α-synuclein fibrils. However, many different molecular pathways of dysfunction have been proposed leading to PD [1]. Diagnosis is usually based on clinical features, but radiological methods such as dopamine transporter scan and positron emission tomography are useful diagnostic tools [5, 6].

Multiple factors may be associated with the prevalence of PD. The frequency of PD and PD subtypes differ in different ethnic groups. One of the most common observations is that PD occurs much more frequently in Western populations. However, there are specific ethnic groups in Asia and Africa where PD is common [7]. The factors impacting upon disease prevalence also differ across populations [8, 9]. One difference in prevalence may be associated with the most important risk factor for PD: age [10]. Western European ethnic groups are usually older than subgroups from low-income countries, so the prevalence of PD is higher. Also, diagnostic and therapeutic options are more available in high-income countries [11, 12]. Furthermore, genetic background is characteristic for different ethnic groups [10]. Most PD cases are sporadic; however, about 15% are familial [1]. The genetic cause of PD

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is usually determined in patients with early-onset PD (EOPD) or in those with a positive family history. Many genetic loci associated with PD have been identified.

In the Online Mendelian Inheritance in Man (OMIM) database, 23 genes have been associated with monogenic forms of PD. The last genome-wide association study (GWAS) identified more risk genes than the 23 already in the OMIM database; > 90 risk loci [13].

Poland is ethnically homogenous; the current population is 38 million and 97.1% declare Polish nationality. However, in the past, many different minority groups have lived in current Polish territories; the borders have changed many times, resulting in massive migrations of people. These factors have led to the presence of a unique genetic background in this country. Poland has a substantial older population and the occurrence of PD is increasing; approximately 75,000 cases were reported in 2016 [14].

Many genetic PD loci associated with different pathways have been studied in the Polish population. Patients have been recruited in five main PD centres in Poland (Supplementary Fig. 1).

The aim of this review was to summarise the genetic studies that have been conducted in Polish patients with PD. The electronic database, PubMed, was searched for articles published between January 1995 and June 2020 relating to studies that evaluated genetics in Polish patients with PD. Review articles and meta-analyses were also investigated, and their reference lists were examined for possible inclusion. Our search was limited to human studies. We used the following search terms: 'Parkinson's disease', 'Polish', 'genetics', 'mutations', and 'variants'. We also describe a new Polish patient with *SNCA* duplication. The blood specimen from this patient was collected with institutional review board approval, and informed consent was signed.

Monogenic forms of PD

In monogenic forms of PD, the disease is inherited dominantly or recessively by mutation of a single gene. The monogenic forms of PD are responsible for about 30% of familial forms and 3-5% of sporadic cases [15]. Several genes from this group have been reported in Polish populations (Tab. 1) [16–30].

Autosomal recessive PD genes

Many studies of monogenic PD forms in Polish populations have analysed the three most common autosomal recessive genes reported in EOPD: *PRKN, PINK1*, and *DJ1* [16, 20, 23, 24, 31]. Though typical age at onset for PD is above 60 years, EOPD is defined in different ways. While the European Parkinson's Disease Association defines 'early' as age at onset younger than 40, the American Parkinson's Disease Association defines it as age at onset younger than 50. EOPD is reported in about 5% of patients [32]. Summaries of monogenic PD forms are provided in Table 1 and Figure 1.

PRKN (OMIM 602544, PARK2)

The *PRKN* gene is associated with the autosomal recessive form of EOPD [33]. *PRKN* encodes the protein responsible for quality control of mitophagy. PRKN is an E3 ubiquitin ligase that participates in ubiquitin-proteasome interaction. Mutations in *PRKN* result in degradation of damaged mitochondria, leading to oxidative stress that can damage the substantia nigra dopaminergic cells [15]. According to published data, the mutations in *PRKN* are present in a large proportion of EOPD worldwide (up to 18% of patients) [15]. *PRKN* PD type is characterised by a broad range of clinical phenotypes, some atypical signs, but generally has early onset, slower progression, better response to levodopa, and often more severe drug-induced adverse effects [34]. Sometimes in the clinical phenotype in carriers, parkinsonism is not a dominant symptom [31].

Several studies have analysed *PRKN* in Polish populations. The first case-control study of 79 patients with EOPD (onset < 40 years) and 204 controls revealed two patients with homozygous or compound heterozygous mutation and one with heterozygous mutation (3.8%) [24]. A study of 150 patients with EOPD (onset < 45 years) reported *PRKN* mutations in 4.7% [23]. Gaweda-Walerych et al. [20] identified only one heterozygous *PRKN* deletion; however, from 344 patients with PD (171 EOPD), Ambroziak et al. [16] identified five compound heterozygous and three heterozygous mutations.

PINK1 (OMIM 608309, PARK6)

PINK1 (phosphatase and tensin homolog-induced putative kinase 1) is another common cause of early-onset parkinsonism worldwide. It was first described in a large Italian family and is the second most commonly identified mutation in patients with autosomal recessive EOPD [35]. *PINK1* protein strongly cooperates with *PRKN* in mitochondrial quality control to identify, label, and remove damaged organelles. *PINK1* is responsible for ubiquitin phosphorylation at Ser65. The endogenous Ser65 phosphopeptide is only detected with *PINK1* and together cause a decrease in mitochondrial membrane potential [27].

In the first Polish *PINK1* genetic study, only four patients with EOPD (2.67%) were carriers of *PINK1* mutations (one homozygote) [23]. Another study analysed molecular characteristics of *PINK1* p.Gln456Ter mutation present in two family members. This mutation can lead to a decrease in mRNA and loss of protein function [29, 36]. One molecular study revealed that previously described *PINK1* p.Ile368Asn cannot be stabilised on the outer mitochondrial membrane upon mitochondrial stress, and due to conformational changes in the active site, does not exert kinase activity towards ubiquitin [17]. In 748 Polish patients with PD, 0.94% were carriers of *PINK1* p.Gly411Ser mutation, which increased PD risk via dominant-negative mechanism [27].

Table 1. Autosomal recessive and autosomal dominant inherited genes analysed in Polish populations

Gene	Chromosome localisation	Results	Study group
Autosomal recessive			
PRKN	6q26	2 homozygotes/compound heterozygotes and 1 heterozygote	79 EOPD, age < 40 y [24]
		5 compound heterozygotes, 2 heterozygotes	150 EOPD, age < 45 y [23]
		5 compound heterozygotes, 3 heterozygotes	344 PD (171 EOPD, age < 45 y; 173 LOPD) [16]
		No pathogenic mutations	104 EOPD, age ≤ 50 y [20]
PINK1	1p36.12	1 homozygote, 3 heterozygotes	150 EOPD, age < 45 y [23]
		PINK1 p.Gln456Ter in both patients	2 family members affected [29]
		PINK1 p.Ile368Asn in both patients	2 family members affected [17]
		0.94% p.Gly411Ser *PINK1* mutation carriers	748 PD [27]
DJ1	1p36.23	No pathogenic mutations	150 EOPD, age < 45 y [23]
Autosomal dominant			
LRRK2	12q12	1 G2019S heterozygote	100 sporadic PD [22]
		No pathogenic variants	174 sporadic PD [18]
SNCA	4q22.1	No p.Ala30Pro, p.Glu46Lys, p.Ala53Thr, or multiplication p.Ala18Thr in 1 patient, p.Ala29Ser in 1 patient	629 PD [21]
		SNCA duplication in patient with EOPD[a]	1 sporadic PD[a]
VPS35	16q11.2	No pathogenic mutations	346 PD [30]
DNAJC13	3q22.1	No pathogenic mutations	702 PD (9.23% positive family history) [25]
CHCHD2	7p11.2	No pathogenic mutation	394 PD [26]
EIF4G1	3q27.1	p.Ala502Val in 1 patient (variant of uncertain pathogenicity)	397 PD [19]
HTRA2	2p13.1	No pathogenic mutations	101 PD [28]

EOPD — early-onset PD; LOPD — late-onset PD; PD — Parkinson's disease; [a]New patient

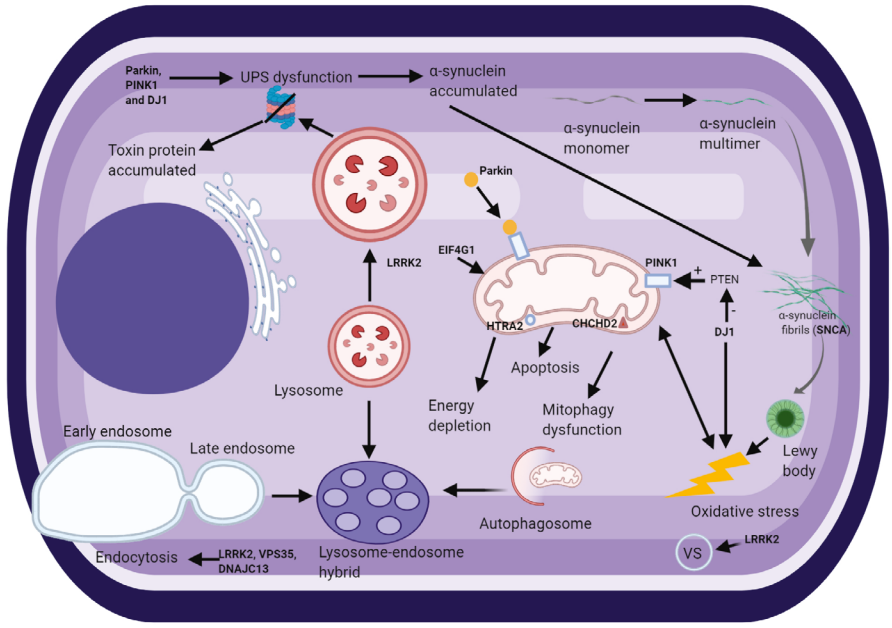


Figure 1. Main pathways associated with Parkinson's disease pathophysiology explored in Polish patients. Bold indicates protein encoding by genes responsible for monogenic forms of PD
ER — endoplasmic reticulum; SV — synaptic vesicle; UPS — ubiquitin-proteasome system

DJ1 (OMIM 602533, PARK7)

The third most commonly reported EOPD gene is *DJ1*; however, it is much rarer than *PRKN* and *PINK1*. It has been reported in only a few populations [37]. As with *PRKN* and *PINK1, DJ1* participates in mitochondrial quality control. *DJ1* increases the expression of two mitochondrial proteins, UCP4 and UCP5, which decrease mitochondrial membrane potential, reduce reactive oxygen species production, improve mitochondrial functions, and protect the neuronal cells [38]. No *DJ1* variants have been reported in Polish populations [23].

Autosomal dominant PD genes

Autosomal dominant inherited genes generally cause medium-onset to late-onset parkinsonism or PD, with few or no additional symptoms. The characteristic feature is incomplete penetrance of these genes [1].

LRRK2 (OMIM 600907, PARK8)

LRRK2, a large (7,584 bp) gene that encodes leucine-rich repeat kinase 2, is the most common genetic cause of PD. The main purpose of this protein remains unknown, but it may involve such cellular functions as neurite outgrowth, cytoskeletal maintenance, vesicle trafficking, autophagic protein degradation, and the immune system. The well-established association with autosomal dominant PD had six variants. The first families identified with mutation in *LRRK2* were in Japan and the US [39, 40]. The most commonly reported *LRRK2* mutation is the p.Gly2019Ser variant, detected in 30% and 13% of Arab-Berber and Ashkenazi Jewish familial cases of PD, respectively [41, 42]. It has also been reported in up to 6% of familial and 2% of sporadic European PD cases [43]; however, in the Polish population it is rather rare. A study screening for *LRRK2* variants in a European population only found them in one Polish family [22], while another study performed in 174 Polish patients did not reveal any pathogenic variants in this gene [18].

VPS35 (OMIM 601501, PARK17)

VPS35 (vacuolar protein sorting 35 homolog) is a rare cause of autosomal dominant PD. The first reported variant, p.Asp620Asn, was described in Swiss and Austrian families with late-onset PD [30, 44]. The encoding protein is responsible for transmembrane receptor recycling and protein transport between the endoplasmic reticulum and the trans Golgi network. The functional protein cooperates with two other proteins, VPS26 and VPS29, to create a highly conservative, active complex. All three genes were analysed in 356 Polish patients with PD, but no variants in *VPS26* and *VPS29* were found [45]. The original paper describing a *VPS35* variant in a PD family also included analysis of 346 patients with PD and did not reveal any other pathogenic variants [30].

SNCA (OMIM 163890, PARK1)

SNCA mutation was first described in mixed Greco-Italian and Greek families [46]. Initially, point mutations were reported, then multiplications [47]. The clinical phenotype is consistent with late-onset PD with a positive family history and is associated with a good response to levodopa treatment. Occasionally, patients have multiple system atrophy phenotype. Fifty-nine families with *SNCA* duplications have been described worldwide [48]. In some patients with duplications, there is no family history and the phenotype is variable. Patients with triplications usually have earlier age at onset and more severe clinical symptoms [49]. From 629 Polish probands, two sporadic cases with variants, p.Ala18Thr and p.Ala29Ser, were reported, but P.Ala30Pro, p.Glu46Lys, and p.Ala53Thr and multiplication variants were not discovered [21]. The clinical phenotype was characterised by a good response to levodopa, at least at the beginning of the disease. Post mortem of the patient with p.Ala29Ser mutation revealed Lewy bodies and neuritis [21].

We recently identified the first Polish patient with *SNCA* duplication. A 43-year-old right-handed man was referred to the neurology clinic. He had been suffering from right hand tremor for two years. Neurological examination revealed hypomimia, slow speech with dysarthria, bradykinesia, rigidity, and rest tremor on the right side. He reported anosmia and mild drooling, but denied any sleep disturbances. Family history was negative for PD. The patient was diagnosed with PD and initial levodopa treatment (200 mg daily) was implemented, with good response. Because of the younger age at onset (< 50), multiplex ligation-dependent probe amplification in *PRKN*, *PINK1*, *DJ1*, *LRRK2*, and *SNCA* and Sanger sequencing in *PRKN* were performed, revealing a heterozygous *SNCA* duplication (Fig. 2).

Candidate familial PD genes

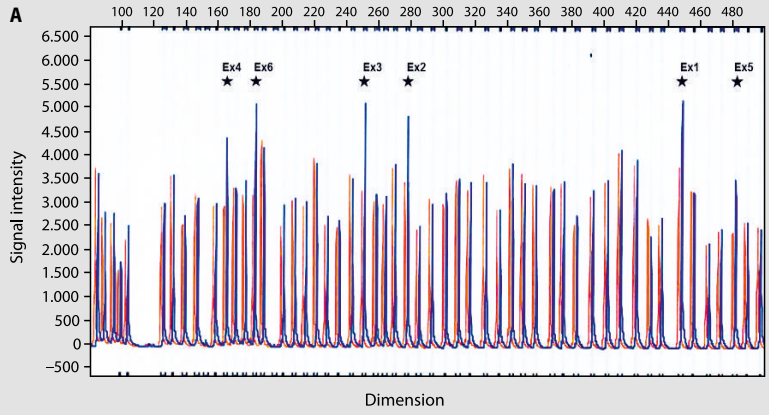
Additional genes have been identified as possible causes of PD. Analyses of autosomal-dominant PD families initially identified *DNAJC13*, *CHCHD2*, *EIF4G1*, *LRP10*, *NUS1*, and *HTRA2* as causative genes; however, data from the case-control study did not support this observation [50]. These genes were also analysed in Polish populations (Tab. 1).

DNAJC13 (OMIM 616361, PARK21)

The first variant in this gene was observed in a Dutch-German-Russian Mennonite family [51]. *DNAJC13* (DnaJ [Hsp40] homolog, subfamily C, member 13 protein) is associated with recycling and functioning of the lysosomal system. In a population of 702 Polish patients with PD with 9.23% positive family history, no pathogenic variants were observed [25].

CHCHD2 (OMIM 616710, PARK22)

Heterozygous mutations in *CHCHD2* (coiled-coil-helix-coiled-coil-helix domain containing 2) were identified first in Japanese families with autosomal dominant patterns of inheritance of PD. The protein is responsible for cytochrome c oxidase activity by acting as a transcription factor to regulate cytochrome c oxidase expression, thereby facilitating mitochondrial electron transport chain flux under low oxygen



B

	Probe Name	Bin Size	Height Ratio
1	X	100.5	1.102
2	Y	105.0	1.128
3	**Ctr1_05q31**	127.2	1.023
4	TNFRSF9	133.0	1.011
5	PINK1_ex05	140.2	1.065
6	PINK1_ex01	148.0	0.971
7	SNCA A 30P	153.5	–1
8	**Ctrl 15q21**	159.3	1.010
9	SNCA_ex04	166.0	1.482
10	PINK1_ex02	171.7	0.998
11	ATP13A2_ex09	177.8	1.094
12	SNCA_ex06	183.8	1.609
13	Ctrl_9p13	189.0	0.965
14	LRRK2_G2019S	196.2	–1
15	PARK2_ex 4	201.4	1.182
16	PINK1_ex03	208.4	1.019
17	ATP13A2_ex 02	215.2	1.033
18	**Ctr1_8q12**	221.7	0.975
19	PINK1_ex07	228.5	1.071
20	PARX2_ex1	235.8	1.054
21	PARK7_ex06	244.0	0.978
22	SNCA ex 03	251.5	1.566
23	Ctr1_18q21	258.9	1.044
24	LPA Downstream	264.6	1.052
25	**PARK7_ex2**	270.5	1.016
26	SNCA_ex2	278.2	1.402
27	PARK2_ex02	285.6	1.028
28	**Ctr1_3p22**	293.4	0.969
29	PARK2_ex8	302.1	1.082
30	PINK1_ex06	309.8	1.007
31	PARK7_ex4	317.0	1.049
32	PARK2_ex09	326.9	0.951
33	**Ctrl_2q36**	335.4	0.997
34	PARK2 ex6	343.1	1.028
35	**PARK7_ex1**	350.5	0.948
36	PARK2_ex10	357.8	0.994
37	PARK7_ex 5	368.5	0.981
38	PARK2_ex11	375.0	1.013
39	**Ctr1_11p14**	383.1	1.076
40	PARK2_ex12	393.1	1.048
41	PINK1_ex4	402.2	1.014
42	PARK7_ex03	410.5	1.015
43	**PARK2_ex5**	420.3	1.027
44	PARK7_ex01	428.9	0.856
45	**Ctrl_6p12**	435.8	1.053
46	SNCA_ex1	448.1	1.377
47	PARK7_ex7	455.6	0.991
48	PINK1_ex08	465.0	1.012
49	PARK2_ex3	472.6	1.011
50	**SNCA_ex5**	481.4	1.463
51	PARK2_ex 7	488.9	1.004
52	**Ctrl_2p13**	497.1	0.983

Figure 2. Detection of the *SNCA* gene duplication in EOPD patient with multiplex ligation-dependent probe amplification (MLPA) method. Reaction was performed with SALSA MLPA Probe mixes P051 (MRC Holland). Dosage analysis was performed with GeneMarker Software v.2.7.0 (SoftGenetics, LLC). **A**. Trace comparison — overdosage of all SNCA exons of patient's sample in relation to control. This panel shows the differences in peak height between patient's sample (blue) and control (red) for all SNCA exons. **B**. Report table — reporting peak ratio for all probes, duplication of SNCA exons (high ratio > 1.3) are indicated in positions 9,12, 22,24, 26,46 and 50. **C**. Ratio plot — visualization of the peak ratios. Normal relative probe signals are between the green lines (0.7–1.3), and are depicted in green. Aberrant relative probe signals are depicted in red

conditions and inhibiting mitochondria-mediated apoptosis. In a study of 394 Polish patients with PD, there were no definite pathogenic variants in this gene [26].

EIF4G1 (OMIM 614251, PARK18)

EIF4G1 encodes the protein, eIF4F, a component of the translation initiation complex. In a cohort of 397 Polish patients with PD, p.Ala502Val variant with unknown pathogenicity was identified in a single case [19]. However, further analysis of this locus did not support its pathogenicity [52].

HTRA2 (OMIM 610297, PARK13)

The Htra2 protein, a serine protease located in mitochondria, is responsible for apoptosis, especially during stress conditions. This protein is also an element of Lewy bodies. *HTRA2* was first reported in German familial and sporadic PD cases [53], but in 101 Polish patients with PD, no pathogenic variants were reported [28].

Risk factor genes

In addition to the genes responsible for familial forms of PD listed in the OMIM database, other genetic loci have been identified that increase the risk of PD occurrence. Some genes can be included as both monogenic and risk factor genes. Most mutations of *SNCA* are responsible for monogenic forms of PD, but some polymorphisms (e.g. rs356219) are risk factors for PD [54]. The last GWAS revealed about 90 genomic regions that can be associated with PD prevalence [13]. However, risk factor genes were analysed in a population of less than 1,000 Polish patients with PD, and so the study was underpowered [55]. While GWAS PD studies are conducted mainly in European populations, Polish patients with PD are not often included in the analysis [13].

GBA

GBA encoding glucocerebrosidase is one of the first risk factors described in PD. The encoding protein is a lysosomal hydrolase located in the lysosomal membrane and is involved

in the degradation of a sphingolipid glucocerebroside. Mutations in both alleles are responsible for Gaucher's disease, which is characterised by glucocerebroside accumulation and secondary macrophage accumulation [56]. Heterozygous carriers of *GBA* variants had increased risk of PD, and the highest prevalence of *GBA* mutations occurred in Ashkenazi Jewish patients. *GBA* variants were found in 19% of patients with PD and 3% of the general population [56]. In the first study conducted in a Polish population, 4.07% of *GBA* carriers were reported in a group of 270 non-demented patients with PD [57]. The second study revealed 16 carriers (11.6%) among 138 Polish patients with PD [58]. It is known that dementia occurs more often in *GBA* mutation carriers (60.0% *vs.* 19.6%) [58].

APOE

Apolipoprotein E plays a key role in lipid metabolism. *APOE* is considered one of the most important genetic risk factors for Alzheimer's disease (AD). Three common polymorphisms (ε2, ε3, and ε4) and six genotypes (ε2/ε2, ε3/ε3, ε4/ε4, ε2/ε3, ε2/ε4, ε3/ε4) have been identified in *APOE*, and ε3 is the most common allele. The potential impact of these variants was studied in the context of the occurrence of dementia in PD, rather than disease prevalence [59]. In a Polish population with PD, Pierzchlinska et al. [60] revealed no statistically significant correlation between *APOE* genotypes and dementia. Another study of 407 Polish patients with PD found no statistically significant differences in the distribution of *APOE* genotypes [60].

Other genetic analysis in Poland

We found other studies of Polish populations that do not fit into the gene groups described above. They describe mutations in mitochondrial DNA and genes associated with the immune system or with dopamine metabolism. All pathways analysed in Polish populations are set out in Table 2 [57, 58, 60–78].

Mitochondrial dysfunction has been implicated in PD pathogenesis [79]. The mutations causing mitochondrial dysfunction in nuclear DNA also risk variants in mitochondrial DNA [70]. Some changes in mitochondrial DNA may modify risk of PD. Mitochondrial transcription factor A (*TFAM*) has been shown to decrease reactive oxygen species [80]. The intronic variant rs2306604 increased risk of PD in an analysis of 326 patients with PD [67]. Mitochondrial DNA can be divided into haplogroups, restricted to particular populations and geographical areas.

Multiple European haplogroups, including J, K, U, and some super-haplogroups (e.g. UK and JT), have been associated with a reduced risk of PD [70]. This observation was also made in a Polish population [81]. Haplogroup J was associated with a lower PD risk in men. Subcluster K1a was more prevalent in healthy controls, while K1c was more frequent in patients with PD ($p = 0.025$ and $p = 0.011$, respectively). Furthermore, the sublineages (U4 + U5a1 + K + J1c + J2) previously proposed to partially uncouple oxidative phosphorylation decrease PD risk ($p = 0.027$) [81]. No impact of *TOMM40* on disease occurrence was observed in 407 PD patients [71].

Oxidative stress is one of the best-known potential pathomechanisms of PD. *NFE2L2* encoding nuclear factor-erythroid 2–related factor 2 is responsible for regulation of the expression of many antioxidant pathway genes in the so-called *phase II response*. In a Polish case-control study, *NFE2L2* haplotypes decreased the risk of PD for heterozygous and homozygous carriers [78]. Matrix metalloproteinases are huge families of endopeptidases important in inflammation. One of these families is macrophage metalloelastase (*MMP12*), first identified as an elastolytic metalloproteinase secreted by inflammatory macrophages [82]. In 241 patients with PD, rs652438 G allele genotypes of *MMP12* decreased the risk of the disease [65].

One of the pathways previously associated with PD and strictly connected with oxidative stress is the immune system. In an analysis of the human leukocyte antigen region polymorphism *HLA-DRA* rs3129882 in 343 Polish patients with PD, the recessive model of GG genotype was observed to be protective [73]. In another case-control study (341 patients with PD and 315 controls), polymorphisms in *IL-10* (-1082G > A and -592C > A) were not risk factors for sporadic PD [63]. Although semaphorins are the proteins responsible for regulation of the immune system and tumour progression, rs7702187 SNP in SEMA5A was not a marker of PD risk in 235 Polish patients with PD [64]. The triggering receptor expressed on myeloid cells 2 (*TREM2*) is a member of the innate immune receptor of the TREM family. It is found on activated macrophages, immature dendritic cells, osteoclasts and microglia. While the *TREM2* p.Arg47His (rs75932628) variant has been associated with increased risk of PD in a Polish study, this variant was rare in patients with PD and no variants were reported in controls [74].

A few studies have been conducted on the variants encoding enzymes associated with dopamine metabolism pathway [61, 62, 75, 83]. Lack of dopamine in synapses is a main clinical indication of PD. Because levodopa is a basic treatment for PD, polymorphisms in these enzymes may impact upon response to this treatment. A couple of studies in Polish patients have analysed genes encoding enzymes associated with dopamine metabolism [61, 62]. Catechol-O-methyltransferase (COMT) and monoamine oxidase B (MAOB) are involved in dopamine degradation in synapses. A study of 210 Polish patients with PD found a significantly lower frequency of the *COMT* LL genotype responsible for high enzyme activity [61]. The combined haplotype of the *MAOB* G (G/G) and *COMT* HL genotypes showed a four-fold increase ($p < 0.05$) in the risk of PD in women [61]. Bialecka et al. [62] analysed the impact of these polymorphisms on response to treatment. Their five-year observational study of 95 Polish patients with PD analysed

Table 2. Genetic risk factors associated with PD analysed in Polish populations

Gene	Mechanism	Results
APOE	Responsible for lipid metabolism; pathological aggregation of proteins	No impact on PD and PDD occurrence [60]
GBA	Lysosomal hydrolase responsible for degradation of a sphingolipid glucocerebroside	2 studies: –4.07% in 270 non-demented patients with PD [57] –11.6% in 138 patients with PD [58]
Mitochondrial dysfunction		
TFAM	Mitochondrial DNA transcription factor	Intronic variant rs2306604 increased risk of PD in analysis in 326 patients with PD (OR, 1.789; 95% CI, 1.162-2.755; $p = 0.008$) [67]
TOMM40	Translocase of the outer mitochondrial membrane 40 homolog	No impact on PD occurrence [71]
Haplo-group J	Mitochondrial DNA	Associated with lower PD risk in men (OR, 0.19; 95% CI, 0.069-0.530; $p = 0.0014$) [70]
Oxidative stress and immune system		
NFE2L2	Regulation of expression of many antioxidant pathway genes	*NFE2L2* haplotypes decrease risk of PD-heterozygous (OR, 0.4; 95% CI, 0.3-0.6; $p < 0.001$), homozygous (OR, 0.2; 95% CI, 0.1-0.4; $p < 0.001$) [78]
MMP12	Matrix metalloproteinase secreted by inflammatory macrophages, responsible for inflammatory reaction	rs652438G allele genotypes decrease risk of disease (OR, 0.47; 95% CI, 0.26-0.85; $p = .013$) [65]
HLA-DRA	Human leukocyte antigen	rs3129882 GG genotype protective for PD occurrence (OR, 0.67; $p = 0.04$) [73]
IL-10	Modulatory effects against proinflammatory cytokines, especially INF-γ and TNF-α	No impact on PD occurrence [63]
SEMA5A	Regulation of immune system and tumour progression	No impact on PD occurrence [64]
TREM2	Found on activated macrophages, immature dendritic cells, osteoclasts, and microglia	No impact on PD occurrence [74]
Dopamine and other neurotransmitter metabolism		
COMT	Catecholo-O-metylotransferase, responsible for dopamine metabolism	Lower frequency of *COMT* LL in PD [61]
MAO-B	Monoamine oxidase B responsible for dopamine metabolism	*MAOB* G (G/G) and *COMT* HL genotype → fourfold increased risk of PD in women ($p < 0.05$) No impact on response to treatment [62]
DBH	Noradrenaline synthesis from dopamine in plasma	rs1611115 was observed more often (OR, 2.01; $p = 0.01$) [75]
MDR1	Responsible for regulating environmental xenobiotics concentration	No impact on PD occurrence [77]
Pathways associated with other neurodegenerative disorders		
STH	Impact on AD pathogenesis	No impact on PD occurrence [72]
GRN	Impact on FTD occurrence	No impact on PD occurrence [68]
MAPT	Microtubule-associated protein	No impact on PD occurrence [69]
CALB1	L-type voltage-operated calcium channels	No impact on PD occurrence [76]
DAPK1	Ca2 +/ calmodulin-dependent serine/threonine kinase that plays a proapoptotic role in programmed cell death cascade	No impact on PD occurrence [66]

AD — Alzheimer's disease; FTD — frontotemporal dementia; INF — interferon; OR — odds ratio; PD — Parkinson's disease; PDD — Parkinson's disease dementia; TNF — tumour necrosis factor

the presence of *COMT* L and *MAOB* G polymorphisms in two study groups: those receiving less than 500 mg/day of levodopa, and those receiving 500 mg/day or more during the observational period. No statistical differences were observed between these groups [62]. Another study examined differences in polymorphism distribution in dopamine B-hydroxylase (*DBH*), responsible for noradrenaline synthesis from dopamine in plasma [75]. In a study of 224 Polish patients, *DBH* -1021C > T; rs1611115 was observed more often in the study group than in controls [75]. Michalowska et al. analysed the occurrence of polymorphisms in genes associated with dopaminergic metabolism and their impact on risk of PD and motor levodopa-induced adverse effects. They found that rs6265 *BDNF* (p.Val66Met) was associated with risk of PD. Additionally, they observed a synergic effect of rs6265 *BDNF* (p.Val66Met), rs397595 *DAT* (SLC6A3), and rs4680 *COMT* (p.Val158Met) polymorphisms on the occurrence of motor levodopa-induced adverse effects [83]. In a study of 158 patients with PD and 139 controls, Tan et al. [77] analysed seven SNPs from *MDR1* responsible for regulating environmental xenobiotics, but found no significant differences between the two groups.

The correlation of eight SNPs localised in the chromosomal region 2q24.3, previously associated with PD risk, was analysed; however, a study of 713 Polish patients revealed no association with PD risk [84]. The saitonin p.Gln7Arg polymorphism previously associated with AD was analysed in 100 patients with PD, but no association with disease occurrence was observed [72]. An SNP in the progranulin gene (*GRN*; 3'UTR+78C > T; rs5848) associated with frontotemporal dementia was not found to be a risk factor for PD in 364 Polish patients [68]. Microtubule-associated protein τ was previously reported to be associated with AD and frontotemporal dementia; however, a study of 832 Polish patients with PD found no impact on disease presence with *MAPT* p.Ala152Thr variant [69]. Death-associated protein kinase 1, previously reported in AD, was also not observed in patients with PD patients [66]. Calbindin belongs to L-type voltage-operated calcium channels. It has been reported that rs1805874 SNP may increase the risk of PD in Japanese patients [85]; however, this observation was not confirmed in Polish or other European populations (Tab. 2) [76]. Locus 5q23 (D5S1462 and D5S2501) was identified in two large Polish families with levodopa responsive parkinsonism [86, 87].

Clinical implications

Our report summarises the prevalence of PD genetic factors in the Polish population, and presents the first case of *SNCA* duplication in this population. Many genes responsible for both familial forms of PD and increased risk of disease have been established in the Polish population. Data indicates that PD genes reported in other countries are rarely observed in this population.

The diagnosis of PD is still based on clinical examination. Detailed genetic characteristics of specific populations may lead to the discovery of new PD biomarkers [86]. With the increasing availability of personalised medicine, the number of clinical trials calling for specific mutation carriers will increase. Currently, there is an ongoing phase I clinical trial for *LRRK2* p.Gly2019Ser mutation carriers. Antisense oligonucleotide BIIB094 binds to *LRRK2* mRNA and causes its degradation (NCT03976349). Another trial analysed DNL201 particle inhibition of the LRRK2 protein (NCT03710707) [87]. The most explored gene in the context of clinical trials is *GBA*. There are six ongoing clinical trials (three phase 1 and three phase 2) with different mechanisms, including glucocerebrosidase activators, glucosylceramide synthases inhibitors, and adeno-associated virus gene therapy [87, 88]. In 2019, the Michael J. Fox Foundation announced funding for development for *PRKN* and *PINK1* [89]. In the 2019, the Michael J. Fox Foundation announced funding for development for *PRKN* and *PINK1* targeted therapy.

Future perspectives

Many PD genes have been extensively screened in the Polish population. The frequency of variants in known genes is low. However, some methodological approaches (GWAS or clinical exomes analysis) have not been conducted yet. Furthermore, there are new sequencing methods, such as long-read sequencing, which can directly sequence single molecules of DNA in real time, often without the need for amplification. This direct sequencing approach enables the production of reads that are considerably longer than those resulting from classical short-read sequencing, allowing the sequencing of parts of the genome that are yet to be discovered. Long-read sequencing will facilitate better genetic characterisation of all patients with PD.

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Pneumocephalus as a rare complication: a systematic review plus clinical vignette

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ABSTRACT

Introduction. Pneumocephalus is a clinical entity characterised by the presence of gas in the intracranial space. It can result from many different causes. The most common cause is head or facial trauma. Other causes include neoplasms, infections, and surgical or diagnostic procedures. Spontaneous non-traumatic pneumocephalus is a rare condition caused by bone defects, malformations, infections, tumours, intravenous air injection, and other causes. This review, supplemented with a case presentation, aims to summarise the current state of knowledge regarding non-traumatic pneumocephalus.

