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CONTENTS

Volume 13, Number 3, 2024

Policy Forum

Review

Original Article

Brief Report

Correspondence

Letter

Policy Forum

DOI: 10.5582/irdr.2024.01026

Guardians of memory: The urgency of early dementia screening in an aging society

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SUMMARY The global aging population has led to a significant rise in the prevalence of age-related non-communicable diseases such as dementia and other cognitive disorders. In 2019, there were 57.4 million people with dementia worldwide, and this number is projected to triple by 2050. Intervening in and managing 12 potentially modifiable dementia risk factors can prevent or delay the onset and progression of about 40% of dementia cases. Neuroimaging, biomarkers, and advanced neuropsychological testing offer promising pathways for the early detection of dementia. Emphasis should be placed on educating the public about the importance of brain health and the early signs of cognitive impairment, as well as promoting dementia prevention measures. Adopting a healthy lifestyle - including a balanced diet, regular physical exercise, active social engagement, cognitive activities, and avoiding smoking and excessive alcohol consumption - can help reduce the risk of cognitive decline and prevent cognitive disorders. Government policies on dementia prevention and health care, along with early and regular dementia screening programs, can enhance the early identification and management of individuals at risk. In addition, integrating cognitive health assessments into routine medical check-ups is essential for the early screening and management of dementia.

Keywords dementia, aging, biomarker, neuroimaging, neuropsychological testing, diagnosis

The global elderly population is undergoing a notable expansion in both size and proportion. According to projections by the World Health Organization, the number of individuals age 60 and above is expected to reach 1.4 billion by 2030, and this figure is forecasted to rise to approximately 2.1 billion by 2050. Moreover, from 2020 to 2050, the number of individuals age 80 and above is predicted to double, reaching approximately 426 million (*1*). As individuals age, the incidence of age-related non-communicable diseases like dementia and other cognitive disorders rises notably. Statistics indicate that in 2019, there were 57.4 million dementia patients worldwide, with projections suggesting this figure will triple by 2050 (*2,3*). Presently, almost 60% of dementia patients reside in low- and middle-income countries, and this proportion is expected to climb to 71% by 2050 (*4*). Dementia has emerged as the seventh leading cause of death globally, accounting for approximately $1.3 trillion in societal costs (*5*). A point worth noting is that around 75% of dementia cases worldwide are not diagnosed in a timely manner (*6*). Moreover, 80% of the population expresses concerns about developing dementia in the future, with approximately one-quarter believing it to be unpreventable (*4*).

As the population ages, there has been a notable surge in dementia cases. Most people believe that a definitive diagnosis will not change clinical management; therefore, the majority of dementia patients do not undergo relevant pathological examinations. A point worth noting is that the US Food and Drug Administration (FDA) recently approved two monoclonal antibody therapies targeting amyloid for individuals with mild cognitive impairment or mild dementia (*7*). The findings suggest that removing amyloid could alleviate the decline in cognitive function in individuals with mild dementia (*8,9*). Moreover, a 2020 study revealed that intervening in and managing 12 modifiable risk factors for dementia could prevent or delay its onset and progression by around 40%, and especially in regions with a high prevalence of dementia (*10*). Hence, early identification of at-risk individuals through diagnostic techniques could potentially alter the course of dementia progression.

Research has revealed the pathology of the disease in that amyloid deposits begin accumulating at least

Table 1. Neuroimaging, fluid biomarkers, and neuropsychological screening for dementia

Biomarkers	Significance	Key findings
Neuroimaging biomarkers		
Amyloid-PET/PiB PET	Identifying amyloid pathology in the brains of patients with AD.	Appears approximately 20 years before the earliest clinical symptoms of AD (*13*).
Tau-PET	Identifying tau accumulation as a biomarker for disease staging.	Reflects the progression of AD pathology and is associated with disease severity (*14*).
FP-CIT SPECT	Brain dopamine transporter imaging is highly sensitive and specific for diagnosis.	A mature biomarker for diagnosing DLB (*15*).
PK-PET	Shows the distribution of neuroinflammation in the brain across different types of dementia.	Neuroinflammation is a part of the pathophysiology of familial FTD (*16*).
Structural MRI	Evaluating the decline in brain structural regions facilitates dementia subtyping.	Structural decline occurred 4.7 years before symptom onset (*13*).
Functional MRI	Demonstrates differences in resting-state functional connectivity and identifies specific networks and regions affected in each type of dementia.	Abnormal activity in the DMN in AD (*17*).
FDG-PET	Indicates reduced local metabolism in the brain.	Metabolic reductions can be detected as early as more than 10 years before symptom onset (*11,13*).
Fluid biomarkers (CSF, blood)		
Aβ42, Aβ42/Aβ40	Aβ deposition.	The ratio of Aβ42/Aβ40 in plasma has a high concordance with the CSF Aβ42/Aβ40 ratio and amyloid PET status (*18*).
P-tau231, P-tau181, P-tau271, P-tau205	Neurofibrillary tangle formation.	P-tau181 and P-tau271 start to increase as early as 20 years before symptom onset (*19*).
T-tau, BD-tau, NfL	Synaptic dysfunction/neural degeneration.	Increased levels of NfL in CSF serve as a biomarker of neurodegeneration and can be assessed in blood (*20*).
GFAP	Neuroinflammation.	The increase in plasma GFAP concentration in patients with AD is greater than that in CSF (*21*).
Neuropsychology		
IQCODE, p-AD8, CAMCI	Self-administered tools.	The sensitivity or specificity in evaluating mild cognitive impairment is over 80% (*22*).
MMSE, MoCA, HDS-R, DemTect, MES, MEFO, CANTAB-PAL	Interview tools.	

Abbreviations: AD, Alzheimer's disease; CANTAB-PAL, Cambridge Neuropsychological Test Automated Battery-Paired Associates Learning; CAMCI, Computerized Assessment of Mild Cognitive Impairment; CSF, cerebrospinal fluid; DLB, dementia with Lewy bodies; DMN, default mode network; FDG, ^{18}F-Fluorodeoxyglucose; FTD, frontotemporal dementia; GFAP, glial fibrillary acidic protein; HDS-R, Revised Hasegawa's Dementia Scale; IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly; LMT, Logical Memory Test; MEFO, Memory, Fluency, and Orientation; MES, Memory and Executive Screening; MMSE, Mini–mental State Examination; MoCA, Montreal Cognitive Assessment; MRI, magnetic resonance imaging; NfL, neurofilament light; PiB PET, Pittsburgh compound B positron emission tomography; PK-PET, ^{11}C-PK11195 positron emission tomography; FP-CIT, ^{123}I-Ioflupane.

10 to 20 years before the onset of symptoms. By the time clinical manifestations of dementia appear, the disease is already at an advanced stage (*11*). In Europe, raising awareness about Alzheimer's disease (AD), implementing early screening, and facilitating prompt diagnosis are considered essential steps to implementing future disease management strategies, improving patient quality of life, and addressing the growing burden of the condition (*12*). Researchers are currently working to explore a range of new methods for early detection and diagnosis aimed at preventing and delaying the clinical progression of dementia. Recent technological advances have revolutionized the early diagnosis of cognitive impairment (Table 1).

Neuroimaging, biomarkers, and advanced neuropsychological testing offer promising ways to detect dementia in its early stages. Significant progress has been made in developing neuroimaging methods for AD biomarkers. These advances offer great potential in identifying underlying pathophysiological changes associated with dementia, such as structural decline (*e.g.*, volume reduction and cortical thinning), functional decline (*e.g.*, fMRI activity and network connectivity), decreased connectivity (*e.g.*, diffusion anisotropy), and pathological accumulation (*e.g.*, amyloid and tau positron emission tomography (PET)) (*13,23*) (as shown in Figure 1). Molecular imaging with PET is used to identify amyloid plaque deposition and tau tangle pathology patterns, thereby aiding in the diagnosis of AD (*24*). Molecular imaging with PET can not only reveal the

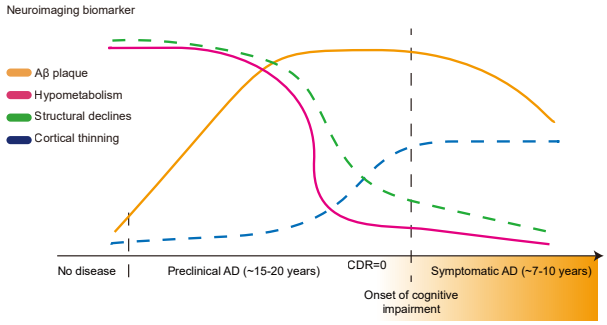

Figure 1. Neuroimaging assessment of Aβ aggregation, metabolism, and structural changes prior to the onset of Alzheimer's disease symptoms.

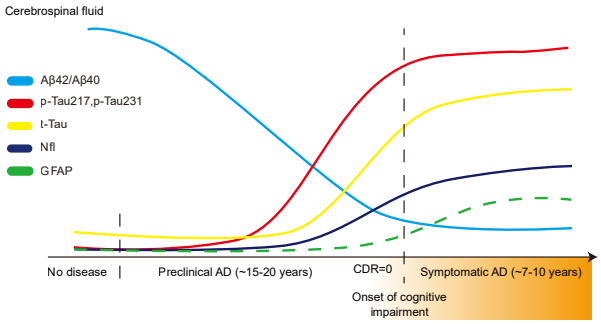

Figure 2. The pathological changes of Alzheimer's disease in cerebrospinal fluid, including amyloid deposition, tau accumulation, neuroinflammation, and neuronal degeneration.

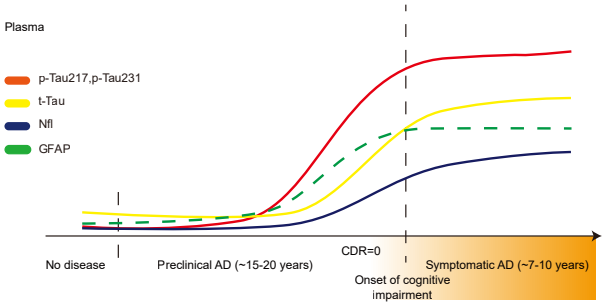

Figure 3. The occurrence of plasma tau accumulation, synaptic dysfunction, and neuronal degeneration before the onset of dementia symptoms.

pathological features of AD that begin decades before the onset of symptoms and assess the loss of dopaminergic terminals in Parkinson's disease, but it can also, with the development of new tracers for neuroinflammation and synaptic density, further elucidate the pathobiological changes characteristic of dementia (*14*). The further development of neuroimaging techniques requires more longitudinal studies with larger sample sizes, combined with advanced imaging modeling methods (such as artificial intelligence (AI)), to establish their clinical utility.

Using one or more AT(N) biomarkers has proven to be advantageous in diagnosing early dementia (*25*). Fluid biomarkers are less invasive and have a sensitivity similar to that of PET imaging in diagnosing dementia. By detecting forms of Aβ, tau, neuroinflammatory proteins, as well as markers of neuronal dysfunction and degeneration in cerebrospinal fluid (CSF) and plasma, AD can be distinguished and diagnosed from other neurodegenerative diseases, and these fluid biomarkers have significant value in predicting future cognitive deterioration (*26*). The soluble properties and post-translational modifications of tau in CSF and plasma are emerging as sensitive and reliable biomarkers for detecting tau pathology in AD. Research has shown that pThr181, pThr217, and pThr231 tau can accurately differentiate between amyloid PET-positive and amyloid PET-negative individuals (*27*). These changes help reflect the alterations in neuronal tau metabolism in the preclinical stages of AD when Aβ has aggregated (*28*).

Glial fibrillary acidic protein (GFAP) expressed by astrocytes in plasma is currently the only AD-related biomarker that outperforms its corresponding CSF measurement (*21*) . Another biomarker is neurofilament light (NFL), which is a component of the axonal cytoskeleton. NFL is released during axonal injury, leading to increased concentrations in CSF and blood. It has been found to be elevated in the CSF and plasma of individuals with mild cognitive impairment (MCI) and preclinical AD (*20*). Elevated levels of structural proteins appear to reflect synaptic loss during AD and neurodegeneration (*29*). In the CSF of patients with AD, synaptic proteins such as neuronal pentraxin 2 (NPTX2), neurogranin, and SNAP-25 are changed and may serve as biological markers for early diagnosis of AD (*30*). Therefore, monitoring fluid biomarkers helps reflect Aβ aggregation, glial activation, tau metabolic changes, synaptic dysfunction, and neurodegenerative changes in patients with preclinical dementia (*21,31*) (as shown in Figure 2,3). Using established and emerging biomarker technologies can reveal the mechanisms underlying AD and identify the earliest biological changes in the brain, marking different aspects of AD pathology for diagnostic and prognostic purposes.

Moreover, digital health technologies, including wearable devices and mobile applications, aid in the continuous monitoring of cognitive function (*32*). Digital spatial navigation can monitor early behavioral changes associated with dementia, while gait measurements facilitate early screening for dementia patients exhibiting prominent motor impairments such as dementia with Lewy bodies (DLB), vascular dementia (VaD), and Parkinson's disease dementia (PDD) (*33*). AI and machine learning algorithms are used to analyze the large datasets generated by these technologies, thus enhancing the sensitivity, accuracy, and specificity of early diagnosis (*34,35*).

Preventive measures are equally crucial in addressing cognitive impairment. At the individual level, adopting a healthy diet, engaging in regular physical exercise,

maintaining active social interactions, participating in cognitive activities, refraining from smoking, and abstaining from alcohol help mitigate the risk of cognitive decline (*36*). Public health initiatives should stress the significance of brain health and early signs of cognitive impairment and advocate for preventive measures against dementia. Notably, governments implementing healthcare policies for dementia prevention and conducting early and regular dementia screening programs aid in early identification and management of individuals at risk (*37*). Moreover, integrating cognitive health assessments into routine medical check-ups is essential for early screening and dementia management.

As we address the challenges of an aging society, what is imperative is to prioritize early diagnosis and prevention strategies for cognitive impairment. Use of new technologies for early detection and promoting preventive measures can significantly enhance individuals' quality of life and alleviate the societal and economic burdens associated with cognitive impairment. Collaborative efforts among healthcare providers, policymakers, and the medical technology industry will be vital in effectively managing cognitive impairment in our aging population.

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Review

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Spontaneous pneumomediastinum: A comprehensive review of diagnosis and management

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SUMMARY Pneumomediastinum is a rare condition defined by the presence of air in the mediastinum. In the absence of traumatic injury, iatrogenic injury, or clear etiology, it is called spontaneous pneumomediastinum (SPM). Spontaneous pneumomediastinum most commonly occurs in younger individuals and has a self-limiting course with a good outcome. The purpose of the present manuscript is to systematically review the existing literature on SPM evaluation and management for updated clinical understanding of this condition. A literature search was conducted of publications about SPM on MEDLINE/PubMed and Google Scholar by identifying all the articles with key search terms "pneumomediastinum" and "spontaneous pneumomediastinum". Inclusion criteria were case series published in English between 1980 and 2023. In total, 24 case series were selected and reviewed to determine presenting symptoms, clinical signs and predisposing factors associated with spontaneous pneumomediastinum. Most patients were male; the average age at diagnosis was 26.3 years. The most common presenting symptoms were chest pain and dyspnea. The most common exam finding was subcutaneous emphysema, in 35.4% of patients. Only 5.9% had the classic Hamman's sign. Risk factors include history of asthma, history of smoking, and recent physical activity. This manuscript presents an extensive review of relevant literature highlighting the diagnosis and essential management of spontaneous pneumomediastinum.

Keywords spontaneous pneumomediastinum, mediastinal emphysema, chest pain, subcutaneous emphysema, systematic review

1. Introduction

Pneumomediastinum (PM), or mediastinal emphysema, was first described in 1819 and is defined as the presence of air within the mediastinal cavity (*1*). PM often occurs in the setting of trauma, blunt or penetrating, or conditions causing changes in intrathoracic pressure, lung disease, childbirth, physical activity, *etc*. Spontaneous pneumomediastinum (SPM) is the presence of air in the mediastinum without a clear etiology. The most common presenting symptoms are dyspnea and chest pain. SPM may be associated with subcutaneous emphysema on physical exam and Hamman's sign on cardiac auscultation (*2*). While previous studies enumerate the presenting clinical characteristics of SPM, there is a paucity of work describing the various triggering events or predisposing risk factors of this interesting clinical entity. In addition, although SPM has been reviewed by many authors, over the last few years there has been a greater appreciation for atraumatic SPM, particularly from viral infection (*e.g*. COVID-19) and underlying collagen vascular disorders (*3*).

Thus, the purpose of the present manuscript is to systematically review the existing literature on SPM evaluation and management to create an up-to-date understanding of this condition, as well as a schema for clinical use.

2. Literature search strategy

We systemically searched clinical literature databases, including MEDLINE/PubMed and Google Scholar, for case series on SPM published in English between 1980 and 2023 following PRISMA guidelines (Figure 1). Key search terms included "spontaneous pneumomediastinum" and "pneumomediastinum". Single case reports and manuscripts reporting on less than 10 patients were not considered. Abstracts of all these

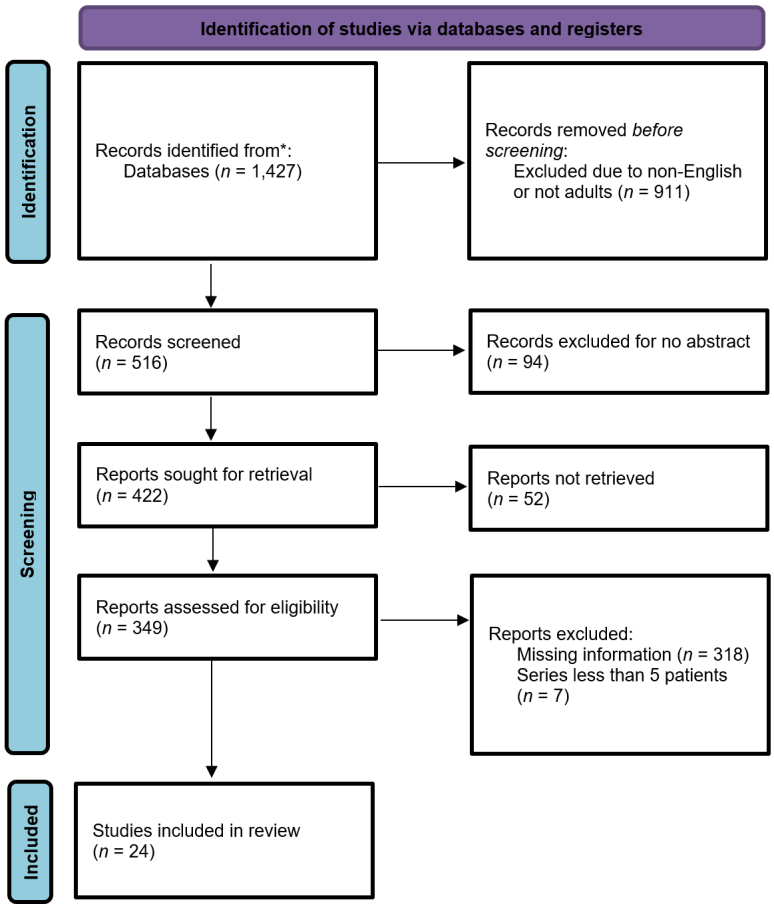

Figure 1. PRISMA flow diagram.

articles were independently screened by two authors (SR, SK) to assess eligibility, with discrepancies resolved by senior author (AT). Full texts of selected articles were then reviewed, and reference list were examined for additional relevant studies.

All data was accessed between August to September 2023. Extracted Information included demographic population, symptoms, clinical findings, triggering events, comorbidities and management strategies for each included patient. Data (means age and range for variable studied) were calculated using Microsoft Excel.

3. Results

The abstracts of 1,427 publications were screened for inclusion criteria eligibility (Figure 1). After selecting for case series published in the English language reporting at least 10 cases of SPM in the adult population, we found 24 articles for full text review. These case series included a total of 1,134 patients who had an eventual diagnosis of spontaneous pneumomediastinum (*4-27*). Clinicodemographic information for these patients was outlined in Table 1. The mean age, of this cohort was determined to 26.33 years, with an age range spanning from 2 to 87. Notably, most cases, 73% (n = 828) were male patients, while 27% (306) were female patients.

3.1. Features of presentation

Of the 24-case series reviewed, the most common presenting symptom was chest pain, occurring in 59% of patients (n = 674) (Table 2). The occurrence of chest pain as a presentation varied from 100% (*14*) in some cases to 16% (*16,27*) in others across different series. Other common symptoms included dyspnea, reported in 31% (n = 352), cough in 10% (n = 112), neck pain in 23% (n = 259), and dysphagia in 10% (n = 118). There were also sporadic complaints of throat pain, odynophagia, dysphonia, lightheadedness, hoarseness, weakness, fever, nausea, back pain, nasally sounding voice (rhinolalia), shoulder pain, swelling of the face, swollen neck, throat discomfort, asthenia and central abdominal pain.

Clinical examination revealed the presence of subcutaneous emphysema in 35.4% (n = 402) of the patients. Various authors have reported the presence of subcutaneous emphysema ranging from 100% (*14,26*) to 3% of the cases (*9*). However, the presence of Hamman's sign, a crunching or bubbling sound over the mediastinum synchronous with the heartbeat was identified in only 5.9% (n = 67) of the patients (*1,28,29*). Associated pneumothorax (*24*) was present in 5.2% (n = 60). Other less commonly reported findings were vomiting, pneumopericardium, pulsus paradoxus,

Table 1. Demographic data on patients diagnosed with spontaneous pneumomediastinum

Ref.	Sample (*n*)	Mean age (Range) (years)	Male/Female	Length of stay (days)	Follow up (months)	Recurrence
Potz *et al.* (*4*)	249	38.7 (17-81)	151/98	2.8	0.46	0
Al-Mufarrej *et al.* (*5*)	17	25.5 (19-39)	11/6		6.72	0
Bakhos *et al.* (*6*)	49	19	26/23	1.8	24-84	1
Dionisio *et al.* (*7*)	18	35.4 (18-87)	12/6	10.5	1-76	0
Yamairi *et al.* (*8*)	71	19.3 (7-48)	53/18	6.3		2
Yu *et al.* (*9*)	237	23.4	222/15	7.5	48.6	11
Freixinet *et al.* (*10*)	32	21.4	25/7	3.2	12-228	0
De Giacomi *et al.* (*11*)	25	(18-82)	10/15			0
Iyer *et al.* (*12*)	62	(20-69)	41/21			1
Okada *et al.* (*13*)	20	22 (13-41)	19/1	7		0
Mondello *et al.* (*14*)	18	25 (5-34)	10/8	6	1	0
Abolnik *et al.* (*15*)	25	18.8 (8-31)	21/4	6.3	87.4	2
Newcomb *et al.* (*16*)	18	(11-58)	14/4			0
Koullias *et al.* (*17*)	24	17.5 (15-26)	18/6	2	36-120	0
Kobashi *et al.* (*18*)	17	19.5	12/5			
Halperin *et al.* (*19*)	10	21.2 (2-56	7/3			
Jougon *et al.* (*20*)	12	25 (16-46)	11/1	4	19	0
Song *et al.* (*21*)	45	18.96	35/10	3.93		0
Weiss *et al.* (*22*)	14	22.5 (18-30)	9/5	2.2		
Macia *et al.* (*23*)	41	21.3 (14-35)	34/7	5		1
Caceres *et al.* (*24*)	28	27 (3-71)	16/12	3	12-120	0
Kaneki *et al.* (*25*)	33	17.6 (13-27)	26/7			0
Perna *et al.* (*26*)	47	27.3 (16-42)	33/14	3.5		
Weissberg *et al.* (*27*)	22	(15-37)	12/10	3.5	12	0
Total	1134	26.33	828/306	4.76		18

Percentages are given in parentheses. Blank cells indicate this information was omitted from the reference.

Table 2. Chief complaints at the time of diagnosis of spontaneous pneumomediastinum

Ref.	Chest pain	Dyspnea	Neck pain	Dysphagia	Cough	Throat pain	Odynophagia	Dysphonia
Potz *et al.* (*4*)								
Al-Mufarrej *et al.* (*5*)	10 (59)	7 (41)	2 (12)				3 (18)	
Bakhos *et al.* (*6*)	32 (65)	25 (51)	14 (29)	4 (8)				
Dionisio *et al.* (*7*)	14 (78)	15 (83)	10 (56)	5 (28)	10 (56)		3 (17)	
Yamairi *et al.* (*8*)	51 (72)	23 (32)	31 (44)	29 (41)		31 (44)		
Yu *et al.* (*9*)	211 (89)	78 (33)	123 (52)					
Freixinet *et al.* (*10*)	25 (78)	13 (41)		2 (6)	3 (9)			
De Giacomi *et al.* (*11*)	7 (28)	11 (44)			3 (12)			
Iyer *et al.* (*12*)	39 (63)	27 (44)	11 (18)	3 (5)	28 (45)			3 (5)
Okada *et al.* (*13*)	15 (75)	8 (40)	2 (10)	10 (50)	1 (5)	5 (25)		
Mondello *et al.* (*14*)	18 (100)	16 (88)	8 (44)	4 (22)	14 (77)			12 (66)
Abolnik *et al.* (*15*)	22 (88)	15 (60)	12 (48)	10 (40)				
Newcomb *et al.* (*16*)	16 (89)	12 (67)	2 (11)	3 (17)				1 (6)
Koullias *et al.* (*17*)	16 (67)	2 (8)		2 (8)	10 (42)	6 (25)		
Kobashi *et al.* (*18*)	14 (82)			1 (6)				
Halperin *et al.* (*19*)	5 (50)	6 (60)						
Jougon *et al.* (*20*)	6 (50)		3 (25)				1 (8)	
Song *et al.* (*21*)	33 (41)	15 (19)		1 (1)	1 (1)	31 (38)		
Weiss *et al.* (*22*)	11 (79)	8 (57)					5 (36)	
Macia *et al.* (*23*)	35 (85)	20 (49)	18 (44)	5 (12)	10 (24)		15 (37)	5 (12)
Caceres *et al.* (*24*)	15 (54)	11 (39)			9 (32)		1 (4)	
Kaneki *et al.* (*25*)	33 (100)	19 (58)	23 (70)	13 (39)				
Perna *et al.* (*26*)	28 (60)	11 (26)		18 (38)	15 (33)			
Weissberg *et al.* (*27*)	18 (16)	10 (9)		8 (7)	8 (7)			
Total	674 (59)	352 (31)	259 (23)	118 (10)	112 (10)	73 (6)	28 (2)	21 (2)

Percentages are given in parentheses. Blank cells indicate no information was given regarding that chief complaint. The less common complaints were Fever (1%), Lightheadedness (1%), Weakness (1%), Back pain (1%), Nausea/Emesis (0.4%), Hoarseness (0.4%), Abdominal pain (0.1%), Shoulder pain (0.1%), Swollen neck (0.1%), Rhinolalia (0.1%), Asthenia (0.07%).

hemoptysis, neck swelling (*i.e.* cervical emphysema), pneumorrhachis and pneumoperitoneum (Table 3).

3.2. Predisposing factors

Of the 24-case series reviewed, the most common triggering event or predisposing risk factor identified was a history of asthma, present in 21.9% (n = 248) of the patients (*5-8*), with reported prevalence ranging from 42% (*9*) to 2% (*21*) (Table 4). A history of smoking or tobacco use was identified in 16% (n = 182) of the patients. Physical activity or sports-related

pneumomediastinum was reported in 14.5% (n = 164) cases. A detailed analysis revealed that emesis occurred in 8% (n = 93) of patients, and bouts of cough in 15% (n = 172) of patients. A recent history of respiratory infection was present in 9% patients (n = 102). 53 patients (4.7%) had a history of interstitial lung disease (ILD), and a history of COPD was identified in 1.7% of the patients (n = 19).

Table 3. Clinical findings in spontaneous pneumomediastinum patients

Ref.	Subcutaneous emphysema	Hamman's sign	Pneumothorax	Crepitus	Pleural effusion	Esophageal perforation
Portz *et al.* (*4*)	39 (16)				27 (11)	24 (10)
Al-Mufarrej *et al.* (*5*)	7 (41)					
Bakhos *et al.* (*6*)	7 (16)		5 (11)	15 (30)		
Dionisio *et al.* (*7*)	15 (83)	1 (6)				
Yamairi *et al.* (*8*)	26 (37)	4 (6)				
Yu *et al.* (*9*)	6 (3)		9 (4)			
Freixinet *et al.* (*10*)	25 (78)		2 (6)			
De Giacomi *et al.* (*11*)	15 (68)		4 (18)	4 (16)		
Iyer *et al.* (*12*)	28 (45)		20 (32)			
Okada *et al.* (*13*)	9 (45)	2 (10)				
Mondello *et al.* (*14*)	18 (100)	8 (44)				
Abolnik *et al.* (*15*)	15 (60)	10 (40)				
Newcomb *et al.* (*16*)	14 (78)	4 (22)				
Koullias *et al.* (*17*)	12 (50)		2 (8)			
Kobashi *et al.* (*18*)	8 (47)	6 (35.3)				
Halperin *et al.* (*19*)	5 (50)	5 (50)	1 (10)			
Jougon *et al.* (*20*)	11 (92)					
Song *et al.* (*21*)	17 (38)		2 (4)			
Weiss *et al.* (22)				8 (57)		
Macia *et al.* (*23*)	29 (71)	5 (12)				
Caceres *et al.* (*24*)	11 (40)		2 (7)			
Kaneki *et al.* (*25*)	26 (79)	17 (52)				
Perna *et al.* (*26*)	47 (100)		7 (15)			
Weissberg et al.(27)	12 (11)	5 (4)	6 (5)			
Total	402 (35)	67 (6)	60 (5)	27 (2)	27 (2)	24 (2)

Percentages are given in parentheses. Blank cells indicate no information was given about patients presenting with that clinical finding. Less commonly reported findings were Hematemesis (1%), Pneumopericardium (1%), Pulsus paradoxus (1%), Hemoptysis (0.5%), Neck swelling (0.4%), Pneumorachis (0.08%), Decreased heart sounds (0.08%), and Pneumoperitoneum (0.08%).