Methodology. This review involved an electronic search (PubMed, Scopus, Embase, and Web of Science) to identify studies regarding non-traumatic pneumocephalus. In addition, reference lists of identified articles were screened for other potentially relevant papers.

Results. In total, 1,107 articles were retrieved by searching databases with the selected query. Based on the selection process, 134 articles were included. These articles were then classified into 'otogenic', 'bone defect', 'malformations', 'infectious', 'tumours', 'associated with intravenous air injection', and other categories.

Conclusion. Spontaneous non-traumatic pneumocephalus is a rare condition. Symptoms, clinical courses, and prognoses vary depending on the underlying cause of the disease. To the best of our knowledge, this review's example is the first case report of spontaneous pneumocephalus due to air embolism secondary to lung cancer.

Key words: pneumocephalus, spontaneous, non-traumatic, atraumatic

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Introduction

Pneumocephalus is characterised by the pathological collection of gas within the cranial cavity [1]. Pneumocephalus was first described in 1741 by Lecat et al. [2]. The main cause of pneumocephalus is head trauma, which is responsible for up to 74% of all cases. Other causes of pneumocephalus include: (1) intracranial neoplasms; (2) infections; (3) neurosurgery; (4) paranasal sinus surgery; and (5) diagnostic or neurosurgical interventions such as pneumoencephalography or lumbar puncture [3].

However, in rare cases, pneumocephalus can occur spontaneously. Spontaneous occurrence represents only 0.6% of all

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cases and was first described in 1954 by Jelsma and Moore [4]. Based on anatomical localisation, pneumocephalus is classified as 'extradural', 'subdural', 'subarachnoidal', 'intraparenchymal' or 'intraventricular'. Two factors contribute to the pathomechanism of pneumocephalus: a decrease in intracranial pressure and coexisting defects in the dura. These factors are caused either by a ball-valve mechanism which causes a rapid increase in the volume of air inside the skull cavity without compensatory cerebrospinal fluid (CSF) outflow, or by CSF leakage which creates intracranial hypotension resulting in an aspiration of air [3, 5, 6].

Spontaneous non-traumatic pneumocephalus is a rare condition. Few case studies have been published to date [5]. The most common causes include (1) otogenic pneumocephalus, (2) bone defects, (3) malformations (birth defects), (4) infections, (5) tumours, (6) other causes, and (7) an intravenous injection of air. Here, we present the case of a patient with non-traumatic pneumocephalus, and provide a literature review on other non-traumatic cases.

Clinical vignette

Patient 1

A 66-year-old right-handed man with a past medical history of lung cancer was found at home, unconscious. His family reported a persistent hiccup, which had occurred the day before admission. Upon neurological examination, the patient was unconscious. He was intubated due to respiratory insufficiency, and his pupils were small, with anisocoria R > L and a poor response to light. He did not respond to pain stimuli. Computer tomography (CT) of the patient's brain showed a massive cerebral air embolism, which was later classified as an example of intra-axial intravascular pneumocephalus (Fig. 1). A thoracic CT scan revealed fluid and solid components of the left hilus with an obstruction of the left main bronchi (Fig. 2). After contrast enhancement, clear infiltration was observed, including the left pulmonary veins, left atrium, and left ventricle. An intra-infiltrating mass included lucencies, indicating the presence of gas or air. Left cardiac lung cancer infiltration with airway communication was strongly suspected. The patient died a few hours after his admission to the emergency department.

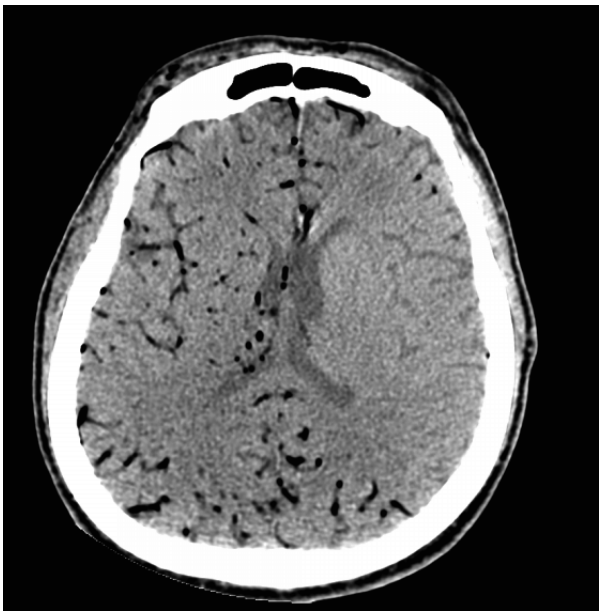

Figure 1. Brain CT. On axial CT, scan shows brain vessels with low density that indicates intravascular pneumocephalus

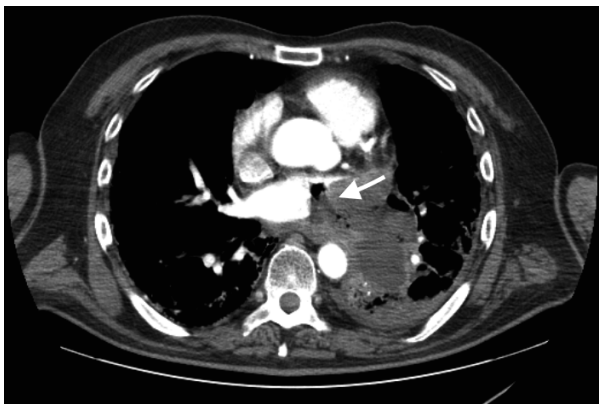

Figure 2. Chest CT. Axial CT-scan. Arrow indicates neoplasmic lung cancer and pulmonary vein infiltration with cancer mass, resulting in bronchial-vein fistula

Methodology

The references used in the current review were primarily identified by performing a systematic search of the PubMed and Web of Science databases. The search queries used included 'pneumocephalus', 'spontaneous', 'non-traumatic', 'atraumatic', and 'cancer'. The title/abstract filter was used to broaden the search. The final search was performed in April 2020. In addition, reference lists of identified articles were screened for other potentially relevant papers. Articles were selected based on eligibility criteria. Studies were included if (1) they presented original research, (2) they were conducted on pneumocephalus, and (3) they were written in English.

Selection process

In total, 1,107 articles were retrieved from the database search using the chosen queries (Fig. 3). Based on the selection process, 134 articles were included. These articles were then categorised as follows: (1) 'otogenic' (44 studies), (2) 'bone defect' (10 studies), (3) 'malformations' (five studies), (4) 'infectious' (38 studies), (5) tumours (30 studies), (6) others (four studies), and (7) associated with intravenous air injection (three studies).

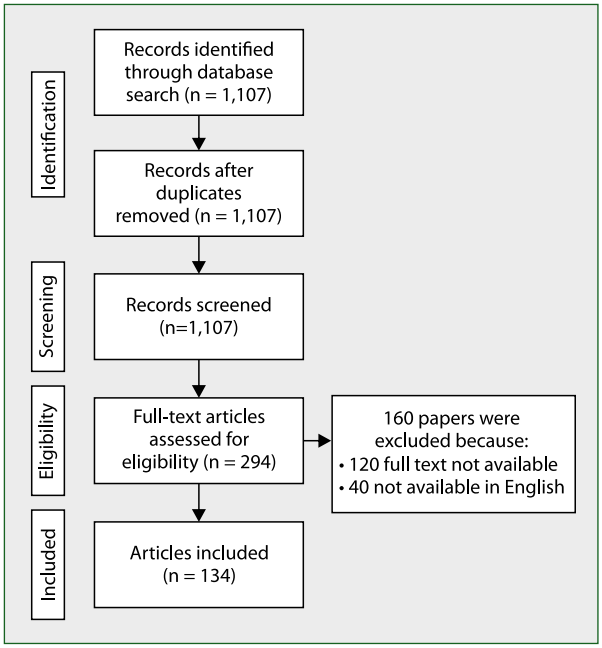

Figure 3. Selection process

Results

Spontaneous otogenic pneumocephalus (SOP)

A total of 44 articles were retrieved from the databases (Table 1). These articles were case studies reporting interesting patients and mechanisms of spontaneous otogenic pneumocephalus (SOP). The most common cause of SOP is a congenital bone defect in the pneumatised cells surrounding the middle ear. This defect develops into a fistulous communication with the intracranial compartment. Various factors can contribute to this mechanism, including: the Valsalva manoeuvre (coughing, sneezing, nose blowing, exhaling), the Politzer manoeuvre (blowing air through the nose during swallowing), and exposure to significant pressure changes (e.g. air travel) [7–11]. However, in a significant number of cases, the trigger remains unknown [12]. Wilkinson et al. reported a patient with intraventricular SOP related to barotrauma, which was due to tegmen defects that were confirmed in neuroimaging studies [13]. As such, bone defects in the middle cranial fossa have been proven to be associated with a risk of SOP occurrence [13]. Additionally, in SOP, hyperpneumatisation mastoids are a relatively common phenomenon [14]. Dowd et al. claimed that both a defect in the bones surrounding the middle ear (e.g. tegmen tympani defect) and a pressure difference on both sides of the defect are necessary for the formation of otogenic pneumocephalus [12]. Other descriptions of SOP in the literature support this theory [9,10,15]. Headache is the most common symptom of SOP, followed by aphasia [12, 16]. Villa et al. and Krayenbühl et al. reported a similar case of intraparenchymal pneumocephalus which manifested clinically with abnormal acoustic sensations, aphasia, and visual-field disturbances [16,17]. The symptoms often described in the literature also include tinnitus, fullness in the ear, rhinorrhoea, nausea, and confusion [11,13,18,19].

Pneumocephalus associated with bone defects

A total of 10 articles were retrieved from the databases (Tab. 1). These articles contained case reports of pneumocephalus resulting from bone defects other than those related to SOP. In this group, the most frequent were defects of sphenoid sinus and cribriform plate causing hyperpneumatisation of the cranium [20,21]. This process leads to communication between the inside of the skull and the intracranial compartment, which allows air to penetrate the cranium. Symptoms may be preceded by the Valsalva manoeuvre (e.g. blowing the nose, sneezing) [22, 23], though in a significant number of cases the triggers remain unknown [24]. Similarly to SOP, headache is the most commonly described symptom, along with rhinorrhoea [20, 24]. Nash et al. presented the case of a 27-year-old woman diagnosed with intraparenchymal pneumocephalus which manifested with a sudden onset of alien limb syndrome [9]. This rare phenomenon is most commonly described in cortico-basal syndrome and associated with frontal lobe and corpus callosum changes. For the reported patient, alien limb syndrome was related to pneumocephalus localised in the right frontal lobe, due to a cribriform plate defect. After treatment (pneumocephalus aspiration and bone defect repair), the patient recovered completely. Tension pneumocephalus is a relatively rare condition: only two articles contain case reports of tension pneumocephalus associated with bone defects [20, 25].

Pneumocephalus associated with malformations

A thorough search of the selected databases yielded a total of five articles on pneumocephalus associated with malformations. In all of these cases, pneumocephalus was associated with malformations related to an open myelomeningocele with accompanying CSF leakage [26]. In three of the five cases, pneumocephalus was localised intraventricularly [26–28]. The other locations were the posterior fossa and infratentorial area [29,30]. Spontaneous pneumocephalus occurs in a mechanism called the 'reverse bottle effect', which is attributed to CSF leakage through the neural tube defect. The air passes into the subarachnoid space and then through the spinal canal into the cranial cavity. In the case of open myelomeningocele, the air enters the spinal canal (e.g. when a child cries), which is associated with an increase of intracranial pressure accompanied by an outflow of CSF, leading to the formation of negative pressure in the ventricular system and an aspiration of air from outside [26,28]. The most commonly described symptoms are breathing difficulties and paresis of the lower limbs [27, 28].

Pneumocephalus associated with infections

A total of 37 articles on infections and pneumocephalus were retrieved from the databases (Table 1). Infections are a relatively common cause of pneumocephalus, and they

Table 1. Non-traumatic pneumocephalus: studies included in review

Spontaneous otogenic pneumocephalus					
Authors [year]	**Aetiology**	**Location**	**Symptoms**	**Location of air entry**	**Other**
Barry [2019] [57]	Spontaneous	Posterior cranial fossa	Confusion, lethargy, myalgia, cough, fever	Mastoid	Mastoiditis, sinusitis, S. pneumoniae
Eggerstedt [2019] [58]	Spontaneous	Left temporal	Confusion, aphasia, otalgia, aural fullness	Mastoid	
	Spontaneous	Intraventricular, right middle cranial fossa	Altered mental status	Tegmen tympani	
	Spontaneous	Intraventricular	Headache, nausea, photophobia	Tegmen tympani, mastoid	
	Spontaneous	Left frontoparietal	Otalgia, hearing loss, tinnitus	Tegmen tympani	
Harth [2019] [59]	Spontaneous	Intraventricular	Balance problems, tinnitus, aural fullness, rhinorrhoea, weakness of right lower limb	Petrous bone	CSF leak
Pollaers [2019] [60]	Barotrauma (air travel)	Extradural	Headache, tinnitus, hearing loss, nasal obstruction, rhinorrhoea, postnasal drip	Mastoid	CSF leak
Voldřich [2019] [10]	Politzer manoeuvre	Middle cranial fossa	Vertigo, tinnitus, instability, aphasia	Tegmen tympani	Bilateral spontaneous pneumocephalus
Stewart [2018] [15]	Barotrauma (air travel)	Right middle cranial fossa	Vertigo, nausea, vomiting, headache, otalgia	Tegmen tympani, petrous	
Wu [2018] [61]	Valsalva manoeuvre (sneezing)	Left occipital lobe	Headache, dizziness, epistaxis, tinnitus, otorrhoea	Mastoid	CSF leak; subdural haemorrhage, maxillary hemosinus
Young [2018] [62]	Spontaneous	Intraventricular, right temporal lobe	Headache, rhinorrhoea, acute lethargy	Temporal bone	CSF leak
Arai [2017] [63]	Spontaneous	Intraventricular	Incontinence, dysarthria, imbalance, ear fullness	Petrous bone	Bilateral pneumocephalus
Guleria [2017] [64]	Spontaneous	Subarachnoid	Headache, vomiting, neck rigidity, otorrhoea	Chronic otitis media	
Wannemuehler [2016] [65]	Spontaneous	Frontal lobes	Anosmia, headaches, confusion	Tegmen tympani	'Mount Fuji sign'
Odani [2015] [66]	Spontaneous	Intracranial	Dizziness, fever	Gas forming bacteria	Streptococcus pneumoniae
Ratre [2015] [67]	Spontaneous	Left cerebellopontine angle	Fever, headache, vomiting, decreased hearing, neck rigidity	Chronic otitis media	
Ginat [2014] [68]	Barotrauma (air travel)	Intracranial	Dizziness, hearing loss	Dehiscent right superior semi-circular canal	
Pishbin [2014] [69]	Valsalva manoeuvre (coughing)	Intracranial	Headache, nausea	Pneumosinus dilatans	
Remenschneider [2014] [70]	Barotrauma (air travel)	Extradural	Vertigo, hearing loss,	Superior canal dehiscence	
Rabello [2013] [71]	Valsalva manoeuvre	Extradural parietal	Aural fullness, nasal obstruction, nasal pruritus, headaches	Mastoid	
Tamura [2012] [72]	Spontaneous	Intraventricular	Otalgia, otorrhoea, headache	Superior external auditory canal, mastoid	Brain abscess
Javan [2011] [73]	Barotrauma (air travel)	Cisternal	Confusion, fever, nausea, vomiting, weakness, headache	Bony defect	Air-fluid level in right sphenoid sinus

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Table 1 cont. Non-traumatic pneumocephalus: studies included in review

Spontaneous otogenic pneumocephalus					
Authors [year]	**Aetiology**	**Location**	**Symptoms**	**Location of air entry**	**Other**
Mohammed [2011] [74]	Valsalva manoeuvre (sneezing, nose blowing)	Extradural	Headache	Temporal bone	Subcutaneous emphysema
Zhao [2011] [75]	Spontaneous	Epidural	Mass in left occipital region, bilateral hearing deficits	Tegmen tympani	Tension pneumocephalus, occipital subcutaneous emphysema
Lee [2010] [76]	Spontaneous	Multifocal	Headache, nausea	Pneumosinus dilatans	
Murugesan [2010] [77]	Barotrauma (scuba diving)	Extradural	Headache, nausea, vomiting	Ethmoid	
Abbati [2009] [78]	Spontaneous	Left temporal lobe	Otalgia, aphasia, headache	Tegmen tympani, temporal bone	
Pennings [2009] [79]	Spontaneous	Extradural	ND	Mastoid	
Roberts [2009] [18]	Spontaneous	Intraventricular	Confusion, rhinorrhoea, urinary incontinence	Fistula - right sphenoethmoidal recess, subsequently localised to right sphenoid sinus	CSF leak
Singh [2009] [9]	Valsalva manoeuvre	Left middle cranial fossa	Confusion	Tegmen tympani, temporal bone	
Hyam [2008] [80]	Spontaneous	Right middle cranial fossa, intraventricular	Confusion, aphasia	Mastoid, tegmen tympani	
Mathai [2008] [81]	Spontaneous	Intracranial	Fever, headache, vomiting, hearing loss	Chronic otitis media; 'gas forming' bacteria	
Tucker [2008] [19]	Valsalva manoeuvre (nose blowing)	Epidural	Headache, nausea	Mastoid	
Villa [2008] [17]	Spontaneous	Parenchymal (left temporal lobe)	Abnormal acoustic sensation, aphasia, visual field disturbances	Mastoid	
Bhattacharyya [2007] [82]	Spontaneous	Intracranial	Headache	Pneumosinus dilatans of frontal sinus	
Ciorba [2007] [83]	Spontaneous	Intracranial	Otalgia, headache, fever, otorrhoea, lethargy, nuchal rigidity, aphasia	Acute otitis media	Meningitis
Wilkinson [2007] [13]	Barotrauma (air travel)	Intraventricular	Tinnitus, hearing loss, aural fullness	Tegmen tympani	Temporal lobe fluid collection
Hage [2005] [11]	Change in altitude	Intraparenchymal, subdural, intraventricular	Abnormal acoustic sensations, deterioration, headache	Temporal bone	
Krayenbühl [2005] [16]	Spontaneous	Left temporal lobe	Abnormal acoustic sensations, aphasia, visual field disturbances	Mastoid	With intracerebral haemorrhage
Jensen [2004] [84]	Barotrauma (air travel)	Intraventricular	Headache, tinnitus	Mastoid	
Richards [2004] [8]	Valsalva manoeuvre (nose blowing)	Extradural	Swelling in ear area, otalgia	Mastoid	With collection of subcutaneous air
	Valsalva manoeuvre (coughing, sneezing, nose blowing)	Extradural	Pain and paraesthesia over left cheek and lower jaw	Mastoid	

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Table 1 cont. Non-traumatic pneumocephalus: studies included in review

Authors [year]	Aetiology	Location	Symptoms	Location of air entry	Other
Spontaneous otogenic pneumocephalus					
Schrijver [2003] [85]	Valsalva manoeuvre	Epidural	Asymptomatic	Mastoid	
Añorbe [2000] [86]	Valsalva manoeuvre	Right parietooccipital	Retroauricular swelling	Mastoid, petrous	With pneumatocele
Babl [1999] [87]	Valsalva manoeuvre	Subarachnoid	Headache	Sphenoid sinus	
Vallejo [1999] [14]	Valsalva manoeuvre	Right temporo-occipital	Headache	Mastoid	
Dowd [1998] [12]	Spontaneous	Intraventricular, left temporal cyst	Headaches, confusion, forgetfulness, aphasia, hemiparesis	Tegmen tympani	Abnormally low ICP
Maier [1996] [7]	Valsalva manoeuvre	Right parieto-temporo-occipital region, epidural	Headache, visual scotoma	Mastoid	
Pneumocephalus associated with bone defects					
Boninsegna [2019] [88]	Valsalva manoeuvre (nose blowing)	Subdural, intraventricular	Headaches	Cribriform plate	
Baba [2016] [23]	Valsalva manoeuvre (sneezing)	Extraaxial air (right cerebral hemisphere)	Headache, rhinorrhoea	Sphenoid sinus	CSF leak, CSF collection
Mirone [2015] [22]	Valsalva manoeuvre (nose blowing)	Intraparenchymal (left frontal lobe)	Loss of consciousness, hemiparesis, anosmia	Ethmoid sinus	
Gaberel [2012] [89]	Spontaneous	Intraventricular, intraparenchymal	Confusion	Sphenoid sinus	Arachnoid cyst
Nash [2012] [90]	Spontaneous	Intraparenchymal (right frontal lobe)	Alien limb syndrome	Cribriform plate	
Lefranc [2009] [20]	Spontaneous	Subarachnoid, intraventricular	Rhinorrhoea, headaches, epistaxis, anosmia	Cribriform plate	CSF leak; tension pneumocephalus
Tedeschi [2007] [21]	Spontaneous	Intraventricular, intraparenchymal	Headache, vomiting, fever, rhinorrhoea, nuchal rigidity, nystagmus	Sphenoid sinus	CSF leak
Kuo [2005] [25]	Spontaneous	Subarachnoid	Headache, rhinorrhoea, neck rigidity	Sphenoid sinus	Tension pneumocephalus, CSF leakage
Hogg [1998] [91]	Spontaneous	Intracranial	Rhinorrhoea, left hemiparesis, headache	Sphenoid sinus	
Park [1998] [24]	Spontaneous	Epidural	Headaches	Bone defect	Hyperpneumatisation of cranium
Pneumocephalus associated with malformations					
Kutty [2018] [28]	Spontaneous	Intraventricular	Apnoea, muscular hypotonia in lower limbs and sphincter	Open myelomeningocele	CSF leak
Erol [2004] [29]	Spontaneous	Infratentorial area	Apnoea attacks	Open lumbosacral myelomeningocele	CSF leak
Oedemis [2004] [27]	Spontaneous	Intraventricular	Lower limb paralysis	Open lumbosacral myelomeningocele	CSF leak; with infection
Garonzik [2001] [30]	Spontaneous	Posterior fossa	Open lumbosacral myelomeningocele	CSF leak	
Trawöger [1994] [26]	Spontaneous	Intraventricular	Respiratory distress	Open lumbosacral myelomeningocele	CSF leak; with infection

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Table 1 cont. Non-traumatic pneumocephalus: studies included in review

Pneumocephalus associated with infections					
Authors [year]	**Aetiology**	**Location**	**Symptoms**	**Location of air entry**	**Other**
Mirzai [2019] [92]	Spontaneous	Intracranial	Abdominal pain, weakness, confusion, constipation	Gas-forming Clostridium septicum	DM type 1
Saleem [2019] [93]	Spontaneous	Intracranial	Confusion, disorientation, fever, headache, vomiting	Bacterial infection	Sinusitis, pansinusitis, subdural empyema
Sun [2019] [94]	Spontaneous	Intracranial	Headache, nasal congestion, vertigo	Gas-forming Haemophilus influenzae	Mucopyocele
	Spontaneous	Intracranial	Fever, frontal headaches, cough, rhinorrhoea	Gas-forming Staphylococcus aureus and Escherichia coli	Meningitis
Ansari [2018] [95]	Spontaneous	Intracranial	Headache, lethargy, deterioration	Gas-forming Streptococcus salivarius	CPAP use complication, sinus wall osteomyelitis
Kumari [2017] [96]	Spontaneous	Subdural and subgaleal	Refusal to feed, progressive increase in head size, hypothermic	Possible bacterial infection	Neonatal meningitis, 'air bubble sign'
Srikumar [2017] [97]	Spontaneous	Intracranial	Left arm clumsiness, altered mental status, fever	Aspergillus terreus	Angiosarcoma
Lin [2014] [98]	Spontaneous	Intracranial	Fever, confusion, anisocoria	Bacterial meningitis with gas-forming organisms	Air embolism
Subasree [2014] [99]	Spontaneous	Intracranial	Headache, fever, neck rigidity	Gas-forming Bacteroides fragilis	
Kim [2013] [31]	Spontaneous	Intraventricular	Night sweats, confusion, headache	Streptococcus pneumoniae	Meningitis
Kosac [2013] [100]	Spontaneous	Intracranial	Headache, vomiting, persistent aqueous rhinorrhoea	Opacification of left sphenoid sinus and bone defect of left pterygoid process	Chronic sinusitis
Rota [2013] [101]	Spontaneous	Intracranial	Comatose state, fever	Gas-forming Streptococcus pneumoniae	Pneumococcal meningitis, right mastoiditis and left ethmoid sinusitis
Shenoi [2013] [37]	Spontaneous	Subarachnoid, intraparenchymal, intravascular	Hypothermia, lethargy, feeding difficulty	Citrobacter koseri	Late-onset neonatal Citrobacter meningitis
Baig [2012] [32]	Spontaneous	Subarachnoid space	Headache, rhinorrhoea, vomiting, fever, meningeal signs, bilateral papilledema	Bony erosion around olfactory cleft	Nasal tuberculosis
Kaur [2012] [102]	Spontaneous	Subdural	Fever, headache, convulsions, neck rigidity, positive Kernig sign	Meningitis	
Bhogal [2011] [103]	Spontaneous	Intraparenchymal	Diarrhoea, vomiting, altered level of consciousness, generalised weakness	Gas-forming Clostridium septicum	Cerebritis
Kumari [2011] [104]	Spontaneous	Intraparenchymal, intraventricular	Abnormal behaviour, fever, headache, back and neck pain vomiting, coughing	Gas-forming Staphylococcus aureus	Staphylococcal pneumonia and meningitis
Martin [2011] [35]	Spontaneous	Intraparenchymal	Anuria, bloody diarrhoea	Clostridium septicum gas – producing bacteria	Haemolytic uremic syndrome

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Table 1 cont. Non-traumatic pneumocephalus: studies included in review

Pneumocephalus associated with infections					
Authors [year]	**Aetiology**	**Location**	**Symptoms**	**Location of air entry**	**Other**
Oh [2011] [105]	Spontaneous	Intracranial	Fever, fatigue	Bony erosion, Tuberculosis meningoencephalitis	Lupus erythematosus
Redhu [2011] [33]	Spontaneous	Intracranial	Headache, dizziness, fever, unconscious with tonic extensor response to pain	Escherichia coli	Subdural empyema
Damergis [2010] [106]	Spontaneous	Intracranial	Headache, photophobia, nausea, jaw discomfort, subjective fever	Streptococcus pneumoniae	Otogenic meningitis
Lasboo [2010] [107]	Spontaneous	Subarachnoid	Altered mental status, urinary incontinence, diarrhoea, lower back pain	Gas-forming Escherichia coli	Septic discitis, meningitis, solid organ abscesses, urosepsis
Chung [2009] [108]	Spontaneous	Subarachnoid	Right hypertonia, hyperreflexia, confusion, dysphasia, right facial droop	Fistula between subarachnoid space crania extension of cervical spine	Nasopharyngeal carcinoma, osteoradionecrosis, osteomyelitis
Hama-Amin [2009] [109]	Spontaneous	Subarachnoid, intraparenchymal, intraventricular	Headache, confusion, somnolentia, meningeal signs	Gram-negative bacterial meningitis	
Lin [2009] [34]	Spontaneous	Intracranial	Headache, vomiting, fever, nasal congestion, neck stiffness, meningeal sign	Candida albicans sphenoid sinusitis	Pus drained from right sphenoid cavity
Kuo [2008] [38]	Spontaneous	Intracranial	Consciousness loss, hypothermia	E. cloacae	Nosocomial meningitis
Sreejith [2008] [110]	Spontaneous	Subarachnoid	Headache, fever, vomiting, ear discharge, hearing impairment, meningeal sign	Klebsiella pneumoniae meningitis	
Alviedo [2006] [39]	Spontaneous	Intracranial	Lethargy, apnoea, pallor, hypothermia	Citrobacter koseri	Meningitis
Townend [2005] [111]	Spontaneous	Intraventricular	Confusion, fever	Streptococcus pneumoniae	Sinusitis
Parmar [2004] [112]	Spontaneous	Intracranial	Lower back pain, fever, night sweats	Gas-forming Bacteroides fragilis	Meningitis
Pooboni [2004] [113]	Spontaneous	Intracranial	Poor feeding, state of collapse, hypothermia, hypotension	Gas-forming Citrobacter koseri	Pneumatosis oculi
Sedaghatian [2004] [114]	Spontaneous	Intracranial	Fever, irritability, convulsions	Gas-forming Enterobacter cloacae	Enterobacter cloacae septicemia and neonatal meningitis
Kassim [2003] [115]	Spontaneous	Intracranial	Poor feeding, lethargy, hypotensive, generalised convulsions	Gas-forming Proteus mirabilis	Meningitis
Goyal [1996] [116]	Spontaneous	Intracranial	Fever, hypotensive, altered sensorium	Gas-forming Clostridium perfringens	Meningitis
Randall [1993] [117]	Spontaneous	Intracranial	Bloody diarrhoea, abdominal pain, seizure, coma	Gas-forming Clostridium septicum	Retinal haemorrhages
Finelli [1991] [118]	Spontaneous	Intraventricular, subarachnoid	Focal left-sided seizure, eye deviation to left, confusion, hyperreflexia	Gas-forming Streptococcus pneumoniae	Meningitis
Candan [1990] [36]	Spontaneous	Intracranial	Otorrhoea, fever, nausea, vomiting, headache, epilepsy attack, left hemiparesis, neck stiffness	Gas-forming organisms: Proteus mirabilis, Veillonella spp.	Abscess in right temporal lobe

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Table 1 cont. Non-traumatic pneumocephalus: studies included in review

Authors [year]	Aetiology	Location	Symptoms	Location of air entry	Other
Pneumocephalus associated with infections					
Holtby [1990] [119]	Spontaneous	Intracranial	Earache, headache, nausea, vomiting, confusion, neck rigidity	Gas-forming Streptococcus pneumoniae	
Klein [1989] [120]	Spontaneous	Intraparenchymal, intraventricular, subarachnoid	Irregular respiration, hypotension	Gas-forming Clostridium perfringens meningoencephalitis	
Pneumocephalus associated with tumour					
Ghimire [2019] [50]	Spontaneous	Intraventricular	Mutism, right hemiparesis, deterioration	Defect in sphenoid sinus	Ecchordodid physaliphora (EP)
Iplikcioglu [2019] [121]	Spontaneous	Intracranial	Headache, drowsiness, vomiting	Posterior wall of frontal sinus	Front ethmoid osteoma
Elabd [2018] [122]	Spontaneous	Intraventricular	Headache, nausea, rhinorrhoea	'One-way valve mechanism' spontaneous to cabergoline treatment	
Albert [2017] [123]	Spontaneous	Intracranial	Cough, haemoptysis, right chest and back pain, right arm weakness	Bronchopleural-dural fistula	Chemoradiation, superior sulcus tumour
Hackenbroch [2017] [124]	Spontaneous	Intraparenchymal, right frontal lobe	Headache, difficulty with concentration, left arm weakness	Erosion of posterior wall of frontal sinus	Sinonasal osteoma, 'one-way valve mechanism'
Umredkar [2017] [125]	Spontaneous	Intracranial	Headache, left side hemiparesis	Erosion of posterior wall of frontal sinus	Frontal sinus osteoma, 'ball valve mechanism'
Jimenez [2014] [126]	Spontaneous	Intraventricular	Aqueous rhinorrhoea, altered mental condition	Nasocranial fistula	Nasopharyngeal carcinoma, radiotherapy
Marchant [2013] [127]	Spontaneous	Intraventricular, air in thoracic spinal canal	Headache	Oesophageal-arachnoid fistula	Non-small cell lung carcinoma
Nanba [2013] [128]	Spontaneous	Intraventricular and subarachnoid spaces	Headache, vomiting, delirious. CSF rhinorrhoea	Cabergoline treatment	MEN-1, macro-prolactinoma, treatment with cabergoline
Patel [2013] [49]	Spontaneous	Intraventricular	Headache	Oesophageal-subarachnoid fistula	Oesophageal cancer
Iacoangeli [2012] [48]	Spontaneous	Subdural (bifrontal), pneumorrhachis	Headache, confusion	Sacral bone	Colon adenocarcinoma
Lehmer [2012] [41]	Spontaneous	Intracranial	Headache, nausea, vomiting	Destruction of posterior wall of frontal sinus	Osteoma, one-way valve mechanism
Machicado [2012] [43]	Spontaneous	Subarachnoid, intraventricular	Rhinorrhoea, vomiting, headache	Cabergoline treatment	Macroprolacti-noma
Guedes [2011] [129]	Spontaneous	Intraparenchymal	Headache, right hemiparesis, seventh cranial nerve palsy	Bone erosion	Fronthoethmoidal osteoma
Qu [2010] [45]	Spontaneous	Subdural (bifrontal), vertebral canal	Headache, altered consciousness	Subarachnoid-oesophagus fistula	Oesophageal cancer; 'Mount Fuji sign'
Chung [2009] [130]	Spontaneous	Intracranial, subarachnoid	Confusion, expressive dysphasia, right facial droop, right-sided hypertonia and hyperreflexia	Osteoradionecrosis of cervical spine	Nasopharyngeal cancer, meningitis
Kamide [2008] [40]	Spontaneous	Intraparenchymal	Headache, left hemiparesis	Erosion of upper wall of ethmoid sinus	Osteoma in ethmoid sinus

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Table 1 cont. Non-traumatic pneumocephalus: studies included in review

Authors [year]	Aetiology	Location	Symptoms	Location of air entry	Other
Pneumocephalus associated with tumour					
Torres [2008] [131]	Spontaneous	Spinal canal	Tightness in thigh muscles, pain in perineal area	Gas communication between carcinoma of sigmoid colon into spinal canal	Carcinoma of sigmoid colon
Wang [2006] [44]	Spontaneous	Intraparenchymal, intraventricular	Headache, vomiting, blurred vision, rhinorrhoea, consciousness disturbances	Skull base defect; high-dose radiotherapy	Nasopharyngeal carcinoma
Bramley [2001] [132]	Spontaneous	Intracranial	Left hemiparesis	Erosion of right ethmoid sinus wall and right orbit	Ethmoid osteoma
Wu [1999] [133]	Spontaneous	Intraventricular, intracranial	Nasal discharge, loss of consciousness	Bony defect in right sphenoid body	Ventricular fluid revealed Staphylococcus infection
Jakubowski [1997] [134]	Spontaneous	Intracranial	Forgetfulness, left hemiparesis, anisocoric pupils	Bony destruction	Diploic epidermoid cyst
Kiu [1996] [135]	Spontaneous	Intraventricular, subarachnoid, subdural	Headache, rhinorrhoea, deterioration, neck stiffness	Destruction of skull base by tumour	Tension pneumocephalus; nasopharyngeal carcinoma, meningitis
Ng [1995] [136]	Spontaneous	Intracranial	Headache, fever, multiple nerve palsy	Noso-cranial fistula, bone necrosis	Nasopharyngeal carcinoma, radiotherapy
Kinsley [1993] [42]	Spontaneous	Intracranial	Left-side weakness, headache, confusion, drowsiness, decreased taste and smell	Bone defect in sinus	Epidermoid tumour of ethmoid sinus origin
Swaid [1983] [46]	Spontaneous	R frontal fossa	Headache, neck pain, confusion	Bronchial-subarachnoid fistula	Lung carcinoma
Lynn [1978] [51]	Spontaneous	Intracranial	Fever, irritability, poor feeding	Neurenteric fistula	Benign teratoma, meningitis
Banerjee [1975] [137]	Spontaneous	Intracranial	Headache, vomiting	Dural defect	Osteoid osteoma of ethmoid
Wilson [1968] [138]	Spontaneous	Intracranial	Brown-coloured discharge from left nostril	Destruction of frontal sinus wall	Epidermoid tumour
Holmes [1957] [47]	Spontaneous	Intraventricular	Nasal obstruction, exophthalmos, headache	Cribriform plate	Sarcoma of ethmoid
Pneumocephalus associated with intravenous air injection					
Laurent [2014] [53]	Spontaneous	Right cavernous sinus, right inferior ophthalmic vein, left cavernous sinus, right sphenoid sinus	Asymptomatic	Peripheral intravenous catheter	Ripped out intravenous catheter
Tran [2010] [52]	Spontaneous	Right cavernous sinus, right superficial temporal veins, left intraorbital veins	Asymptomatic	Peripheral intravenous catheter	
	Spontaneous	Right cavernous sinus, right superficial temporal veins, right superior orbital veins	Asymptomatic	Peripheral intravenous catheter	
	Spontaneous	Bilateral cavernous sinus, behind dorsum sella, right superficial temporal veins	Asymptomatic	Peripheral intravenous catheter	

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Table 1 cont. Non-traumatic pneumocephalus: studies included in review

Authors [year]	Aetiology	Location	Symptoms	Location of air entry	Other
Pneumocephalus associated with intravenous air injection					
Syed [2008] [54]	Spontaneous	Intracranial	Visual disturbances, headache, dizziness	Intravenous catheterisation	Gas emboli
Pneumocephalus associated with other aetiology					
Isikay [2018] [55]	Spontaneous	Subarachnoid	Generalised seizures	Interruption in cervical dural sheath	Positive end ventilation
Akashi [2015] [139]	Spontaneous	Intracranial	Headache, nasal hydrorrhoea	Nasal fistula	Granulomatosis with polyangiitis
Miyaji [2013] [140]	Spontaneous	Subarachnoid, intraventricular, pneumatocele in spinal canal	Headache, fever	Fistula in sacral region	Pressure ulcer
Park [2010] [56]	Spontaneous	Epidural	Headache	'One-way valve' mechanism	

represent 8.8% of all cases [3]. Bacterial infections are the most common, and they can cause subdural empyema with meningitis, leading to spontaneous pneumocephalus [31–33]. Two possible pathways were described: (1) an infection with 'gas-forming' bacteria and (2) a bony erosion caused by bacteria. Redhu et al. described a case of spontaneous tension pneumocephalus associated with subdural empyema caused typically by Escherichia coli. Pneumocephalus, therefore, resulted from an accumulation of gases in the nerve tissue as a result of bacterial metabolism [33]. Baig et al. presented a case of spontaneous pneumocephalus associated with nasal tuberculosis. As a result of a Mycobacterium tuberculosis infection, bone erosion around the olfactory cleft occurred, with subsequent dura mater damage and CSF leakage [32]. Lin et al. described a case of spontaneous pneumocephalus associated with sphenoid sinusitis caused by Cancida albicans. Extensive fungal osteomyelitis caused bone erosion of the right sphenoid sinus and resulted in intracranial pneumocephalus [34]. Martin et al. presented a case of spontaneous pneumocephalus associated with haemolytic uremic syndrome. As a result of Clostridium septicum-induced sepsis, many pockets of pneumocephalus created a 'Swiss cheese' appearance on brain CT scans [35]. The symptoms are typical for meningitis: headache, fever, vomiting, and night sweats (Tab. 1) [31, 33, 36]. Tension pneumocephalus as a result of bacterial meningitis is usually fatal [37–39].