Table 4. Triggering events and risk factors for spontaneous pneumomediastinum

Ref.	Asthma	Smoking/ Tobacco	Cough	Physical activity	Respiratory Infection	Vomiting/ Retching	Illicit drug use
Portz *et al.* (*4*)	32 (13)						
Al-Mufarrej *et al.* (*5*)	4 (24)			2 (12)		4 (24)	1 (6)
Bakhos *et al.* (*6*)	20 (41)	11 (22)	14 (29)	3 (6)		8 (16)	12 (24)
Dionisio *et al.* (*7*)	5 (28)	12 (67)	9 (50)	1 (6)	8 (44)	2 (11)	2 (11)
Yamairi *et al.* (*8*)	13 (18)		3 (4)	18 (25)		2 (3)	
Yu *et al.* (*9*)	99 (42)	92 (39)	72 (30)	56 (24)	66 (28)	34 (14)	
Freixinet *et al.* (*10*)	9 (28)	9 (28)		11 (34)			3 (9)
De Giacomi *et al.* (*11*)		9 (36)	4 (16)			2 (8)	
Iyer *et al.* (*12*)	9 (15)		5 (8)	2 (3)	5 (8)	4 (6)	6 (10)
Okada *et al.* (*13*)	4 (20)		3 (15)	8 (40)		2 (10)	
Mondello *et al.* (*14*)	8 (22)		12 (66)	6 (33)			
Abolnik *et al.* (*15*)	6 (24)	6 (24)	2 (8)	6 (24)	3 (12)		
Newcomb *et al.* (*16*)	7 (39)	6 (33)	3 (17)	3 (17)		2 (11)	4 (22)
Koullias *et al.* (*17*)	4 (17)		4 (17)	6 (25)	4 (17)	2 (8)	6 (25)
Kobashi *et al.* (*18*)			6 (35)				
Halperin *et al.* (*19*)	2 (20)		2 (20)	1 (10)	3 (30)	2 (20)	
Jougon *et al.* (*20*)	1 (8)			4 (33)			
Song *et al.* (*21*)	1 (2)		4 (9)	3 (7)		2 (4)	
Weiss *et al.* (*22*)	1 (7)	2 (14)	6 (43)		4 (29)	8 (57)	14 (100)
Macia *et al.* (*23*)	9 (22)	14 (41)	3 (7)	5 (12)	3 (7)	4 (10)	4 (10)
Caceres *et al.* (*24*)	6 (21)	8 (29)	2 (7)	1 (4)		10 (36)	
Kaneki *et al.* (*25*)			6 (18)	21 (61)			
Perna *et al.* (*26*)	8 (17)	13 (26)	4 (7)	7 (12)	6 (12)	3 (7)	26 (55)
Weissberg *et al.* (*27*)			8 (36)			2 (2)	
Total	248 (22)	182 (16)	172 (15)	164 (14)	102 (9)	93 (8)	78 (7)

Table 4. Triggering events and risk factors for spontaneous pneumomediastinum (continued)

Ref.	Invasive Procedures	Trauma	Vocal exercise	Thoracic Surgery	Interstitial lung disease	COPD
Portz *et al.* (*4*)						
Al-Mufarrej *et al.* (*5*)						
Bakhos *et al.* (*6*)						
Dionisio *et al.* (*7*)					2 (11)	
Yamairi *et al.* (*8*)			11 (15)			
Yu *et al.* (*9*)						
Freixinet *et al.* (*10*)						
De Giacomi *et al.* (*11*)					23 (92)	
Iyer *et al.* (*12*)					11 (18)	5 (8)
Okada *et al.* (*13*)			3 (15)			
Mondello *et al.* (*14*)						
Abolnik *et al.* (*15*)						
Newcomb *et al.* (*16*)					1 (6)	
Koullias *et al.* (*17*)					1 (4)	
Kobashi *et al.* (*18*)			3 (18)			
Halperin *et al.* (*19*)						1 (10)
Jougon *et al.* (*20*)						2 (17)
Song *et al.* (*21*)						
Weiss *et al.* (*22*)						
Macia *et al.* (*23*)						2 (17)
Caceres *et al.* (*24*)					2 (7)	1 (4)
Kaneki *et al.* (*25*)						
Perna *et al.* (*26*)						4 (7)
Weissberg *et al.* (*27*)	36 (32)	34 (30)	7 (6)	19 (17)		
Total	36 (3)	34 (3)	24 (2)	19 (2)	40 (3)	13 (1)

Percentages are given in parentheses. Blank cells indicate no information was given about patients presenting with that clinical finding. Less commonly reported findings were Hematemesis (1%), Pneumopericardium (1%), Pulsus paradoxus (1%), Hemoptysis (0.5%), Neck swelling (0.4%), Pneumorachis (0.08%), Decreased heart sounds (0.08%), and Pneumoperitoneum (0.08%).

4. Discussion

Spontaneous pneumomediastinum is a complex clinical entity with a reported incidence of less than 1:44,000 (*23*). It occurs most commonly in young males and generally has a benign course (*30*). The pathogenesis of SPM (termed the "Macklin Effect") involves alveolar rupture leading to air dissection along bronchovascular sheaths with eventual spread into the mediastinum (*31,32*). This process explains the occurrence of SPM in patients with a sudden increase in intrathoracic pressure (*12,24,25,33,34*). The absence of a discernable etiological factor in SPM presents a diagnostic challenge and opportunity for heightened comprehension. The present study is the most comprehensive review of SPM to date and reports a unique diagnostic framework for this condition. We have reviewed 24 published studies which included 1,134 patients with spontaneous pneumomediastinum (*4-27*) (Figure 1). To the best of our knowledge, our review comprises of the largest number of reported spontaneous pneumomediastinum cases. In doing so, this work beckons a broader discourse on the need for standardized diagnostic criteria, prognostic indicators, and therapeutic modalities tailored to the distinctive etiologies of SPM.

In our review, SPM most often presented as chest pain and dyspnea. Neck pain was also a prevalent symptom, which is under appreciated in existing reviews of SPM (Table 5). Common physical exam findings were subcutaneous emphysema, Hamman's sign, and associated pneumothorax, which is consistent with previous reports (Table 5). Given the nonspecific nature of SPM symptoms, the differential diagnosis for patients presenting with this condition tends to be broad, and may lead to delayed diagnosis (*25*). Once confirmed, effort should be made to investigate potential predisposing factors. In the present analysis, the common factors included asthma, history of smoking, cough, and sudden change in intrathoracic pressure (related to physical activity/sports) (Table 5).

Table 5. Most common clinical signs, symptoms and predisposing events reported in literature

	Highest- Lowest %
Subcutaneous emphysema (402/1134) (35.4)	100-2.5
Hamman's sign (67/1134) (5.9)	52-4.4
Pneumothorax (60/1134) (5.2)	32.2-3.8
Chest Pain (674/1134) (59.4)	100-15.8
Dyspnea (352/1134) (31.04)	88-8.3
Neck Pain (259/1134) (22.8)	70-10
Asthma (248/1134) (21.9)	41.7-7.1
Smoking/ tobacco (182/1134) (16)	100-22
Cough (172/1134) (15.2)	42.9-4.2
Sudden change in intrathoracic pressure (*i.e.* physical activity/ sports related) (164/1134) (14.5)	58.3-3.2

Percentages are given in parentheses.

The most salient result of our study was a

Table 6. Risk factors for pneumomediastinum

SMOKING AND SUBSTANCE USE ASCOIATED WITH SPONTANEOUS PNEUMOMEDIASTINUM
Smoking or tobacco use (*26*)
Vaping (*44*)
Other inhalational substance use (*e.g.*: Hookah smoking (*45*), Cocaine (*35*), Methamphetamine (*46*), Marijuana (*47*), Heroin (*48*))
INTRINSIC LUNG DISEASES AND AIRWAY CAUSES
Asthma (*49*)
Chronic obstructive pulmonary disease (*26*)
Interstitial lung disease (*e.g.*: Dermatomyositis (*50*), SLE (*51*))
Cystic fibrosis (*52*)
Lung cancer (*53*)
Foreign body in the airway (*54*)
Metastatic cancers (*55*)
Thoracic endometriosis (*56*)
Mounier-Kuhn syndrome (*57*)
INFECTIOUS CAUSES
Bacterial (*e.g.*: Pertussis (*58*), Tuberculosis (*59*), Mycoplasma pneumoniae (*60*))
Viral pneumonias (*e.g.*: COVID-19 (*3*), Influenza (H5N6) (*36*), HIV infected pneumonia) (*61*)
Fungal (*e.g.*: Pneumocystis jirovecii) (*62*)
CONDITIONS CAUSING CHANGES IN THE INTRATHORACIC PRESSURE
Valsalva maneuver (*e.g.*: Coughing (*63*), Forceful sneezing/inhalation (*64*), Shouting (*65*), Persistent yelling (*66*), Inflation of party balloons (*67*), Forceful blowing into a bottle (*68*))
Pregnancy and labor (*69*)
Vomiting (*e.g.*: Anorexia Nervosa (*70*), Boerhaave syndrome (*71*), Cannabinoid hyperemesis syndrome (*72*), Hyperemesis gravidarum (*73*))
Strenuous physical activities (*e.g.*, Weightlifting (*74*), sports, sex (*75*), pushup exercise (*76*))
Playing musical instruments/Vocal training (*e.g.*, Baritone Practice (*77*))
Pulmonary function testing (*78*)
High flow nasal canula (*79*)
Scuba diving (*80*)
Air travel (*81*)
Mechanical Ventilation (*82*)
IATROGENIC
Drug related (*e.g.*: Bleomycin induced interstitial pneumonitis (*83*))
Procedures (*e.g.*: Bronchoscopy (*84*), Endoscopy Procedures (*85*))
Head and neck surgeries (*e.g.*: Dental (*86*), Adenotonsillectomy (*87*))
Thoracic surgeries (*e.g.*: Esophageal surgery (*88*))
Infra diaphragmatic surgeries (*e.g.*: Laparoscopic surgeries (*89*), Whipple surgery (*90*))
Graft Vs Host disease (*91*)
Tracheobronchial injury (*92*)
OTHER RARE CAUSES
Inflammatory bowel disease (*93*)
Intestinal perforation (*94*)
Dress syndrome (*95*)
Poisonings (*e.g.*: Paraquat (*96*))
Ecstasy ingestion (*97*)

comprehensive review of the conditions associated with SPM. We confirm several well-known associations such as smoking, tobacco use, asthma, and chronic obstructive pulmonary disease (Table 6). Sudden changes in intrathoracic pressure (playing wind instrument, physical activity, Valsalva maneuver, pregnancy/labor induced) also remain an important cause and should be considered. However, other associations which have been reported more recently in the literature are much less appreciated in clinical practice. These include inhalation substance abuse (*35*), collagen vascular disorders (*e.g.* dermatomyositis), and viral infections (*e.g.* COVID-19 (*3*), Influenza (*36*)). We hope these important findings serve as a primer for physicians when faced with a case of SPM and helps guide workup/treatment strategies.

Our study is limited by its design as a systematic review of the literature. A more thorough understanding of SPM will likely require retrospective evaluation of several hospitals' EMRs, as it is a rare condition. In addition, our search did not encompass single case studies. With time, and especially with the onset of COVID-19, our analysis may underrepresent the population of SPM due to viral illness.

A definitive diagnosis of SPM is made with imaging evidence of air in the mediastinum. Chest radiography is the imaging modality of choice and can identify up to 70% of cases (*33*). In cases where additional workup is necessary, CT chest is diagnostic

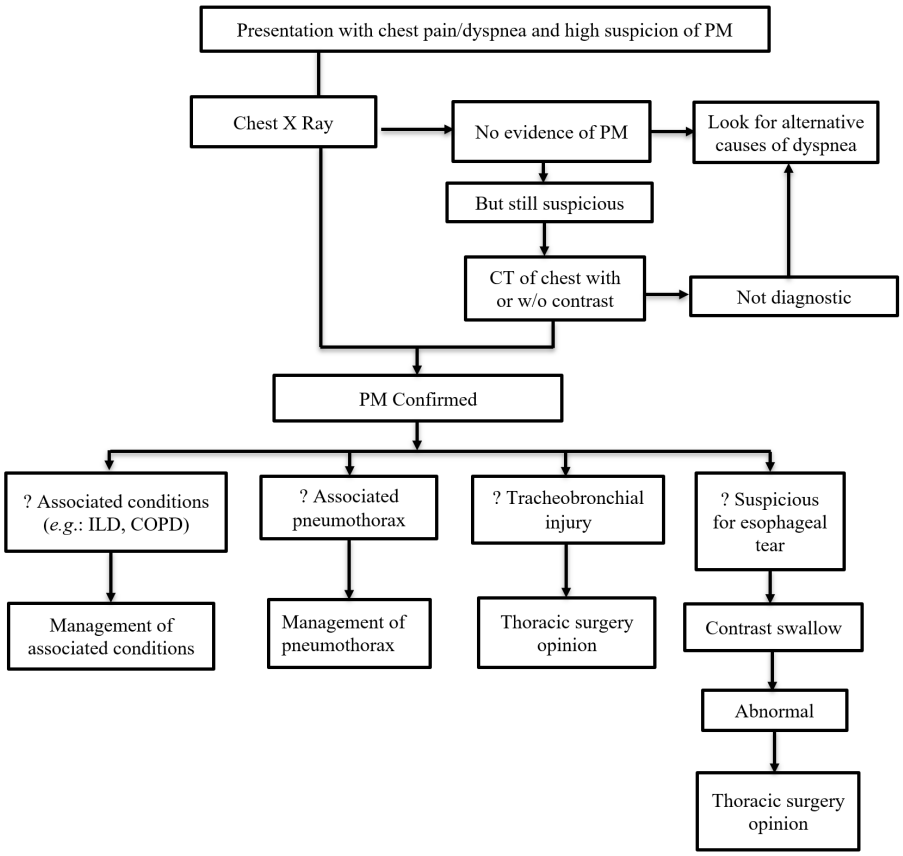

Figure 2. Evaluation and management of pneumomediastinum (PM).

(*37*). The CT scan not only helps confirm the diagnosis but also provides information about the extent of pneumomediastinum as well as evaluation of associated conditions (*e.g.* mediastinal compartment of air, presence of subdiaphragmatic air, presence of subcutaneous air, presence of pleural effusion, presence of acute pulmonary airspace opacification or infiltrate, presence of pulmonary interstitial emphysema, and presence of pneumothorax) (*38*). Those suspected with esophageal injury may require esophagogram.

The goals of SPM treatment are to *i*) promote resorption of free air and *ii*) prevent progression of free air. The most common management strategy is conservative including analgesia, rest, and cough control. Supplemental oxygen has been recommended in many previous reports (*39*) as it provides relief by increasing the diffusion pressure of nitrogen in the interstitium and promoting absorption of free air in the mediastinum. Additional treatment is directed and based on associated conditions. For example, patients with asthma and COPD may benefit from bronchodilators. Patients with associated pneumothorax may necessitate a chest tube placement. If there is tracheobronchial injury or evidence of esophageal injury on esophagogram, surgical consultation is appropriate. However, in the majority of patients, SPM has a benign course and most patients can be discharged for outpatient follow up after 24 to 48 hours of observation in the hospital (*17,30*). Symptomatic relief is typically observed within 24 hours, but complete radiological resolution may take up to 3 weeks (*40*). The authors of this paper recommend documenting complete resolution of the process with a follow up chest x-ray around 2-3 weeks in patients with SPM. A simple, clinically relevant algorithm for workup and management is provided in Figure 2.

Complications of SPM that clinicians should be aware of include progressive respiratory distress, extension of air into the retropharyngeal/retroperitoneal spaces, extension of air into the spinal canal (pneumorhachis), or tension pneumomediastinum (accumulation of air causing tracheal obstruction, compression of the great vessels, or decreased venous return) (*41-43*). SPM related mortality is rare but if it does occur, it is related to underlying associated conditions (pneumonia, COVID-19 (*3*)).

In conclusion, SPM is a unique clinical diagnosis with several associated conditions. SPM typically has a benign course and generally resolves with conservative management. The breadth of associated predisposing conditions is important to understand as they may guide additional treatment.

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Original Article

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Impact of chronic pain and depressive symptoms on the quality of life of adults with Chiari Malformation type I: A comparative study

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SUMMARY Chiari Malformation type I (CM-I) is a neurological disorder characterized by cerebellar tonsillar herniation. Chronic pain, particularly headaches, is a prevalent symptom in CM-I patients, significantly impacting their quality of life. The objective of this study was to evaluate the perceived quality of life in adults with CM-I and examine the influence of chronic pain and comorbid symptoms on their well-being. 26 CM-I patients (8 with decompressive surgery) and 26 matched healthy controls were recruited. Participants completed the following questionnaires: WHOQOL-BREF, HDI, NDI, OLBPDQ and HADS. CM-I patients exhibited significantly lower scores across all domains of quality of life when compared to healthy controls. Chronic pain, including headache, neck pain, and low back pain, was more pronounced among CM-I patients and demonstrated a significant correlation with depressive symptoms. Notably, after controlling for chronic pain, the differences in quality of life between CM-I patients and controls diminished. The results suggest that chronic pain, especially headaches, and comorbid depressive symptoms exert a substantial impact on the quality of life of CM-I patients. Surgical intervention alone may not fully address these issues, highlighting the importance of considering psychological interventions as part of the comprehensive treatment. Further research with larger samples and pre-post-surgery assessments is needed to validate these findings and explore the potential benefits of psychological therapies in enhancing the quality of life for CM-I patients.

Keywords Chiari Malformation type I, chronic pain, quality of life, psychological symptoms

1. Introduction

Chiari Malformation type I (CM-I) is a rare neurological disorder characterized by a downward herniation of the cerebellar tonsils (> 3-5mm) through the foramen magnum into the spinal canal (*1*). The current prevalence rate is uncertain due to the lack of appropriate data as well as the wide disparity of analysis and results among different studies (*2*). Tonsillar ectopia is the most characteristic sign of CM-I, which leads to posterior cranial fossa crowdedness (*3,4*). The hindbrain compression may explain the majority of signs and symptoms, among which headache and neck pain are the most frequently reported by CM-I patients (*5,6*). However, there is a remarkable heterogeneity in the clinical expression of the disease, from asymptomatic patients to cases with a variety of systems affected, including auditory, vestibular, visual, oropharyngeal, gastrointestinal and sleep disorders (*6*).

Meeker *et al.* (*7*) analyzed the impact that CM had on daily activities, suggesting high rates of vulnerability even when minimal symptoms were reported. Although pain is the most frequent complaint, CM-I can also present neurocognitive (*8*) and psychological symptoms (*9*). Considering cognitive deficits, attention, executive functioning and visuospatial abilities appeared to be the most affected domains (*10*). Regarding psychiatric morbidity, anxiety and depression were the most outstanding disorders reported by CM-I patients (*11,12*).

The adverse impact which the set of symptoms has on CM-I patients leads to a lower quality of life and a significant decrease in their general well-being (*9,7,13*).

Mestres *et al.* (*14*) analyzed the perceived quality of life in a cohort of 67 CM-I patients noting that the impact of this malformation was mild in the 53.7%, moderate in 25.4%, severe in 11.9% and the 9% of participants indicated no effect on their lives. Moreover, these authors reported high rates of anxiety (86.6%) and depressive symptoms (25.4%). These figures reveal the importance of considering together the physical, psycho-emotional and social consequences of CM-I. Although there is scarce literature that assesses globally these aspects, the vast majority analyzed the effect of surgical procedures on the quality of life, suggesting a positive outcome for patients who underwent surgery (*15,16*). Considering the effect of pre-surgical conditions, Mueller and Oro' (*15*) recruited 112 patients and stated that having syringomyelia and the level of tonsillar descent did not correlate with self-perceived quality of life, which was assessed using the Sickness Impact Profile (SIP). In a more recent study, Almotairi *et al.* (*17*) reported similar conclusions. These authors also found a significant improvement after decompressive surgery in 11 patients when EQ-ED-5L measurements were compared, while the Life Satisfaction (LiSat-11) questionnaire did not report any differences before and after surgery (*17*). Overall, the existing literature that compares pre and post-surgical status suggests a positive outcome for CM-I patients on their quality of life, however, generalizability is limited due to the variability in the procedures and assessments.

According to previous works, chronic pain is the most limiting symptom reported by CM-I patients, which contributes greatly to a decrease in their quality of life. A recent study conducted by Garcia (*18*) provided preliminary evidence supporting the Acceptance and Commitment Therapy (ACT) as a valid intervention to treat this clinical symptomatology. To our knowledge, after an extensive literature search through databases (PubMed, Scopus and Web of Science) using different combinations between CM-I and psychological therapy keywords, this is the only study that addresses this symptomatology with psychological interventions for CM-I patients, although it did not demonstrate sufficient effectiveness for all target symptoms.

There is a remarkable lack of scientific research focused on chronic pain and its effect on quality of life in CM-I patients. The aim of this study is to analyze the perceived quality of life in a sample of CM-I adult patients and the impact of pain and comorbid symptoms. Further efforts are necessary to identify key symptoms in order to find and develop effective treatments.

2. Patients and Methods

2.1. Participants

Twenty-six patients diagnosed with CM-I were recruited from the Neurology Service of the Marqués de Valdecilla University Hospital and the Chiari and Syringomyelia Association of the Principality of Asturias. Twenty-six gender-, age- and education-matched healthy controls participated in the study. The inclusion criteria were as follows: *i*) available diagnosis of CM-I, *ii*) at least 12 months after surgery (if applicable), *iii*) age ≥ 18, *iv*) Spanish as the primary language (only *iii* and *iv* were applied to the control group). Exclusion criteria included: *i*) any other neurological, psychological or psychiatric diagnosis not secondary to CM-I, *ii*) illiteracy, *iii*) non-compensated sensory deficits. Sociodemographic data and clinical features are presented in Table 1.

2.2. Instruments

Sociodemographic and clinical data were collected through a brief interview with each participant. After collecting them, the following questionnaires were administered to assess physical and psychosocial status using the adapted version for Spanish population.

WHOQOL-BREF (*19*): this questionnaire is the World Health Organization's quality of life short version scale. It consists of 24 items that evaluate four domains including: physical, psychological, social relationships and environmental, in addition to two more items that measure the individual's overall perception of quality of life and their health.

Hospital Anxiety and Depression Scale (HADS) (*20*): this instrument is used to evaluate anxious-depressive symptomatology in clinical populations. It contains 14 items, divided into two subscales, evaluating anxiety and depression respectively.

Headache Disability Inventory (HDI) (*21*): this questionnaire measures the individual's perception of the impact of headache on daily living. It consists of 25 items.

Neck Disability Index (NDI) (*22*): this scale consists of 10 items. It is used to evaluate self-perception of the effect that cervical pain has on daily activities.

Oswestry Low Back Pain Disability Questionnaire (OLBPDQ) (*23*): this questionnaire evaluates the individual's perception of the impact that lumbar pain has on daily activities.

2.3. Procedure

CM-I patients were recruited from the Neurology Service at the Marqués de Valdecilla University Hospital and the Chiari and Syringomyelia Association of the Principality of Asturias. Healthy controls were recruited among adult volunteers. Those who met the inclusion criteria were called to be assessed. All participants completed the informed consent document before their enrolment. Each session was individual and lasted around 45 minutes. Both clinical group and control group were administered the test protocol similarly by a trained researcher.

The study was approved by the Ethics Committee of the University of Deusto (ETK-20/17-18) and it was conducted according to the guidelines of the Declaration of Helsinki.

2.4. Data analyses

Statistical analyses were run with the Statistical Package for Social Sciences (SPSS) 28.0. Means and frequencies were obtained for sociodemographic data and clinical features. For comparisons between clinical and control group, raw scores were converted into *z* scores. To analyze differences between clinical and control group, *Chi*-square and Mann-Whitney *U* tests were used. For multiple comparisons, Kruskal-Wallis test was used together with the Bonferroni test as *post hoc* analysis. Multivariate analysis of covariance (MANCOVA) was used to control for the effect of chronic pain on psychosocial variables. Effect sizes were calculated based on Kramer's *V* or eta squared (η^2), as appropiate. Correlations were performed using Spearman's *Rho* statistic. Statistical level of significance was established at $p < 0.05$.

3. Results

CM-I patients and healthy controls were gender-, age-, and education-matched ($p > 0.05$). Sociodemographic information and clinical features of clinical group are shown in Table 1. It should be noted the high comorbidity with other diagnosis, especially with syringomyelia and scoliosis, along with the age of onset, which is around the third decade, similar to what literature reports. Likewise, it is worrying the average delay to get the diagnosis, which is around five years.

3.1. Effect of underlying symptomatology

The comparison between decompressed and non-decompressed CM-I patients about their clinical symptomatology is presented in Figure 1. Participants were asked about the occurrence of symptoms and their frequency (low or high). As it can be observed, a general overview revealed higher percentages of symptomatology in patients who had undergone posterior fossa decompression (PFD), except for headache, dizziness and visual disturbances. However, statistically significant differences were only found for upper limbs pain ($\chi^2 = 7.22$, $p = 0.007$, $V = 0.527$), lower limbs pain ($\chi^2 = 3.87$, $p = 0.049$, $V = 0.386$), instability ($\chi^2 = 7.22$, $p = 0.007$, $V = 0.527$), auditory disturbances ($\chi^2 = 4.26$, $p = 0.039$, $V = 0.405$), and oropharyngeal difficulties ($\chi^2 = 3.97$, $p = 0.046$, $V = 0.391$), where non-decompressed patients indicated lower occurrence. No differences

Table 1. Sociodemographic and clinical features of the sample

Variables	CM-I patients		Control group	*χ2/U*	*p*
	PFD	Non-PFD			
	n (%) / M (SD)		*n* (%) / M (SD)		
SOCIODEMOGRAPHIC DATA					
N	8 (30.8%)	18 (69.2%)	26 (100%)		
Gender				0	1
Female	7 (87.5%)	15 (83.3%)	22 (84.6%)		
Male	1 (12.5%)	3 (16.7%)	4 (15.4%)		
Age	54.38 (9.74)	43.94 (15.47)	46.42 (13.53)	317.5	0.707
Years of education	13.88 (2.1)	13.33 (3.1)	14.62 (2.73)	247.0	0.090
CLINICAL FEATURES					
Age at diagnosis (y)	41.88 (13.54)	34.22 (12.65)			
Age of onset (y)	36.13 (14.44)	29.22 (14.72)			
Diagnosis delay (y)	5.75 (11.73)	5.0 (6.28)			
Disease duration (y)	12.50 (8.14)	10.06 (8.34)			
Tonsillar ectopia (mm)	7.13 (1.55)	7.94 (4.52)			
Time elapsed from surgery (mo)	88.13 (56.80)	-			
COMORBIDITY					
Syringomyelia	6 (75%)	1 (5.6%)			
Hydrocephalus	-	2 (11.1%)			
Basilar impression	-	1 (5.6%)			
Platybasia	-	1 (5.6%)			
Scoliosis	5 (62.5%)	9 (50%)			
Other cranial malformations	-	1 (5.6%)			

PFD: posterior fossa decompression. *Note:* The information shown in this table has been reported by Chiari patients.

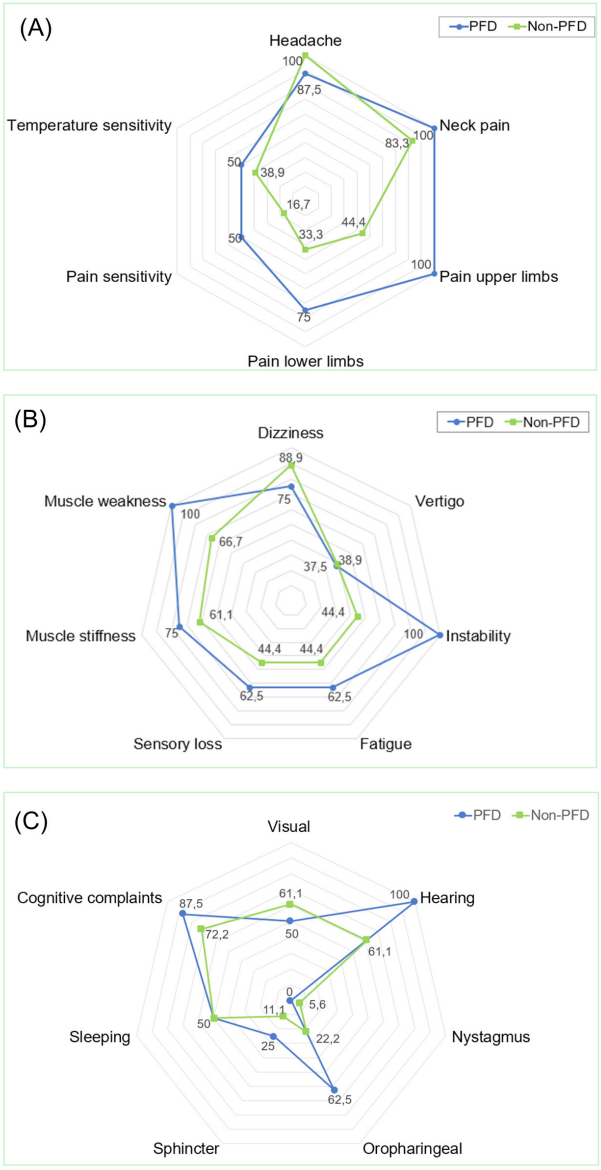

Figure 1. Clinical features of CM-I patients. (A), Pain and sensitivity. **(B)**, Muscie and vestibular symptoms. **(C)**, Other systems.

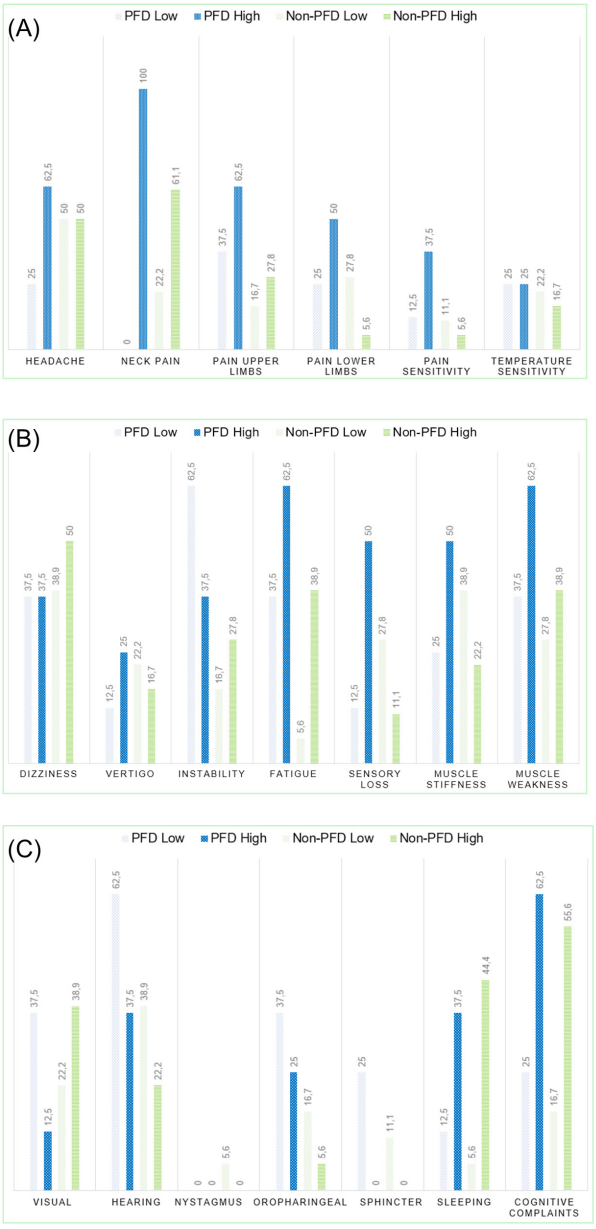

Figure 2. Frequency of clinical symptoms in CM-I patients. (A), Pain and sensitivity. **(B)**, Muscular and vestibular. **(C)**, Other systems.

were found when frequency of appearance was analyzed between both groups ($p > 0.05$) (Figure 2).