Pneumocephalus associated with tumours

A thorough search of the databases revealed a total of 30 articles of pneumocephalus associated with tumours (Tab. 1). Pneumocephalus related to tumours has mostly been associated with sinus osteomas, followed by epidermoids, pituitary tumours, and nasopharyngeal carcinomas [40–44]. Different factors can destroy the tissues surrounding tumour cells, such as fistulae between the posterior wall of the oesophagus and the subarachnoid space of the spinal cord, which contribute to trapping air in the cranial cavity or in subdural spaces [45]. Additionally, pneumocephalus can be caused by radionecrosis as a result of treatment (e.g. subarachnoid bronchial fistulae as a result of irradiation therapy) [44, 46]. Furthermore, tension pneumocephalus can occur spontaneously as a result of treating dopaminergic agents with cabergoline [47, 48]. The main symptoms include headache, rhinorrhoea (presumably CSF), and confusion [45–49]. Interestingly, Torres et al. described a patient with stiffness in their thigh muscles and perineal areas as a result of gas collection in the subdural layer of the sacral nerve root [50]. Lynn et al. presented a case of spontaneous pneumocephalus with neurenteric communications in the form of a neurenteric fistula [51]. Wang et al. presented an interesting case of tension spontaneous pneumocephalus due to intensive high-dose radiotherapy, which caused osteoradionecrosis of the skull base and CSF leakage [44]. Interestingly, Kinsley et al. described a patient with tension pneumatocele originating from an epidermoid tumour. The patient developed symptoms for six months prior to his admission, which shows that the signs of pneumocephalus can develop either severely, within the first 24 hours of occurrence, or slowly progress over weeks or even months [42].

Pneumocephalus associated with an intravenous injection of air

The database search revealed only three articles on intravenous-related pneumocephalus (Tab. 1). This is considered a rare cause of pneumocephalus, usually related to the placement of an intravenous catheter [52]. Laurent et al. reported a patient with pneumocephalus resulting from an air embolism caused by a ripped-out peripheral intravenous access [53]. Tran et al. suggested that the diagnosis of intravenous-induced pneumocephalus should be considered in every case of incidentally detected air in the cranial cavity venous system on CT scans unless a medical reason is known [52]. In the reported cases, the most common location of pneumocephalus was the sinus cavernous [52, 53]. Intravenous-related pneumocephalus is associated with a good prognosis [54]. Pneumocephalus associated with an air embolism usually remains asymptomatic [52, 53].

Pneumocephalus associated with other aetiologies

Four case reports on the occurrence of spontaneous pneumocephalus related to other factors which could not be classified as belonging to the above-mentioned groups, were found in the chosen databases (Tab. 1). Isikay et al. reported an infant with bronchopneumonia who developed pneumocephalus as a result of positive ventilation during treatment for bronchopneumonia [55]. A generalised inflammatory reaction can disrupt the nerve sheaths, which – in combination with positive pressure ventilation – can result in air entering the subarachnoid spaces. Park et al. reported a case of pneumocephalus resulting from pneumatocele in the frontal sinus [56]. Air entered through the frontal sinus ostium but was unable to exit, which increased pressure in the sinus and caused subsequent expansion. The most common symptoms include headache and nausea, but also generalised seizures [55, 56].

Discussion

To the best of our knowledge, ours is the first case report of spontaneous pneumocephalus due to air embolism secondary to lung cancer. In our case, lung cancer caused infiltration of the left atrium and the left ventricle; therefore, air from the left ventricle entered the aorta and then travelled through the brachiocephalicus trunk and carotid arteries into cerebral circulation, causing a massive air embolism.

Spontaneous intravenous pneumocephalus caused by an air embolism is a rare condition – so far, only three cases have been described [51–53]. An air embolism underlying pneumocephalus can affect both the arterial and venous vessels. In contrast to our case, the remaining reports presented pneumocephalus due to venous embolism as a result of intravenous air injection through a peripheral venous catheter [51, 52]. Patients with intravenous pneumocephalus can show acute symptoms within the first 24 hours of occurrence, similar to the patient presented here, or can show slowly progressing signs within weeks [47]. The course of the disease in the presented patient was acute, accompanied by disturbances of consciousness and respiratory failure resulting in death. In contrast, Laurent et al. describe a mild, asymptomatic course of pneumocephalus related to intravenous air injection through a peripheral venous catheter [51, 52]. However, Syed et al. reported that the patient experienced visual disturbances, headache, and dizziness due to gas embolism during intravenous catheterisation [53].

Our review of the literature confirms that the most common causes of pneumocephalus are otogenic and infectious, followed by tumours. A minority of pneumocephalus cases are caused by intravenous air injection and other cases.

Symptoms and the clinical course of pneumocephalus can vary, depending primarily on aetiology and the location of intracranial gas. The most frequent symptoms, regardless of pneumocephalus aetiology, are headaches and consciousness disturbances [30, 59, 99, 120]. In otogenic spontaneous pneumocephalus, otalgia, aural fullness, hearing loss, tinnitus, otorrhoea, and abnormal acoustic sensations are also often described. In pneumocephalus caused by infections, fever and meningeal symptoms are the most common symptoms [31, 92, 98, 108].

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Trends in glioblastoma treatment research: an analysis of clinical trials and literature

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ABSTRACT

Introduction. Glioblastoma is the most common, and the most lethal, primary malignant brain tumour in adults. The aim of the study was to present a comprehensive, data-based review of glioblastoma treatment research, considering all clinical trials and peer-reviewed journal publications.

Materials and methods. Data regarding all glioblastoma clinical trials that was available on 7 August 2019 on ClinicalTrials.gov was analysed. Information on interventions' mechanisms of action was obtained from AdisInsight. A PubMed search for 'glioblastoma' was performed in September 2019. Citation counts were gathered from Scopus. Custom software for obtaining and analyzing data was developed by the authors.

Results. 1,388 clinical trials on glioblastoma with a start date between 1979 and 2020 were identified. The distribution of glioblastoma clinical trial phases differs significantly from that of other high-mortality cancers. 526 unique interventions of clinical trials and 206 molecular targets have been isolated. 32,410 publications on glioblastoma have been found, the number having increased especially since 2006. Publications on identified treatment options comprised 32.2%. Publications on glioblastoma are cited on average 4.27 times per year. The average specificity of treatment options' publications for glioblastoma is 6.9%.

Conclusions. Glioblastoma treatment options and their molecular targets can be quantitatively ranked according to their scientific research output. To the best of our knowledge, no such registries have been elaborated before.

Key words: glioblastoma, treatment, research, clinical trial, literature, review

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Introduction

Glioblastoma is the most common, and the most lethal, primary malignant brain tumour in adults. Each year, more than 3 per 100,000 people in the United States [1], Europe [2–4], Israel [5], Australia [6] and elsewhere are diagnosed with this WHO grade IV glioma, especially so in areas of higher socioeconomic status [7, 8]. Although there are regions with a lower incidence, such as Japan, Korea [9, 10] and Jordan [11], the reason for this is not yet fully understood. The overall incidence is increasing [4, 9, 12], yet this has not been convincingly attributed either to growing mobile phone use [13, 14], nor to improved diagnostic techniques [15], nor merely to the ageing of society.

The tumour manifests itself predominantly in patients aged 55–85 [1], with a median age at diagnosis of 63 [12]. Incidence rates increase with age up to 80–85 years [12, 16]. WHO-recognised [17] risk factors include: risk increase after exposure to ionising radiation to the head and neck, and risk decrease with history of allergies and atopic disease [14]. Additionally, the diagnosis is not always straightforward and swift, as symptoms are often vague, including headache (27%), fatigue (20%), confusion (27%), and drowsiness (35%), before progressing to more neurologically distinctive ones such as

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seizures (37%) and motor deficits (21%) [18]. Furthermore, the disease progresses quickly: Stensjøen et al. observed daily untreated tumour growth of 1.4% [19].

Consequently, the rapidly growing glioblastoma causes an enormous psycho-socioeconomic burden [20] to its often working-age and unsuspecting victims and their families.

The prognosis is highly unfavourable, with a five-year survival of no more than 5% [21]. This makes glioblastoma one of the most deadly cancers [22, 23]. Accordingly, survival is commonly given in months instead of years.

Palliative treatment (mostly with corticosteroids and anticonvulsants) offers survival ranging from a few weeks to a few months [21]. Decades of research on surgical resection and radiotherapy have prolonged this to about 6–10 and 12 months respectively [21]. The 2005 introduction of concomitant and adjuvant temozolomide chemotherapy further increased survival by 2.5 months [24]. Refinement of these interventions has resulted in a current median survival of 16 months [25]. The remaining most frequently used treatment options include nitrosoureas (such as lomustine and carmustine), bevacizumab [26] and tumour treating fields [27]. The outcomes are especially promising for the latter, with reports of expanding median overall survival to almost 21 months [28].

Despite these encouraging new treatment modalities emerging just since 2005, the results are far from satisfactory, given that there is an average of more than 20 years of life lost due to premature mortality caused by glioblastoma [29]. Further research is highly desirable.

Over time, scientific research has provided us with significant advances in our understanding of the histopathological characteristics of glioblastoma. In 1926, 62 years after Rudolf Virchow coined the term 'glioma', Percival Bailey and Harvey Cushing were able to distinguish 'glioblastoma multiforme' (GBM) as a separate entity, although the name acknowledged the variable gross appearance of the tumour [30]. Nowadays, glioblastoma is still classified as a high-grade glioma with predominant diffuse astrocytic differentiation exhibiting hypercellularity, nuclear atypia, mitotic activity with microvascular proliferation and/or tumour necrosis [31]. Although the histopathology of the tumour remains extremely variable, with abundant cellular and nuclear polymorphisms alongside significant regional heterogeneity, the recent discoveries of molecular mechanisms and genetic alterations established the basis for the 2016 fourth edition of the *WHO Classification of Tumours of the Central Nervous System* [17] (Fig. S1, see supplementary materials). This changed the way we perceive glioblastoma. The discoveries are so profound that after 90 years we now see a new shift in nomenclature. The popularity of the broad term 'glioblastoma multiforme' has waned, giving way to more specific entities such as IDH-wildtype glioblastoma, giant cell glioblastoma, gliosarcoma, epithelioid glioblastoma, IDH-mutant glioblastoma, etc. [32] (Fig. S2, see supplementary materials). Despite the limited therapeutic success, it would be unfair to say that glioblastoma treatment research is scarce. On the contrary: plentiful clinical trials are conducted [33–36] and articles published [37].

In fact, the amount of research is so abundant that it is challenging for any non-expert to grasp the glioblastoma therapeutic interventions outlook without becoming confused [38]. The scientific community has recently recognised this problem, and summaries of past studies are beginning to emerge. In 2012, Nieder et al. [37] analysed patterns of citations and reviewed articles published on glioblastoma between 2006 and 2010. They showed glioblastoma research activity increasing over time. The Journal of Clinical Oncology and the Journal of Neuro-Oncology were the major scientific journals in the field. Among the top 10 cited articles, seven reported on genomic analyses, molecular subclasses or stem cells, with only two articles on phase II or III clinical trials. In 2017, Cihoric et al. [34] analysed 2005–2015 phase II and III clinical trials, providing insight into, inter alia: experimental interventions, clinical trials' funding, enrollment, and phase distribution. They found a high initiation of glioblastoma clinical trials, suggesting that this was caused by the failure of previous early investigative treatments to show satisfactory efficacy. The authors found that 51.9% of trials were funded primarily by the industry, consistent with other oncology clinical trials. They demonstrated, however, insufficient representation of surgery, radiotherapy, and imaging focused trials, with these funded solely by academic institutions. At the time, the most researched topics were treatment options targeting EGFR or VEGF receptors and their pathways, as well as multi-TKIs. In 2018, Vanderbeek et al. [35] reviewed interventional glioblastoma clinical trials initiated between 2005 and 2016, analysing, inter alia: clinical trials' duration, enrollment, phase distribution, selected endpoints, and connected publications as linked by ClinicalTrials.gov. The authors found that a significant minority of glioblastoma patients were enrolled in clinical trials, although this observation did not differ much from general oncology trials. They showed long development times to be characteristic of glioblastoma research, and suggested suboptimal decision making that led to too many patients with ineffective therapies engaging significant financial resources. Also in 2018, Paolillo et al. [33] reviewed 2015–2017 clinical trials and selected therapeutic strategies. They reported multiplying strategies against glioblastoma and a growing knowledge of genetic profiling and mutations. The authors considered new immunotherapy strategies to show the most promise. They stressed, however, that traditional therapies such as surgery, chemotherapy, and radiation remain the first line approaches to glioblastoma. In 2019, Zanders et al. [36] reviewed the 2016–2018 glioblastoma clinical trials. They concluded that small-molecule interventions have not significantly improved the standard of care, pointing out, nonetheless, that the vast majority of new clinical trials continue to focus on small-molecule therapy. They suggested however that together with smarter combination therapy selection and adaptive clinical trial design,

small-molecule interventions could still show success. These authors felt the best hope lay in novel immunotherapies and developments in the modulation of T cells.

The aim of the current study is to present a comprehensive, data-based review of glioblastoma treatment research, considering all past clinical trials and scientific journal publications. This article is intended to serve as a roadmap for anyone interested in the subject, and as a guide for further research in the pursuit of effective glioblastoma treatment.

Methods

Clinical trials

Glioblastoma

Complete XML data for all clinical trials found by searching for the condition of 'glioblastoma' was downloaded from ClinicalTrials.gov on 7 August 2019. Sought after information was extracted and saved: this included data on clinical trial status, phase, enrollment, intervention(s), study type, start, and completion dates. Calculations in the current study involved only clinical trials started after 1993, due to incomplete data for older entries.

Other cancers

Analogous information about clinical trials was gathered for cancers that had data on incidence, mortality, and five-year survival rate available on the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Programme website [23]. The cancers selected are listed in Table S1 (see supplementary materials). This data was used to contrast and compare glioblastoma to other cancers.

Treatment options

Inclusion and exclusion criteria for interventions of glioblastoma clinical trials

Diagnostic interventions were intentionally excluded as they were not the subject of this study. Due to the automatic processing of large amounts of data, we included interventions with consistently identifiable nomenclature. Interventions with names that were not specific enough (e.g. 'surgery', 'radiation therapy') were excluded. Interventions with synonymous names listed as separate entities on ClinicalTrials.gov were merged. Combination interventions were divided into single entities. Interventions that were included in this study are summarised in Table S2 (see Supplementary materials).

Treatment options in scientific literature

We searched PubMed [39] for articles on the treatment of glioblastoma published from 2010 onwards. The search was conducted on 16 February 2019 with a query listed in Appendix 1. Search results were imported to Mendeley Reference Management Software. Glioblastoma treatment options were extracted by reading through the titles and abstracts of the publications found.

Alternative names and categorisation

The National Cancer Institute Thesaurus [40] and Springer AdisInsight [41] were searched for synonyms of treatment options. A comprehensive list of alternative names (i.e. synonyms) warranted the reliability of further automated literature analysis, and was therefore paid much attention.

AdisInsight [41] was also used for categorisation of treatment options according to their 'mechanism of action' and (drug) 'class'. We then combined the mechanisms of action into broader categories based on molecular targets of treatment options. The aim of this approach was to more clearly present numerous results.

Literature

PubMed [39] was selected as a source for data on glioblastoma research [42]. It was searched for each of the identified glioblastoma treatment options in September 2019. Search queries included previously gathered synonymous names. They were targeted to glioblastoma. The results were downloaded in XML format and parsed. Extracted information included publication identifiers (DOI, PMID), publication dates, journal, and publication type.

Accordingly, on 22 September 2019, the authors once again searched PubMed with queries for each of the identified treatment options. These queries were however targeted to neoplasms in general. They, too, included treatment options' alternative names. Total number of publications found and their distribution by year were saved for each treatment option.

Finally, a large-scale PubMed search was performed for the entire field of glioblastoma research on 29 September 2019 [39] to serve as a baseline for further analysis. Publication identifiers, dates, journals, affiliations, and types were saved.

All search queries used are listed in Appendix 1.

Glioblastoma specificity

Research specificity to glioblastoma was calculated for each treatment option as a percentage of publications on glioblastoma to all publications on neoplasms in general (as explained in the previous section).

Distribution of studies by country

Country information was extracted from the affiliations of glioblastoma publications gathered from PubMed.

Citation analysis

Citation counts for all publications on glioblastoma (including publications on identified glioblastoma treatment options) were gathered. Scopus [43] was selected as the most suitable source for citation data. This was primarily due to its advantageous coverage of newer (i.e. after 1995) publications

[42] that were deemed more valuable for the current study than older ones. In addition, the authors recognised Scopus's advantage over Google Scholar and Web of Science in terms of website architecture and suitability for automated data collection. Given the large amount of data to gather (citation counts for over 30,000 publications), the process took six days.

Other cancers

Data on the number of publications on cancers selected from the SEER Programme website [23] (Tab. S1, see supplementary materials), and their distribution by year, was downloaded from PubMed.

Glioblastoma treatment research rating

The authors created an original algorithm for rating glioblastoma treatment options based on their research data. Treatment options were ordered by quantitative criteria and then ranked according to the sum of their relative positions in each criterion. These criteria consisted of: 1) the number of clinical trials conducted, 2) total enrollment, 3) average number of publications per year, 4) average citation count per publication per year, 5) specificity of publications to glioblastoma, and 6) year of introduction in a clinical trial, favouring newer treatments because past treatments have shown limited efficacy despite more time for fine-tuning the regimes. The items were ordered in descending order for all the criteria.

Mechanisms of action and their common molecular targets were selected in the same fashion, with an additional criterion of: 7) number of treatment options in each category, likewise in descending order.

Data gathering

All steps of data gathering were accompanied with C# .NET applications that saved the data in appropriate SQLite databases. This tailor-made software was developed by the authors.

Statistical analysis

Basic descriptive analysis was carried out programming directly in SQL. The remaining descriptive, and all regression, analysis was performed in R programming language. The 'segmented' R package [44] was used for change-point regression (Fig. S2, S5, S7, see supplementary materials). Obtaining p-values from confidence intervals was accomplished using the method proposed by Altman and Bland [45].

Results

Clinical trials

1,388 clinical trials on glioblastoma were identified, with start years ranging between 1979 and 2020. There has been a constant increase of almost four additional new trials started each year ($R^2 = 0.94$, $p < 0.001$) since 1990 (Fig. S3, see supplementary materials). This number now exceeds 90 yearly trials. The vast majority (91.6%) of clinical trials are of interventional study type, although there has been a slight decrease over time in favour of observational clinical trials ($p < 0.01$).

There are currently 379 (27.3%) active (*recruiting, not recruiting* or *enrolling by invitation*) clinical trials with completion dates after August 2019. Their enrollment totals 45,225. Early phase I (phase 0) trials comprise 16 (4.2%) trials, phase I — 168 (44.3%) trials, phase II — 164 (43.3%) trials, phase III — 23 (6.1%) trials, and phase IV — one (0.3%) trial. The distribution of phases in currently active clinical trials does not differ statistically significantly ($p = 0.17$) from the distribution of all-time interventional glioblastoma clinical trials (Fig. S4 B, see Supplementary materials). There is also no statistically significant annual change in the relative distribution of glioblastoma clinical trial phases. Nevertheless, each year, more clinical trial interventions are tested in phase I (increasing by 2.8 per year, $p < 0.001$, $R^2 = 0.83$) and phase II (increasing by 3.0 per year, $p < 0.001$, $R^2 = 0.78$) clinical trials. There seems to be also a moderate increase (by 0.3 per year, $p < 0.05$, $R^2 = 0.39$) in interventions tested in phase IV trials (Fig. S4 C, see supplementary materials). No statistically significant increase was found for phase III ($p = 0.07$, $R^2 = 0.18$).

The distribution of glioblastoma clinical trial phases differs significantly from that of clinical trials for other examined high-mortality cancers ($p < 0.001$) (Fig. S4 A, see Supplementary materials). However, no statistically significant correlation between phase distribution and five-year survival or mortality rates was observed for the analysed 30 neoplasms.

Treatment options

1,583 unique entries for interventions of clinical trials have been identified. ClinicalTrials.gov categorises interventions into 11 categories (Tab. S2, see supplementary materials). 'Other' interventions were largely composed of neuroradiological (e.g. 'MRI'), diagnostic (e.g. 'blood draw'), and research (e.g. 'questionnaire'). 'Procedure' category contained mostly surgical and diagnostic interventions. 'Device' interventions included many neurodiagnostic devices. 'Genetic' interventions were almost exclusively laboratory techniques (e.g. 'DNA analysis', 'polymorphism analysis'). 'Behavioural' category predominantly comprised activities such as 'psychoeducation' and 'exercise'.

526 unique interventions of clinical trials have been isolated after data clean-up according to the procedure described in the Methods section. These interventions were undertaken 2,072 times in clinical trials. Each intervention was tested, on average, in 3.94 clinical trials (SD = 16.77). However, both the median and the mode are equal to 1, as more than half of the interventions were tested in single trials (278/526 = 52.8%).

Treatment options in scientific literature

1,487 publications were retrieved from the search on glioblastoma treatment. Investigation of the publications allowed for the identification of 181 treatment options. 172 of them

were listed as interventions in clinical trials. Not tested in clinical trials were nine treatment options (two nutritional and seven drugs). A total of 535 (526 + 9) glioblastoma treatment options further analysed in this study are summarised in Table S2 (see Supplementary materials).

Alternative names and categorisation

4,452 alternative names were identified for 515 (96.2%) treatment options, with an average of 8.64 names per treatment option (SD = 7.65). No synonyms were found for the remaining 20 treatment options, mostly immunological (10/20, e.g. Ad-hCMV-TK) and radiotherapeutic (4/20).

427 mechanisms of action were found for 503 (94.0%) treatment options. There was an average of 2.34 mechanisms of action per treatment option (SD = 1.95), and 2.75 treatment options per mechanism of action (SD = 5.04, median = 1). Molecular target-based categorisation allowed for a reduction of category number to 206. 407 (drug) classes were identified for 486 treatment options, with an average of 4.13 classes per treatment option (SD = 2.35), and 4.93 (SD = 21.8, median = 2) treatment options per class. Mechanisms of action and classes were unavailable in AdisInsight for radiotherapeutic and surgical treatment modalities as well as for therapeutic devices (e.g. Optune) and dietary supplements.

Novel treatment options

There have been, on average, 18 novel interventions introduced annually in clinical trials (SD = 11, median = 18). This number increases each year by an average of 1.2 more novel treatment options, and currently exceeds 30 (Fig. 1). Every year, there are new mechanisms of action introduced as well as new common molecular targets. The latter, however, show a somewhat steadier annual rate (Fig. 1).

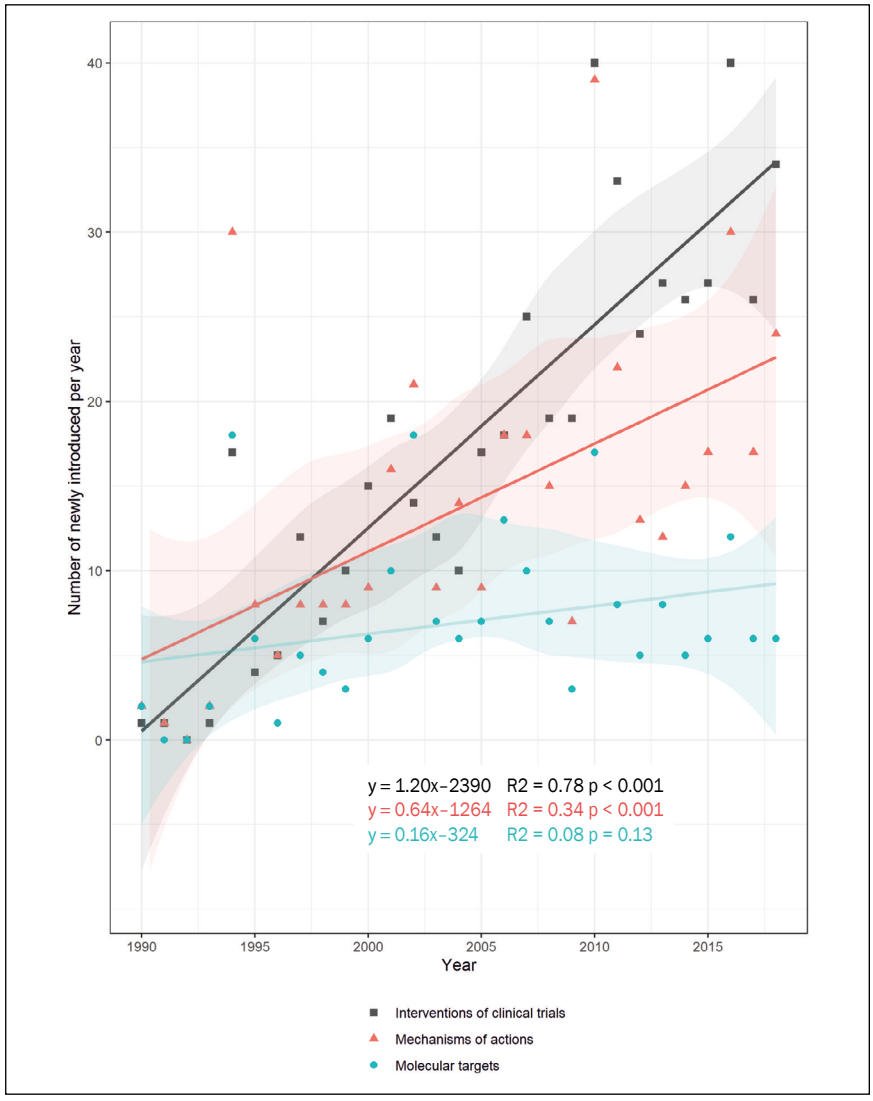

Figure 1. New treatment options, mechanisms of action, and molecular targets by year of first introduction in clinical trials

Literature

32,410 publications on glioblastoma have been identified. The number of publications is increasing each year and has especially accelerated since 2006 (Fig. S5 A, see supplementary materials). A summary of the most popular publication types is presented in Table S3 (see supplementary materials). Most papers on glioblastoma have been published in the journals listed in Table S4 (see supplementary materials). Similarly to that shown by Nieder et. al in 2012 [37], both the Journal of Clinical Oncology and the Journal of Neuro-Oncology continue to contain the highest numbers of glioblastoma articles. The latter, however, is approached by PloS one, Oncotarget and Cancer research (see Table S4 in supplementary materials).

10,435 publications on treatment options were found. This number increases each year and has especially accelerated since 2004. Publications on identified treatment options comprise 32.2% of all glioblastoma research. This percentage has increased statistically significantly ($p < 0.001$) since 1961 at an average yearly rate of 0.69% (Fig. S6, see supplementary materials). No publications were found for 129 treatment options. Each of the remaining 406 treatment options was presented in an average of 39 publications, although the median was equal to 7, mode to 1, and standard deviation was 196 publications. Treatment options are mentioned in publications targeted to glioblastoma, on average, 14.2 years (SD = 14.6 years, median = 8 years) after they were first mentioned in publications targeted to neoplasms in general. However, there seems to be a tendency towards shortening this timespan over time, especially after 1980, at an average rate of 0.29 years difference per year ($p < 0.001$) (Fig. S7, see supplementary materials).

Publication preceded clinical trial in the case of 200 treatment options (on average by 10.9 years, SD = 10.8, median = 7 years). In 161 cases, publications appeared after the first clinical trial started (on average after 3.7 years, SD = 3.2, median = 3 years). In 32 cases, the first clinical trial started in the same year as the first publication (Fig. S8, see supplementary materials). There is no statistically significant change over time of the overall average timespan between the first clinical trial and the first publication ($p = 0.61$). There seems to be, however, a tendency to shorten this timespan in the clinical-trial-first group by an average of 0.2 years per year ($R^2 = 0.14$, $p < 0.001$).

Glioblastoma specificity

Average specificity of publications on treatment options for glioblastoma is 6.9% (SD = 16.0%, median = 1.5%). There is no statistically significant trend for change over time when treatment options are analysed according to their clinical trial introduction year ($p = 0.88$, $R^2 < 0.01$).

Distribution of studies by country

A world map with the numbers of cumulative glioblastoma articles (co)authored in given countries is presented in Fig. S9 (see supplementary materials). Over 81% of all glioblastoma articles have been published in just 11% of countries (n = 16). Moreover, articles from just seven countries (USA, China, Germany, Japan, Italy, France, and Canada) represent over 53% of all the glioblastoma articles available on PubMed. The percentage shares of articles on the analysed treatment options relative to all glioblastoma articles remain fairly constant across countries: they are approximately normally distributed with a mean of 34% (SD = 5%).

An up-to-date world map showing the numbers of glioblastoma clinical trials is available online at https://clinicaltrials.gov/ct2/results/map?cond=Glioblastoma

Citation analysis

Citation data was available in Scopus for 30,267 publications on glioblastoma (94%). There is an average of 4.27 citations per publication per year (SD = 11.85, median = 2.08).

9,855 publications on treatment options for glioblastoma (94,4%) had citation data available in the Scopus database. There are statistically significantly ($p < 0.05$) more citations per publication per year in the publications on the treatment options group than in all publications on the glioblastoma group, an average for the former being 4.73 (SD = 12.04) with a median of 2.50 citations.

Changes over time of citations per publication per year of publications on glioblastoma and on treatment options in glioblastoma are presented in Figure 2.

It is possible that publications on glioblastoma generally favour the growth of journals' impact factor, as the overall yearly citation effect is approaching its impact factor multiplied by 1.5 [46] (Tab. S4). However, a separate study would be needed for conclusive results. Factors to be taken into account should include, at least, publication type (article/review), yearly citations separated since publication, and yearly journal impact factor.

Of the top 10 articles with the most citations per year (Tab. S5, see supplementary materials), the majority report on glioblastoma characteristics rather than treatment, similarly to how it was described by Nieder et al. in their 2012 review [37].

Glioblastoma treatment research rating

The top 30 treatment options selected according to the proposed algorithm are presented in Table 1. Molecular targets of the most researched treatment options are summarised in Table 2.

Limitations

Despite great attention to detail, planning and organisation, the authors were unable to eliminate all limitations.

Clinical trials

The authors' aspiration not to omit treatment options by limiting the study to subjective selection has, at times, led to overinclusion and posed a threat of overestimation. For example, the clinical trial NCT04028479 investigating chimeric antigen receptor T-cell therapy in 76 cancers (including glioblastoma) must have been manually excluded from the

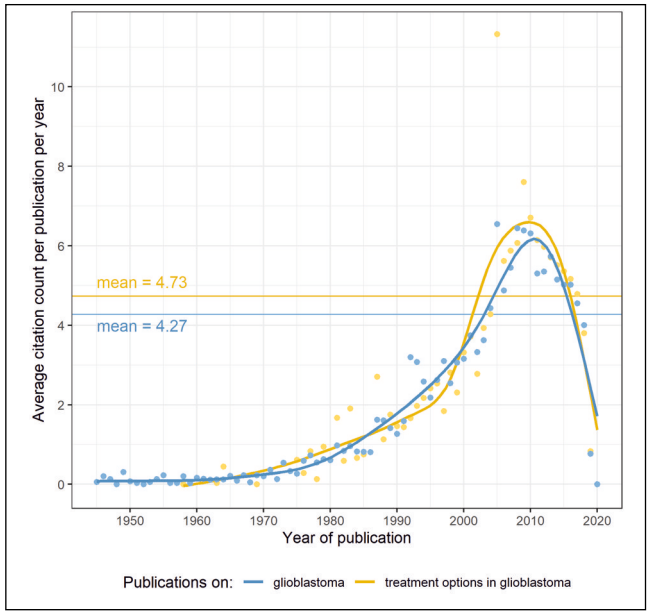

Figure 2. Average citations of publications on glioblastoma and on glioblastoma treatment options

calculations of total enrollment due to the outlined value (enrollment of 100,000 when there is a total enrollment for all the remaining glioblastoma trials of 104,752). But not including this trial altogether because it is not glioblastoma-specific (as practiced by some authors) would however in itself induce bias by underestimating CAR-T research in glioblastoma. Although great attention was given to identifying and correcting any instances of such bias, some may have escaped our scrutiny.

Alternative names and categorisation of interventions

There is no one ideal way to categorise treatment options according to their mechanisms of action. The approach of presenting them in narrow categories, such as listed in the AdisInsight database, risks underestimation. For example, drugs targeting topoisomerase enzymes are listed under separate categories of type I and type II inhibitors. When the number of drugs is calculated independently for each category, topoisomerase is the 27th most numerous treatment target. When, however, both mechanisms of action are regarded as one category of topoisomerase-targeting drugs, topoisomerase becomes the 16th most numerous target. The authors believe the latter approach more faithfully represents the whole picture of glioblastoma treatment research, but we nonetheless must warn the reader of the risk of overestimation.

Epidemiological statistics

Readers should be warned that Table S2 (see supplementary materials) may be biased since, due to the limited epidemiological data availability, a rather specific entity of glioblastoma is compared to broader disease definitions like breast or lung cancer (instead of e.g. small-cell lung cancer). However, the observation that there are more clinical trials on brain cancers than would be expected from the disease incidence is consistent with the results of Hirsch et al. [47]. Our intention was to illustrate the noninferiority of glioblastoma research compared to other neoplasms, and also the ones of higher incidence.

Distribution of studies by country

Data for the analysis was gathered by means of a PubMed search, as explained in the Methods section. This may understate the number of articles from countries other than the USA and/or not written in English, as there is research published in local scientific journals not (fully) indexed on PubMed [48].

Conclusions

Glioblastoma treatment options can be quantitatively ranked according to their scientific research output, as presented in Table 1. Similarly, molecular targets can be ordered (Tab. 2). To the best of our knowledge, no such registries have been elaborated before.

Additionally, to the best of our knowledge, this study is the first to have conducted an essential bibliometric analysis on all PubMed-found glioblastoma-related publications. Publications on glioblastoma are expected to be cited on average 4.27 times per year. Publications regarding treatment options are expected to be cited slightly more often (on average 4.73 times per year). Although further research would be desirable, these results suggest that journals may welcome publications on glioblastoma not only for their readability, but also for their citation advantage.

Table 1. Top 30 treatment options as ranked by most popular research (see text). Values in [square brackets] represent relative position of treatment option in each criterion

Order	Treatment option	Times tested in clinical trials		Total enrollment		Publications per year		Citations per publication per year		Specificity to glioblastoma		First tested in a clinical trial	
1	**Bevacizumab** binds to vascular endothelial growth factor (VEGF); inhibits binding to its receptor; prevents growth and maintenance of tumour blood vessels	140	[2]	11,608	[2]	76.31	[2]	5.83	[101]	9.50%	[56]	2005	[16]
2	**Tumour Treating Fields** are low-intensity, intermediate-frequency, locally delivered, alternating electrical fields inducing selective toxicity to proliferating cells; cause mitotic arrest and apoptosis of rapidly dividing cells [27]	27	[6]	2,548	[6]	12.00	[5]	4.83	[146]	86.34%	[3]	2006	[15]
3	**Temozolomide** causes methylation of DNA at O6 and N7 positions of guanine; inhibits DNA replication	347	[1]	34,778	[1]	146.28	[1]	5.24	[125]	54.55%	[9]	1997	[24]
4	**Nivolumab** binds to and inactivates programmed death receptor 1 (PD-1) by blocking its ligands; inhibits its negative regulation of T-cell activation and cell-mediated immune responses against tumour cells	26	[7]	3,286	[4]	8.75	[11]	10.56	[33]	1.01%	[239]	2014	[7]
5	**Cediranib** inhibits all three vascular endothelial growth factor receptor (VEGFR 1–3) tyrosine kinases; prevents growth and maintenance of tumour blood vessels	10	[18]	1,013	[22]	4.67	[30]	11.10	[27]	17.55%	[27]	2006	[15]
6	**Cilengitide** binds to and inhibits alpha v beta 3 and alpha v beta 5 integrins; inhibits endothelial cell-cell interactions, endothelial cell-matrix interactions, and angiogenesis	14	[14]	1,240	[15]	4.17	[36]	11.29	[24]	36.89%	[16]	2000	[21]
7	**Lomustine** alkylates and crosslinks DNA; inhibits DNA and RNA synthesis	31	[5]	5,449	[3]	5.53	[23]	4.43	[167]	10.83%	[49]	1998	[23]
8	**Erlotinib** binds to intracellular catalytic domain of epidermal growth factor receptor (EGFR) tyrosine kinase; reversibly inhibits EGFR phosphorylation; blocks signal transduction and tumourigenic events associated with EGFR activation	23	[9]	1,434	[12]	8.12	[14]	7.72	[57]	2.45%	[146]	2001	[20]
9	**Irinotecan** is an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor; blocks signal transduction and tumourigenic events associated with EGFR activation	42	[4]	2,055	[9]	9.32	[9]	6.36	[83]	2.17%	[158]	1997	[24]
10	**5-Aminolevulinic Acid** is converted to intracellular protoporphyrin IX in glioblastoma cells; allows for intraoperative fluorescence-assisted resection and photodynamic treatment [46]	13	[15]	512	[48]	8.08	[15]	4.71	[154]	7.35%	[69]	2008	[13]
11	**Carmustine** alkylates and crosslinks DNA during all phases of cell cycle; carbamoylates proteins	43	[3]	3,272	[5]	10.76	[7]	3.52	[216]	12.75%	[42]	1990	[30]
12	**Depatuxizumab Mafodotin** is an epidermal growth factor receptor (EGFR) inhibitor	6	[22]	1,241	[14]	1.00	[117]	7.07	[66]	51.16%	[11]	2013	[8]
13	Dendritic Cell present or process antigens; stimulate cellular anti-tumour immunity	31	[5]	1,483	[10]	14.35	[4]	5.25	[124]	1.07%	[230]	2001	[20]
14	**Rindopepimut** is a cancer vaccine stimulating cellular immunity against tumour cells expressing EGFRvIII (originally discovered in glioblastoma multiforme, not expressed in normal tissues)	3	[25]	954	[24]	2.20	[70]	9.13	[43]	95.65%	[2]	2007	[14]
15	**Pembrolizumab** binds to and inactivates programmed death receptor 1 (PD-1) by blocking its ligands; inhibits its negative regulation of T-cell activation and cell-mediated immune responses against tumour cells	23	[9]	1,470	[11]	2.75	[56]	11.33	[22]	0.43%	[307]	2010	[11]
16	**Enzastaurin** selectively inhibits protein kinase C beta — an enzyme involved in induction of vascular endothelial growth factor (VEGF)-stimulated glioma angiogenesis	6	[22]	877	[28]	2.07	[71]	7.33	[60]	14.72%	[33]	2006	[15]

↓

Table 1 cont. Top 30 treatment options as ranked by most popular research (see text). Values in [square brackets] represent relative position of treatment option in each criterion

Order	Treatment option	Times tested in clinical trials		Total enrollment		Publications per year		Citations per publication per year		Specificity to glioblastoma		First tested in a clinical trial	
17	**Abemaciclib** is a Cyclin-dependent kinase 4 (CDK4) and CDK6 inhibitor (with greater selectivity for former); prevents G1/S transition; suppresses DNA synthesis; inhibits cancer cell growth	2	[26]	322	[79]	2.25	[69]	11.85	[14]	4.81%	[94]	2017	[4]
18	**O6-Benzylguanine** binds DNA repair enzyme O6-alkylguanine DNA alkyltransferase (AGT); inhibits AGT-mediated DNA repair; potentiates cytotoxicity of DNA-damaging agents (e.g. temozolomide)	17	[11]	539	[44]	1.59	[84]	8.48	[48]	10.41%	[52]	1997	[24]
19	**Vorinostat** binds to catalytic domain of histone deacetylases (HDACs); leads to accumulation of hyperacetylated histones (followed by upregulation of cyclin-dependant kinase p21 and G1 arrest) and transcription factors	14	[14]	876	[29]	4.21	[34]	4.81	[149]	3.02%	[129]	2005	[16]
20	**Disulfiram** after complex formation with copper (that selectively accumulates in cancer cells), generates reactive oxygen species and inhibits proteasome activity after complex formation with copper (that selectively accumulates in cancer cells)	8	[20]	342	[75]	3.10	[51]	5.24	[126]	6.37%	[77]	2013	[8]
21	**Temsirolimus** binds to and inhibits mammalian target of rapamycin (mTOR); decreases expression of mRNAs and G1-phase arrest	13	[15]	1,125	[17]	2.00	[72]	9.51	[39]	2.42%	[147]	2001	[20]
22	**CAR-T Cells** are autologous or allogeneic T-lymphocytes that are engineered to contain a chimeric antigen receptor (CAR) that specifically targets a particular tumour antigen	4	[24]	81	[178]	9.67	[8]	8.54	[47]	4.10%	[101]	2017	[4]
23	**Ipilimumab** binds to CTLA4 expressed on T-cells; inhibits CTLA4-mediated downregulation of T-cell activation	10	[18]	898	[26]	2.75	[56]	10.88	[29]	0.42%	[308]	2014	[7]
24	**Ketogenic diet's** mechanism not fully understood; diet characterised by low carbohydrate intake, high fat and balanced protein content; higher blood glucose levels may be associated with worse prognosis in patients with glioblastoma [47]	9	[19]	230	[105]	3.78	[40]	5.83	[100]	8.95%	[60]	2007	[14]
25	**Buparlisib** targets all four isoforms of class 1 phosphoinositide 3-kinase (PI3K); inhibits activation of PI3K signalling pathway	6	[22]	389	[64]	1.86	[74]	6.51	[79]	4.74%	[95]	2011	[10]
26	**Veliparib** inhibits poly (ADP-ribose) polymerases (PARPs); prevents DNA repair; potentiates cytotoxicity of DNA-damaging agents (e.g. temozolomide)	5	[23]	902	[25]	1.40	[95]	6.90	[69]	4.86%	[92]	2009	[12]
27	**Galunisertib** binds to kinase domain of TGF beta receptor 1 (TGFBR1); prevents activation of TGF-ß-mediated signalling pathways	2	[26]	255	[97]	2.00	[72]	8.63	[46]	11.36%	[45]	2011	[10]
28	**Multi-glioblastoma-peptide-targeting Autologous Dendritic Cell Vaccine ICT-107** exposes immune system to GBM-associated antigens (AIM-2, MAGE-1, TRP-2, gp100, HER-2, IL-13Ra2); activates a specific cytotoxic T-lymphocyte response against GBM cells	5	[23]	570	[41]	0.33	[170]	30.67	[2]	100.00%	[1]	2006	[15]
29	**Everolimus** inhibits activation of mTOR; inhibits T lymphocyte activation and proliferation that is associated with antigen and cytokine (IL-2, IL-4, IL-15) stimulation and inhibition of antibody production	12	[16]	882	[27]	5.76	[21]	5.12	[131]	1.48%	[197]	2003	[18]
30	**Antibody-like CD95 Receptor/Fc-fusion Protein CAN-008** binds to CD95 ligand and blocks its receptor binding; prevents initiation of apoptosis in healthy cells and invasive growth of cancer cells	4	[24]	504	[49]	0.75	[132]	6.60	[78]	66.67%	[6]	2009	[12]

AIM-2 — interferon-inducible absent in melanoma 2 protein; MAGE-1 — melanoma-associated antigen 1; TRP-2 — tyrosinase-related protein 2; gp100 — glycoprotein100; HER-2 — epidermal growth factor receptor 2; IL-13Ra2 — interleukin-13 receptor subunit alpha-2; mTOR — mammalian target of rapamycin; IL-2, IL-4, IL-15 — interleukin 2, 4, 15; CD95 — cluster of differentiation 95 aka Fas receptor

Table 2. Top 30 molecular targets of glioblastoma treatment options as ranked by most popular research (see text). Values in [square brackets] represent relative position in each criterion

Order	Molecular target	Treatment options		Times tested in clinical trials		Total enrollment		Publications per year		Citations per publication per year		Specificity to glioblastoma		First tested in a clinical trial	
1	T lymphocyte	46	[1]	154	[4]	10,500	[5]	41.89	[5]	8.40	[8]	16.92%	[7]	1997	[24]
2	mTOR	13	[12]	48	[17]	3,277	[16]	32.27	[10]	6.46	[21]	8.59%	[29]	2010	[11]
3	Epidermal growth factor receptor	19	[9]	79	[11]	6,176	[8]	33.04	[9]	7.22	[17]	10.53%	[21]	1998	[23]
4	Vascular endothelial growth factor receptor	23	[6]	84	[9]	4,907	[13]	31.44	[11]	6.11	[24]	4.48%	[66]	2000	[21]
5	Microtubule	23	[6]	74	[12]	5,268	[12]	29.36	[13]	4.55	[54]	11.85%	[16]	2002	[19]
6	c-KIT	15	[11]	80	[10]	5,430	[10]	33.90	[8]	5.82	[33]	3.98%	[68]	2006	[15]
7	Natural killer cell (NK)	9	[16]	39	[18]	1,893	[20]	4.47	[56]	7.83	[12]	14.24%	[10]	2008	[13]
8	Vascular endothelial growth factor	4	[21]	147	[5]	12,054	[4]	79.22	[4]	5.90	[31]	3.89%	[71]	2008	[13]
9	Phosphatidylinositol 3 kinase	10	[15]	24	[24]	1,745	[21]	9.95	[32]	5.87	[32]	11.46%	[17]	2011	[10]
10	RET proto-oncogene	8	[17]	50	[16]	3,165	[17]	15.53	[17]	7.70	[13]	2.58%	[88]	2009	[12]
11	Platelet-derived growth factor receptor	16	[10]	73	[13]	4,534	[14]	29.60	[12]	4.94	[46]	2.74%	[85]	2006	[15]
12	Platelet-derived growth factor	3	[22]	16	[30]	1,739	[22]	13.15	[24]	7.93	[10]	6.16%	[46]	2017	[4]
13	Programmed cell death protein 1	6	[19]	64	[14]	5,827	[9]	14.50	[20]	10.94	[3]	0.35%	[163]	2017	[4]
14	Protein kinase B (PKB)	7	[18]	15	[31]	1,333	[31]	15.18	[18]	5.97	[30]	12.00%	[15]	2010	[11]
15	Cyclin-dependent kinases (CDKs)	5	[20]	12	[34]	1,170	[36]	10.03	[31]	6.10	[25]	7.99%	[32]	2017	[4]
16	Topoisomerase	21	[8]	101	[8]	5,290	[11]	36.15	[7]	3.87	[69]	2.74%	[84]	1999	[22]
17	FMS-like tyrosine kinase	9	[16]	29	[21]	1,420	[30]	13.17	[23]	5.25	[40]	5.25%	[57]	2009	[12]
18	Integrin alpha	2	[23]	15	[31]	1,322	[32]	4.17	[61]	11.29	[2]	18.45%	[6]	2006	[15]
19	Histone deacetylase (HDAC)	7	[18]	26	[22]	1,468	[29]	15.13	[19]	4.46	[57]	6.91%	[39]	2005	[16]
20	Protein kinase C (PKC)	1	[24]	6	[39]	877	[44]	2.07	[87]	7.33	[14]	14.72%	[9]	2019	[2]
21	Toll-like receptor	6	[19]	18	[28]	627	[50]	11.46	[29]	10.90	[4]	2.36%	[93]	2005	[16]
22	Mitogen-activated protein kinase (MAPK)	5	[20]	14	[32]	1,301	[33]	11.94	[28]	5.03	[44]	3.16%	[79]	2013	[8]
23	Bcr-Abl	7	[18]	33	[19]	2,802	[18]	12.09	[27]	4.97	[45]	1.10%	[126]	2007	[14]
24	BRAF	5	[20]	26	[22]	2,250	[19]	9.70	[34]	4.83	[47]	0.79%	[141]	2013	[8]
25	Bcl-2	6	[19]	12	[34]	988	[40]	13.11	[25]	4.75	[48]	4.73%	[64]	2008	[13]
26	Glycogen synthase kinase 3 beta	2	[23]	7	[38]	599	[51]	5.74	[49]	4.47	[55]	9.02%	[27]	2018	[3]
27	Hepatocyte growth factor receptor (HGFR)	6	[19]	12	[34]	703	[48]	6.40	[42]	6.01	[28]	2.53%	[90]	2010	[11]
28	Poly (ADP-ribose) polymerase	5	[20]	13	[33]	1,600	[25]	4.44	[57]	6.06	[26]	1.67%	[105]	2011	[10]
29	NF-kB	4	[21]	10	[36]	571	[53]	13.73	[21]	3.78	[72]	6.25%	[45]	2013	[8]
30	Peptide Elongation Factor 2	2	[23]	6	[39]	194	[92]	1.81	[92]	4.61	[53]	27.68%	[4]	2017	[4]

Scientists' interest in publishing on glioblastoma has been increasing significantly since 2006, possibly due to the encouraging 2005 introduction of the Stupp protocol of temozolomide treatment [24] (Fig. S5 A, see supplementary materials). It is interesting to note, however, that clinical trials have not followed that trend, with a rather steady annual increase rate (Fig. S3, see supplementary materials).

Nonetheless, the number of clinical trials on glioblastoma is constantly growing at a relative increase rate not inferior to that of other cancers. The best predictor for clinical trial number seems to be the cancer incidence rate. Five-year survival rates help to better explain clinical trial count, with more trials for cancers with shorter survival. Cancer prevalence in the USA is only a weak predictor of research output. Cancer publication count is strongly positively correlated with clinical trial count. It, too, depends on cancer incidence and five-year survival rate. Marginally more than half of glioblastoma treatment options have had glioblastoma-targeted scientific publications before having a clinical trial conducted. This observation remains constant over time.

The number of new glioblastoma treatment options as well as new mechanisms of action is rapidly growing each year. However, the specificity of their publications for glioblastoma is low, with no clear trend of increase over time. The results reveal also that interventions are tested in glioblastoma, on average, 10 years after they were first published on other neoplasms. More than half of clinical trial interventions have only been tested in single trials. The vast majority was tested in earlier phase clinical trials, which is consistent with what Cihoric et al. observed in their 2017 review [34]. However, there seems to be a trend for a moderate increase in the number of glioblastoma treatment options studied in phase IV clinical trials, and no clear negative trend for phase III was demonstrated.

Searching ClinicalTrials.gov website is a faster, more convenient, and reliable way of gathering a comprehensive list of glioblastoma treatment options than analysing publications on PubMed. Given the strong correlation between clinical trials and publications for glioblastoma and other cancers, the same premise might hold true for the latter. However, data on glioblastoma clinical trials' interventions could be better organised on ClinicalTrials.gov. Categorisation into intervention types ('drug', 'device', etc.) in its current form cannot be used to reliably filter interventions. Separating treatment from dosage and maintaining a list of unique treatments would prevent categorising the same treatment as different entities. There have been successful attempts to overcome this issue by privately-run databases such as Springer's AdisInsight [41].

However, they usually demand a fee and, most importantly, still depend on data from ClinicalTrials.gov.

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The relation between plasma miR-126 levels and cerebral collateral circulation in patients with intracranial arterial stenosis

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ABSTRACT

Objective. This study aimed to investigate the correlation between the circulating miR-126 regulation pathway and the cerebral collateral circulation (CCC), and to test whether miR-126 could serve as a potential biomarker for CCC formation in patients with intracranial arterial stenosis or occlusion.

Material and methods. This single-centre cross-sectional study enrolled patients who underwent cerebral angiography with severe stenosis (≥70%) or occlusion in at least one major intracranial artery. Collateral degree was graded according to the ASITN/SIR classification. The patients were divided into a good CCC group (grade 3–4) or a poor CCC group (grade 0–2). We investigated the plasma levels of miR-126, VEGF, Spred-1 and PIK3R2 by using qRT-PCR, ELISA and Western blot methods, respectively. In addition, we assessed the correlations of plasma miR-126 with VEGF, Spred-1, PIK3R2 and ASITN/SIR grade using the Spearman correlation test and investigated its predictive power for CCC status by using the receiver operating characteristic curve.

Results. A total of 68 patients were enrolled (44 with good CCC and 24 with poor CCC). Data showed that plasma miR-126 and VEGF were significantly higher in the good CCC group than in the poor CCC group. Plasma Spred-1 and PIK3R2 level were lower in the good CCC group than in the poor CCC group. In addition, miR-126 and VEGF were positively correlated with ASITN/SIR (miR-126: $R = 0.595$, $P < 0.01$; VEGF: $R = 0.595$, $P < 0.01$), whereas Spred-1 and PIK3R2 were negatively correlated with ASITN/SIR (Spred-1: $R = -0.817$, $P < 0.01$; PIK3R2: $R = -0.513$, $P=0.01$). However, the area under the curve of miR-126 level for CCC status was only 0.328 (95% CI: 0.158–0.498; $p = 0.067$).

Conclusions. Plasma miR-126 level may be related to better CCC formation, one of the mechanisms that may be explained by upregulation of VEGF and reduction of Spred-1 and PIK3R2 protein expression. However, miR-126 might not be an independent predictor for CCC, given its low predictive value.

Key words: miR-126, VEGF, cerebral collateral circulation, ASITN/SIR, biomarker

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Introduction

Acute ischaemic stroke can result in severe neurological disability and/or death [1]. The cerebral collateral circulation (CCC) is a network of blood vessels designed to preserve cerebral blood flow when primary routes fail [2]. Several recent studies have provided information about the role of collaterals in stroke pathophysiology, and it has been recognised that collateral circulation influences arterial recanalisation, reperfusion, haemorrhagic transformation and neurological outcomes after stroke [3–7]. To date, the CCC has mainly been assessed by using expensive or invasive imaging techniques.

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The strength of the CCC varies among patients and partly depends on genetic and modifiable risk factors. Therefore, the identification of a biomarker of CCC status would be of considerable clinical significance.

MiRNAs are small non-coding RNAs (~22 nucleotides) that suppress translation or induce degradation of downstream mRNA targets, thereby modulating gene expression at the post-transcriptional level [8]. Studies have shown that miRNAs play an important role in arteriogenesis and vascular remodelling [9, 10]. Among the several miRNAs, miR-126 has appeared to be enriched in tissues with a high vascular component and to be specific to the vascular system [11, 12]. miR-126, an endothelial cell-specific miRNA, modulates angiogenesis in vivo by enhancing the proangiogenic actions of VEGF, and repressing the expression of Spred-1 and PIK3R2 [13, 14]. It has been reported that there is a close relationship between miR-126 and coronary collateral vessel function, and these are considered as circulating biomarkers to identify insufficient or sufficient collateralisation in patients with chronic coronary occlusion [15, 16]. Although miR-126 plays an important role in collateral circulation formation of the coronary artery, the correlation between miR-126 and the CCC has not been investigated.

In this single-centre cross-sectional study, we enrolled patients who underwent cerebral angiography with severe stenosis (≥ 70%) or occlusion in at least one major intracranial artery. We aimed to investigate the correlation between the circulating miR-126 regulation pathway and the CCC, and to test whether miR-126 could serve as a potential biomarker for CCC formation.

Materials and methods

Study design and population

Inclusion criteria of this single-centre cross-sectional study were: (1) age 35–80 years; (2) hospitalised in the neurological department and having undergone cerebral angiography at Baotou Central Hospital; (3) having severe stenosis (≥ 70%) or occlusion in at least one major intracranial artery between December 2017 and July 2018.

Patients with any of the following factors, which were confirmed according to self-report, available medical records, and the judgement of physicians, were excluded: (1) acute cerebral infarction within the previous week; (2) various liver diseases and elevated transaminases (three times the normal level); (3) severe systemic organic disease (dysfunction of heart, lung, liver, kidney or other organs, and where survival time may be less than 12 months in the opinion of experienced physicians); (4) various tumours; (5) refusal to sign an informed consent form.

The CCC was graded using the American Society of Interventional and Therapeutic Neuroradiology/Society of Interventional Radiology (ASITN/SIR) Collateral Flow Grading System, which permitted dichotomisation into 'good' (grades 3–4) and 'poor' (grades 0–2) collateral flow: grade 0 (no collaterals visible at the ischaemic site); grade 1 (slow collaterals visible at the periphery of the ischaemic site with persistence of some of the defect); grade 2 (rapid collaterals at the periphery of the ischaemic site with persistence of some of the defect in only a portion of the ischaemic territory); grade 3 (collaterals with slow but complete angiographic blood flow of the ischaemic bed by the late venous phase); and grade 4 (complete and rapid collateral blood flow to the vascular bed in the entire ischaemic territory by retrograde perfusion).

We categorised ASITN/SIR into one of two classes (either poor or good) rather than the conventional three classes (poor, moderate or good) because of the small study sample and limited statistical power. Patients were divided into two groups according to their ASITN/SIR grade: patients with grade 3–4 (the good CCC group, n = 44), and patients with grade 0–2 (the poor CCC group, n = 24). Written consent was obtained from each subject, and the study protocol was approved by the Ethics Committee of Baotou Central Hospital.

Determination of plasma miR-126

Fasting blood samples (5 mL) were collected from the subjects via direct venous puncture, put into tubes containing sodium citrate, and then centrifuged at 2,000 × g for 10 min. The supernatant (plasma) was then transferred carefully into tubes, which were stored in the refrigerator at -80°C. mRNAs were extracted from the plasma samples using the miRNeasy Plasma Kit (Qiagen). Both poly-(A) tailing and reverse transcription were performed on the samples with the miScript Reverse Transcription Kit (TIANGEN). miR-126 was quantified by quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay, and U6 RNA was used as the miRNA internal control. Each reaction was performed using a miRNA-specific forward primer and a universal reverse primer according to the protocol of the manufacturer (TIANGEN). The primers were miR-126 forward: 5'-CGGGCCATTATTACTTTTG -3'; U6 forward: 5'-CTCGCTTCGGCAGCACA-3'. Each reaction was performed in a total volume of 20 uL: 10.0 µL TaqMan® Fast Advanced Master Mix (2×), 0.5 µL miRNA-F (10 µM), 0.5 µL Universal miRNA-R (10 µM), 0.5 µL Universal Taqman probe (10 µM), 1.0 µL template DNA and nuclease-free H_2O to adjust the volume. The PCR reaction was performed as follows: 50°C for 2.0 min; 95°C for 20 sec, followed by 40 cycles (95°C for 5 sec; 60°C for 25 sec). Relative gene expression levels were analysed using the formula $2^{\Delta CT}$, where ΔCT = CT (target gene) – CT (control), and this was repeated three times for each sample.

Western blot method for Spred-1 and PIK3R2

The whole blood total protein extraction kit (BB-3410, BestBio) was used to extract the total protein of the fasting blood sample. The bicinchoninic acid (BCA) quantitative kit was used to quantify the total protein. Protein concentrations were determined using a BCA protein assay kit (P0010,

Beyotime). Each protein sample (30 μg) was loaded for the assay. Nonspecific bands were blocked in TBS-T containing 5% non-fat milk for 1 h at room temperature. Membranes were subsequently incubated with the primary antibodies of rabbit anti-sprouty-related EVH1 domain containing protein 1 (SPRED-1) polyclonal antibody (1:500; ab64740, Abcam), rabbit anti-phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2) polyclonal antibody (1:1,000; PA5-15268, Thermo Fisher), followed by goat anti-mouse IgG (H+L) secondary antibody (1:5,000; 31160, Thermo Pierce) and goat anti--rabbit IgG (H+L) secondary antibody (1:5,000; 31210, Thermo Pierce) for 1 h at room temperature. The immunocomplexes were visualised by SuperSignal®West Dura Extended Duration Substrate. The film signals were digitally scanned and then quantified using BandScan5.0 software (Bio-Rad, USA). An antibody for transferrin (1:2,000; MAB5746, R&D Systems) was used as an internal control. Data was provided in terms of the mean ± standard deviation of the percentage ratio of the control. Each band was repeated three times, and the relative expression of target protein was expressed as {target protein/ /internal parameters (optical density value)} × 10^n. Results are expressed as mean ± standard deviation.

ELISA assay for plasma VEGF levels

All fasting blood samples (5 mL per patient) were collected via a direct venous puncture, placed into tubes containing sodium citrate, and then centrifuged at 2,000 × g for 10 min. The layer of the supernatant (plasma) was carefully transferred into other tubes and stored at −20°C. We used the Human VEGF ELISA Kit (R&D Systems) to measure the plasma levels of VEGF, following the manufacturer's instructions. Absorbance was measured at 450 nm (primary wavelength).

Other variables of interest

Several clinical characteristics were compared between the groups:

(1) demographics (age and sex) and medical histories, including hypertension (according to self-report, available medical records, taking hypotensive medication, systolic blood pressure ≥ 140 mmHg on a different day, or diastolic blood pressure ≥ 90mmHg on a different day), diabetes (according to self-report, available medical records, taking antidiabetic medication, or fasting blood glucose ≥ 7.0 mmol/L), coronary heart disease (diagnosed by self-report or available medical records), and hyperlipidemia (diagnosed according to in-hospital tests or medical records, meeting at least one of the following criteria: blood cholesterol ≥ 6.2mmol/L; LDL-C ≥ 4.1mmol/L; blood triglyceride ≥ 2.3 mmol/L; or HDL-C < 1.0mmol/L);
(2) BMI (kg/m^2); smoking, defined as regularly smoking (smoking ≥ 1 cigarette/day on average for longer than six months) or occasionally smoking (smoking more than four times a week and but less than one cigarette/day on average); drinking, defined as regular drinking (drinking ≥ 1 time/ /week, drinking ≥ 50 ml alcohol each time, for more than half a year), or occasional drinking (drinking 1-3 times/month, drinking ≥ 50 ml alcohol each time, for more than half a year);
(3) fasting blood sample tests, including cholesterol, triglyceride, LDL-C, HDL-C, glucose, creatinine, and homocysteine;
(4) medication histories, including aspirin, clopidogrel, ACEI/ /ARBs, statins, CCB, and beta-blockers.

Statistical analyses

The continuous variables are expressed as mean ± standard deviation, and the categorical variables are expressed as frequencies. The Kolmogorov–Smirnov test was used to test whether the data were normally distributed. The differences in continuous clinical characteristics between the groups were assessed using the Student t-test or Mann–Whitney U test. Associations between categorical variables were tested by Pearson's $\chi 2$ test. Associations of miR-126, Spred-1, PIK3R2, VEGF-A with collateral grade were assessed using the Spearman correlation test. Associations of miR-126 with VEGF, Spred-1 and PIK3R2 were also analysed with Spearman correlation test because: (1) data on miR-126, Spred-1, and PIK3R2 do not have a normal distribution; (2) to keep consistency with the previous analysis (association of miR-126 and other markers with CCC status).

Receiver operating characteristic (ROC) curves were established to evaluate the predictive power of circulating miR-126 for the CCC status of patients. The area under the curve (AUC) was used to assess the predictive power. We only performed univariate analysis for three reasons: firstly, patients with severe organ dysfunction, liver diseases, tumours and other diseases which might affect circulating biomarkers of interest were excluded from the study; secondly, the baseline characteristics, including demographic factors, medical histories and laboratory tests which might have an influence on miR-126 level were generally comparable between the good CC group and the poor CCC group; and thirdly, the small study sample further restrained our statistical power to perform multivariate analysis. Statistical analyses were conducted using IBM SPSS Statistics 19, and $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics

Of 80 patients examined for eligibility, 10 were excluded (eight with acute cerebral infarction, one with tumour, and one with severe systemic organ disease). Of the 70 patients confirmed eligible, two were excluded due to refusing consent.