3.2. Effect of chronic pain

To analyze differences between clinical and control group in their physical and psychosocial status, Kruskal-Wallis statistic was run. Results are detailed in Table 2. CM-I patients showed statistically significant worse scores in quality of life than healthy controls in physical, psychological, social relationships and environmental domain, as well as in anxious-depressive symptomatology ($p < 0.01$). When chronic pain-related tests were analyzed, CM-I patients also showed statistically significant worse status compared to control group ($p < 0.001$). Moreover, the effect sizes were high ($\eta^2 > 0.14$). The *post hoc* analysis revealed results for multiple comparisons (Table 3). When clinical group was compared to healthy controls, undergoing patients showed poorer perceived quality of life and worse psychopathological status except for psychological domain and anxious symptomatology, respectively. Non-operated patients did not differ from controls in social relationships domain ($p = 0.341$), individual's overall perception of quality of life ($p = 0.064$) and depressive symptomatology ($p = 0.060$). Comparison between decompressed and non-decompressed CM-I patients showed no differences in quality of life nor pain-related measures ($p > 0.05$). According to these results, it can be observed that chronic pain has a significant impact

Table 2. Physical and psycho-emotional status of the sample

Status	CM-I patients		Control group	*H*	*p*	Effect size η^2
	PFD	Non-PFD				
	M (SD)[a]		M (SD)			
Quality of life (WHOQOL-BREF)						
Physical domain	43.75 (12.63)	52.78 (16.37)	78.43 (13.03)	26.50	< 0.001	0.51
Psychological domain	54.69 (17.46)	51.16 (16.47)	68.59 (16.17)	12.19	0.002	0.23
Social relationships domain	46.88 (26.33)	62.50 (16.97)	69.23 (11.25)	9.85	0.007	0.19
Environment domain	58.20 (13.87)	60.07 (11.72)	72.12 (11.12)	11.59	0.003	0.22
Individual's overall perception of QoL	46.88 (16.02)	54.17 (23.09)	69.23 (20.38)	8.97	0.011	0.17
Individual's overall perception of health	34.38 (22.90)	40.28 (22.91)	63.46 (25.72)	11.70	0.003	0.23
Psychopathological status						
HADS-Anxiety	9.63 (4.72)	11.33 (4.86)	7.08 (3.53)	9.32	0.009	0.18
HADS-Depression	7.75 (3.50)	5.61 (3.87)	3.04 (3.12)	12.04	0.002	0.23
HADS-Total score	17.38 (7.58)	16.94 (8.45)	10.12 (6.33)	11.76	0.003	0.23
Physical status						
HDI-Functional	24.0 (14.62)	25.72 (12.06)	0.08 (0.40)	43.10	< 0.001	0.83
HDI-Emotional	16.5 (14.80)	22.56 (13.42)	0.00 (0.00)	39.10	< 0.001	0.75
HDI-Total score	40.5 (29.04)	48.28 (24.60)	0.08 (0.40)	43.21	< 0.001	0.83
NDI	23.88 (7.92)	14.11 (7.90)	1.65 (2.86)	37.05	< 0.001	0.71
OLBPDQ	17.13 (6.00)	8.72 (6.14)	2.85 (6.71)	25.44	< 0.001	0.49

H: Kruskal-Wallis test; HADS: Hospital Anxiety and Depression Scale; HDI: Headache Disability Index; η^2: eta squared; NDI: Neck Disability Index; OLBPDQ: Oswestry Low Back Pain Disability Questionnaire; PFD: Posterior Fossa Decompression; WHOQOL-BREF: World Health Organization's Quality of Life short version scale. [a]Data are shown in raw scores.

Table 3. Post hoc results of physical and psycho-emotional status between clinical and control group (Bonferroni test)

Status	PFD *vs.* Controls		Non-PFD *vs.* Controls		PFD *vs.* Non-PFD	
	M (SD)	*p*	M (SD)	*p*	M (SD)	*p*
Quality of life (WHOQOL-BREF)						
Physical domain	-25.53 (6.11)	0.000	-19.26 (4.64)	0.000	-6.27 (6.42)	0.987
Psychological domain	-12.96 (6.10)	0.101	-15.24 (4.63)	0.003	2.29 (6.41)	1.000
Social relationships domain	-18.55 (6.05)	0.006	-7.25 (4.59)	0.341	-11.30 (6.36)	0.227
Environment domain	-15.24 (6.11)	0.038	-13.78 (4.63)	0.009	-1.46 (6.42)	1.000
Individual's overall perception of QoL	-14.61 (5.76)	0.034	-10.06 (4.37)	0.064	-4.54 (6.06)	1.000
Individual's overall perception of health	-16.03 (5.91)	0.020	-12.65 (4.48)	0.014	-3.38 (6.21)	1.000
Psychopathological status						
HADS-Anxiety	8.86 (6.10)	0.439	13.90 (4.63)	0.008	-5.04 (6.41)	1.000
HADS-Depression	19.32 (6.09)	0.005	10.75 (4.62)	0.060	8.57 (6.41)	0.543
HADS-Total score	16.26 (6.12)	0.024	13.39 (4.64)	0.012	2.88 (6.43)	1.000
Physical status						
HDI-Functional	25.12 (5.78)	0.000	26.39 (4.38)	0.000	-1.26 (6.07)	1.000
HDI-Emotional	21.31 (5.63)	0.000	25.19 (4.27)	0.000	-3.88 (5.91)	1.000
HDI-Total score	24.38 (5.78)	0.000	26.72 (4.38)	0.000	-2.35 (6.07)	1.000
NDI	30.89 (6.05)	0.000	21.71 (4.59)	0.000	9.18 (6.36)	0.446
OLBPDQ	27.14 (5.95)	0.000	15.61 (4.51)	0.002	11.54 (6.25)	0.195

HADS: Hospital Anxiety and Depression Scale; HDI: Headache Disability Index; η^2: eta squared; NDI: Neck Disability Index; OLBPDQ: Oswestry Low Back Pain Disability Questionnaire; PFD: Posterior Fossa Decompression; WHOQOL-BREF: World Health Organization's Quality of Life short version scale. *Note:* Data are shown in raw scores.

on daily living for CM-I patients, and therefore, it could be a negative factor to their quality of life. Therefore, a MANCOVA analysis was run considering HDI, NDI and OLBPDQ total scores as covariates. After controlling for the effect of chronic pain, the differences between CM-I patients and healthy controls were eliminated for physical domain ($F = 0.367$, $p = 0.548$), psychological domain ($F = 2.245$, $p = 0.141$), social relationships domain ($F = 0.242$, $p = 0.0625$), environmental domain ($F = 0.219$, $p = 0.642$), individual's perception of quality of life ($F = 0.700$, $p = 0.407$), and individual's perception of their health ($F = 0.247$, $p = 0.622$).

Correlation analyses were also performed to study the association between physical and psychosocial measures. The *HDI score* showed a significant correlation with the HADS's anxiety (*Rho* = 0.598, $p = 0.001$), depression (*Rho* = 0.571, $p = 0.002$), and total score (*Rho* = 0.680, $p < 0.001$), physical domain (*Rho* = -0.553, $p = 0.003$), psychological domain (*Rho* = -0.800, $p < 0.001$), environmental domain (*Rho* = -0.494, $p = 0.010$), individual's perception of quality of life (*Rho* = -0.430, $p = 0.028$), and perception of their health (*Rho* = -0.396, $p = 0.045$). No correlation was found between the HDI score and social relationships domain (*Rho* = -0.293, $p = 0.146$). The *NDI score* showed significant correlation with HADS's depression score (*Rho* = 0.427, $p = 0.030$), physical domain (*Rho* = -0.657, $p < 0.001$), social relationships domain (*Rho* = -0.403, $p = 0.041$), individual's perception of quality of life (*Rho* = -0.405, $p = 0.040$), and perception of their health (*Rho* = -0.617, $p < 0.001$). No correlation was found between the NDI score and the HADS's anxiety (*Rho* = 0.154, $p = 0.452$) and total score (*Rho* = 0.337, $p = 0.092$), psychological domain (*Rho* = -0.285, $p = 0.158$), and environmental domain (*Rho* = -0.361, $p = 0.070$). The *OLBPDQ score* showed significant correlation with HADS's depression score (*Rho* = 0.503, $p = 0.009$), physical domain (*Rho* = -0.641, $p < 0.001$), social relationships domain (*Rho* = -0.574, $p = 0.002$), environmental domain (*Rho* = -0.444, $p = 0.023$), individual's perception of quality of life (*Rho* = -0.504, $p = 0.009$), and perception of their health (*Rho* = -0.640, $p < 0.001$). No correlation was found between the OLBPDQ score and the HADS's anxiety (*Rho* = 0.149, $p = 0.468$) and total score (*Rho* = 0.375, $p = 0.059$), and psychological domain (*Rho* = -0.297, $p = 0.140$).

As it can be observed, the HADS's depression score showed significant correlation with all pain-related measures, including headache, neck and low back pain. Likewise, HDI, NDI and OLBPDQ total scores showed negative significant correlations with physical domain, individual's perception of quality of life and individual's perception of their health. The largest correlation was between HDI score and psychological domain.

3.3. Effect of demographic variables

Considering the associations between sociodemographic and clinical data, only age at diagnosis showed a significant correlation with HADS's anxiety score (*Rho* = -0.426, $p = 0.030$). No significant correlations were found between tonsillar ectopia and chronic pain or quality of life-related measures ($p > 0.05$).

4. Discussion

In this study, the quality of life, level of chronic pain, and psychopathological status of 26 patients with CM-I (of whom eight underwent decompressive surgery) were evaluated and compared with 26 gender, age, and education-matched healthy controls. Clinical group showed lower scores than control group in all domains of their perceived quality of life, including physical, psychological, social relationships and environmental ($p < 0.001$). Likewise, CM-I patients showed higher scores in disability caused by chronic pain, such as headache, neck pain and low back pain ($p < 0.001$). The comparison between both surgical status (decompressed and non-decompressed) showed no differences in their physical and psychosocial profile. However, when chronic pain was controlled for, the differences between both groups in quality of life-related measures were eliminated. Patients also reported higher scores in anxious-depressive symptomatology, but only depression showed a significant correlation with all pain-related measures, while anxiety and the HADS's total score correlated with headache, but not with neck and low back pain.

The term "quality of life" refers to the physical, psychological, and social domains of health (*24*). On the one hand, the relationship between chronic pain and quality of life is close and multifactorial (*25*), having a negative impact on overall health and psychological well-being (*26*). To our knowledge, there is scarce literature that has examined quality of life in CM-I patients, taking into consideration the negative effect that chronic pain has on daily living, perhaps due to the asymptomatic nature of the disease in some individuals (*27,28*). In cases where patients experience pain, headaches are the most common manifestation, and sometimes the only one (*29*). Therefore, pain management in CM-I patients is crucial as it may largely determine the perceived quality of life. On the other hand, the present study also supports the well-established comorbidity between chronic pain and affective symptoms (*30*), which may affect the perception of pain itself. Depressive disorders are common among patients with chronic pain, resulting in a substantial disease burden and a barrier to effective pain relief (*31*), which can also amplify the experience and perception of pain (*32,33*). Historically, the relationship between depression and pain has been a significant area of study in the health field, being two main reasons to address this relationship in CM-I patients. First, around 45% of patients exhibit depressive symptoms (*11*). Second, increased pain is related to higher levels of depression (*34,35*), forming a negative feedback which

affects quality of life.

Considering our findings, a recent work has also suggested high levels of disability in adult patients, identifying chronic pain and depression as significant factors, regardless of surgical status (*36*). Our results are in accordance with Labuda *et al.*'s (*36*) study because depressive symptomatology is the only variable that showed significant correlations with chronic pain-related measures. Another recent work published by Balasa *et al.* (*13*) also suggested a strong relationship between depression and pain in CM-I adults, leading to worse quality of life. Moreover, previous literature supported these findings, stating that chronic pain could be a key factor for negative assessment of the CM disease impact (*7,9*). There seems to be a consensus in the accumulated evidence regarding the identification of chronic pain and affective disorders as remarkable factors influencing quality of life in CM-I patients.

Taking into account the research to date, one of the main focus has been the study of the reported outcomes after undergoing surgical procedures (*15,16,37,38*). The most referred intervention is the PFD, which consists in a suboccipital craniectomy. Generally, pain-related symptoms constitute the primary motivation for undergoing surgery, making symptom-related quality of life a key outcome measure for effectiveness studies (*39*). Perhaps, the lack of differences found in our study between non-decompressed patients and controls in social domain, overall perception of quality of life and depressive symptomatology could be related with that fact. The baseline status of non-operated patients is more optimal or have minor comorbid conditions such as syringomyelia than those requiring surgery, and therefore more similar from healthy controls. However, this is an idea which needs further research because pre-post analyses were not conducted. This is an important aspect of clinical management since it has been noted that headaches range between 50% (*40,41*) and 81% of cases (*42*). In general, this type of surgery has shown positive results, but there is scarce literature considering patients' follow-ups done appropriately (*15,17*). Similarly, the procedure is not without controversy, as symptoms sometimes persist along with medical complications (*43,44*). Therefore, it is important to individualize treatments and select the most appropriate procedure for each patient according to their symptomatology (*16,45*). Moreover, as it has been aforementioned and also found in our study, when decompressed and non-decompressed patients are compared on their perceived quality of life and reported symptomatology, similar results have been found, probably related to the chronicity of the disease, regardless of the therapeutic alternatives (*11,13*). This is particularly worrying and shows that surgical intervention alone is not fully effective, therefore, further studies should consider complementing treatments with other approaches such as psychological therapies.

Exclusive biomedical approach for the burden of living with pain is often criticized for its weakness to account for psychosocial variables. In fact, the psychosocial health of the patient has been somewhat overlooked, perhaps with the expectation that reducing pain would lead to improve mental health and subsequently, the quality of life. However, chronic pain is both a psychosocial and physiological problem: anxiety, depression, insomnia, loss of financial independence, disability, and family instability are closely associated with long-term pain (*46*). Our results support this statement, pointing to the chronic pain as the primary mediator of altered quality of life in these patients, but without underestimating the influence of affective disorders.

Often, when no clear neurological deficits are present, individuals with CM-I are labeled as depressed or considered to be suffering from psychosomatic symptoms. Additionally, a significant portion of these patients are initially diagnosed with fibromyalgia, chronic fatigue, or anxious-depressive disorders, which slows and complicates the process of establishing a clear diagnosis of the CM-I. Among general medical conditions, psychiatric comorbidity has been associated with reduced physical functioning and quality of life. To that, it may be added the possible concurrence of mood disorders after brain surgery (*47*), however, in our study no differences were observed comparing both surgical status regarding affective variables. In addition to surgical treatments, psychological therapy has shown some beneficial effects on chronic pain in different conditions (*48,49*). Some of the most widely studied therapies are cognitive-behavioral therapy (CBT), Acceptance and Commitment Therapy (ACT), and Pain Management Programs. All the previously mentioned therapeutic approaches have demonstrated efficacy in the treatment of chronic pain and have the common goal of reducing the influence of pain on daily life, improving the quality of life (*50*). In the case of CM-I, an online intervention based on ACT has shown preliminary evidence for improvement in mental flexibility, pain acceptance and willingness and activity engagement, but still without enough impact on sleep disturbances, anxious-depressive symptoms and pain interference (*18*). Therefore, future studies addressing psychological treatment for pain management in patients with CM-I, such as CBT-based programs, can take into consideration these findings for other pathologies and try to study the potential benefits of these therapies among Chiari-type diseases.

Our study has notable limitations. First of all, sample size is small and not equally distributed between both surgical status (decompressed and non-decompressed patients), which reduces the representativeness and validity of the results. Moreover, quality of life has been measured with a general instrument (WHOQoL-BREF) and is not specifically designed for CM-I population. This aspect does not allow to identify specific symptomatology or features related to CM diagnosis

appropriately. Another important limitation in our study is the lack of comparative measures before and after surgical intervention, which should be managed in further research. Likewise, there is a lack of information about decompression details from medical records, because the recruited data were obtained from patients' interviews. All these limitations should be addressed in future studies, considering pre-post-surgery comprehensive assessments that include physical, psychological and psychosocial measures.

5. Conclusion

In sum, this study delves into the components of quality of life in individuals with CM-I and the significant role that chronic pain plays on their life. Pain seems to be the factor that explains the differences in quality of life reported between CM-I patients and healthy population. Furthermore, this study found no differences in pain or quality of life measures between operated and non-operated patients. With that in mind, future studies should investigate whether systematic approaches to pain, such as those based on psychological therapies, can significantly improve the quality of life of patients.