So, in total 68 patients were included in this study, 44 with good CCC and 24 with poor CCC. All enrolled patients were found > 70% stenosis or occlusion, via TCD, CTA, or MRA,

Table 1. Clinical characteristics and biochemical parameters of patients

Variables	ASITN/SIR grade		P-value
	Good CCC (n = 44)	Poor CCC (n = 24)	
Clinical characteristics			
Age (y)	59.52 ± 9.45	62.29 ± 8.75	0.240
Male n (%)	35 (79.5%)	18 (75%)	0.666
BMI (kg/m2)	25.39 ± 3.21	25.54 ± 3.24	0.850
Hypertension n (%)	30 (68.2%)	20 (83.3%)	0.176
Diabetes n (%)	11 (25%)	10 (41.7%)	0.155
Coronary heart disease n (%)	10 (22.7%)	4 (16.7%)	0.555
Smoking n (%)	26 (59.1%)	13 (54.2%)	0.695
Drinking n (%)	16 (36.4%)	11 (45.8%)	0.446
Hyperlipemia n (%)	18 (40.9%)	6 (25%)	0.190
Laboratory index			
TC (mmol/l)	4.03 ± 0.93	4.15 ± 1.14	0.635
TG (mmol/l)	1.84 ± 1.11	1.60 ± 0.68	0.691
LDL-C (mmol/l)	2.34 ± 0.71	2.29 ± 1.02	0.836
HDL-C (mmol/l)	0.98 ± 0.21	1.10 ± 0.44	0.273
Glu (mmol/l)	5.98 ± 2.02	6.08 ± 1.77	0.657
Creatinine (μmol/l)	66.23 ± 13.64	60.08 ± 12.92	0.075
Homocysteine(umol/l)	14.56 ± 8.99	16.81 ± 10.33	0.229
Medication history			
Aspirin n (%)	44 (100%)	24 (100%)	NA
Clopidogrel n (%)	38 (86.4%)	24 (100%)	0.083
ACEI/ARBs n (%)	9 (20.5%)	4 (16.7%)	1.000
Statin n (%)	44 (100%)	24 (100%)	NA
CCB n (%)	8 (18.2%)	0 (0%)	0.043
Beta-blocker n (%)	8 (18.2%)	7 (29.2%)	0.364

in at least one major intracranial artery, and were advised to perform digital subtraction angiography (DSA) to verify if interventional therapy was justified. The distribution of cerebral vascular stenosis/occlusion among all patients in both groups are shown in the supplement. Their clinical characteristics, biochemical parameters and medication history, including age, BMI, sex, rates of hypertension, coronary heart disease, diabetes mellitus and hyperlipemia, smoking history, drinking history, fasting glucose, and lipid profiles including LDL, HDL, TG, TC, homocysteine and creatinine, are set out in Table 1. The number of patients who were taking aspirin, clopidogrel, ACEIs/ARBs, statins and beta-blockers did not differ between the two groups. The difference in the calcium antagonist of medication history between the two groups was statistically significant ($p < 0.05$).

Levels of plasma miR-126, VEGF, Spred-1 and PIK3R2 in patients classified by CCC status

The concentrations of plasma VEGF-A in the good CCC group and the poor CCC group were 103.66 ± 8.00 pg/ml and 75.82 ± 15.69 pg/ml, respectively. Compared to the poor CCC group, the plasma level of VEGF was significantly higher in the good CCC group ($p < 0.05$) (Fig. 1A).

Subsequently, we tested the levels of plasma miR-126, Spred-1 and PIK3R2 in patients with intracranial artery occlusion or stenosis of ≥ 70% according to their CCC status. Results showed that the level of plasma miR-126 was 1.81-fold higher in the good CCC group (14.76 ± 11.91 pg/ml) than in the poor CCC group (8.17 ± 4.45 pg/ml, $p < 0.05$) (Fig. 1B). Moreover, the protein level of Spred-1 and PIK3R2 in plasma was lower in the good CCC group than in the poor CCC group (Spred-1:0.23 ± 0.09 pg/ml for good CCC group, 0.83 ± 0.12 pg/ml for poor CCC group; PIK3R2: 0.22 ± 0.15 pg/ml for good CCC group, 0.65 ± 0.23 pg/ml for poor CCC group) (Fig. 2).

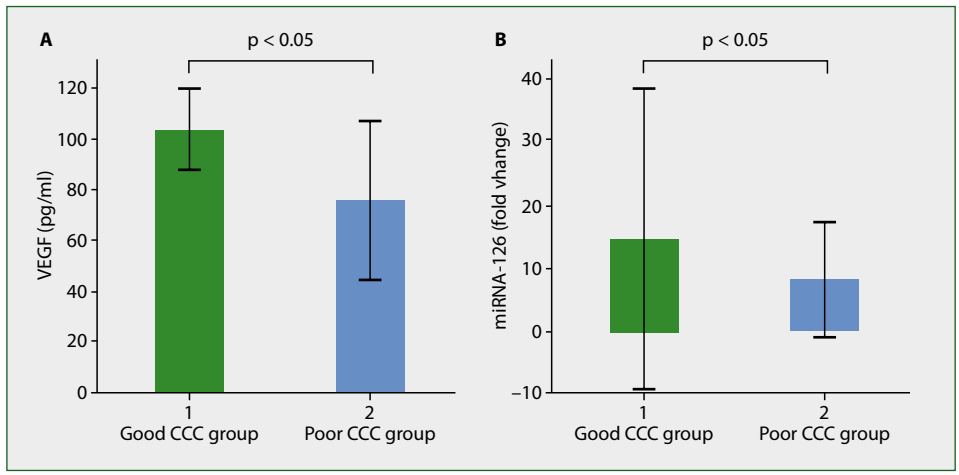

Figure 1. Comparison of plasma miR-126 and VEGF levels between good and poor CCC groups. **A.** Plasma level of VEGF in patients; **B.** Plasma level of miR-126 in patients

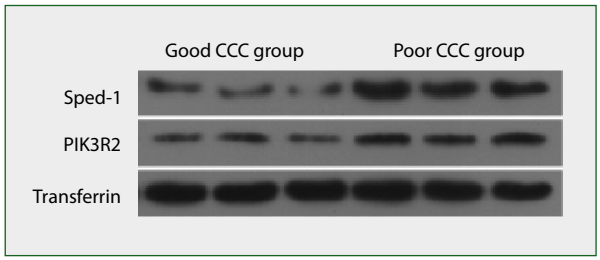

Figure 2. Protein level of Spred-1 and PIK3R2

correlation between miR126 and VEGF and ASITN/SIR score (R = 0.595, p < 0.01). Conversely, ASITN/SIR score was negatively correlated with Spred-1 (R = -0.817, p < 0.01) and PIK3R2 (R = -0.513, p = 0.01) (Fig. 4).

Correlation between miR-126 and VEGF, Spred-1 and PIK3R2

To explore the mechanism of miR-126 in improving collateral circulation, we analysed the correlation

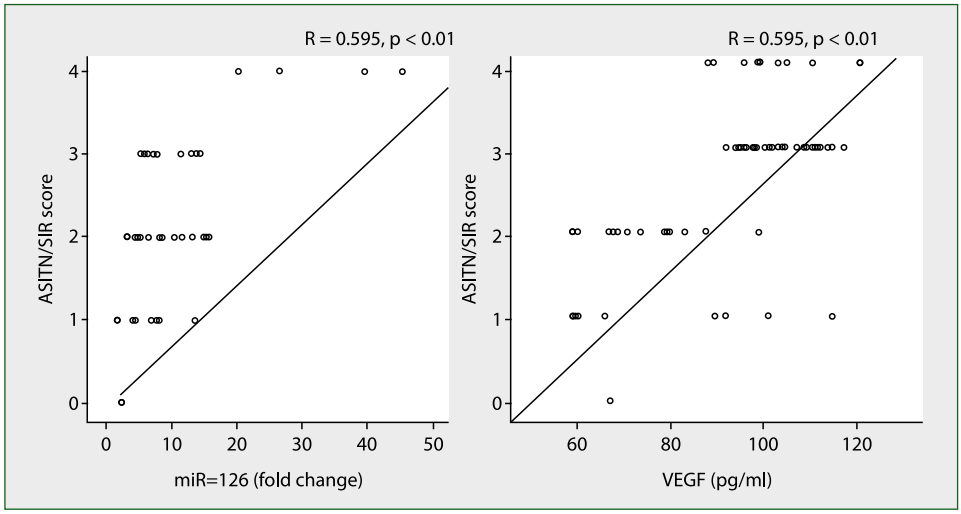

Figure 3. Correlation between miR-126 and VEGF and ASITN/SIR score

Correlation of miR-126, VEGF, Spred-1 and PIK3R2 with ASITN/SIR score

We further investigated the correlation of miR-126 and VEGF with ASITN/SIR score, which was used to evaluate the degree of CCC. As shown in Figure 3, there was a positive

between miR-126 and VEGF, Spred-1 and PIK3R2. There was a positive correlation between miR-126 and VEGF (R = 0.333, p = 0.041) (Fig. 5). However, the correlation between plasma miR-126 and Spred-1 and PIK3R2 was not significant (Fig. 6, 7).

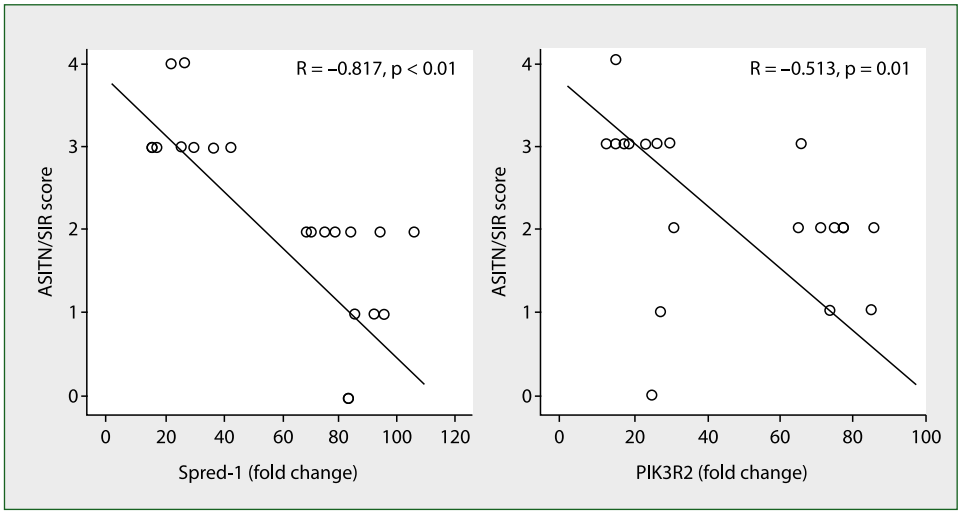

Figure 4. Correlation between Spred-1 and PIK3R2 and ASITN/SIR score

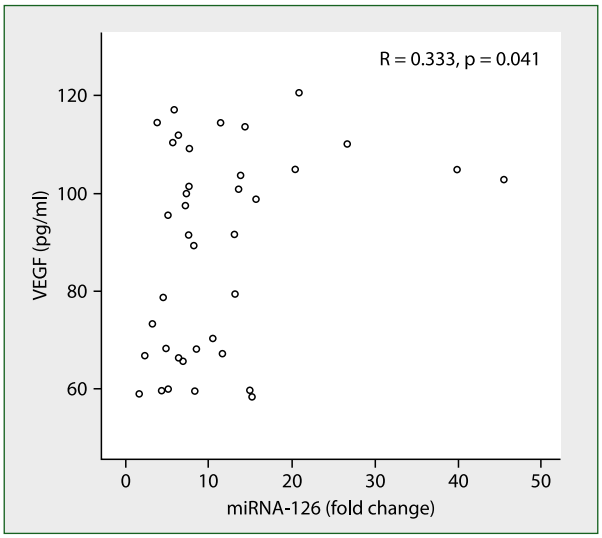

Figure 5. Correlation between plasma miR-126 and VEGF

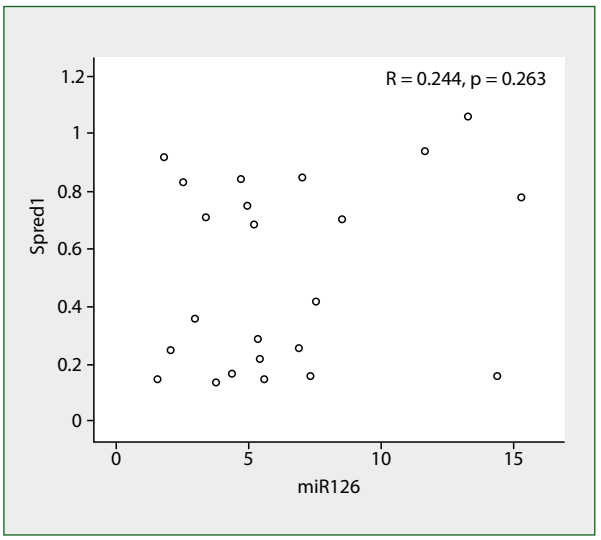

Figure 6. Correlation of plasma miR-126 with Spred1

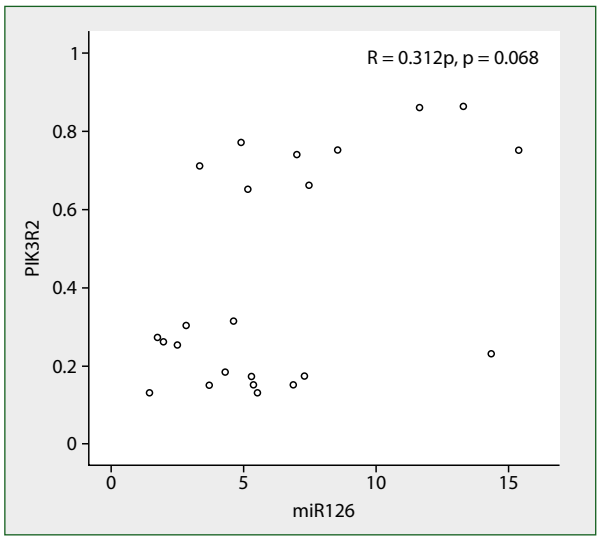

Figure 7. Correlation of plasma miR-126 with PIK3R2

Comparison of predictive power of miR-126 for CCC status

To compare the predictive power of plasma miR-126 for CCC status, we performed ROC analysis for the patients. As shown in Figure 8, the AUC for miR-126 was 0.328 (95% CI: 0.158–0.498; p = 0.067).

Discussion

In this study, we found that plasma miR-126 and VEGF were significantly higher in patients in the good CCC group than in those in the poor CCC group. In contrast, plasma Spred-1 and PIK3R2 levels were lower in patients in the good CCC group. In addition, miR-126 and VEGF were positively correlated with ASITN/SIR, whereas Spred-1 and PIK3R2 were negatively correlated with ASITN/SIR. However, miR-126 might not be an independent predictor for CCC, as the AUC of the marker for CCC status was only 0.328 (95% CI: 0.158–0.498; p = 0.067).

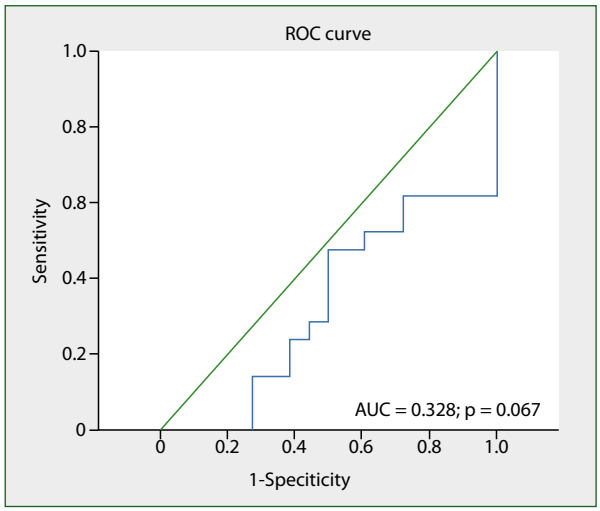

Figure 8. Predictive power of miR-126 for CCC

miR-126, the angiogenesis-related miRNA, is regarded as one of the main regulators of physiological angiogenesis. [13, 17, 18]. Several recent studies have identified that miR-126 promotes VEGF expression by suppressing Spred-1 and PIK3R2 expression [14], which contributes to angiogenesis [19–22]. Nie et al. [23] reported that plasma miR-126 level is positively correlated with CCC formation and is an independent predictor of its development in patients with severely narrowed coronary arteries. To date, it has not been reported whether miR-126 can be used as a biomarker for cerebrovascular collateral circulation. We first found that plasma miR-126 and VEGF were significantly higher in patients in the good CCC group than those in the poor CCC group among patients with at least one major intracranial artery severe stenosis (≥ 70%) or occlusion. Plasma miR-126 and VEGF were positively correlated with ASITN/SIR, whereas Spred-1 and PIK3R2 were negatively correlated with ASITN/SIR. Although our ROC analysis found insignificant AUC value, the abovementioned findings still support our hypothesis that higher miR-126 was associated with better CCC status, possibly mediated by increasing VEGF and reducing Spred-1 and PIK3R2 levels.

Our study has several limitations. Firstly, we did not analyse the effects of other factors on miR-126, for which the reasons are mentioned above (in the Statistical Analysis). It is known that hypoxia can induce miR-126 expression [24]. So, it is possible that more severe stenosis of cerebral arteries promotes better CCC status in the good CCC group, and simultaneously increased the miR-126 level. But the severity of stenosis was generally comparable between the groups in our study (see Supplement). Moreover, it has also been reported that proatherogenic metabolic factors, such as diabetes and hyperlipidemia, might down-regulate miR-126 expression [24].

Therefore, association between miR-126 and collateral flow might be complicated by arterial stenosis and metabolic disorders. The relationship between miR-126 level, metabolic risk factors, cerebral artery stenosis and collateral circulation remains to be elucidated.

Secondly, the small sample size in our study restrained statistical power to fully evaluate association of miR-126 with CCC status. In combination with the single-centre cross-sectional design and male-predominant population (more than 70%), our findings might not be fully generalisable to other studies.

Finally, this is an exploratory study that lacks evidence of repeated comparisons, and therefore further research should be conducted to confirm the results.

Conclusions

Plasma miR-126 level may be related to better CCC formation accompanied by upregulation of VEGF and reduced Spred-1 and PIK3R2 protein expression. However, miR-126 might not be an independent predictor for CCC, given its low predictive value.

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RESEARCH PAPER

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Nusinersen treatment of spinal muscular atrophy type 1 — results of expanded access programme in Poland

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ABSTRACT

Aim of the study. This study aimed to evaluate the effects of nusinersen therapy in Polish children with SMA type 1.

Clinical rationale of study. Spinal muscular atrophy (SMA) is a neuromuscular disorder that is characterised by the loss of motor neurons, progressive muscle weakness and atrophy, leading to increased disability and mortality. Nusinersen, an antisense oligonucleotide that promotes production of the functional survival motor neuron protein is approved for the treatment of SMA 5q in the European Union. In 2017, an early access programme (EAP) for nusinersen was launched in Poland. In this study, we present the results of nusinersen treatment in Polish patients participating in the EAP.

Materials and methods. We collected prospectively clinical data including mutational analysis of *SMN1* and *SMN2* genes, motor function outcomes as measured on a standardized scales, ventilatory and nutritional status, on SMA type 1 patients receiving nusinersen in three EAP centres in Poland. Scores on the CHOP-INTEND scale after 18–26 months of treatment were compared to baseline.

Results. We analysed data from 26 patients with SMA type 1, mean age 4.79 (2–15) years. The mutational analysis revealed two SMN2 gene copies in the majority of patients (61.54%). Three and four copies were found in 34.62% and 3.84%, respectively. Median disease duration was 21 months. Half (n = 13) of the patients required mechanical ventilation at baseline and 57.69% (n = 15) were fed by nasogastric tube or percutaneous endoscopic gastrostomy. No patient worsened during the follow-up. Mean improvement in CHOP-INTEND from baseline to the last follow-up was 7.38 points ($p < 0.001$). CHOP-INTEND scores did not decline for any patient. Patients with three or more *SMN2* gene copies had higher scores than did the patients with two copies ($p = 0.013$), and they tended to show greater improvement over time, but the difference was not significant ($p = 0.324$). Shorter disease duration and higher CHOP-INTEND baseline score were associated with a better response ($p = 0.015$). Patients with a CHOP-INTEND score above the median had higher scores overall than the rest ($p < 0.0013$), and they improved significantly more than the rest ($p = 0.037$). Nusinersen was well tolerated, no new safety findings were identified.

Conclusions and clinical implications. Our data indicates that nusinersen treatment might be effective in SMA type 1 patients, regardless of their age and functional status.

Key words: nusinersen, spinal muscular atrophy, antisense oligonucleotide, expanded access programme

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Introduction

Spinal muscular atrophy (SMA), a rare genetic disease belonging to a group of neuromuscular disorders, is the leading mono genetic cause of mortality in infants. It is characterised by the progressive loss of motoneurons resulting in progressive muscle weakness and atrophy [1–4]. SMA results from a homozygous mutation or deletion in the SMN1 (survival motor neuron) gene located on the long arm of chromosome 5. In affected individuals, the production of functional survival motor neuron (SMN) protein, which is necessary for motor neuron function and survival, is severely impaired [4–7]. Nusinersen, an antisense oligonucleotide has been approved in the United States in the European Union and elsewhere, for the treatment of 5q spinal muscular atrophy. Nusinersen increases the production of the full-length SMN protein by modifying splicing of the pre-mRNA of the SMN2 gene, almost identical to SMN1, located on the same chromosome [4, 8, 9]. Spinal muscular atrophy incidences of 10.3 and 13.5/100,000 live births were recorded in Poland in 2005 and 2015, respectively [10, 11]. According to reports collected from 122 laboratories in 27 European countries, the median incidence was 11.9/100,000 [6.3–26.7/100,000, (1: 3,900–16,000)] of live births in 2011–2015. In 2015, the number of newly diagnosed patients with SMA in Poland was 54, and in total in 2011–2015 it was 240 [11]. Type 1 SMA (Werdnig-Hoffmann Disease) is the most prevalent and accounts for over 50% of all new cases. It is characterised by the occurrence of symptoms up to six months of age, as well as the patient's inability to sit unsupported. Patients usually do not survive beyond the age of two years [12–14]. Alternative classification for type 1 SMA is also recognized and utilizes subtypes 1A-1C. The most severe subtype 1A is associated with symptoms onset at birth or within 2 weeks of infancy, symptoms occurring later on, but before 3 months of age refer to subtype 1B and thereafter, up to 6 months of age to subtype 1C [10–12]. Severe hypotonia as well as paradoxical breathing with a bell-shaped chest, characteristic for type 1, have been observed in studied patients [14]. Motor development is disturbed; patients not only do not acquire extra skills, but also their existing skills worsen by an average of 1.27 points on the CHOP INTEND (Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders) scale per year [15, 16]. Before reimbursement, only a small subgroup of type 1 patients was treated in Poland within an expanded access programme. The reimbursement of nusinersen, granted on 1 January 2019, has provided wide access to treatment, including patients of any SMA type and functional status and those diagnosed presymptomatically. 518 patients were being treated in this drug programme as of 29 February 2020 [17] The aim of this study was to show the impact of nusinersen treatment received in an expanded access programme in type 1 SMA patients in a Polish population.

Methods

Patients and procedures

We conducted prospective, longitudinal collection of data from three centres that participated in the expanded access programme in Poland. Health Ministry approval of the programme was given in December 2016, with the first patients beginning treatment in February 2017. Patients in the EAP had been dosed until March 2019, after which they were moved to the reimbursed treatment programme. According to global EAP inclusion criteria, only patients with type 1 SMA, confirmed by genetic testing (biallelic deletion of SMN1 gene) were approved for the treatment [18]. Additionally, as agreed by sites, the number of SMN2 copies had to be confirmed as well. Lumbar puncture contradictions were considered as a treatment exclusion factor [19]. An interview with parents was conducted before patients were qualified, with an explanation of the regime of applied standard of care, and clear treatment expectations were set. The attending physician or team leader also collected informed consent from the parents or legal guardians. Prior to drug administration, assessments were made of the patient's motor function using the CHOP-INTEND scale, physical state, respiratory, orthopaedic and nutritional status. Laboratory tests, i.e. morphology, arterial blood gas measurement, coagulation, biochemistry, general urine test, general CSF and CSF culture test were also performed. Patients received 12 mg nusinersen via intrathecal administration on days 1, 15, 30, 60 and thereafter every four months. Most procedures were done without general or local anaesthesia. If required, local anaesthesia such as EMLA cream was used. Prior to administration, 3–5 ml of cerebrospinal fluid was withdrawn as recommended. Administration of nusinersen followed after carrying out a CHOP-INTEND 16-item functional measurement, used to evaluate children's motor skills on a 0-64 point scale, plus ventilatory and feeding status assessments. These assessments were performed prior to the first dose, and thereafter before the sixth (month 10) and at each subsequent 4 month dose. Laboratory tests and physical condition assessments were performed at each visit. Patients were kept under observation, as per site guidance, with a minimum of 24 hours required as per any lumbar puncture procedure. The cohort of patients under investigation had treatments initiated at different timepoints, thus the available last follow-up differed with minimum observation prior to the eighth dose up to the tenth dose. All three SMA treatment centres obtained approval from their ethics committee to conduct the study.

Statistical analysis

Data was presented with descriptive statistics. Mixed effects models for repeated measures were used to study the effect of time on the CHOP INTEND score (baseline *vs.* follow-up), with time as a fixed effect and intercept as a random

Table 1. Cohort characteristics

Variable	Statistic
Number of patients	26
Female, n (%)	13 (50)
Age, years	
Mean ± SD	4.79 ± 3.23
Median (IQR)	3.58 (3–5)
Min-Max	2.00–15.00
p for Shapiro-Wilk test*	< 0.001
Age at disease onset, months	
Mean ± SD	2.00 ± 1.72
Median (IQR)	2.00 (1.00–3.00)
Min-Max	0–6.00
p for Shapiro-Wilk test	0.007
Age at diagnosis, months	
Mean ± SD	11.38 ± 25.28
Median (IQR)	6.50 (3.00–8.75)
Min-Max	1–134
p for Shapiro-Wilk test	< 0.001
Age at treatment start, months	
Mean ± SD	36.57 ± 39.25
Median (IQR)	23.00 (12.25–41.25)
Min-Max	3.00–165.00
p for Shapiro-Wilk test	< 0.001
SMN2 copies, n (%)	
2	16 (61.54)
3	9 (34.62)
4	1 (3.84)
SMN subtype	
1A	1 (3.84)
1B	21 (80.76)
1C	4 (15.38)
Body weight	
Mean ± SD	16.27 ± 9.30
Median (IQR)	13.55 (11.00–16.57)
Min-Max	9.50–50.50
p for Shapiro-Wilk test	< 0.001
Body weight < 3 centile, n (%)	9 (34.61)

*when p < 0.05, the distribution of variable differs significantly from normal distribution, thus non-parametric statistics are preferred (like median and IQR rather than mean and SD); IQR – inter-quartile range; SD – standard deviation

effect (Model A). Additionally, the effect of time was analysed depending on the number of SMN2 copies (2 *vs.* 3 or more copies, Model B) and baseline CHOP INTEND score (below median *vs.* above median, Model C). Spearman rank correlation coefficient was used to study the relationship between baseline CHOP INTEND scores and its on-treatment change. P < 0.05 was considered significant. All analyses were completed in the R software (version 3.52).

Results

Baseline characteristics

The mean age of analysed patients was 4.79 (2–15) years. The majority 61.54% (16) had two SMN2 copies, 34.62% (9) had three SMN2 copies, and 3.84% (1) had four SMN2 copies. Mean CHOP-INTEND score at baseline was 19.11 ± 14.28 points. Most, 80.76% (21), patients were subtype 1B, with symptoms onset before age three months, but not immediately after birth that was associated with subtype 1A. Subtype 1C patients experienced an onset of weakness after the neonatal period, but, unlike other subtypes of type 1 SMA, these children acquired good head control. Characteristics of the cohort are summarized in Table 1. Regarding ventilatory status, 13, 50%, patients required tracheostomy and respirator support, five, 19.23%, required non-invasive support, and eight, 30.76%, did not require ventilatory support. Feeding support by NGT (nasogastric tube) or PEG (percutaneous endoscopic gastrostomy) was required in 15, 57.69%, patients. Data on the baseline functioning of patients is captured in Table 2.

Follow-up

According to agreed monitoring timelines, effectiveness for this analysis was focused on data starting from the eight dose as a minimum up to ninth dose, based on the last follow-up visit. Data considers CHOP-INTEND assessment at the 18th up to 26th months of treatment duration, which is one of the longest observation period since nusinersen treatment has started in real world setting. Mean score in CHOP-INTEND at last follow-up was 26.50 ± 18.04, which is a mean improvement of 7.38 points from baseline (p < 0.001; 95% CI, 4.69–10.07). Respirator support with tracheostomy at last follow-up (as a result of an acute lung infection) was required by three more patients versus baseline, while two patients who were previously on 24 hour support had decreased their dependency to only nighttime ventilation or while on acute infection. Follow-up data on functioning are summarized in Table 2.

Patients with three or more SMN2 copies had higher CHOP INTEND scores (15.25, 95% CI: 4.02–26.47, p = 0.013) and tended to improve in functional tests more than the rest of the patients, but this effect was non-significant (2.78, 95% CI: –2.63–8.20, p = 0.324). The effect of time remained significant (p = 0.015), patients with CHOP INTEND baseline score above median had higher scores (p < 0.001) and improved significantly more than the rest of the patients (5.53, 95% CI: 0.62–10.44, p = 0.037). First improvements were observed, starting from the administration of the fifth dose. Some improvements in patient physical status were not captured by the scales, including longer endurance and easier conduction of the exercises reported by leading physiotherapists. Summary of the data is shown in Figure 1 and in Model A-C. None of the patients worsened, one (no. 25) did not change, and the rest improved. Similarly to Model C, patients with higher baseline

Table 2. Baseline and follow-up data on functioning

Variable	Baseline	Follow-up
CHOP INTEND		
Mean ± SD	19.11 ± 14.28	26.50 ± 18.04
Median (IQR)	17.50 (6.00-32.5)	25.50 (12.25-40.50)
Min-Max	0-48.00	1-56.00
p for Shapiro-Wilk test	0.097	0.084
Respiratory support, n (%)		
Noninvasive	5 (19.23)	5 (19.23)
None	8 (30.76)	5 (19.23)
Ventilation > 16 h per day, n (%)	13 (50.00)	11 (42.30)
Tracheostomy, n (%)	13 (50.00)	16 (61.54)
NGT or PEG, n (%)	15 (57.69)	13 (50.00)

Follow-up was led for 18-26 months NGT-Nasogastric tube; PEG-percutaneous endoscopic gastrostomy

Model A

Effect	Estimate	Std. error	95% CI	t	p
Intercept	19.11	3.191	12.76–25.46	5.99	< 0.001
Time	7.38	1.35	4.69–10.07	5.47	< 0.001

Change in CHOP-INTEND score was significant (p < 0.001; an increase of 7.38 points from baseline, 95%CI, 4.69–10.07)

Model B

Effect	Estimate	Std. error	95% CI	t	p
Intercept	13.250	3.574	6.29–20.20	3.708	< 0.001
Time	6.313	1.720	2.94–9.67	3.670	0.001
SMN2 copies (≥ 3)	15.250	5.762	4.02–26.47	2.647	0.013
Time by no. of SMN2 copies interaction	2.787	2.774	-2.63–8.20	1.005	0.324

Effect of time was significant (6.31, 95%CI: 2.94–9.67, p = 0.001); patients with three or more SMN2 copies had higher CHOP-INTEND scores (15.25, 95%CI: 4.02–26.47, p = 0.013) and tended to improve more than rest of patients, but this effect was non-significant (2.79, 95%CI:-2.63–8.20, p = 0.324)

Model C

Effect	Estimate	Std. error	95% CI	t	p
Intercept	6.769	2.313	2.28–11.24	2.927	0.006
Time	4.615	1.777	1.14–8.08	2.598	0.015
Baseline score (above median)	24.692	3.271	18.35–31.02	7.549	< 0.001
Baseline score by time interaction	5.538	2.512	0.62–10.44	2.204	0.037

Effect of time remained significant (p = 0.015); patients with CHOP INTEND baseline score above median had higher scores (p < 0.001) and improved significantly more than rest of patients (5.54, 95%CI: 0.62–10.44, p = 0.037)

CHOP INTEND scores improved more than those with lower baseline scores (rho = 0.41, p = 0.033) as shown in Figure 2.

Safety

Through the study period, we did not observe any adverse events related to nusinersen itself. Drug administration was tolerated well, without any major complications. The adverse events observed in patients were: post-lumbar puncture syndrome in four patients (15.38%), respiratory tract infection in four patients (15.38%), increased liver enzymes after gastrointestinal infection in two patients (7.69%), and unsealed puncture site with temporary CSF leakage in two patients (7.69%). We evaluated the safety profile to be favourable, and this was similar to the latest findings from a broad review of the nusinersen clinical trial programme [20].

Discussion

We conducted a prospective, longitudinal observation of type 1 SMA patients treated within the expanded access programme for nusinersen. We analysed 26 patients over a minimum 18 month follow-up period. We observed improved motor function in 25 patients with stabilisation in one, which, compared to the natural history of the disease and an annual deterioration of minus 1.27 points, can be considered to be a positive treatment outcome [15].

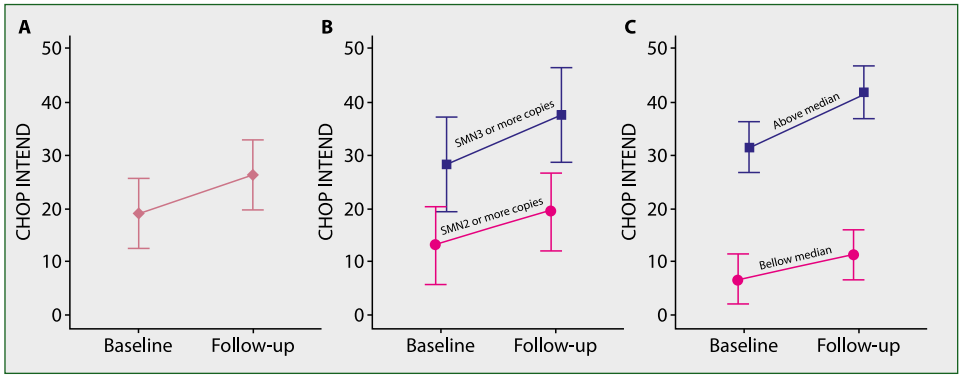

Figure 1. Change in CHOP INTEND score from baseline to follow-up among all patients (**A**) and depending on number of SMN2 copies (**B**) and baseline CHOP INTEND score (**C**). Plots show mean values and 95% confidence intervals from linear mixed models for repeated measures with a random intercept and fixed effects of time (A, B, C), number of SMN2 copies (B), and baseline CHOP INTEND score (C)

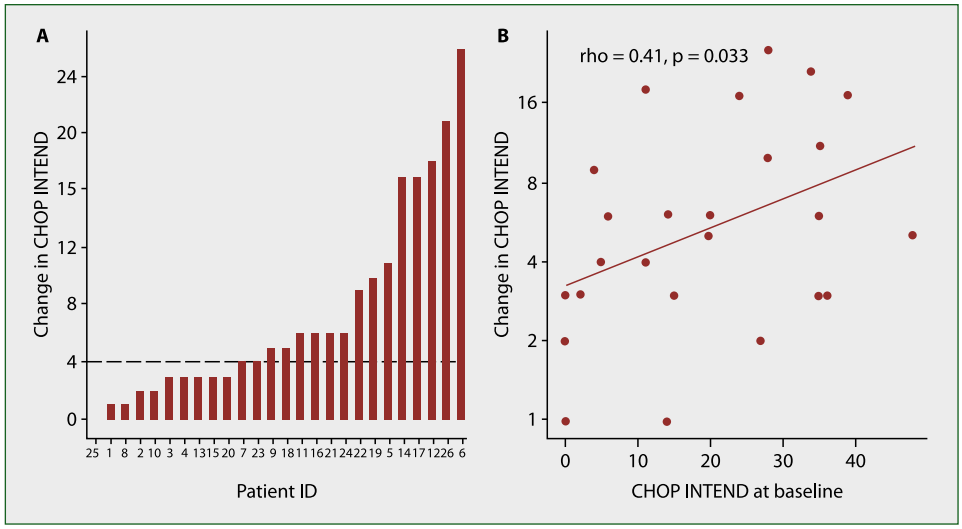

Figure 2. Change in CHOP INTEND score for individual patients (**A**) and correlation between baseline CHOP INTEND scores, with on-treatment change. No patient worsened, one (no. 25) did not change, and the rest improved. Similarly to Model C, patients with higher baseline scores improved more than those with lower baseline scores (rho = 0.41, p = 0.033)

In our cohort, 16 patients (61.54%) improved, measured by the clinically meaningful threshold of 4 or more points in CHOP-INTEND, with an individual change in one patient of up to 26 points. We acknowledge the potential gaps in the nusinersen clinical treatment programme, with very rigid criteria including patient ventilatory status and maximum age at screening [21]. The presented results can to some extent address those gaps. The cohort we analysed had more advanced stage of the disease, with 50% of the patients on tracheostomy and more than 16/24 hours use of a respirator, something which was an exclusion criterion in the phase III randomised, sham-control ENDEAR study [7]. Additionally, patients in our cohort had 21 months mean duration of the disease, whereas patients in ENDEAR at screening had a mean 13.2 weeks. Regarding body weight, nine (34.61%) patients were also below the third percentile for age-adjusted body weight. We acknowledge remaining differences between the studies and limitations of our data related to the small group of patients, and differences in SMN2 copy number, study design or open label approach.

Nevertheless, we believe the results from the analysed cohort, together with the reports published by Pechman et al. and Farrar et al., can support a treatment rationale for type 1 patients, based on real-world data and clinical experience outside of the clinical trial programme [22, 23]. In these two expanded access programme studies, improvement was seen in 77% and 100% respectively. Despite the baseline status of patients, we observed positive treatment effect regarding motor function in all. Our results also showed that patients with higher than median CHOP-INTEND scores at baseline and 3+ SMN2 copies have generally better treatment outcomes, although this did not reach the point of statistical significance. We did not report major changes in patient respiratory status.

Throughout the treatment period, three more patients underwent tracheostomy. At the same time in two patients we had decreased time needed on respirator support to nighttime ventilation and during the acute illness period.

Clinical implications / future directions

Our data shows that none of the analysed patients worsened, one stabilised, and the rest improved, despite the more advanced status of the disease, due to progressed ventilatory or feeding status. We found that patients with higher than median CHOP-INTEND scores at baseline and 3+ SMN2 copies presented better treatment responses.

Therefore, it is very important that patients suffering from spinal muscular atrophy receive treatment in the early stages of the disease. In Poland, this is now possible thanks to a national drug programme that provides treatment for all types of SMA patients. We hope to be able to treat patients with SMA before they develop clinical symptoms, which will be possible thanks to the implementation of newborn screening as standard.

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4C Mortality Score correlates with in-hospital functional outcome after COVID-19-associated ischaemic stroke

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ABSTRACT

Aim of the study. The 4C Mortality Score was created to predict mortality in hospitalised patients with COVID-19 and has to date been evaluated only in respiratory system disorders. The aim of this study was to investigate its application in patients with COVID-19-associated acute ischaemic stroke (AIS).

Clinical rationale for study. COVID-19 is a risk factor for AIS. COVID-19-associated AIS results in higher mortality and worse functional outcome. Predictors of functional outcome in COVID-19-associated AIS are required.

Materials and methods. This was a retrospective observational study of patients with AIS hospitalised in seven neurological wards in Małopolska Voivodship (Poland) between August and December 2020. We gathered data concerning the patients' age, sex, presence of cardiovascular risk factors, type of treatment received, and the presence of stroke-associated infections (including pneumonia, urinary tract infection and infection of unknown source). We calculated 4C Mortality Score at stroke onset, and investigated whether there was a correlation with neurological deficit measured using the National Health Institute Stroke Scale (NIHSS) and functional outcome assessed using the modified Rankin Scale (mRS) at discharge.

Results. The study included 52 patients with COVID-19-associated AIS. The 4C Mortality Score at stroke onset correlated with mRS ($r_s = 0.565$, $p < 0.01$) at discharge. There was also a statistically significant difference in the mean 4C Mortality Score between patients who died and patients who survived the stroke (13.08 ± 2.71 *vs.* 9.85 ± 3.47, $p = 0.04$).

Conclusions and clinical implications. 4C Mortality Score predicts functional outcome at discharge in COVID-19-associated AIS patients.

Key words: acute ischaemic stroke, COVID-19, 4C Mortality Score, modified Rankin Scale

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Introduction

As the COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread across the globe, researchers found growing evidence that several neurological conditions, including stroke, are associated with the disease [1].

A recent systematic review and meta-analysis has shown that acute cerebrovascular disease occurs in about 1.4% of all COVID-19 patients (ranging from 0.4% to 8.1% in different observational cohort studies) [2]. Acute ischaemic stroke (AIS) is the most common cerebrovascular complication of SARS-CoV-2 infection, but cases of COVID-19-associated haemorrhagic stroke and cerebral venous thrombosis have also been described in the literature [3, 4].

SARS-CoV-2 infection is proven to be an independent risk factor for AIS [5]. The suggested mechanisms in which SARS-CoV-2 increases the risk of AIS are hypercoagulation, vasculitis and cardiomyopathy. Various laboratory markers of coagulopathy are found in patients with COVID-19, including elevated D-dimer levels and abnormalities in prothrombin time, platelet count or fibrinogen level; the presence of antiphospholipid antibodies has been detected in some patients, although their impact remains uncertain. Because angiotensin-converting enzyme 2 (ACE2) receptors, through which SARS-CoV2 enters the cells, are also present in the vascular endothelium, the virus can affect them causing lymphocytic endothelitis. Cardiomyopathy can be a direct effect of viral infection or can occur due to concomitant inflammation or hypoxia [6, 7]. Recent studies show that cases of AIS in patients with COVID-19 are more severe at onset [8] and result in higher mortality and worse functional outcome [9].

The 4C Mortality Score is a validated tool for predicting mortality in hospitalised patients with COVID-19 [10], but no studies have been performed thus far to assess its application in patients with COVID-19-associated AIS.

Clinical rationale for study

The aim of this study was to determine whether 4C Mortality Score calculated at the onset of COVID-19-associated AIS could be a predictor of in-hospital death, and whether it correlated with neurological deficit and functional outcome at discharge. As SARS-CoV-2 is highly infectious and spreads quickly across different communities, the coming months may increase the burden of COVID-19-associated ischaemic stroke cases, meaning that there is an urgent need for research on prognostic tools in AIS patients.

Materials and methods

In this retrospective observational study, we analysed the medical documentation of patients diagnosed with stroke who were hospitalised in seven neurological wards in five cities in Małopolska Voivodship (Poland) between 14 August and 16 December 2020.

The study included patients with AIS associated with COVID-19 infection, confirmed by detecting SARS-CoV-2 RNA by reverse transcription polymerase chain reaction (RT-PCR) from a nasopharyngeal swab.

We considered AIS to be associated with COVID-19 in three cases:

1. AIS in a patient with ongoing symptomatic COVID-19 infection confirmed before admission
2. AIS in a patient without symptoms of infection with a positive SARS-CoV-2 test on admission
3. AIS in a patient with a positive SARS-CoV-2 test obtained during hospitalisation in the stroke unit with no potential source of infection on that ward.

The 4C Mortality Score was calculated on admission for each patient. This score ranges from 0 to a possible 21 points and it includes eight parameters: age, gender, number of comorbidities, peripheral oxygen saturation, respiratory rate, level of consciousness (assessed using the Glasgow Coma Scale) and results of laboratory tests: serum urea and C-reactive protein levels [10].

Each patient was followed according to the standard protocol of the Krakow Stroke Data Bank, a single-centre registry of clinical, radiological and genetic data of hospitalised patients with AIS. For the purposes of this study, we analysed the presence of cardiovascular risk factors (Tab. 1) and concomitant stroke-associated infections requiring antibiotic therapy including pneumonia, urinary tract infection and infections of unknown source. We also noted the type of treatment i.e. intravenous thrombolysis (IVT), mechanical thrombectomy (MT), or no reperfusion therapy. We collected data concerning in-hospital mortality, neurological deficit measured in the National Institute of Health Stroke Scale (NIHSS) at discharge,

Table 1. Frequency of cardiovascular risk factors and stroke-associated infections requiring antibiotic therapy in patients with COVID-19-associated AIS

Cardiovascular risk factor	N (%)
Arterial hypertension	43/52 (83%)
Diabetes mellitus	19/52 (37%)
Atrial fibrillation	19/52 (37%)
Coronary artery disease	16/52 (31%)
Overweight/obesity	12/52 (23%)
History of stroke/TIA	9/52 (17%)
Dyslipidemia	9/52 (17%)
History of smoking	9/52 (17%)
Peripheral arterial disease	8/52 (15%)
Carotid artery atherosclerosis	30/40 (75%)
Stroke-associated infections requiring antibiotic therapy	**N (%)**
Pneumonia	17/52 (33%)
Pneumonia + urinary tract infection	4/52 (8%)
Infection of unknown source	9/52 (17%)

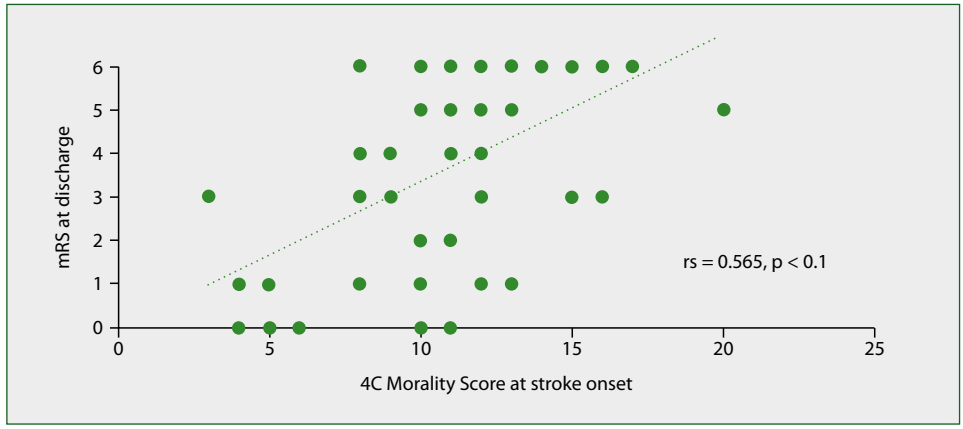

Figure 1. Scatterplot showing results of mRS at discharge in patients with different 4C Mortality Score results at stroke onset with trendline

and functional outcome at discharge assessed with the modified Rankin Scale (mRS).-

The data we collected was put into a database and analysed using a PS Imago Pro 6.0 program. Categorical data was presented as counts and percentages, and continuous data as mean and standard deviation (SD) or median and interquartile range (IQR). Continuous variables were tested for normality using a Shapiro-Wilk test and compared between groups by a Mann-Whitney U test. The correlations between continuous variables were assessed using Spearman's rank-order correlation. A p-value of less than 0.05 (two-sided) was considered to be statistically significant.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of the District Medical Council in Krakow (opinion number 143/KBL/OIL/2020).

Results

We identified 60 patients with COVID-19-associated stroke: 54 (90%) with AIS, five (8%) with haemorrhagic stroke, and one (2%) with cerebral venous thrombosis. Seventeen (31%) patients with COVID-19-associated AIS received reperfusion therapy: 12 (22%) were treated with IVT, two (3.5%) with MT, and three (5.5%) with both methods.

In one patient with AIS there was no follow-up available regarding neurological outcome because they had been transferred to another hospital. In one patient calculation of 4C Mortality Score at stroke onset was impossible because they had been hospitalised in another centre and the documentation data was incomplete. Therefore, the final analysis included 52 patients with COVID-19-associated AIS.

The patients were aged 49 to 97 years with a mean age of 75 (SD = 10.8). 32 of them (61.5%) were male. Forty-six patients (88%) had at least two concomitant cardiovascular risk factors. The most common risk factor was arterial hypertension (N = 43, 83%) (Tab. 1). The presence of carotid artery atherosclerosis could be assessed in 40 patients (in others the diagnostics of stroke causes was performed after discharge from a COVID ward, and they were lost to follow-up), and it was present in 30 (75%) of those 40 patients.

A concomitant infection requiring antibiotic use was present in 30 (58%) patients: pneumonia in 17 patients (33%), both pneumonia and urinary tract infection in four (8%), and nine patients (17%) received antibiotics due to infection of unknown source. Seven (13%) patients were diagnosed with viral pneumonia due to COVID-19 and did not receive antibiotic therapy.

The 4C Mortality Score at the onset of stroke varied from 3 to 20 points with a median of 11 (IQR = 4).

The mortality rate in our group was 23% (N = 12). There was a significant difference in the mean 4C Mortality Score between patients who died and patients who survived the stroke (13.08 ± 2.71 *vs.* 9.85 ± 3.47, p = 0.04).

There was a statistically significant ($p < 0.01$) moderate positive correlation between 4C Mortality Score and the in-hospital functional outcome after stroke assessed with mRS (Spearman's Rank Correlation Coefficient = 0.565). For a scatterplot showing results of mRS at discharge in patients with different 4C Mortality Score results at onset, see Figure 1.

The correlation between 4C Mortality Score and the neurological deficit at discharge measured using the NIHSS scale was also statistically significant (p = 0.038) but weak (Spearman's Rank Correlation Coefficient = 0.329).

Discussion

Our study is the first to assess the significance of 4C Mortality Score in patients with COVID-19-related AIS. The score was created to predict mortality in hospitalised patients with COVID-19 [10] and further studies suggest that it could be applied to other respiratory system infections [12]. Our study shows that in the specific group of patients with COVID-19-associated AIS, who are in danger of not only death but also lifelong disability, 4C Mortality Score at onset could be a predictor of functional outcome after stroke. What's

more, patients who died of COVID-19-associated AIS had a statistically higher 4C Mortality Score at onset than those who survived.

A case definition of COVID-19-associated stroke was recently proposed [13]. All of the patients included in this study fulfilled both major criteria of this definition i.e. clinical and neuroradiological evidence of acute stroke and SARS-CoV-2 detection by PCR testing. Twelve patients (23%) fulfilled two minor criteria (allowing us to diagnose probable COVID-19-associated stroke) and 29 (56%) fulfilled one minor criterion (allowing us to diagnose possible COVID-19-associated stroke). However, a full assessment of minor criteria was in some cases impossible because the levels of D-dimers and lactate dehydrogenase were not routinely assessed in some hospitals and information concerning mild infection symptoms preceding the stroke could be missing from the source medical documentation.

Moreover, the minor criteria do not cover those patients who were asymptomatic during onset of the stroke (but tested positive for COVID-19 at admission) or those who tested positive a few days after developing stroke symptoms while being hospitalised (we included those patients in the study if there was no proof of an in-hospital epidemiological outbreak, assuming that the stroke may have occurred during the 'window period' for COVID-19) [14]. We combined AIS patients with symptomatic and asymptomatic SARS-CoV-2 infection patients in a unified study group to reflect the real-life clinical diversity of COVID-19-associated AIS.

Our study has some important limitations. Firstly, it was of retrospective character and our observations need to be confirmed by prospective studies in larger cohorts of patients. Secondly, we did not analyse the impact of other factors (such as the type of reperfusion therapy received or the physical rehabilitation of the patient). Thirdly, the assessment of Glasgow Coma Scale (GCS) may be flawed in patients with aphasia, thus modifying the result of 4C Mortality Score. However, as the Score does not require a specific result of GCS, but only information if the score was 15 points or less, in patients with aphasia we assessed only the quantitative disturbances of consciousness, whereas in patients without aphasia we could fully assess the GCS score. Fourthly, in some cases it is possible that some of the 4C Mortality Score components (such as low GCS score) were positive due to stroke, rather than due to the infection itself, thus impacting upon our results.

Clinical implications/future directions

4C Mortality Score predicts functional outcome at discharge in COVID-19-associated AIS patients, making it potentially a promising prognostic tool. However, further prospective studies are needed to confirm our observations.

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RESEARCH PAPER

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Movement disorders associated with chromosomal aberrations diagnosed in adult patients

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ABSTRACT

Introduction. Chromosomal aberrations are rare but important causes of various movement disorders. In cases of movement disorders associated with dysmorphic features, multiorgan involvement and/or intellectual disability, the identification of causative chromosomal aberrations should be considered.

Aim of the study. The purpose of this article was to summarise clinical findings in six patients with dystonia and two with parkinsonism and identified chromosomal aberrations in a single-centre prospective study.

Materials and methods. 15 adult patients with dystonia or parkinsonism were referred to array comparative genomic hybridisation (aCGH) testing from our Department of Neurology between 2014 and 2019. Additionally, one patient had a karyotype examination. Detailed clinical, psychological and radiological diagnostics were performed in each case.

Results. Chromosomal aberrations were identified in six patients with dystonia and two with parkinsonism. Two patients were identified with aberrations associated with de Grouchy syndrome. We also reported generalised dystonia in patients with deletion in 3q26.31 and duplication in 3p26.3, as well as dystonia and hypoacusis in a patient with duplication in Xq26.3. One patient was diagnosed with duplication in 21q21.1. Early-onset parkinsonism was a manifestation of deletion in the 2q24.1 region. Late onset parkinsonism was also present in the patient with the most severe aberrations (duplication 1q21.1q44; deletion 10p15.3p15.1; deletion 10q11.21).

Conclusions. Dystonia and parkinsonism are possible manifestations of chromosomal aberrations. Chromosomal aberrations should be excluded in patients with early-onset movement disorders and concomitant dysmorphic features and/or intellectual disability. It is important to include this cause of movement disorders in future classifications. aCGH can be a valuable diagnostic tool in the evaluation of movement disorder aetiology.

Key words: dystonia, Parkinson's disease, chromosomal aberrations, microarray, parkinsonism

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Introduction

Dystonia and parkinsonism are among the most commonly diagnosed movement disorders. There is, however, no specific mention of chromosomal aberrations as a cause of dystonia in the IPDMS dystonia classifications [1, 2]. In parkinsonism, a genetic background is usually observed in those patients with disease onset before the age of 50 or with a positive family history [3, 4].

Structural aberrations are caused by a rearrangement of the genetic material. They can be associated with a very broad clinical spectrum, from asymptomatic or benign to lethal. The study by Dale et al. suggested that up to 28% of children with movement disorders of suspected genetic aetiology have chromosomal abnormalities [5].

Chromosomal aberrations in selected movement disorder patients are important to identify. They cause multiorgan

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involvement, and extended diagnostic measures need to be undertaken in order to exclude cardiac and endocrine system abnormalities. Assessment of chromosomal imbalances may be performed using standard karyotyping or molecular diagnostics with array comparative genomic hybridisation (aCGH).

aCGH is based on identifying differences in fluorescence signal intensity between the patient and reference DNA samples hybridised to the array. Its main limitation is an inability to detect balanced aberrations. It has a higher resolution of ~100 kbp *vs.* 5 Mbp for karyotype examination.

An American expert consensus statement from 2010 identified aCGH as a first-line diagnostic tool for the postnatal testing of patients with autism-spectrum disorder, intellectual disability, and multiple congenital anomalies [6]. aCGH is often performed in children with developmental delays, epilepsy or multiorgan manifestations, but rarely in adult patients with movement disorders. Some reports have suggested that it may be of value, particularly when atypical features appear in patients with otherwise acknowledged syndromes. This includes the paper by Lohmann et al. on the identification of causes of dopa-responsive dystonia and eye and skeletal abnormalities in a large family, in which aCGH examination revealed deletion on chromosome 6, including the *CGH1* gene [7]. Similarly, a case of brain-lung-thyroid syndrome was described in a girl with additional immunodeficiency. Negative PCR and Sanger sequencing led to an aCGH examination, where the deletion of 3.32 Mbp in the chromosome 14q13.2-q21.1 region was detected, including genes *NKX2-1* but also genes involved in immunological response [8].

A few other studies have reported chromosomal aberration as a potential cause of early-onset PD. An aCGH study in patients with early-onset levodopa-responsive parkinsonism and intellectual disability revealed partial trisomy in 4q [9]. The best-characterised chromosomal aberration associated with PD is 22q11 deletion. The study by Mok et al. revealed eight carriers from a total cohort of 9,387 PD patients confirmed by aCGH examination [10]. In a recent publication, an aCGH examination revealed cases with duplication of the *SNCA* gene and heterozygous intragenic deletion of the *GBA* gene in 99 PD subjects with a positive family history [11].

In our paper, we describe a cohort of adult patients with neurodevelopmental disorders and concomitant dystonia or parkinsonism and chromosomal aberrations.

Materials and methods

We prospectively identified and examined patients hospitalised in the Department of Neurology between 2006 and 2019 in whom dystonia or parkinsonism caused by karyotype abnormalities were suspected. Early onset of neurological symptoms, the progressive nature of symptoms, neurodevelopmental delay or negative metabolic investigations led to aCGH or karyotype examinations. Fifteen adult patients with dystonia or parkinsonism were referred to cytogenetic testing from our Department of Neurology between 2014 and 2019. In 7/15 patients, aCHG testing indicated pathogenic aberrations or variants of uncertain significance. We also included in our study one patient with significant karyotype findings from an examination performed in 2006. Each patient with chromosomal aberration underwent comprehensive clinical, psychological and radiological evaluations. The Polish version of MDS-UPDRS was performed in parkinsonian patients [12].

aCGH was performed using a 60 K oligonucleotide microarray (CytoSure, ISCA v3 Oxford Gene Technology, Oxford, UK). The patient's DNA was hybridised against the control DNA. Labelling and hybridisation were performed following the manufacturer's protocols. 500 ng of DNA was labelled and purified on the column's centrifugal filters. After probe denaturation and prehybridisation with Cot-1 DNA, hybridisation was performed at 65°C with rotation for 20 hrs. After washing, the array was analysed with Agilent scanner and Feature Extraction software (Agilent Technologies, Santa Clara, CA, USA) and text file outputs from the quantisation analysis were imported to CytoSure Interpret software (Oxford Gene Technology) for copy number analysis.

This study was approved by the Ethics Committee of the Medical University of Warsaw (KB/56/2018) and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants signed an informed consent form for genetic examination prior to their inclusion in the study.

Results

Patients with CNV and karyotype aberrations were analysed in detail. Family history of dystonia or parkinsonism was negative in all cases. All the dystonia patients had been born from uneventful pregnancies and 5/6 received 10-point Apgar scores. In all patients, the onset of dystonia was in childhood. The pattern of the progression of dystonia was highly variable but in most (5/6) cases it led to generalised dystonia. The key clinical features we identified in our cohort, apart from dystonia, were dysmorphic features of the face, which were present in five out of six patients (Patients 1–5), hearing impairment in two (Patients 4 and 6), and epilepsy in one (Patient 2). The pattern of dystonia involvement varied greatly among patients, from focal foot dystonia to severe generalised dystonia affecting speech and walking. Patients from the PD group had later manifestation of movement disorder than did those in the dystonia group, developing their first parkinsonian symptoms when aged 37 (Patient 7) and 62 (Patient 8). Both had good responses to levodopa treatment without motor fluctuations and dyskinesia. The clinical characteristics of all patients are set out in Table 1.

Detailed psychological assessment revealed intellectual disability in most of the patients, regardless of the type of movement disorder. Intellectual disability varied from mild to severe.

Table 1. The clinical characteristics of patients

Dystonia patients									
Aberration (size)	**Gene**	**Significance of variant**	**Gender F/M (age at examination in years)**	**Age at dystonia onset**	**Localisation of dystonia (chronological order)**	**Other neurological features**	**Phenotypic features**	**Additional testing**	**Familial testing**
46,XX,der(18)t(7;18) (p22.3;q11.2) mat (Karyotype examination)		Pathogenic	F (18)	18 yrs	1. Blepharospasm 2. Lower limbs 3. Oromandibular dystonia	Intellectual disability, nystagmus, strabismus of right eye	Short stature, facial dysmorphic features, short fingers and toes	N/A	Balanced translocation between chromosomes 7 and 18 in mother and grandmother
arr[GRCh37] 18q21.2q21.32 (49051715_56330398)x1 (7.28 Mbp)		Pathogenic	F (33)	Childhood	Left foot	Profound intellectual disability, epilepsy, self-harming	Facial dysmorphic features, short stature, atresia of gastrointestinal tract corrected in infancy	Hypoplasia of truncus and splenium of corpus callosum in MRI	Parents unavailable for testing
arr[GRCh37] 3p26.3 (274156_1038528)x3 pat (764 Kbp)	Exons 2–28 of *CHL1* gene	VOUS	F (27)	8 yrs	1. Lower limbs 2. Upper limbs	Mild intellectual disability, dysphagia, drooling	Facial dysmorphic features	Excluded *TOR1A*, *THAP1* mutation	Father an asymptomatic carrier of mutation
arr[GRCh37] 21q21.1 (20233081_20872960)x3 dn (640 Kbp)	-	Likely benign	F (24)	6 weeks	1. Oculogyric crisis 2. Lower limbs 3. Upper limbs and neck	Myoclonus, profound intellectual disability, autoaggression, absence of speech, sensorineural hypoacusis	Short stature, facial dysmorphic features	Excluded *SLC2A1* mutation exonic rearrangements in *GCH1* and *TH* excluded	*De novo* mutation, excluded in parents
arr[GRCh37] 3q26.31 (175105886_175719996)x1 (614 Kbp)	*NAALADL2* gene	VOUS	M (24)	5 months	1. Right upper limb 2. Left upper limb and lower limbs 3. Neck	Mild intellectual disability, spastic tetraparesis, anarthria	Facial dysmorphic features	Excluded *TOR1A* mutation	Parents unavailable for testing
arr[GRCh37] Xq26.3 (134674451_134831619)x3 (157 Kbp)	Exons 3–17 of *INTS6L* gene	Likely benign	F (70)	Childhood	1. Neck 2. Trunk 3. Upper and lower limbs	Hypoacusis, mild intellectual disability	Mild facial dysmorphic features	Cortical and subcortical atrophy (Global cortical atrophy scale-1) in MRI	Parents dead
Chromosomal aberration (size)	**Genes associated with parkinsonism**	**Significance of variant**	**Gender (F/M)**	**Age at parkinsonism onset**	**First symptoms of parkinsonism**	**Other neurological features**	**Phenotypic features**	**Additional examinations**	**Familial testing**
arr[GRCh37] 2q24.1 (156857370_158312882)x1, (1.4 Mbp)	*GPD2* and *NR4A2*	Pathogenic	M (39)	37 yrs	Left-side bradykinesia	Anxiety, mild intellectual disability	Normal phenotype	Substantia nigra hyperechogenicity in transcranial sonography	Parents unavailable for testing
arr[GRCh37] 1q21.1q44 (144289645_249203359)x3 (104.91 Mbp)	*GBA*	Pathogenic	F (67)	63 yrs	Left-side bradykinesia and rest tremor	Intellectual disability	brachydactyly, hand deformity (lack of two fingers), thyroid nodular goitre, polycythemia vera	*PRKN* mutation excluded, exonic rearrangements in *PRKN*, *PINK1*, *DJ1*, *SNCA* excluded, agenesis of corpus callosum, bilateral nodular paraventricular grey matter heterotopy, bilateral polymicrogyria in frontoparietal lobes in MRI	Parents dead
arr[GRCh37] 10p15.3p15.1 (136145_4459452)x1 (4.32 Mbp)		Pathogenic							
arr[GRCh37] 10q11.21 (42651599_46091287)x1 (3.44 Mbp)		VOUS							

All patients underwent magnetic resonance imaging (MRI) studies. Some patients underwent other genetic testing prior to aCGH. In three cases, family members also underwent aCGH. In other cases, the parents were lost to follow-up. Outcomes of these examinations are summarised in Table 1.

Genetic imbalance previously associated with dystonia was found in two patients (Patients 1 and 2). Patient 7 was diagnosed with deletion involving *GPD2* and *NR4A2* genes, previously discussed in the pathology of PD.

Genetic findings in Patients 3, 4, 5, 6 and 8 were variants of uncertain clinical significance. In Patient 3, aCGH examination revealed duplication involving exons 2–28 of the *CHL1* gene. In Patient 4, duplication in the long arm of chromosome 21 did not include any protein-coding genes. Patient 5 was diagnosed with a deletion involving part of the *NAALADL2* gene. He was affected by severe dystonia and intellectual disability. Duplication of exons 3–17 of the *INTS6L* gene was identified in Patient 6, who was suffering from dystonia, hearing impairment and intellectual disability. Patient 8 had the most severe aberrations. All the findings in karyotype and aCGH examinations are summarised in Table 1.

The remaining patients with dystonia or parkinsonism who underwent aCGH in our Department had normal results.

Discussion

Our study indicates that patients with movement disorders and genetic imbalance are a very heterogeneous group in terms of clinical manifestation.

We present eight patients with complex neurological symptoms, including early-onset dystonia or parkinsonism. Vast differential diagnosis was performed in each case. Another important feature present in the majority of patients was intellectual disability of varying degree. In some cases, chromosomal aberrations included genes previously associated with dystonia or parkinsonism which may have effects similar to monogenic disorders. The co-occurrence of the symptoms of movement disorders, intellectual disability and dysmorphic features, after exclusion of common genetic causes, led to testing for chromosomal abnormalities.

Two patients in our cohort (Patients 1 and 2) were diagnosed with De Grouchy syndrome (deletion in the 18p or 18q region). 18p deletion (historically known as De Grouchy syndrome type 1) is a recognised cause of dystonia as well as other movement disorders, including tics, myoclonus and ataxia. The incidence is estimated to be about 1:50,000 live-born infants [13]. The syndrome is characterised by short stature, developmental delay and dysmorphic features — all of which were present in our patients. It is considered that epilepsy appears more often in cases of the 18q syndrome than the 18p syndrome, which is consistent with our observations. Dystonia is much less frequent in the 18q syndrome, with only anecdotal reports [14].

An absence of the *GNAL* gene may contribute to dystonia in patients with the 18p deletion syndrome. However, the frequency of dystonia in 18p deletion patients involving *GNAL* has been estimated at only 3% [15].

The other patients in our group (Patients 3 and 4) were diagnosed with chromosomal aberrations in which no linkage to dystonia or parkinsonism has been made so far. The significance of these findings is therefore uncertain but, in the absence of other causes, possible. This is also supported by the presence of the additional features of hearing impairment, dysmorphic facial features and intellectual disability, strongly suggesting the clinical importance of the detected aberrations.

Patient 3 was diagnosed with a microduplication of the *CHL1* gene. This aberration has already been reported in the literature in patients with neurodevelopmental delay, learning and language difficulties, and seizures. Mild dysmorphic features and autism spectrum disorder were also described. To the best of our knowledge, neither dystonia nor other movement disorders have been described among patients with *CHL1* duplication. Interestingly, Patient 3's father was an asymptomatic carrier of the mutation.

In one patient (Patient 4) pronounced myoclonus, hearing deficits, dysmorphic facial features, intellectual disability and psychiatric symptoms were also observed. Duplication in the long arm of chromosome 21 did not encompass regions with known genes, therefore this CNV is most likely benign. We can however speculate, as several long non-coding RNA are encoded in that region, that these could play a role in disease mechanisms [16].

Patient 5 was diagnosed with partial deletion in the long arm of chromosome 3, which included the *NAALADL2* gene. A breakpoint of *de novo* balanced translocation has been mapped to that gene in a Cornelia de Lange (CdL) syndrome patient. However, further sequencing and screening of the gene did not reveal any pathogenic variants in a cohort of patients with CdL syndrome [17]. Our patient had dysmorphic craniofacial features which were inconsistent with the CdL phenotype (steeple skull, high-arched palate), intellectual disability, and speech problems. There was no involvement of other organs.

Patient 6 (a female) had a duplication on chromosome X. The duplicated region is not located in a pseudoautosomal region, and therefore this raises a question as to whether X chromosome inactivation could have an effect on clinical expression. In some cases involving larger duplications on the X chromosome, a non-random, skewed preferential inactivation of the aberrant X chromosome has been observed. However, several cases of smaller duplications have been reported where the normal X chromosome was preferentially inactivated, leading to severe phenotype [18]. The X chromosome inactivation process may lead to a mosaic distribution of the active region, harbouring the duplication within the body, organs and tissues. The X chromosome inactivation status of the specific brain areas should be considered. A brain tissue sample was not available for inactivation testing. Madrigal et al.

described two patients with partial duplications involving the long arm of the X chromosome and intellectual disability, short stature, microcephaly and hypopituitarism among others [19].