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Cost-utility analysis of romiplostim for the treatment of chronic primary immune thrombocytopenia in China

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SUMMARY This study aimed to assess the cost-utility of romiplostim (ROMI) compared to eltrombopag (EPAG) as a second-line treatment for chronic primary immune thrombocytopenia (cITP) in Chinese adults. A decision tree-embedded Markov model with a lifetime horizon was used to estimate the quality-adjusted life years (QALYs) and costs for ROMI versus EPAG from the perspective of the Chinese health care system. The model was driven by platelet response with a 4-week cycle. Both QALYs and costs were discounted 5% per year. Clinical data comparing ROMI and EPAG were obtained by matching-adjusted indirect comparison (MAIC), utilizing individual patient data on ROMI and published Chinese Phase III trial data on EPAG. Costs were reported in 2022 US dollars and included drug acquisition costs, monitoring costs, bleeding-related costs, and costs associated with adverse events. Deterministic and probabilistic sensitivity analyses were performed. The CEA model indicated that treatment with ROMI resulted in an average of $4,344.4 higher costs for 0.004 QALYs. One-way sensitivity analysis (OSA) indicated that the model was most sensitive to the high bleeding rate in response (Markov stage) for EPAG and ROMI. Probabilistic sensitivity analysis (PSA) indicated that ROMI was likely to be cost effective in 0.16% cases at a willingness-to-pay threshold of $12039.1 (China per capita GDP in 2022) per QALY. If the price of ROMI is either lower than or equal to that of EPAG, ROMI could likely be considered cost-effective as a second-line treatment for Chinese adults with cITP.

Keywords immune thrombocytopenia, romiplostim, eltrombopag, cost-utility analysis

1. Introduction

Primary immune thrombocytopenia (ITP) is a hematological disorder characterized by isolated thrombocytopenia (platelet count < 100×10^9/L (< 100×10^3/microliter)) in the absence of a clear cause. The annual incidence of ITP in adults is (2-10)/100,000 worldwide (*1*), and the condition predominantly affects people over 60 years old (*2*). cITP (one of ITP, ≥ 12 months' duration) is an autoimmune disease, and a significant proportion of patients may suffer from other complications, such as recurrent and persistent disease, a high risk of bleeding, and unpredictable disease progression. These complications, along with cITP, lead to a serious reduction in the quality of life (QoL) of patients. At the same time, frequent use of medications to maintain platelet counts at safe levels poses a significant long-term economic and QoL burden on patients with cITP in China (*2*).

According to ITP guidelines and an expert consensus in China (*2*), the US (*3*), Japan (*4*), South Korea (*5*), Italy (*6*), and Spain (*7*), intravenous immunoglobulin (IVIg), prednisolone, or anti-D immunoglobulin are commonly regarded as first-line therapies for cITP. These drugs have a rapid onset of action, but they do not result in durable remission in most patients. If first-line therapy is ineffective or not tolerated as a result of long-term use, patients with cITP need to be switched to a second-line regimen. The goal of second-line therapy is to maintain platelet counts at safe levels in order to achieve disease remission. Thrombopoietin receptor agonists (TPO-RAs) are recommended as the first choice for second-line treatment of cITP, including recombinant human thrombopoietin (rhTPO), EPAG, ROMI, herombopag, and avatrombopag.

ROMI is a long-acting TPO-RA that is administered subcutaneously once a week. It works by stimulating the production of platelets, which are blood cells that help to clot blood. Clinical studies have demonstrated ROMI's effectiveness in increasing platelet counts and reducing bleeding episodes in patients with cITP. Alongside ROMI, eltrombopag (EPAG) is another TPO-RA that

is generally accepted as a therapeutic alternative with comparable safety and efficacy(*8*). However, despite their widespread use, ROMI and EPAG have not been directly compared in head-to-head clinical trials to treat cITP.

Given the growing attention on the value of innovative cITP drugs, it is essential to evaluate their economic impact. Understanding the cost-utility of these treatments can inform healthcare decision-makers and ensure optimal resource allocation. This study aims to fill this gap by assessing the cost-utility of ROMI compared to EPAG in the treatment of cITP in Chinese adults. The findings furnish evidence supporting the clinical use of ROMI and offer empirical data for cost-effectiveness assessments conducted by healthcare technology assessment (HTA) agencies globally. By evaluating both the clinical and economic aspects of ROMI and EPAG, this study aims to support informed decision-making and contribute to the broader discussion on the value of innovative treatments for cITP.

2. Patients and Methods

2.1. Population and perspective

Subjects were consistent with the instructions for ROMI, *i.e.*, adults (≥ 18 years of age) with cITP who have not responded well to other treatments (*e.g.*, corticosteroids and immunoglobulins). Potential subjects with a mean body weight of 60 kg were included. An analysis was performed from the perspective of the Chinese health care system.

2.2. Comparators

A model compared ROMI versus EPAG. ROMI dosing data were obtained from a Chinese phase III clinical trial (CTR20150395) where patients were given intramuscular injections once a week at a mean dose of 3.1 μg/kg (*9*). The average daily dose of EPAG was 42.1 mg, which was based on a Chinese phase III clinical trial (*10*).

2.3. Model construction

A short-term decision tree was embedded in a Markov model over a lifetime horizon (33 years, a cohort from 45 years of age and older). The model cycle was 4 weeks. Both QALYs and costs were discounted at a rate of 5% per year. The incremental cost-effectiveness ratio (ICER) of ROMI versus EPAG for the second-line treatment of adults with chronic ITP was calculated using Microsoft Excel (Version 16.72).

As shown in Figure 1, the 6-week decision-tree stage considered two treatment options: ROMI and EPAG. Based on treatment results, participants were divided into platelet response and non-response branches, and the definition of platelet response is any platelet count ≥ 50 × 10^9/L. Platelet response was further divided into non-bleeding and mild bleeding branches; platelet non-response was divided into non-bleeding, mild bleeding, and severe bleeding branches. Non-bleeding patients were those with a score of 0 on the World Health Organization Bleeding Scale, while scores of 1 and 2 were considered mild bleeding and scores of 3-4 were considered severe bleeding.

As shown in Figure 2, the long-term Markov model included three health states: "Response", "Non-response", and "Dead". In this model, a "Response" state is defined as patients achieving a platelet count of ≥ 50 × 10^9/L, while a "Non-response" state refers to patients with a platelet count of < 50 × 10^9/L.

Figure 3 shows the model of the drug treatment pathway. After 4 weeks of nonresponse to treatment with ROMI or EPAG; participants were switched to rhTPO combined with rituximab (rhTPO + RTX) and then switched to all-trans retinoic acid combined with danazol (ATRA + danazol). After 4 consecutive cycles of ATRA + danazol (*11*), participants were finally switched to best

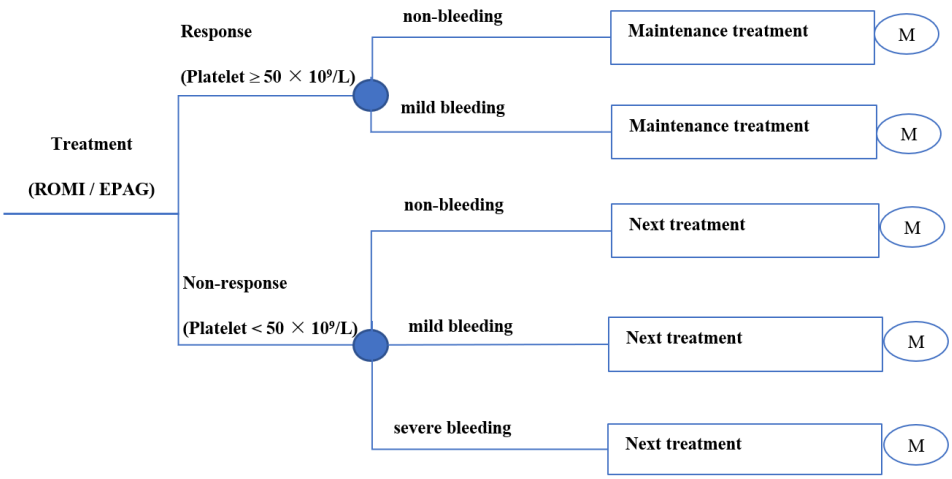

Figure 1. Overview of an embedded decision tree. EPAG: eltrombopag; ROMI: romiplostim; Next Treatment: rhTPO + Rituximab.

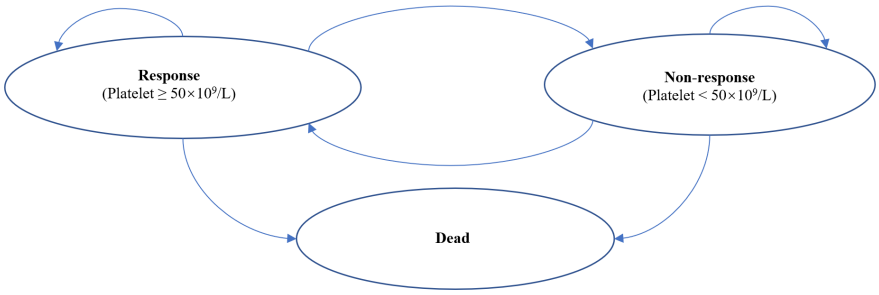

Figure 2. Overview of a long-term Markov model driven by platelet response.

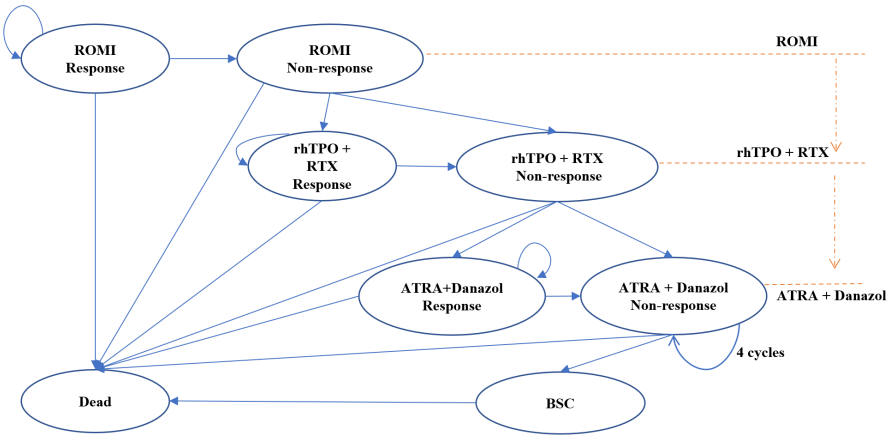

Figure 3. Treatment pathway (ROMI as an example). ROMI: romiplostim; RTX: rituximab; ATRA: all-trans retinoic acid; BSC: best supportive care.

Table 1. Clinical efficacy inputs for the short-term decision tree stage

Inputs	ROMI	Reference	EPAG	Reference
Probability of response	47.6%	(*11*)	48.8%	P_{ROMI}*ORs
Low bleeding rate in response	11.4%		13.2%	
Low bleeding rate in non-response	35.1%		40.4%	
High bleeding rate in non-response	1.30%		1.9%	

EPAG: eltrombopag; ROMI: romiplostim.

supportive care (BSC).

2.4. Assumptions

i) Splenectomies: According to expert clinical opinion, splenectomy for treatment of ITP declined in prevalence (less than 10%) in recent years. All patients in this study cohort were assumed to have not undergone a splenectomy.

ii) Treatment pathway: The drug treatment was assumed to be platelet non-response for one cycle before moving on to the next treatment. The sequence of drug changes was only from ROMI/ EPAG to rhTPO + RTX and then to ATRA+ danazol.

iii) Mortality: Patient mortality was not considered in either group during the decision tree stage. It was assumed to be equivalent to natural mortality for patients without bleeding or those experiencing mild bleeding. The risk ratio (RR) between severe bleeding and the natural population is 2.6 (*12*). Mortality was higher in the non-responders treated with ATRA combined with danazol after several lines of treatment, consequently, we assumed that the mortality rate ratio (RR) of this group and the BSC group versus the natural mortality rate was 2.6.

2.5. Clinical inputs

Clinical efficacy data on ROMI were obtained from a clinical trial (*11*) in China, including the platelet response rate, low bleeding rate, and high bleeding rate in each health state. Due to the lack of head-to-head clinical RCT studies comparing ROMI and EPAG, the odds ratios (ORs) for the clinical effect data between the ROMI and EPAG groups were obtained by MAIC. Efficacy data for the EPAG group were calculated by multiplying the absolute values for the ROMI group (P_{ROMI}) by the ORs (Table 1).

Table 2. Clinical efficacy inputs for the long-term Markov stage

Inputs	To		
	Response	Non-response	Dead
From			
ROMI/EPAG response	0.79983	0.19996	0.00021
ROMI/EPAG non-response; rhTPO + RTX response	0.54379	0.45594	0.00027
rhTPO + RTX non-response; ATRA + Danazol response	0.61984	0.37980	0.00036
ATRA + Danazol non-response; BSC	0	0.99946	0.00054
dead	0	0	1

EPAG: eltrombopag; ROMI: romiplostim; RTX: rituximab; ATRA: all-trans retinoic acid; BSC: best supportive care.

Table 3. Utility inputs

Inputs	Mean	SE	Reference
Non-bleeding in response	0.86	0.15	Agota 2010
Mild bleeding in response	0.73	0.19	Agota 2010
Non-bleeding in non-response	0.84	0.19	Agota 2010
Mild bleeding in non-response	0.73	0.19	Agota 2010
Severe bleeding in non-response	0.45	0.06	Leontiadis 2007
Dead	0.00	/	
Disutility	-0.1	0.03	Jamali 2009

The analysis indicated that ROMI had a slightly lower platelet response rate compared to EPAG (ROMI *vs*. EPAG, OR: 0.976, 95% confidence interval (CI): 0.13-6.83). However, ROMI was superior to EPAG in terms of the low bleeding rate (ROMI *vs.* EPAG, OR: 0.85, 95% CI: 0.27-2.74) and high bleeding rate (ROMI *vs.* EPAG, OR: 0.67, 95% CI: 0.02-15.05).

In the long-term Markov stage, the effectiveness of ROMI was assumed to be the same as that of EPAG because MAIC of the response rates suggested no significant differences between the 2 TPO-RAs, as evinced by a wide CI that included 1. The response rate to EPAG was based on the RAISE trial (*12*), and ROMI performed as well as EPAG. The response rates for all other treatments (*13*) were obtained from the published literature (Table 2). As mentioned in the previous hypothesis, the model included both natural mortality and high-risk mortality.

2.6. Utility

The patient utility values were collected in different bleeding groups (Table 3). Due to lack of utility data for Chinese adults with ITP, utility values from a time trade-off (TTO) survey (*13*) conducted in the UK were included in this study, and disutility values associated with serious bleeding and adverse effects were obtained from the published literature (*14,15*).

2.7. Costs

The full course of treatment for a patient with cITP was considered in this study (Table 4). Drug prices were from the Yaozhi database (*16*), and drug daily doses from clinical trials (*2,11,13,17*). The costs of adverse effects were derived from the published literature in China. Administration and monitoring costs were obtained from a catalog of prices for medical care in representative cities, such as Shanghai, Guangzhou, Beijing, Zhengzhou, and Chengdu. All costs were assessed in US dollars (USD), using the average RMB/USD exchange rate of 6.7261 in 2022. This comprehensive cost assessment ensures an accurate and realistic evaluation of the economic impact of cITP treatments within the Chinese healthcare system.

2.8. Sensitivity analysis

One-way and probabilistic sensitivity analyses were used to test the uncertainty of the model. OSA was performed to identify the most sensitive parameters of this model. In OSA, all key model inputs were varied around the base-case values by ± 20%. PSA was performed using 5,000 iterations by simultaneously sampling from estimated probability distributions of model parameters to examine parameter uncertainty over the entire model, and cost-effectiveness acceptability curves (CEACs) were then calculated.

3. Results

3.1. Base case

The lifetime horizon Markov model indicated that treatment with ROMI resulted in an average of $4,344.4 higher costs for 0.004 QALYs (Table 5). The ICER in this model was $1,135,779.46/QALY, which was higher than $36,117.2/QALY (3 GDP per capita) in China.

3.2. OSA

Figure 4 shows the results of the OSA, presented as the net monetary benefit (NMB) considering a willingness-to-pay threshold of $12,039.1 per QALY. The variables with the largest effect on the model were a high bleeding rate due to EPAG in response (Markov stage), a high bleeding rate due to ROMI in response (Markov stage), the average daily dosage of rhTPO (15,000 U/ampoule), days of weekly rhTPO (7,500 U/ampoule), and the

Table 4. Direct costs per cycle (USD)

Costs	ROMI	EPAG	rhTPO + RTX	ATRA + Danazol/BSC
Drug acquisition cost	2334.2	1307.1	3439.7	138.4
Administration cost	54.5	5.7	152.0	24.9
Costs of a bleeding disposition in response	2.1	2.4	601.8	601.8
Costs of a bleeding disposition in non-response	40.9	58.5	990.4	990.4
Costs of adverse events	3.1	57.9	25.2	-
Monitoring costs	63.2	63.2	63.2	63.2
Total fee in response	1036.3	1436.2	4281.8	828.2
Total fee in non-response	1075.1	1492.3	4670.4	1216.8

EPAG: eltrombopag; ROMI: romiplostim; RTX: rituximab; ATRA: all-trans retinoic acid; BSC: best supportive care.

Table 5. Base case cost-effectiveness

Drug	Total costs ($)	Total QALYs	Incremental costs ($)	Incremental QALYs	ICERs ($/QALY)
EPAG	22,366.2	9.787			
ROMI	26,704.1	9.791	4344.4	0.004	1135779.46

EPAG: eltrombopag; ROMI: romiplostim.

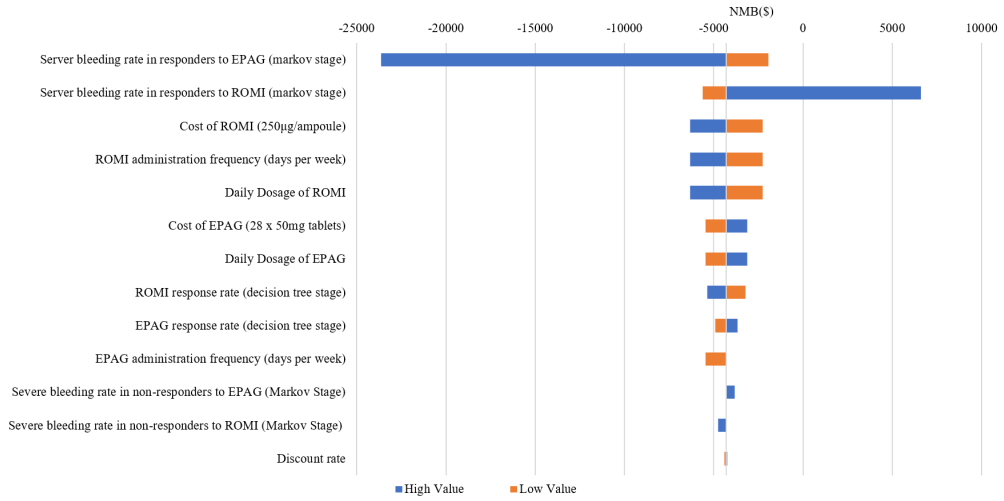

Figure 4. One-way sensitivity analysis. ROMI: romiplostim; EPAG: eltrombopag. NMB: net monetary benefit, NMB=(λ×ΔEffectiveness)−ΔCost, λ=1 times GDP per capita.

average daily dosage of rhTPO (7,500 U/ampoule).

3.3. PSA

PSA results are presented on the cost-effectiveness planes in Figure 5. CAECs demonstrated that at a cost-effectiveness threshold of $0-$36,117.2 (3 times GDP per capita)/QALY, the probability that ROMI is cost-effective versus EPAG was 0.68% and 0.16% (Figure 6).

3.4. Scenario analysis

Based on the pre-negotiation prices, ROMI is not cost-effective compared to EPAG. In the scenario analysis, we examined the probability of ROMI being cost-effective at different prices while keeping other conditions constant. When the monthly drug cost of ROMI ($326.8) is equal to EPAG, under a willingness-to-pay threshold of 0.5 times GDP per capita in China, the probability of ROMI being cost-effective exceeds 50% (Table 6). Moreover, as the price of ROMI decreases, the probability of ROMI being cost-effective increases. This analysis highlights the importance of price adjustments in determining the cost-effectiveness of ROMI in treating cITP.

4. Discussion

4.1. ROMI's cost-effectiveness relative to EPAG under specific pricing conditions

This study aimed to evaluate the cost-utility of romiplostim (ROMI) compared to eltrombopag (EPAG) for the treatment of chronic immune thrombocytopenia (cITP) in Chinese adults, specifically addressing the

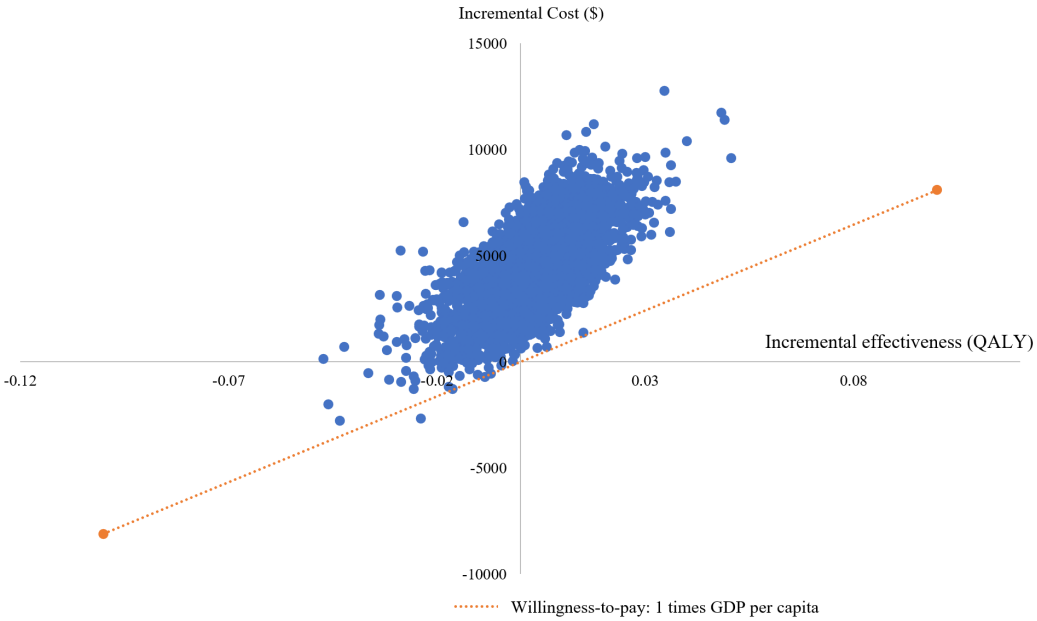

Figure 5. Probabilistic sensitivity analysis (5,000 simulations).

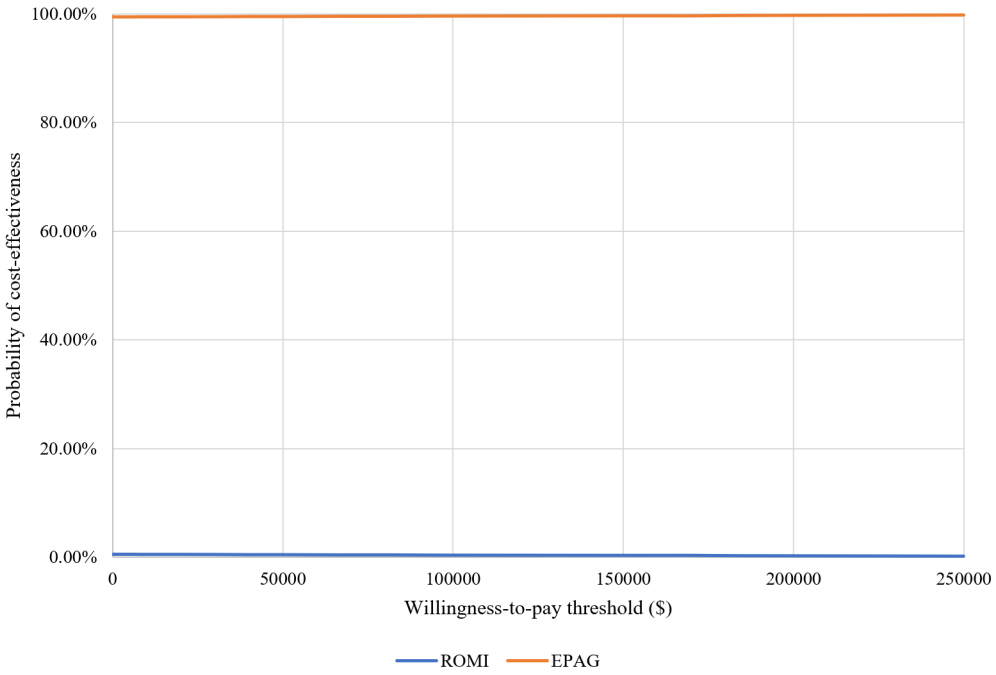

Figure 6. Cost-effectiveness acceptability curves. ROMI: romiplostim; EPAG: eltrombopag.

Table 6. Probability of ROMI being cost-effective compared to EPAG at different price points

Price of ROMI ($/ampoule)	Willingness-to-pay threshold		
	(0.5 times GDP per capita)/QALY	(0.8 times GDP per capita)/QALY	(1 times GDP per capita)/QALY
583.5*	0.54%	0.44%	0.44%
326.8	55.7%	56.4%	56.7%
294.4	74.8%	75.5%	76.2%
264.6	87.5%	88.2%	88.7%
234.9	95.8%	96.2%	96.5%

*$583.5/ampoule: the price used in the base case analysis, resulting in a drug acquisition cost of $2,334.2 per cycle.

economic impact of these treatments. In evaluating the pharmacoeconomic aspects of cITP treatment in China, a point warranting acknowledgement is that this analysis is specifically based on the pre-negotiation list price of ROMI. This context is essential as it underlines that the economic insights and conclusions are contingent upon these initial pricing assumptions before any pricing negotiations or adjustments. If the pricing of ROMI is in line with or lower than that of EPAG, ROMI emerges as a better choice, providing both economic and therapeutic advantages. This dominance of ROMI is predominantly attributed to its comparative affordability, without compromising efficacy, and an enhanced safety profile. Moreover, the similarity in platelet response rates between ROMI and EPAG, as highlighted in a comprehensive review of 14 randomized controlled trials (RCTs) by Puavilai *et al.* (2020) (*18*), corroborates the pharmacoeconomic benefit of ROMI when priced competitively. Our findings provide valuable guidance for clinical practice, emphasizing the importance of cost considerations in therapeutic choices for ITP.

4.2. Contribution to clinical economic evaluation in China

The current findings added to the existing evidence and models while remaining in line with prior models (*19-21*). First, EPAG is commonly used as a comparator in the economic evaluation of ROMI. Second, a lifetime horizon was thought to be appropriate, since adult ITP tends to be a chronic disease and the median age of patients is 45. Third, a one-month cycle was used to match the clinical trial involving ROMI. Costs and outcomes were discounted at a rate of 5% annually, as recommended by the China Guidelines for Pharmacoeconomic Evaluations (*22*). The treatment pathway used in this model was based on the Chinese guidelines on the diagnosis and management of adult ITP (version 2020) (*2*). Above all, this study stands out as one of the few model-based approaches being adopted in developing countries, bridging a critical knowledge gap and offering substantial insights for the evaluation of relevant drugs in this context.

4.3. Limitations

While providing valuable insights, this study had several limitations that warrant consideration. First, the key effectiveness outcomes rely on indirect comparisons between ROMI and EPAG, as direct head-to-head clinical trials are not available. This reliance introduces a degree of uncertainty, but this methodology remains the most feasible and is representative for the Chinese context. Due to simplifications in the model, this study focused on patients who have not undergone a splenectomy, and the cost-effectiveness of ROMI in a small subset of patients who have undergone a splenectomy for ITP remains unknown. More clinical efficacy and cost data are necessary to draw conclusions in this regard. Additionally, this cost analysis is confined to direct medical expenses, drawing primarily from existing literature, and didn't account for broader societal perspective, including the indirect costs related to lost productivity. A more comprehensive analysis incorporating these indirect costs would offer a more holistic understanding of ROMI's economic impact. These limitations highlight the need for ongoing research from diverse perspectives to fully comprehend the pharmacoeconomic implications of ROMI and EPAG in the treatment of cITP.

5. Conclusion

Under the current pre-negotiation pricing, ROMI is not a cost-effective option. However, ROMI could be cost-effective if its price is reduced to be lower than or equal to EPAG. This study highlights the critical role of pricing in treatment cost-effectiveness and suggests that future research and pricing negotiations are needed to make ROMI a viable economic alternative for ITP treatment in China.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Brief Report

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Splenectomy unveils thrombocytosis in underlying myeloproliferative neoplasms with extrahepatic portal vein obstruction

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SUMMARY Extrahepatic portal vein obstruction (EHPVO) is a rare disease with myeloproliferative neoplasm (MPN) as the most common cause. We report that hypersplenic hematologic changes in EHPVO might be eliminated by MPN. Through experience with splenectomy for variceal control with EHPVO, we suspected that spleen might mask MPN-induced thrombocytosis, and that MPN might have a significant influence on excessive thrombocytosis after splenectomy. To clarify the influence of MPN and spleen on platelet trends, we conducted a retrospective hospital database analysis, evaluating 8 EHPVO patients with splenectomy (2 males, 6 females; from 17 years to 64 years, mean 38.3 years). Three (37.5%) of 8 were diagnosed as MPN by JAK2V617F mutation. The perioperative serum platelet counts in EHPVO without MPN were 10.5, 35.4, and 36.6 ($x10^4/\mu L$) preoperatively, after 1 week and 3 weeks, respectively. The platelet counts in EHPVO with MPN were 34.2, 86.4, and 137.0 ($x10^4/\mu L$), respectively. Splenectomy and MPN showed positive interaction on platelet increasing with statistical significance. We also examined the spleen volume index (SpVI: splenic volume (cm^3) / body surface area (m^2) and postoperative platelet elevations ratio (PER: 3-week postoperative platelet counts / preoperative platelet counts). However, both SpVI and PER showed no significant difference with or without MPN. Histological examination revealed splenic congestion in all 8 EHPVO cases, and splenic extramedullary hematopoiesis in 2 of 3 MPN. In EHPVO with MPN, hypersplenism causes feigned normalization of platelet count by masking MPN-induced thrombocytosis; however, splenectomy unveils postoperative thrombocytosis. Spleen in EHPVO with MPN also participates in extramedullary hematopoiesis.

Keywords extrahepatic portal vein obstruction, myeloproliferative neoplasm, splenectomy, thrombocytosis, extramedullary hematopoiesis

1. Introduction

Extrahepatic portal vein obstruction (EHPVO) is a crucial cause of non-cirrhotic and prehepatic portal hypertension. EHPVO is an intractable and rare disease with incidence rates of EHPVO at 3.78 and 1.73 per 100,000 inhabitants in European males and females, respectively (*1*). Japan shows a lower estimated incidence of approximately 0.61 per 100,000 inhabitants (*2*).

Myeloproliferative neoplasm (MPN), including polycythemia vera, essential thrombocythemia, chronic idiopathic myelofibrosis, and unclassifiable type (MPN-U), is the most prominent cause of EHPVO with a prevalence of 15%-30% (*3-9*). The incidence of MPN is also rare with annual incidence rates for polycythemia vera, essential thrombocythemia, and chronic idiopathic myelofibrosis at 0.84, 1.03, and 0.47 per 100,000, respectively (*10*).

We recently suggested that hypersplenic hematologic changes of thrombo-leukocytopenia in EHPVO patients might veil MPN-induced proliferation of blood cells (*9*). Approximately 35% of polycythemia vera, one type of MPN, manifested no hematological sign of myeloproliferations. These are referred to as masked polycythemia vera (*11*). In portal hypertension, including EHPVO and Budd-Chiari syndrome, latent MPN with JAK2V617F mutation and normal blood counts was reportedly more common than overt MPN with elevated hemoglobin, white cell counts and/or platelets (*12*).