The deletion identified in Patient 7 contains two protein-coding genes: *NR4A2* and *GPD2*. *GPD2* encoding mitochondrial glycerophosphate dehydrogenase has not been unambiguously linked to any movement or neurodevelopmental disorder. Deletions encompassing *NR4A2* alone, or with *GPD2,* have been found in a few patients with intellectual disability, epilepsy, language impairment and/or dysmorphic features. Recently, two patients have been reported with loss-of-function variants in *NR4A2* and mild intellectual disability with dystonia-parkinsonism in early adulthood [20]. On the molecular level, the haploinsufficiency caused by deletion in our patient should result in a dosage effect similar to the frameshift mutations in the two patients described recently [20]. This case further strengthens the hypothesis that parkinsonism is a part of the clinical picture associated with NR4A2 dysfunction.

Patient 8 had the most severe aberrations, including many genes which could be responsible for parkinsonism. The patient had a 104 Mbp duplication on chromosome 1 containing more than 1,000 genes, a 34-gene containing deletion encompassing 4.32 Mbp of 10p, and a 3.44 Mbp deletion on 10q containing 46 genes. The patient's aberration on chromosome 1 resulted in a duplication of the *GBA* gene region. Heterozygous point mutations are a known parkinsonism risk factor, and homozygotes present with the more severe Gaucher disease. Some clinical features such as severe depression and dementia are consistent with typical GBA-PD patients. However, full *GBA* gene deletions/duplications have not been described in PD. Copy number variants in the *GBA* gene have been identified in five parkinsonian patients, although none of them had a full gene duplication [21].

Conclusions

Chromosomal aberrations may be an under-recognised cause of movement disorders. When dystonia or parkinsonism is the dominant clinical manifestation, karyotype examination may not be considered early. In cases when other features dominate, including neurodevelopmental problems, autism spectrum disorder, epilepsy or dysmorphic features, aCGH examination is prioritised. It is nevertheless important to keep in mind that karyotype studies may serve as an important first-line diagnostic tool in patients with early-onset movement disorders. In selected cases, such patients may require an examination of the cardiac system, a hearing examination and a gastrological assessment to exclude possible comorbidities caused by chromosomal aberrations.

We have identified some rare aberrations that have not been previously associated with parkinsonism or dystonia. They represent variants of uncertain clinical significance with possible association with the disorder. The lack of functional studies and whole-exome sequencing in patients is a limitation of our study. However, in all cases the general clinical picture and neurological symptoms, as well as an absence of the variant in some patient's parents and gene content, suggest their importance. In 4/8 patients, aberrations contained genes in which mutations have been previously associated with dystonia or parkinsonism. Further studies are needed in the remaining cases to investigate whether they play a pathogenic role in the disease process.

Chromosomal abnormalities should be mentioned along with monogenic autosomal dominant and recessive causes of common movement disorders. Chromosomal aberrations may produce the same effect on gene expression and result in clinical phenotypes of monogenic disorders. It is known that small deletions and duplications (1 kbp to 10 Mbp) constitute up to 15% of all mutations underlying human monogenic diseases [22].

In this burgeoning era of precision medicine and vector-based therapies for the treatment of PD and dystonia, it is increasingly important to identify disease backgrounds [23].

Therefore, we recommend the introduction of an aCGH examination as an important and cost-effective diagnostic tool, especially in cases of early-onset dystonia and selected cases of parkinsonism with intellectual disability in adult patients.

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miRNA-16 as a predictive factor for intracranial aneurysms in autosomal dominant polycystic kidney disease

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ABSTRACT

Introduction. Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic renal disorder. It leads to multiple extra-renal complications, with intracranial aneurysms (IA) among the most serious. Biological markers could become tools in identifying patients at risk of an IA. MicroRNAs 16 (miR-16) and 25 (miR-25) have been proposed as being markers of IAs in the general population. In the current study, we attempted to discover if they may also be considered markers of IAs in ADPKD.

Material and methods. 64 renal transplant recipients with ADPKD were included. After magnetic resonance angiography of the brain, they were divided into a case group (IA+, n = 13) and a control group (IA-, n = 51). Expression of miRNAs in plasma was analysed by qRT-PCR.

Results. The expression of miR-16 was higher in the control (IA-) group. There was no statistically significant difference between the groups in terms of miR-25 expression.

Conclusions and clinical implications. MicroRNA-16 is a potential marker of IAs in renal transplant recipients with ADPKD. It may become a tool to identify patients who should undergo screening for an IA.

Key words: autosomal dominant polycystic kidney disease, biomarkers, intracranial aneurysm, kidney transplantation, micro RNA

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic renal disorder. Apart from end-stage kidney disease (ESKD) in a large proportion of patients, it leads to multiple extra-renal complications; subarachnoid haemorrhages due to the rupture of an intracranial aneurysm (IA) are among the most serious [1]. In fact, the prevalence of intracranial aneurysms in ADPKD patients is approximately five times greater than in the general population, amounting to 10-11.5% [2, 3], and may be further increased in special subpopulations e.g. renal transplant recipients with ADPKD [4]. Despite the fact that universal screening for IAs in ADPKD populations has been proposed [5, 6], it is still not widely accepted [1, 7]. Therefore, there is a need to improve the selection of patients who should undergo screening to enable the detection of the maximum number of IAs in the pre-symptomatic period. Biological markers could become tools to identify patients at risk of an IA.

MicroRNAs (miRNAs) are short molecules of RNA (18–25 nucleotides in length). They are involved in post-transcriptional regulation of gene expression. They are present in plasma, and are relatively resistant to enzymatic degradation. Due to the unique patterns of change in different diseases, miRNAs are considered to be disease-specific biomarkers. MicroRNAs 16 (miR-16) and 25 (miR-25) have been proposed as being markers of IAs in the general population [8]. In the current study, we attempted to discover if they may also be considered markers of IAs in ADPKD.

Material and methods

Our work was a sub-study of a cross-sectional study on the prevalence of IAs in renal transplant recipients with ADPKD;

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its design and results have already been reported [9]. Participants in that study who consented to also participate in this sub-study were included. Participants were renal transplant recipients with ADPKD managed at the outpatient department of the Department of Immunology, Transplant Medicine and Internal Diseases of the Medical University of Warsaw, Poland. The inclusion criteria were: a diagnosis of ADPKD, being a renal transplant recipient, age 18-plus, lack of contraindications for magnetic resonance imaging, and written informed consent. Sixty-four patients met the inclusion criteria and were enrolled. In all patients, a 3D time-of-flight magnetic resonance angiography (MRA) of the brain was performed. All imaging studies were performed between January 2015 and November 2019 using an Ingenia 1.5T HP (Philips Healthcare, Best, the Netherlands) scanner, and in total 20 ml of blood was collected for further examination. Peripheral venous blood was collected into EDTA-containing tubes. Whole blood was first centrifuged at 1,600*g* for 10 minutes, and then the supernatant was transferred into a fresh tube and centrifuged again at 13,000*g* for 10 minutes. The clear plasma was aliquoted and stored at -80°C until use. All procedures were carried out on ice.

Then, according to the design of a case-control study, the patients were divided into two groups according to the presence or absence of IA.

Expression of miRNA-16 and miRNA-25 was analysed by qRT-PCR. A column method miRNeasy Serum/Plasma advanced kit (Quiagen) was used for RNA isolation from previously frozen plasma samples according to the manufacturer's instructions. RNA quality and quantity were determined spectrophotometrically with a NanoDrop ND-2000 (NanoDrop Technologies, Inc.). A Quiagen Spike-In control C.elegans miR39 miRNA mimic was used as an internal control for miRNA expression. Specific TaqMan Gene Expression assays were purchased from Applied Biosystems. A TaqMan MicroRNA reverse transcription kit and Universal Master Mix II (Applied Biosystems) were used for RNA reverse transcription and real-time PCR amplification. RT-PCR was performed on an ABI Prism 7500 Sequence Detection System (Applied Biosystems) using specific TaqMan primers and probes (Applied Biosystems): hsa-miR-16 (UAGCAGCACGUAAAUAUUGGCG) and hsa-miR-25 (AGGCGGAGACUGGGCAAUUG). U6 was used as a housekeeping gene. Each sample was analysed in triplicate. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The results were presented as a fold change of gene expression in patients with an intracranial aneurysm and in those without, where the reference point was expression in the non-aneurysmal group.

Normality of data distribution was measured with a Kolmogorov-Smirnov test. Statistical analysis was performed by comparing ΔCt values using nonparametric tests for independent samples (Mann-Whitney U test). All analyses were performed with Statistica software version 12.5 (StatSoft, Tulsa, OK, USA). A *P* value of < .05 was considered statistically significant.

This study was conducted in accordance with the principles of the Declaration of Helsinki, and the ethics committee of the Medical University of Warsaw approved the protocol. All patients gave written informed consent for inclusion in the study.

Results

64 patients were included in the study, comprising 33 (52%) men and 31 (48%) women. All included patients were white and all were recipients of their first renal transplant. The characteristics of the study group are set out in Table 1. Among this study group, IA was detected in 13 cases, while 51 patients were IA-free.

The expression of miR-25 was found in all patients, and the expression of miR-16 was found in all but one patient; that case was excluded from analysis. We observed statistically significant differences in the expression of miR-16 between patients with and without IA, based on the accepted level of significance $p < 0.05$ and Z statistic of the Mann-Whitney U test ($Z = 2.90616$) with continuity correction $p = 0.01658$ as well as based on accurate U statistics (743). The expression of miR-16 was higher in the control (non-aneurysmal) group, and the ratio of mean miR-16 expression level was 1.00:0.39 (control:cases). A similar relationship was also observed for miR-25, although the results did not reach statistical significance ($Z = -0.1441$; $U = 1{,}126.5$; $p = 0.88866$).

Discussion

The prevalence of IAs in renal transplant recipients with ADPKD has been assessed as being between 14.9% [4] and 22.7% [9]. Developing biomarkers may shed light on the

Table 1. Characteristics of study group

Characteristic	Value		
	Whole group (n = 64)	IA (+) group (n = 13)	IA (-) group (n = 51)
Men/women (n, %)	33/31 52%/48%	6/7 46%/54%	27/24 53%/47%
Median age, years (range, IQR)	60.7 (38–78, 11.1)	55.7 (37.5–67.2, 8.7)	62.1 (39.5–78.2, 10.5)
Median time after transplant in months (range, IQR)	35.6 (1–236, 77.9)	8.9 (1.2 – 100, 49.7)	46 (0.6–236.2, 72.1)
Median eGFR [CKD-EPI formula], ml/min/1.73m^2 (range, IQR)	45.6 (7–97.3, 32.3)	38.9 (22.4–82.6, 22.5)	47.7 (7–97.3, 34.5)

IQR — interquartile range, eGFR — estimated glomerular fraction rate, IA — intracranial aneurysm

pathogenesis of IAs in this group. Additionally, biomarkers may become tools useful in the selection of patients who should undergo imaging for an IA.

miR-16 belongs to the most abundant miRNAs present in human plasma. Because its level was considered to be relatively stable, it was previously used as the housekeeping gene in qPCR assays. However, it was later shown that the plasma levels of miR-16 can change in certain diseases. According to the study by Li et al. [8], expression of miR-16, together with miR-25, is increased in patients with IA compared to healthy controls. miR-16 is expressed by vascular endothelial cells and is involved in angiogenesis [8]. While the function of endothelial cells is disturbed in ADPKD [10], it seemed desirable to verify the results of Li et al. [8] in ADPKD patients.

Our results show that, indeed, plasma expression of miR-16 is altered in ADPKD patients after renal transplantation with IA, but, in contrast to the results of Li et al. [8], we observed **decreased** expression. Additionally, we did not observe altered plasma levels of miR-25 in this particular group of patients. Therefore, according to our results, miR-16, but not miR-25, could become a potential biomarker of IAs in ADPKD patients after renal transplantation.

Our results suggest that the pathogenesis of IAs in ADPKD may be different compared to IAs in the general population. That may explain numerous differences in the clinical picture of IAs between ADPKD patients and the general population, namely their prevalence, age at development, lack of gender differences etc. [2]. Thus, there is a need to take into account the distinct clinical proceedings with these particular cases. For instance, clinical biomarkers of IAs, if developed for the general population, may not be applicable to ADPKD patients, at least after kidney transplantation. Additionally, it would be of interest to compare levels of miR-16 in renal transplant recipients to those in the general population. This could potentially explain the differences between our results and those of Li et al. [8].

The fact that the plasma levels of miR-16 may change in certain diseases could also impact upon our results. For example, blood levels of miR-16 can change during vascular rejection of a renal graft [11]. In all of our patients, the graft function was stable at the moment of sample collection; however, our results should be interpreted cautiously due to the fact that some confounding factors might be omitted in the analysis. Therefore, further studies on this subject are needed.

Some limitations of our study should be noted. Firstly, due to the characteristics of the Polish population, only Caucasians were included in our study; results in other populations may differ. Secondly, the group was relatively small, and our observations should be confirmed in a larger study including populations of different ethnicities. Thirdly, only renal graft recipients were included in this study, and additional factors connected to this fact (e.g. immunosuppressive treatment, asymptomatic infections) might lead to bias. Additionally, renal graft recipients are in general older than patients in the pre-ESKD period, and therefore our results may not reflect the situation in the entire ADPKD population.

The clinical utility of miR-16 as a biomarker of IA in renal transplant recipients with ADPKD, and, possibly, in the whole ADPKD population, requires verification in further studies.

Conclusions and clinical implications

MicroRNA-16 is a potential marker of IA in renal transplant recipients with ADPKD. It may become a tool to identify patients who should undergo screening for IAs.

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Two COVID-19-related video-accompanied cases of severe ataxia-myoclonus syndrome

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ABSTRACT

Aim of the study. The pandemic state of COVID-19 has resulted in new neurological post-infection syndromes. Recently, several papers have reported ataxia-myoclonus syndrome following SARS-CoV-2 infection. The aim of this study was to present our two cases and compare them to previously reported cases.

Materials and methods. We present two video-accompanied new cases with ataxia-myoclonus syndrome following SARS-CoV-2 infection and discuss the studies published so far.

Results. Ataxia-myoclonus syndrome, isolated myoclonus, opsoclonus-myoclonus syndrome as post-COVID-19 syndrome following infection have been described in 16 patients (including our two cases). Patients have been treated with intravenous immunoglobulins and/or steroids except for 4 patients, which resulted in a significant improvement within 1–8 weeks.

Conclusions and clinical implications. The increasing number of patients with a similar symptomatology shows a significant relationship between COVID-19 infection and ataxia-myoclonus syndrome. The subacute onset of neurological symptoms after a resolved COVID-19 infection and prominent response to immunotherapy may suggest that the neurological manifestations are immune-mediated. Although recovery is highly possible, it may take several weeks/months, and clinicians should be aware of this diagnosis and the beneficial effects of immunological treatment administered as soon as possible.

Key words: COVID-19, SARS-CoV-2, ataxia, myoclonus, voice tremor

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The pandemic state of COVID -19, now having lasted more than 12 months, has resulted in new clinical manifestations and neurological post-infection syndromes of SARS-CoV-2 infection, involving both central and peripheral nervous systems [1]. The most commonly occurring in the acute phase of infection include ischaemic and haemorrhagic strokes, cerebral venous thrombosis, posterior reversible encephalopathy (PRES), and those of autoimmunological origin presenting as Guillain-Barre, Miller Fisher syndromes and Bickerstaff's encephalitis, acute demyelinating encephalomyelitis (ADEM), acute necrotising encephalopathy (ANE), generalised myoclonus, acute transverse myelitis, limbic encephalitis, and miscellaneous encephalitis [1, 2]. Recently, several papers have reported ataxia-myoclonus syndrome following SARS-CoV-2 infection (Tab. 1) [6–12]. We present two video-accompanied new cases of this presumably autoimmunological syndrome.

Case 1

A 49-year-old male, without co-morbidities besides lumbar surgery eight years previously, was admitted to hospital with involuntary movements of limbs and trunk accompanied by severe instability (he was almost unable to stand or walk independently). Eleven days before admission, the patient had experienced an episode of one-day fever (38° C) with excessive sweating without any other infection symptoms. These symptoms resolved spontaneously after a few days and he was not tested for COVID-19 infection. One week later, he developed hand tremors, jerky movements of all limbs,

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Table 1. Characteristics of patients with ataxia-myoclonus syndrome associated with COVID-19

Article	Age/ /gender	COVID-19 severity of infectious stage	Neurological symptoms	Time between acute infection and appearance of neurological symptoms	Treatment/outcome
Our cases	49/M	Mild/ asymptomatic	• Myoclonus • Ataxia • Voice tremor	11 days	Methylprednisolone 1 g/day, 5 g total followed by oral prednisone 60mg/day for two weeks; significant improvement after three weeks
	62/M	Mild	• Myoclonus • Opsoclonus • Ataxia	11 days	IVIG 0.4 g/kg for five days, methylprednisolone 1g/day, 5g total followed by oral prednisone (1 mg/kg/day) for two weeks with gradual dose reduction; significant improvement after two weeks
Emamikhah et al. 2021 [6]	51/M	Mild	• Myoclonus • Opsoclonus • Ataxia • voice tremor	2 weeks	Clonazepam 0.5 mg 4x1, levetiracetam 500 mg 2 x 1, IVIG 2 g/kg, total dose 150 g; complete recovery after four weeks
	54/M	Moderate	• Myoclonus • Ataxia	4 days	Levetiracetam 2,000 mg/day, sodium valproate 1,000 mg/day IVIG in total dose 100 g; Partial recovery after one week
	52/M	Moderate	• Myoclonus • Ataxia • Voice tremor	16 days	Sodium valproate 1,000 mg/day. clonazepam 1mg 4 x 1; partial recovery after two months
	42/F	Mild	• Myoclonus • Ataxia • Voice tremor	10 days	Sodium valproate, clonazepam; no data
	44/M	Mild	• Myoclonus • Opsoclonus • Ataxia • Voice tremor	3 days	Sodium valproate, clonazepam, IVIG; complete recovery after two months
	52/M	Mild	• Myoclonus • Ataxia • Voice tremor	3 weeks	Clonazepam, IVIG in total dose of 100 g; significant improvement after four weeks
	39/M	Severe	• Myoclonus • Opsoclonus • Ataxia • Voice tremor	10 days	Levetiracetam, sodium valproate, clonazepam, IVIG, dexamethasone; no data
Wright et al. 2020 [7]	79/M	Mild	• Opsoclonus • Ataxia • Cognitive impairment	13 days	No data; improvement of cognitive function and resolution of eye movement abnormality after 19 days; death after 27 days due to general physical decline
Dijkstra et al. 2020 [8]	44/M	Mild	• Myoclonus • Ataxia • Voice tremor • Transient ocular flutter (no evident opsoclonus) • Cognitive impairment	1 week	Methylprednisolone 1 g daily for five days, IVIG 0.4 g/kg for three days; partial recovery after 15 days, full recovery within two months
Schellekens et al. 2020 [9]	48/M	Mild	• Myoclonus • Ataxia • Saccadic intrusions of eye movement (no opsoclonus)	13 days	Levetiracetam; partial recovery within two months
Foucard et al. [10]	83/M	No data	• Myoclonus • Opsoclonus • Ataxic dysarthria • Confusion	10 days	IVIG 0.4 g/kg for five days, steroids 1g/day for five days, diazepam; significant improvement after one week
	63/M	No data	• Myoclonus • Ataxia • Ataxic dysarthria	6 weeks	IVIG 0.4 g/kg for five days; significant improvement after one week
Shah et al. 2020 [11]	Middle aged/M	No data	• Myoclonus • Opsoclonus • Ataxia • Ataxic dysarthria	3 weeks	Methylprednisolone 1 g/day, sodium valproate 20 mg/kg/day, clonazepam 2 mg/day, levetiracetam 2 g/day; recovery within one week
Grimaldi et al. 2020 [5]	72/M	Moderate	• Myoclonus • Ataxia • Ataxic dysarthria	17 days	IVIG 0.4 g/kg/day for five days, methylprednisolone 1 g/day for five days; recovery within two weeks

and posture and gait instability with diarrhoea lasting for three days. Because of these symptoms, he was admitted to the Emergency Unit (EU). On neurological examination, he presented dysarthria with voice tremor, generalised myoclonus exacerbated by intentional movements, bilateral limb ataxia and wide-based gait (see video, segment 1). In the EU he was treated with clonazepam 1mg i.v., biperiden 2 mg p.o. and levetiracetam 500 mg i.v. but with no effect. Nasopharyngeal RT-PCR test for SARS-CoV-2 was negative. Brain computer tomography (CT) and magnetic resonance imaging (MRI), as well as a general examination of cerebrospinal fluid (CSF), were normal. Antibodies testing (blood and CSF) for NMDAR, VGKC, Borrelia burgdorferi IgG and IgM antibodies were negative. Viral neurological panel (Adenovirus, CMV, EBV, HSV-1, HSV-2, VZV, Enterovirus, Parechovirus, Parvovirus B19, HHV-6, HHV-7) and soluble antigens in CSF were also negative. Oligoclonal bands in CSF showed type 4 according to the Charcot Foundation. Blood laboratory tests for onconeural antibodies (anti-Hu, anti-Ri, Anti-Yo), anti-nuclear antibodies (ANA) were negative, and vitamin B12, B1 and folic acid were within normal ranges.

Due to the infection episode 11 days before admission, serum level of SARS-CoV-2 IgG and IgM antibodies testing was performed, with a positive result for IgG (IgG 38.6AU/ml, IgM 2.2 AU/ml). Treatment with intravenous methylprednisolone (1g/day for five consecutive days, 5 g in total) followed by oral prednisone (60 mg/day) for two weeks and motor rehabilitation resulted in a significant improvement after three weeks (see video, segment 2).

Case 2

A 62-year-old male with a history of arterial hypertension, benign prostatic hyperplasia, and kidney stones, was, 11 days after resolution of a COVID-19 infection confirmed with a nasopharyngeal RT-PCR test, admitted to the EU with imbalance, gait disturbance, generalised tremor and ataxia. On neurological examination, he had jerky oscillatory movements of eyes (diagnosed as opsoclonus), generalised myoclonus and ataxia. This made the patient unable to stand or walk independently (see video, segment 3). Head MRI with MRI-angiography as well as general CSF examination were normal. Fast CSF tests for the presence of viral (CMV, Enterovirus, HSV-1, HSV-2, HHV-6, Parechovirus, VZV) and bacterial (E. coli, H. influenzae, L. monocytogenes, N. meningitidis, S. agalactiae, S. pneumoniae) antigens were negative as well. Auto-immune encephalitis antibodies in serum (NMDAR, AMPAR 1 and 2, CASPR 2, LGI-1, GABA-B receptor), Borrelia IgM and IgG antibodies and onconeural antibodies (anti-Hu, anti-Ri, Anti-Yo, anti-PNMA2, anti-CV2, anti-amphiphysin) were not detected. Ganglioside antibody panel was negative. Chest and abdomen CT were normal. Tumour markers (CA-125, CA-19-9, CEA) were negative. Electroencephalography (EEG) on admission showed bilateral theta waves in the central and frontal areas which resolved at release. Treatment with intravenous methylprednisolone (1g/day, 5g in total) was initiated followed by intravenous immunoglobulins (IVIG) for five days (0.4 g/kg) and oral prednisone (1 mg/kg/day) for two weeks with gradual dose reduction. The administered treatment and motor rehabilitation resulted in a significant improvement after two weeks (see video, segment 4).

Discussion

Understanding regarding the neurological complications of SARS-CoV-2 infection is still limited. Brain damage can be caused by hypoxia, direct brain invasion of the coronavirus, or post-infectious autoimmune mechanisms. The hypothesis of a possible neuroinvasiveness of SARS-CoV-2 causing neurological symptoms during the acute state of the infection has been proposed [2]. Widespread neuronal ACE2 expression in brainstem cardiorespiratory neurons, motor cortex, the raphe nucleus, and others may suggest the direct invasion of virus, which may result in diverse neurological symptoms.

While ACE2's role in the brain is incompletely understood, this diverse central nervous expression pattern may provide SARS-CoV-2, and other CoVs, with a 'port of entry' into the brain [3]. On the other hand, opsoclonus-myoclonus syndrome (OMS) is a rare disorder of apparently autoimmune paraneoplastic or parainfectious aetiology. This presents with opsoclonus, limbs and trunk myoclonus, and ataxia. Paraneoplastic OMS is generally observed in patients with breast adenocarcinoma or small cell lung carcinoma, while parainfectious OMS has been observed in patients with HIV, Mycoplasma pneumoniae, Salmonella enterica, rotavirus, cytomegalovirus, human herpesvirus 6, hepatitis C, and Rickettsia conorii. Treatment with corticosteroids or IVIG is usually successful [4]. Therefore, its aetiology is considered to be autoimmunological.

The subacute onset of neurological symptoms after resolved COVID-19 infections (however, only 11 days after) in both presented cases, and the prominent response to immunotherapy, may suggest that the neurological manifestations are immune-mediated. However, it is difficult to entirely exclude a direct viral effect of COVID-19, given that we know that acute COVID infection can benefit from steroids. The brain ^{18}F-FDG-PET (fluorodeoxyglucose Positron Emission Tomography) study of a patient with ataxia-myoclonus syndrome associated with COVID-19 performed by Grimaldi et al. showed a diffuse pattern compatible with encephalitis and especially cerebellitis. Moreover, nerve tissue immunostaining with serum and CSF showed the presence of autoantibodies directed against the nuclei of Purkinje cells, striatal and hippocampal neurons. The titre of the IgG autoantibodies in serum and CSF was high (serum 1/25,000, CSF 1/96) and both in serum and CSF the same intensity and reactivity were observed at the same IgG concentration [5]. These observations may confirm the autoimmune origin of the post-COVID-19 ataxia-myoclonic syndrome.

Ataxia-myoclonus syndrome, isolated myoclonus, OMS as post-COVID-19 syndrome following infection have been described in several case series and reports (Tab. 1). The increasing number of patients with a similar symptomatology suggests that there may be a significant relationship between infection and the described neurological symptoms, adding SARS-CoV-2 to the list of possible pathogens. Because recovery is highly possible, although it may take several weeks/ /months, clinicians should be aware of this diagnosis and the beneficial effects of immunological treatment administered as soon as possible.

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SHORT COMMUNICATION

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Neurological symptoms in hospitalised patients with COVID-19 and their association with in-hospital mortality

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ABSTRACT

Objectives. To evaluate the spectrum of neurological symptoms in patients with COVID-19 during the first 14 days of hospitalisation and its association with in-hospital mortality.

Material and methods. We included 200 patients with RT-PCR-confirmed COVID-19 admitted to University Hospital in Krakow, Poland. In 164 patients, a detailed questionnaire concerning neurological symptoms and signs was performed prospectively within 14 days of hospitalisation. In the remaining 36 patients, such questionnaires were completed retrospectively based on daily observations in the Department of Neurology.

Results. During hospitalisation, 169 patients (84.5%) experienced neurological symptoms; the most common were: fatigue (62.5%), decreased mood (45.5%), myalgia (43.5%), and muscle weakness (42.5%). Patients who died during hospitalisation compared to the remainder were older (79 [70.5–88.5] *vs.* 63.5 [51–77] years, $p = 0.001$), and more often had decreased level of consciousness (50.0% *vs.* 9.3%, $p < 0.001$), delirium (33.3% *vs.* 4.4%, $p < 0.001$), arterial hypotension (50.0% *vs.* 19.6%, $p = 0.005$) or stroke during (18.8% *vs.* 3.3%, $p = 0.026$) or before hospitalisation (50.0% *vs.* 7.1, $p < 0.001$), whereas those who survived more often suffered from headache (42.1% *vs.* 0%, $p = 0.012$) or decreased mood (51.7% *vs.* 0%, $p = 0.003$).

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Conclusions. Most hospitalised patients with COVID-19 experience neurological symptoms. Decreased level of consciousness, delirium, arterial hypotension, and stroke during or before hospitalisation increase the risk of in-hospital mortality.

Key words: COVID-19, Sars-Cov-2 infection, Neuro-COVID-19, neurological symptoms, in-hospital mortality

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Introduction

Poland is among the eight worst-hit countries in Europe in terms of the prevalence of novel coronavirus causing Coronavirus Disease 2019 (COVID-19), with more than two and a half million affected people, and nearly 60,000 deaths, as of 11 April, 2021 [1].

Numerous infectious diseases with pandemic and epidemic potential can cause neurological symptoms in their course [2]. With an increasing number of patients, neurological manifestations of severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) infection also became apparent, with the first study from Wuhan showing that this could apply to one in every three cases [3]. Later studies revealed that the frequency of neurological symptoms might be even higher, ranging from 57.4% in hospitalised patients with COVID-19 in Spain [4] to 73.0% in a mixed cohort of outpatient and hospitalised patients in a single centre in the state of Washington, USA [5]. However, most of these studies had a retrospective design which consequently might result in underestimation of the frequency of neurological manifestations in SARS-Cov-2 infection.

Recently, two prospective studies on neurological symptoms in COVID-19 patients were performed, in which neurological consultation was used to identify neurological manifestations [6, 7]. Fleischer et al. showed that nearly 60% of 102 patients infected with the SARS-Cov-2 virus had non-specific neurological involvement with general weakness, cognitive decline or delirium [6]. In another study, the most common neurological complaints in a cohort of 873 Iranian patients were: smell and taste dysfunction, myalgia, headache, and dizziness [7].

Therefore, due to discrepancy in the frequency of neurological symptoms among patients infected with the SARS-Cov-2 virus, we aimed to evaluate the frequency and spectrum of neurological symptoms in COVID-19 patients during the first 14 days of hospitalisation, and to seek any possible association with in-hospital mortality.

Material and methods

We recruited patients admitted between March and September 2020 to four different departments of the University Hospital in Krakow — Neurology, Metabolic Diseases and Diabetology, Internal Medicine, and Otorhinolaryngology — which during the pandemic was transformed into the main centre for patients with the SARS-Cov-2 infection in the region of Lesser Poland. All patients had the diagnosis of COVID-19 confirmed by the detection of SARS-Cov-2 RNA by real-time reverse transcription-polymerase chain reaction (RT-PCR) from a nasopharyngeal swab.

Causes of hospital admission, and therefore inclusion criteria for participation in the study, were as follows: dyspnoea, low blood saturation (≤ 92%), chronic disease which had to be treated in hospital, or lack of possibility of isolation. Excluded were: patients younger than 18 years and those who needed mechanical ventilation on hospital admission.

In most patients (n = 164), a detailed questionnaire concerning the presence of 12 neurological symptoms (headache, dizziness, decreased mood, memory or concentration difficulties, fatigue, visual disturbances, anosmia, ageusia, muscle weakness, myalgia, paresthesia, and increased sweating) and eight neurological signs (decreased level of consciousness, delirium, ataxia, seizure, stroke/TIA, autonomic disturbances such as diarrhoea, arterial hypotension < 90/60mmHg or tachycardia > 100/min) was performed prospectively within 14 days of hospitalisation by both patients and physicians. In the remaining 36 patients, such questionnaires were completed retrospectively on the basis of detailed daily clinical records in the computerised hospital system in the Department of Neurology. We additionally collected data concerning respiratory and gastrointestinal symptoms of COVID-19, comorbidities, and in-hospital mortality.

Written or verbal — in the presence of two witnesses — informed medical consent was obtained from each patient followed prospectively. This study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of the District Medical Council in Krakow (opinion number 143/KBL/OIL/2020).

We presented categorical data as counts and percentages and continuous data as mean and standard deviation (SD), median and interquartile range (IQR). We tested continuous variables for normality with the Shapiro-Wilk test and compared, as appropriate, by the Student's t-test or by the Mann-Whitney U test. A p-value of 0.05 (two-sided) was considered statistically significant. We performed all statistical analyses with STATISTICA version 13 (Statsoft Inc, Tulsa, OK, USA).

The data confirming the results of this study is available from the corresponding author upon reasonable request.

Results

This study included 200 consecutive patients hospitalised in four departments of the University Hospital in Krakow – Neurology (29.0%, n = 58), Metabolic Diseases and Diabetology (54.5%, n = 109), Internal Medicine (10%, n = 20), and Otorhinolaryngology (6.5%, n = 13). Patients were admitted from home (62.5%, n = 125), another hospital (27.5%, n = 55), nursing home (9.0%, n = 18), or institutional isolation (1.0%, n = 2).

Median patient age during hospitalisation was 65.0 (interquartile range, IQR 52.0-78.5) years and there were 56.0% females (n = 112). Median time from first COVID-19 symptoms to hospital admission was 5 (IQR 3-7) days, whereas the median time from first positive nasopharyngeal swab test to the onset of hospitalisation was 1 (IQR 1-3) day. In 128 patients (64.0%), the following comorbidities were noted: hypertension (57.5%, n = 115), diabetes mellitus (26.5%, n = 53), ischaemic heart disease (17.5%, n = 35), obesity (16.5%, n = 33), chronic renal disease (4.5%, n = 9), cancer (4.5%, n = 9), and chronic obstructive pulmonary disease or asthma (4.0%, n = 8). Eleven (5.5%) and 19 (14.5%) patients had a history of cancer or stroke, respectively. There were six (3.0%) current and 29 (14.5%) past smokers.

First manifestations of SARS-Cov-2 infection were as follows: cough (57.5%, n = 115), fever (body temperature ≥ 38°C; 52.0%, n = 104), dyspnoea (48.5%, n = 97), loss of appetite (27.5%, n = 55), diarrhoea (24.0%, n = 48), abdominal pain (18.5%, n = 37) and sore throat (12.5%, n = 25). At day 14 since the onset of hospitalisation, 22 (11%) patients were receiving oxygen therapy and seven (3.5%) were mechanically ventilated. There were 16 (8.0%) deaths at a median 12.5 (IQR 8.5–28.5) days of hospitalisation.

During a median 14 (IQR 8-22) days of hospitalisation, 169 patients (84.5%) experienced some form of neurological symptom or sign. In 20 patients (10.0%) neurological symptoms were the first manifestation of SARS-Cov-2 infection accompanying respiratory or gastrointestinal symptoms. The most common neurological symptoms were: fatigue (62.5%), decreased mood (45.5%), myalgia (43.5%), muscle weakness (42.5%) and headache (37.0%) (Tab. 1). Most neurological symptoms had an onset within the first three days of hospitalisation, whereas paresthesia or arterial hypotension < 90/60mmHg occurred later in the disease course (median 6 days, IQR 1-11 or 7.5 days, IQR 3-13, respectively; Tab. 1).

Patients who died during hospitalisation compared to the remainder were older, and more often had a decreased level of consciousness, delirium, arterial hypotension or stroke during or before hospitalisation, whereas those who survived more often suffered from headache or decreased mood (Tab. 2).

When the analysis was restricted to patients followed prospectively, those who died during hospitalisation compared to survivors were older (83 [76–87] *vs.* 63 [50–77] years, p < 0.001) and more often had a decreased level of consciousness (44.4% *vs.* 7.8%, p = 0.006), delirium (50.0% *vs.* 3.9%, p < 0.001), or a history of stroke (44.4% *vs.* 5.8%, p = 0.002) (Tab. 3). Among patients recruited prospectively, those who survived compared to the remainder more often complained of decreased mood (54.7% *vs.* 0.0%, p = 0.021) (Tab. 3).

Discussion

Our study showed that different neurological symptoms exerted a diverse association with in-hospital mortality in patients with COVID-19. Some of them, possible to assess regardless of patient cooperation such as delirium or decreased level of consciousness, were linked to the risk of death during hospitalisation. This observation was in line with a previous study in which a short and simple scale for mortality risk was validated in a cohort of more than 20,000 patients with COVID-19 and which consisted of eight parameters with a level of consciousness among others [8]. A recent Italian study also confirmed that patients with COVID-19 and delirium on admission had a nearly two-fold higher risk of in-hospital mortality compared to those without delirium [9]. In another study, consisting of a retrospective analysis of electronic medical records of 307 COVID-19 patients in Turkey, it was shown that altered mental status was the most common neurological manifestation and associated with a higher mortality rate [10].

On the other hand, in our study some symptoms which could be evaluated only in those patients who were able to fill out the questionnaire, such as headache or decreased mood, increased the chance of survival. Our results were similar to the findings of a prospective study of nearly 900 patients with SARS-Cov-2 infection where the presence of headache was a protective factor against death due to COVID-19 [7]. The frequency of headache in previous studies has been reported as between 7% and 75%, with higher percentages in European populations than in Chinese cohorts [11].

We hypothesise that the protective role of headache and decreased mood in terms of mortality due to COVID-19 could be — at least partially — explained by the inflammatory process occurring in the body of these patients and leading to virus elimination [12]. Indeed, in a recent Chinese study of 77 patients with COVID-19, those who suffered from anxiety had higher serum levels of IL-6 and IL-10, whereas those with depression had higher CD8+ T-cell count and lower CD4+/CD8+ ratio compared to the remainder [13]. What is more, as it was shown in a large American cohort of nearly 4,000 patients with prior SARS-Cov-2 infection, the severity of COVID-19 symptoms and the presence of headache increased the risk of subsequent major depression by 2.6 and 1.3-fold, respectively [14]. Recently, so-called lesser neurological symptoms such as fatigue, inability to concentrate, myalgia, and headache, were found to have the potential to become chronic and result in the syndrome recently labelled as Long Covid [15].

Table 1. Neurological symptoms and signs in patients with COVID-19 during the first 14 days of hospitalisation

Neurological symptom or sign	Presence of neurological symptom or sign	Number of patients n (%)	Onset of neurological symptom or sign (days of hospitalisation, median and IQR)	Duration of neurological symptom or sign (days of hospitalisation, median and IQR)	Number of patients who declared to have neurological symptom or sign before hospitalisation, n (%)
Neurological symptoms					
Headache	No Yes Not possible to assess	111 (55.5) 74 (37.0) 15 (7.5)	2 (1–5)	2 (1–3)	11 (5.5)
Dizziness	No Yes Not possible to assess	136 (68.0) 63 (31.5) 1 (0.5)	3 (1–7)	2 (1–2)	8 (4.0)
Decreased mood	No Yes Not possible to assess	94 (47.0) 91 (45.5) 15 (7.5)	2 (1–8)	4 (2–8)	2 (1.0)
Memory or concentration difficulties	No Yes Not possible to assess	152 (76.0) 34 (17.0) 14 (7.0)	1 (1–8)	3 (2–5)	4 (2.0)
Fatigue	No Yes Not possible to assess	60 (30.0) 125 (62.5) 15 (7.5)	1 (1–4)	5 (2–8)	10 (5.0)
Visual disturbances	No Yes Not possible to assess	174 (87.0) 11 (5.5) 15 (7.5)	1 (1–10)	2 (1–3)	1 (0.5)
Anosmia	No Yes Not possible to assess	142 (71.0) 43 (21.5) 15 (7.5)	1 (1–1)	4 (2–9)	7 (3.5)
Ageusia	No Yes Not possible to assess	131 (65.5) 54 (27.0) 15 (7.5)	1 (1–4)	4 (2–8)	5 (2.5)
Muscle weakness	No Yes Not possible to assess	102 (51.0) 85 (42.5) 13 (6.5)	1 (1–6)	4 (1–8.5)	17 (8.5)
Myalgia	No Yes Not possible to assess	112 (56.0) 87 (43.5) 1 (0.5)	1 (1–6)	2 (1–5)	10 (5.0)
Paresthesia	No Yes Not possible to assess	139 (69.5) 46 (23.0) 15 (7.5)	6 (1–11)	1 (1–4)	3 (1.5)
Increased sweating	No Yes Not possible to assess	116 (58.0) 71 (35.5) 13 (6.5)	3 (1–8)	2 (1–6)	7 (3.5)
Neurological signs					
Decreased level of consciousness	No Yes Not possible to assess	174 (87.0) 25 (12.5) 1 (0.5)	1 (1–4)	5 (3–15)	1 (0.5)
Delirium	No Yes Not possible to assess	184 (92.0) 15 (7.5) 1 (0.5)	3 (1–7)	7 (4–9)	3 (1.5)
Diarrhoea	No Yes Not possible to assess	137 (68.5) 62 (31.0) 1 (0.5)	3 (1–6)	2 (1–3)	8 (4.0)
Arterial hypotension < 90/60 mmHg	No Yes Not possible to assess	156 (78.0) 44 (22.0) 0 (0.0)	7.5 (3–13)	1 (1–2)	0 (0.0)
Tachycardia > 100/min	No Yes Not possible to assess	144 (72.0) 56 (28.0) 0 (0.0)	3.5 (1–9)	2 (1–3)	0 (0.0)
Ataxia	No Yes Not possible to assess	188 (94.0) 11 (5.5) 1 (0.5)	1.5 (1–8)	1 (1–1)	1 (0.5)
Seizure	No Yes Not possible to assess	198 (99.0) 1 (0.5) 1 (0.5)	1 (1–1)	0.5 (0–1)	0 (0.0)
Stroke or TIA	No Yes Not possible to assess	188 (94.0) 5 (2.5) 7 (3.5)	5 (1–11)	1 (1–1)	0 (0.0)

Table 2. Comparison of patients with COVID-19 who survived or died during hospitalisation

	Patients who survived during hospitalisation n = 184	Patients who died during hospitalisation n = 16	P-value
Demographics			
Female sex	103 (55.6)	9 (56.2)	0.982
Age	63.5 (51–77)	79 (70.5–88.5)	0.001
First COVID-19 symptoms			
Fever	94 (51.2)	10 (62.5)	0.381
Cough	107 (58.2)	8 (50.0)	0.527
Sore throat	23 (12.5)	2 (12.5)	1.000
Loss of appetite	52 (28.3)	3 (18.8)	0.413
Dyspnoea	88 (47.8)	10 (62.5)	0.260
Diarrhoea	44 (23.9)	4 (25.0)	0.922
Abdominal pain	35 (19.0)	2 (12.5)	0.519
Neurological symptoms and signs			
Headache	74 (42.1)	0 (0.0)	0.012
Dizziness	49 (27.8)	0 (0.0)	0.065
Decreased mood	91 (51.7)	0 (0.0)	0.003
Memory or concentration difficulties	31 (17.6)	3 (30.0)	0.324
Fatigue	122 (69.3)	3 (33.3)	0.060
Visual disturbances	11 (6.3)	0 (0.0)	0.439
Decreased level of consciousness	17 (9.3)	8 (50.0)	< 0.001
Delirium	8 (4.4)	5 (33.3)	< 0.001
Seizure	0 (0.0)	1 (6.2)	0.080
Ataxia	4 (2.2)	0 (0.0)	1.000
Stroke/TIA	6 (3.3)	3 (18.8)	0.026
Anosmia	42 (23.9)	1 (11.1)	0.687
Ageusia	53 (30.1)	1 (11.1)	0.287
Muscle weakness	80 (44.9)	5 (55.6)	0.532
Myalgia	69 (39.2)	4 (44.4)	0.741
Paresthesia	44 (35.0)	2 (22.2)	1.000
Diarrhoea	59 (32.1)	3 (20.0)	0.400
Increased sweating	68 (38.6)	3 (27.3)	0.538
Arterial hypotension (< 90/60mmHg)	36 (19.6)	8 (50.0)	0.005
Tachycardia (> 100/min)	50 (27.2)	6 (37.5)	0.378
Any neurological symptom or sign	158 (85.9)	11 (68.8)	0.069
Comorbidities			
Hypertension	106 (62.4)	9 (64.3)	0.885
Ischaemic heart disease	31 (18.3)	4 (28.9)	0.312
Diabetes mellitus	47 (27.8)	6 (42.9)	0.233
History of stroke	12 (7.1)	7 (50.0)	< 0.001
Asthma/Chronic Obstructive Pulmonary Disease	8 (4.7)	0 (0.0)	1.000
Cancer	19 (11.2)	1 (7.1)	1.000
— No cancer — Current cancer — History of cancer	150 (88.8) 8 (4.7) 11 (6.5)	13 (92.9) 1 (7.1) 0 (0.0)	0.580
Chronic renal disease	7 (4.1)	2 (14.3)	0.143
Obesity	30 (17.8)	3 (21.4)	0.720
Smoking	33 (19.9)	2 (14.3)	1.000
— No smoking — Current smoking — History of smoking	133 (80.1) 5 (3.0) 28 (16.9)	12 (85.7) 1 (7.1) 1 (7.1)	0.479

Table 3. Comparison of patients with COVID-19 recruited prospectively who survived or died during hospitalisation

	Patients who survived during hospitalisation n = 155	Patients who died during hospitalisation n = 9	P-value
Demographics			
Female sex	94 (60.7)	4 (4.4)	0.485
Age	63 (50–77)	83 (76–87)	< 0.001
First COVID-19 symptoms			
Fever	77 (49.7)	4 (44.4)	1.000
Cough	88 (56.8)	2 (22.2)	0.080
Sore throat	118 (11.6)	1 (11.1)	1.000
Loss of appetite	44 (28.4)	2 (22.2)	1.000
Dyspnoea	67 (43.2)	6 (66.7)	0.169
Diarrhoea	37 (23.9)	0 (0.0)	0.211
Abdominal pain	29 (18.7)	0 (0.0)	0.363
Neurological symptoms and signs			
Headache	65 (43.9)	0 (0.0)	0.073
Dizziness	43 (29.1)	0 (0.0)	0.322
Decreased mood	81 (54.7)	0 (0.0)	0.021
Memory or concentration difficulties	26 (17.6)	3 (50.0)	0.081
Fatigue	110 (74.3)	2 (40.0)	0.112
Visual disturbances	11 (7.4)	0 (0.0)	1.000
Decreased level of consciousness	12 (7.8)	4 (44.4)	0.006
Delirium	6 (3.9)	4 (50.0)	< 0.001
Seizure	0 (0.0)	0 (0.0)	1.000
Ataxia	1 (0.7)	0 (0.0)	1.000
Stroke/TIA	4 (2.6)	1 (11.1)	0.248
Anosmia	39 (26.4)	1 (20.0)	1.000
Ageusia	52 (35.1)	1 (20.0)	0.659
Muscle weakness	68 (45.3)	2 (40.0)	1.000
Myalgia	59 (39.9)	2 (40.0)	1.000
Paresthesia	27 (25.0)	1 (20.0)	1.000
Diarrhoea	52 (33.6)	0 (0.0)	0.057
Increased sweating	65 (43.9)	2 (40.0)	1.000
Arterial hypotension (< 90/60mmHg)	35 (22.6)	4 (44.4)	0.218
Tachycardia (> 100/min)	45 (29.1)	3 (33.3)	0.721
Any neurological symptom or sign	135 (87.1)	6 (66.7)	0.115
Comorbidities			
Hypertension	95 (61.3)	7 (77.8)	0.321
Ischaemic heart disease	27 (17.4)	3 (33.3)	0.213
Diabetes mellitus	43 (27.7)	3 (3.3)	0.711
History of stroke	9 (5.8)	4 (44.4)	0.002
Asthma/Chronic Obstructive Pulmonary Disease	8 (5.2)	0 (0.0)	1.000
Cancer	19 (11.2)	1 (7.1)	1.000
— No cancer — Current cancer — History of cancer	139 (89.7) 5 (3.3) 11 (7.1)	8 (88.9) 1 (11.1) 0 (0.0)	0.332
Chronic renal disease	7 (4.5)	2 (22.2)	0.079
Obesity	29 (18.7)	1 (11.1)	1.000
Smoking	28 (18.4)	2 (22.2)	0.675
— No smoking — Current smoking — History of smoking	124 (81.6) 4 (2.6) 24 (15.8)	7 (77.8) 1 (11.1) 1 (11.1)	0.504

We also revealed that patients with COVID-19 with previous stroke and older age had a greater risk of in-hospital mortality. Stroke during COVID-19 was also associated with increased in-hospital mortality in the whole group but not in the prospective cohort, probably due to severe neurological deficit which prevented these patients from filling out the questionnaire. Previous research has shown that chronic neurological comorbidity increases the risk of in-hospital mortality [16, 17]. A recent meta-analysis of 18 studies, mostly of retrospective design, revealed that the mortality rate in COVID-19 patients with concomitant stroke was concerning and, especially in relation to ischaemic stroke, higher than would be expected due to stroke itself [18].

Several possible aetiologies of stroke in COVID-19 patients include hypercoagulable state, cardioembolism, and direct viral-induced pathology of the endothelium, whereas increased mortality rate of stroke patients could be, at least partly, explained by limited hospital resources [19]. A previous meta-analysis of 109 articles showed that the risk of mortality was higher in older patients, which was similar to the results of our study, but additionally pointed to male gender, dyspnoea, diabetes mellitus, and hypertension as risk factors for death [20]. We were unable to replicate these findings, probably due to the small number of patients who died during our study.

Our study showed that neurological symptoms were found in 84.5% of patients hospitalised due to COVID-19. This proportion was quite high compared to the previous retrospective studies from Wuhan [3] and Washington state [5]. However, in contrast to previous research, 82% of our patients were followed-up prospectively during hospitalisation with a detailed clinical questionnaire. Therefore, physicians should proactively ask patients with COVID-19 about neurological symptoms in the course of hospitalisation. Additionally, most neurological symptoms occurred at the beginning of hospitalisation which was similar to the experience of Chinese authors [21].

The most common neurological symptoms in our cohort were fatigue, decreased mood, myalgia, muscle weakness and headache. A recent review of the literature showed that the majority of COVID-19 patients complained of non-specific neurological manifestations early in the disease course, with headache, dizziness, excessive tiredness, myalgia, anosmia/hyposmia, and ageusia/dysgeusia being the most common [22]. Even in a detailed study of 53 patients encompassing data of cerebrospinal fluid and radiological analysis together with electroencephalography, the most common neurological features of hospitalised COVID-19 patients, apart from abnormalities in neurological examination, were cognitive impairment, hyposmia, headache, general muscle weakness and pain [23]. These symptoms usually occurred during the acute phase of COVID-19 infection, following an incubation period of 5-6 days, but some of them, especially fatigue and cognitive impairment, became chronic during the post-infectious phase [22].

Mechanisms leading to neurological symptoms in COVID-19 might comprise hypoxia and hypercoagulability [12]. Moreover, the SARS-Cov-2 virus exerts its neurotrophic effect by interaction with ACE-2 receptor which is found in the brainstem among other body localisations [24, 25]. As shown recently, invasion of hypothalamic circuits by the SARS-Cov-2 virus may be responsible for mediating both central and peripheral nervous system symptoms [26]. The role of hyperactivity of the immune system with the cytokine storm has also been considered [27].

Our study has several important limitations. Firstly, we performed additional tests to evaluate the presence of subjective neurological symptoms only in a minority of patients. Secondly, we did not analyse the significance of paraclinical tests such as bloodwork or chest X-ray, which was beyond the scope of this report. Thirdly, due to severe neurological state, in some patients it was impossible to assess the presence of subjective neurological symptoms such as headache or decreased mood. Fourthly, there was a risk of selection bias due to the small sample of patients recruited in the Departments of Internal Medicine and Otorhinolaryngology. And fifthly, the small number of patients who died during hospitalisation did not allow for multivariate analysis.

Conclusions

Our study demonstrates that neurological symptoms occur in most hospitalised patients with COVID-19 and that some of them, such as a decreased level of consciousness and delirium, increase the risk of in-hospital mortality. Future studies on larger patient populations are needed to evaluate how the presence of these neurological manifestations could be incorporated into prognostic scales for patients with COVID-19.

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Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia associated with an A792D mutation in the CSF1R gene in a Polish patient

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To the Editors

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare cerebral white matter disease characterised by motor and neuropsychiatric symptoms, including pyramidal and extrapyramidal signs, personality changes, cognitive impairment, depression and seizures.

Cognitive impairment and psychiatric symptoms are the most common initial symptoms. It is prevalent in both sexes equally. The mean age at disease onset is 43, with women developing the disease approximately seven years earlier than men. The most commonly reported magnetic resonance imaging findings in patients with ALSP are: bilateral white matter lesions (96% of cases), thinning of the corpus callosum (88%), abnormal signal of the pyramidal tracts (58%), calcifications in the white matter (54%), and diffusion-restricted lesions (38%). No apparent phenotype-genotype correlations have been found [1].

Colon-stimulating factor-1 receptor (CSF1R) is a transmembrane tyrosine kinase receptor that is expressed in phagocytic cells, including microglia in the brain. The activation of CSF1R through auto-phosphorylation contributes to signal transduction, maintenance, and activation of microglia [2]. ALSP is also known as *CSF1R*-related leukoencephalopathy, and is representative of primary microgliopathies.

Microglia are resident macrophages of the central nervous system, and their unique molecular signature is dependent upon CSF-1 signalling. It has been proved that microglial populations in affected frontal white matter in ALSP differ from microglia in unaffected frontal grey matter and cerebellar white matter. This finding suggests a potential mechanism of disease pathogenesis by linking aberrant CSF-1 signalling to altered microglial phenotype [3].

To date, many different mutations of *CSF1R* have been reported including missense, splice-site, nonsense and deletion mutations. Almost all are located in the tyrosine kinase domain [1]. Furthermore, it has been suggested that frameshift mutations outside the tyrosine kinase domain are able to cause ALSP by haploinsufficiency [4]. This condition is inherited in an autosomal dominant pattern, with sporadic cases due to *de novo* mutation also reported [1].

In 2015, a novel A792D mutation in the *CSF1R* gene was described in two Japanese family members. Their initial symptoms, including cognitive impairment, were likely to correspond with previously reported clinical characteristics. The disease profile of the cases was late onset (age 51 on average), and long duration (> 12 years on average) [5].

We here present the case of a Polish Caucasian patient with A792D mutation and rapid disease progression.

A 35-year-old male, with no family history of neurological disorders, developed gait and postural disturbances, dysarthria and bradykinesia. These were followed by cognitive decline and emotional lability at age 36, and urinary incontinence and erectile dysfunction at age 37.

On neurological examination three years after the onset of symptoms, he demonstrated psychomotor slowing,

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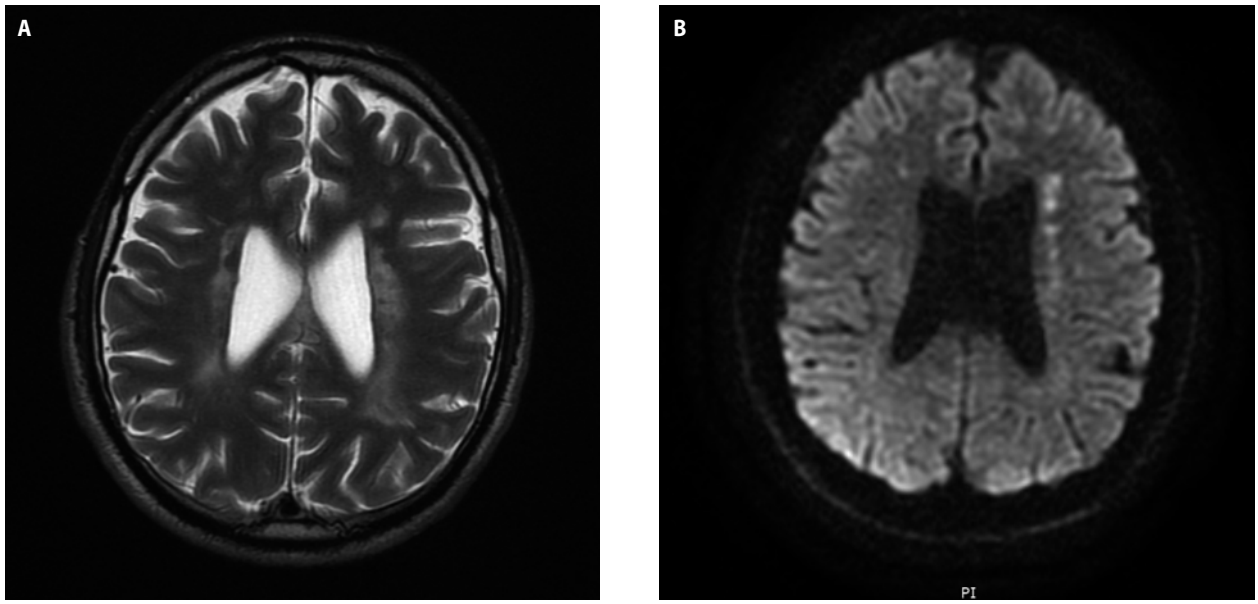

Figure 1. A. Magnetic resonance T2 imaging. Extensive, multifocal signal abnormalities in periventricular and deep white matter. Lateral ventricles and spaces are enlarged; **B.** Diffusion-weighted magnetic resonance images reveal multiple foci of diffusion restriction

a mask-like expression, dysarthria with monotonous speech, spasticity of the lower limbs symmetrically with bilateral positive Babinski sign, rigidity, ataxia of all four extremities, and postural instability. After three years of symptoms, his EDSS (Expanded Disability Status Scale) score was 5 (range 0–10). [Supplementary materials: Video 1–3].

A neuropsychological examination performed three years after the onset of symptoms showed mild cognitive dysfunction, especially in terms of memory, attention and articulacy. In addition, executive and behavioural disorders were revealed, as well as problems with cognitive inhibition, working memory and mental flexibility.

MR imaging showed confluent multiple patchy and confluent T2 hyperintense foci in the deep cerebral white matter bilaterally with associated atrophy. Diffusion-weighted images revealed punctuate foci of restricted diffusion in the deep left frontal white matter with normal signal on ADC maps. Sagittal T2-weighted scanning showed the corticospinal tract to be affected, with thin appearance of corpus callosum [Fig. 1].

Due to the suspicion of ALSP, genetic analysis of the *CSF1R* gene was performed. A c.2375C > A mutation in exon 18 of *CSF1R* was identified. This variant in the *CSF1R* gene was not detected in the patient's parents.

Recently, a case with clinical features suggestive of CSF1R-related leukoencephalopathy was described in the Polish population, but without a genetic confirmation of CSF1R gene mutation status [6, 7]. However, our case is the first genetically confirmed CSF1R-related leukoencephalopathy patient in Poland.

The clinical features of the affected Japanese family members with the A792D mutation in the *CSF1R* gene did not vary from previous reports. However, their age at onset was 51 years on average (range 43–54) and disease duration was > 12 years on average (range 6–29) suggesting a possible link between this type of mutation and the clinical profile of late-onset and long duration of ALSP [5].

We present a Caucasian patient with a *de novo* A792D mutation and different clinical manifestation. With initial symptoms of motor dysfunction at the age of 35 and serious decline within three years of first symptoms, his clinical profile can be described as rapidly progressive.

We believe further studies are required to reveal the phenotype-genotype correlation and to establish risk factors of a rapidly progressive clinical course of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia.

Search terms: *gene expression studies, leukodystrophies*
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Facial onset sensory and motor neuronopathy syndrome — a rare variant of motor neurone disease

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To the Editors

A 65-year-old right-handed woman reported a four-year history of numbness of the left side of her face as well as in her left fingers and forearm. Then, slowly progressive weakness of the muscles of the left hand along with the left wrist flexors appeared, accompanied by global atrophy of the left upper limb muscles. In 2016, an ambulatory nerve conduction study (NCS) revealed selective axonal sensory neuropathy in the left upper limb, although a needle electromyography was not done. In 2017, magnetic resonance imaging (MRI) of the cervical spine showed discopathy at C5-C6 and C6-C7 levels. However, no improvement was noted after surgery, and the patient complained of stiffness and a pulling sensation of the neck, left upper limb and trunk. Due to progressive and mainly distal weakness of the left upper limb, the patient started dropping objects that she was holding, and was unable to lift her arm above the level of her head. In addition, she reported fatigue of the masseter muscles while eating and periodically less clear, slurred speech, without swallowing impairment. Paresthesia of the face, neck and left limb were unresponsive to pregabalin, gabapentin and antidepressants treatment.

In 2018, the patient was for the first time admitted to the Department of Neurology at the Medical University of Warsaw. Neurological examination revealed peripheral left facial nerve paresis, reduced pain sensation, temperature and touch in all divisions of the left trigeminal nerve, atrophy of the shoulder girdle muscles on the left and slightly of the left upper extremity, fasciculations in the left triceps muscle, slight weakness of the wrist flexors and extensors, hand and left hand fingers, and disturbed sense of pain, temperature and touch on the left upper limb. Anti-ganglioside, anti-myelin associated glycoprotein, anti-sulfatide, and anti-acetylcholine receptor antibodies were negative. Results of cerebrospinal fluid analysis, Lyme serology, lung functional tests, motor-evoked potentials for right thumb abductor and right short toe flexor muscles, brain and cervical spine MRI were not relevant. Brachial plexus MRI with contrast revealed asymmetrical atrophy of the rotator cuff, rhomboid, deltoid, serratus anterior, latissimus dorsi and biceps brachii muscles. There were no changes of the brachial plexus cords bilaterally (Fig. 1 A–B). NCS confirmed axonal sensory neuropathy in the left upper limb (Tab. 1). A needle electromyography recording from the muscles of the left upper limb showed significant denervation in the muscles supplied by C5-Th1 segments, with features of chronic reinnervation and marked reorganisation of motor units. Chronic reinnervation was also present in the left vastus lateralis muscle. The blink reflex indicated abnormal conduction at the level of the brainstem, as both sides R2 components had prolonged latency after left stimulation. Treatment with steroids (5 g methylprednisolone i.v. in October 2018) followed by intravenous immunoglobulins (105 g, 2.0 g/kg bw in July 2019 and two maintenance doses: 50 g in September 2019 and 60 g in October 2019) was introduced without any improvement. Finally, a diagnosis of facial onset sensory and motor neuronopathy syndrome (FOSMN) — a rare variant of motor neurone disease (MND) — was established, and riluzole 100mg/d was started.

In June 2020, the patient was readmitted to the Department of Neurology with severe progression of dysphagia. She was underweight, with a body mass index of 18 kg/m^2 (an unintentional loss of 22 kg since April 2019). Neurological

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Table 1. Nerve conduction studies

Motor nerves	Right		Left	
	Amp [mV]	CV [m/s]	Amp [mV]	CV [m/s]
Median	7.0	58.3	4.4	58.3
Ulnar	9.1	62.1	6.9	54.3
Radial	not done		4.5	70.0
Peroneal	1.4	41.3	0,3	54.8
Tibial	not done		13.9	52.7
Sensory nerves	**Right**		**Left**	
	Amp [uV]	CV [m/s]	Amp [uV]	CV [m/s]
Median	32	55.0	2.6	68.8
Ulnar	13	65.1	1.4	73.3
Radial	40	68.6	2.6	67.8
Peroneal superficial	not done		11	57.1
Sural	not done		8.8	59.1
Lateral antebrachial cutaneous	12	74.3	not done	

examination revealed absent corneal reflex on the left and weak on the right, no palatal and pharyngeal reflexes, dysarthria, peripheral left facial nerve paresis and impaired sense of touch on the left side of the face, weakness of the masseter muscles, weakness of axial muscles and head droop, left hemiparesis with global atrophy of the upper limb (the patient was able to abduct the arm to 10 degrees), and left side hypoaesthesia. She was able to walk alone, and could perform the squat manoeuvre with support. NCS results were comparable with the previous set, but needle electromyography showed progression of neurogenic changes in right biceps brachii, with reinnervation of muscles that previously were not affected (right first interosseus dorsalis and vastus lateralis). Percutaneous endoscopic gastrostomy (PEG) was performed.

Facial onset sensory and motor neuronopathy was first described by Vucic et al. in 2006 as a 'syringomyelia-like' condition [1]. It is characterised by facial onset sensory abnormalities which can spread to the scalp, neck, upper trunk and extremities, followed by lower motor neurone involvement. Bulbar symptoms, such as dysarthria and dysphagia, muscle weakness, cramps and fasciculations, can present later. It affects both genders, with a male-to-female ratio of 1.92:1, the mean age at onset is 54.0 years, and the mean disease duration is 8.9 years [2].

The diagnosis of FOSMN is based on medical history and clinical characteristics. Distinctive electrophysiological findings include reduced amplitude of sensory nerve action potentials. The blink reflex is usually abnormal, with either a delayed or absent R2 response. The pathogenesis of FOSMN syndrome is unclear. The possibility of an immunological mechanism was raised after the confirmation of auto-antibodies in a few patients. While some patients have partially responded to various immunotherapies, an immunological mechanism has been considered. There is the possibility of a potential pathophysiological link with amyotrophic lateral sclerosis (ALS), although there have been no familial cases of FOSMN to date [3]. Post-mortem evidence of sensory and motor neuronal degeneration within the trigeminal sensory nuclei, dorsal root ganglion, brainstem and spinal cord motor nuclei has been described [1]. FOSMN should be differentiated neurophysiologically from brachial plexus injury, as well as syringomyelia and other brainstem pathologies, trigeminal sensory neuropathy, motor neurone disease and Kennedy's Disease [4].

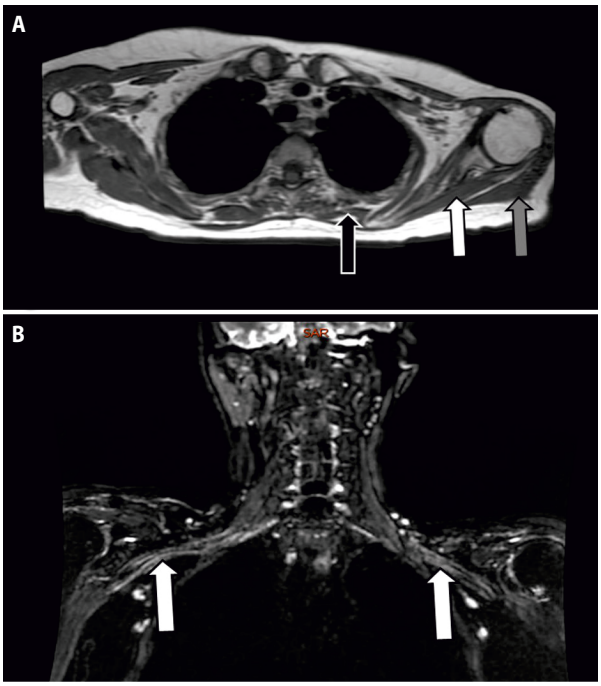

Figure 1. Magnetic resonance imaging. Atrophy of rotator cuff (white arrow), rhomboid (black arrow) and deltoid muscles (grey arrow) on left side on axial T1-weighted image (**A**), and similar thickness of brachial plexus cords bilaterally on STIR image (white arrows) (**B**)

It is worth discussing in more detail damage to the brachial plexus, which poses a serious diagnostic and therapeutic medical challenge. The basis for the diagnosis of brachial plexus function is a clinical examination and neurophysiological studies. It is characteristic that the amplitudes of sensory potentials decrease from five days post-damage, reaching their lowest values after 11 days. In the case of compound muscle action potentials (CMAP) amplitudes, abnormalities can occur 3-7 days after injury. Pathological changes in muscle function appear approximately three weeks after injury. The first NCS examinations should be carried out up to 3–4 weeks after the injury, as the Wallerian degeneration process will end. Incorrect parameters are shown first in motor, rather than sensory, potentials [5]. Maintaining the correct CMAP amplitude with accompanying muscle weakness at least seven days after injury suggests neuropraxia. If the difference in amplitude between the symptomatic and asymptomatic side is 50–75%, this indicates a moderate axons loss; more than 75% unregistered indicates axonotmesis or neurotmesis. F wave study should be performed only with reference to the long nerves. If the sensory nerve action potential (SNAP) is recorded, this suggests proximal damage to the sensory neuron. If the SNAP amplitude is reduced, or the sensory potential has not been recorded, this suggests a postganglionic plexus damage. In needle electromyography studies, denervation activity can be recorded 10–14 days after injury [5].

In conclusion, patients with FOSMN syndrome typically present with insidious, slowly evolving unilateral or bilateral numbness of the face. This is followed by bulbar and proximal (neck and arms) weakness. Despite the fact that FOSMN is mainly a lower motor neurone disease, some upper motor neurone signs make this condition an ALS mimic. Where appropriate, clinicians should consider percutaneous gastrostomy.

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