For the gastroenterologist, diagnosing the underlying MPN in EHPVO patients is extremely difficult because of the almost normalized blood conditions in hematological appearance due to the conflicting effects between hypersplenic hematopenia by EHPVO and blood cell proliferation by MPN. MPN is a potentially life-threatening blood malignancy with thrombotic complications, including myocardial, cerebral infarction and splanchnic vein thrombosis. For EHPVO patients with MPN, the original cause is splanchnic thrombosis due to thrombotic tendency; however, the main symptom of EHPVO is hematemesis or melaena by portal hypertensive bleeding, including esophagogastric or ectopic varix rupture. Further, MPN patients usually need antithrombotic agents for platelet hyperaggregability regardless of the high risk of variceal rupture. EHPVO patients are usually face a dilemma between thrombotic complications and variceal bleeding events, which causes a refractory situation for treatment by gastroenterologists.

For treatment of intractable esophagogastric varices, we performed Hassab's operation including splenectomy with devascularization of the upper half of the stomach and distal esophagus. EHPVO is very rare. Furthermore, treatment with Hassab's operation including splenectomy for esophagogastric varices in EHPVO with MPN is extremely unusual. Based on experience with splenectomy in EHPVO patients, we suspected that the comorbidity of MPN might have a great influence upon the postoperative increase of platelet counts, and further, that the spleen's participation in veiling original MPN-induced blood cell proliferation might also be associated with latent MPN as a feigned normalization of hematological appearance (*9,13*).

This retrospective single-center study was conducted to clarify the influence of MPN on spleen and platelet count trends in EHPVO patients.

2. Patients and Methods

Between January 2000 and February 2024, 17 patients with a diagnosis of EHPVO were treated in our hospital. Eight of 17 EHPVO patients who had undergone splenectomy for treatment of esophagogastric varices were enrolled in this study. Medical records for all 8 patients were identified and reviewed retrospectively. In all patients, the diagnosis of EHPVO was confirmed by imaging modalities, including ultrasonography, contrast-enhanced computed tomography (CT), angiography, or contrast-enhanced magnetic resonance imaging. Patients with hepatocellular carcinoma, other malignancies, liver cirrhosis or operative history including pancreaticoduodenectomy and choledochotomy were excluded as a diagnosis of EHPVO.

The medical records for patient age, gender, esophagogastroduodenoscopy findings were extracted from patient charts. Regular blood tests, hepatic and renal function tests were performed in all patients. JAK2V617F mutations were tested as a screening for MPN. The diagnosis and treatment of MPN was performed by hematologists in our hospital.

In all cases of EHPVO patients with Hassab's operation including splenectomy, CT was scrutinized for preoperative simulation. Preoperative splenic volume was measured by 3-dimensional CT volumetry using Synapse Vincent® (FUJI FILM Medical, Tokyo). The volume analyzer Synapse Vincent® automatically measures splenic volume.

The splenic volume index (SpVI) was calculated as splenic volume (cm^3) / body surface area (m^2) (*14*). Platelet elevation ratio (PER) was calculated as 3-week postoperative platelet counts (/μL) / preoperative platelet counts (/μL). Continuous variables were presented as the mean ± standard deviation and compared using the Mann-Whitney *U*-test since the sample sizes were relatively small. Values without normal distribution were presented as medians with interquartile range (IQR). The effects of splenectomy with and without MPN, and their interaction in perioperative serum platelet counts were investigated using two-way analysis of variance (ANOVA). The data were analyzed using IBM SPSS Statistics® Ver. 28.0.1. A p-value < 0.05 was considered significant.

Histological hematoxylin and eosin-stained sections of spleen in all EHPVO cases were reviewed by pathologists in our hospital. In cases of splenic extramedullary hematopoiesis, immunohistochemical labeling of CD41, CD71 and myeloperoxidase for scrutiny of megakaryocyte, erythroid and myeloid lineage cells, respectively, were also evaluated.

All study participants provided informed consent and the study was carried out in accordance with the ethical standards set by the Declaration of Helsinki. This study was approved by the hospital ethics committee (B-2022-615).

3. Results and Discussion

Eight EHPVO patients who underwent splenectomy were included in the analysis. The clinical presentations of these patients are summarized in Table 1. Two males (25.0%) and 6 females (75.0%); the age distribution for splenectomy ranged from 17 to 64 years, with a mean age of 38.3 years. Indication for splenectomy included 7 esophagogastric varices (87.5%) and 1 gastric varix (12.5%). All 8 EHPVO cases were referred by local gastroenterologists because of refractory esophageal and gastric varices by endoscopy and intervention radiology. Six (75.0%) of 8 cases had a history of variceal rupture, and 2 cases (25.0%) received prophylactic treatment for risky esophagogastric varices. Three patients (37.5%) of 8 were positive for JAK2V617F mutation. All 3 of the JAK2V617F mutated patients with EHPVO were diagnosed as MPN including polycythemia vera, essential thrombocythemia, and myeloproliferative

Table 1. The clinical presentations of EHPVO patients treated by splenectomy for refractory esophagogastric varices

Case	Age	Sex	Indication for splenectomy	Prophylactic or bleeding varices	JAK2V617 mutation	MPN
1	44	M	EGV	Bleeding		-
2	42	F	EGV	Prophylactic	+	MPN-U
3	32	M	EGV	Bleeding		-
4	47	F	EGV	Bleeding	+	ET
5	27	F	EGV	Bleeding		-
6	33	F	GV	Prophylactic		-
7	64	F	EGV	Bleeding	+	PV
8	17	F	EGV	Bleeding		-

EGV: esophagogastric varices, ET: essential thrombocythemia, GV: gastric varices, MPN: myeloproliferative neoplasm, MPN-U: myeloproliferative neoplasm, unclassifiable, PV: polycythemia vera.

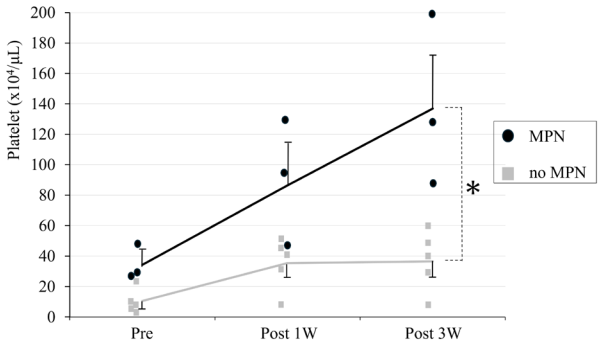

Figure 1. Comparison of serum platelet counts of EHPVO patients with or without MPN before and after splenectomy at 1 week and 3 weeks. Serum platelet counts increased to a greater degree in EHPVO with MPN than without MPN. Splenectomy and MPN showed positive interaction in platelet count trends with statical significance (*: F = 4.14, $p = 0.033$).

neoplasm, unclassifiable (MPN-U), respectively, by hematologists.

Figure 1 shows a comparison of the serum platelet counts of EHPVO patients with or without MPN before and after splenectomy at 1 week and 3 weeks. The perioperative serum platelet counts for EHPVO without MPN were 10.5, 35.4, and 36.6 ($x10^4$/μL) preoperatively, after 1 week and after 3 weeks, respectively. Further, the perioperative serum platelet counts for EHPVO with MPN were 34.2, 86.4, and 137.0 ($x10^4$/μL) preoperatively, after 1 week, and after 3 weeks, respectively. The distribution of preoperative platelet counts in EHPVO patients demonstrated thrombocytopenia without MPN and normal to slightly high platelet count with MPN. The serum platelet trends of both groups were postoperatively increasing; however, splenectomy in MPN patients caused prominent elevation. Two of 3 MPN cases showed thrombocytosis of more than 120×10^4/μL 3 weeks after splenectomy, and exceeded 190×10^4/μL during perioperative periods. The postoperative thrombocytosis of all 3 MPN cases was treated by hematological cytoreduction therapy including hydroxyurea with/without anagrelide by hematologists. All 3 MPN patients also continued anti-platelet agents under appropriate additional endoscopic treatment for esophageal varices. In 3 of 5 patients without MPN, splenectomy contributed to normalization of platelet counts after 3 weeks. The interaction of splenectomy and MPN in perioperative serum platelet counts was investigated using two-way ANOVA. Splenectomy and MPN showed positive interaction in postoperative thrombocytosis with statistical significance ($F = 4.14$, $p = 0.033$).

We then examined preoperative spleen volume and perioperative platelet increasing degree with or without MPN, in EHPVO patients. The median splenic volume determined using 3-dimensional CT volumetry Synapse Vincent® (FUJI FILM Medical, Tokyo, Figure 2A and 2B) was 788.4 (IQR, 622.9-1019.1; range, 187.3-1719.2) cm^3 and the mean splenic volume was 841.2 ± 420.4 cm^3. The median SpVI as splenic volume (cm^3) / body surface area (m^2) was 540.5 (IQR, 367.5-660.8; range, 118.6-859.6) cm^3/m^2. Figure 2C demonstrated the distribution between the SpVI and PER in all 8 EHPVO patients with splenectomy. With or without MPN in EHPVO patients, SpVI was 572.9 ± 73.6 and 488.0 ± 269.3, respectively, and no significant difference was noted (Figure 2D). We also examined the PER calculated as 3-week postoperative platelet counts (/μL) / preoperative platelet counts (/μL) with and without MPN in EHPVO patients. The PER was 4.32 ± 1.85 and 4.79 ± 3.22, with or without MPN in EHPVO patients, respectively, and there were also no significant differences (Figure 2E).

Figure 3 shows resected spleen weighing 574 grams (Figure 3A) and pathological assessment in case 4. Histological examination of the resected spleen demonstrated splenic sinus congestion and decreased white pulp (Figure 3B) in all the 8 EHPVO patients, the 5 without MPN and the 3 with MPN. Furthermore, 2 of 3 EHPVO patients with MPN revealed local splenic extramedullary hematopoiesis (EMH), as shown Fig Figure 3C. Immunohistochemical examination of case 4 revealed CD41-positive megakaryocytes (Figure 3D, white arrowhead), CD71-positive erythroid lineage cells (Figure 3E) and myeloperoxidase-positive myeloid lineage cells (Figure 3F) in spleen. In cases of EHPVO with MPN, not only portal hypertensive change of spleen, but also EMH in spleen might be associated

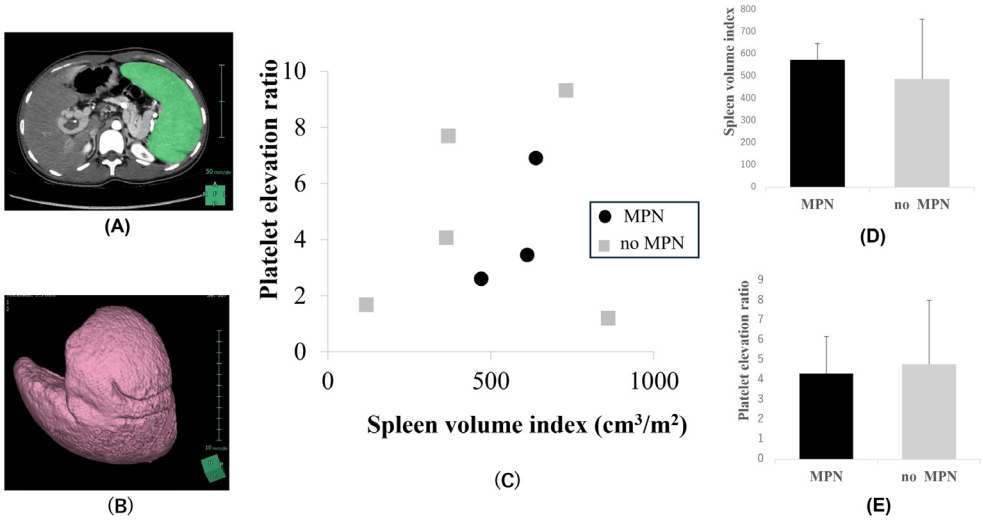

Figure 2. (A, B) Measurement of splenic volume. Splenic volume was automatically measured by 3-dimensional computed tomography volumetry using Synapse Vincent® (FUJI FILM Medical, Tokyo); **(C)** The distribution of the SpVI (Splenic volume index) and PER (Platelet elevation ratio) in all 8 EHPVO patients with splenectomy; **(D, E)** With or without MPN in EHVPO patients, SpVI and PER showed no difference.

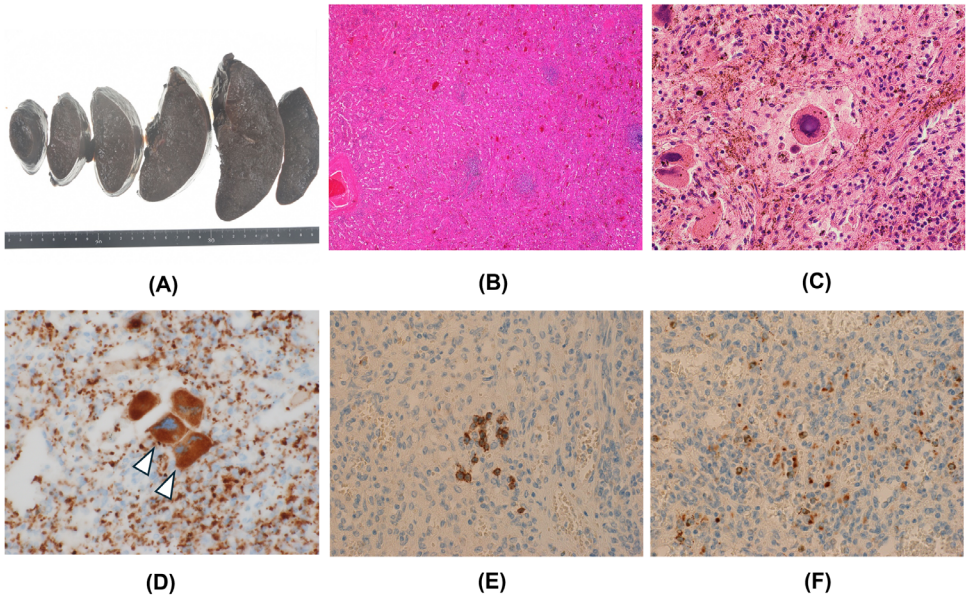

Figure 3. Macroscopic (A) and histological (B-F) findings of the resected spleen in case 4. (A) Photograph of resected spleen weighing 574 gram; **(B, C)** Histological examination demonstrated splenic sinus congestion and decreased white pulp, with local extramedullary hematopoiesis (B: hematoxylin-eosin, x 40, C: hematoxylin-eosin, x 400); **(D, E, F)** Immunohistochemical studies revealed CD41-positive megakaryocytes (D, white arrowhead, x 400), CD71-positive erythroid lineage cells (E, x 200), and myeloperoxidase-positive myeloid lineage cells (F, x 200) in the spleen.

with the pathogenesis of splenomegaly.

MPN is reported to be the most significant factor for EHPVO (*3-9*). The original cause of EHPVO is based on thrombotic tendency; therefore, the association between MPN and increased risk of thrombosis should be considered in screening for EHPVO (*15*). However, awareness of the association between EHPVO and MPN among gastroenterologists is generally insufficient.

A significant relationship between EHPVO and MPN was proven by the high frequency of a clonal mutation of JAK2V617F, and which should be utilized as a diagnostic tool to detect latent MPN in EHPVO patients (*9*). Screening for JAK2V617F mutation in patients with splanchnic vein thrombosis including EHPVO is extremely important, and can assist in both the diagnosis and treatment of MPN. The JAK2V617F mutation is present in more than 90% of patients with polycythemia vera and in approximately 60% of patients with essential thrombocythemia and myelofibrosis (*16,17*). JAK2 tyrosine kinase activates a cytokine-independent JAK–STAT pathway, causing proliferation of mature myeloid cells. The JAK2V617F mutation was shown to be an independent risk factor for splanchnic vein thrombosis (*3,18-20*). None of the risk factors could be identified in a third of patients with EHPVO; however, more than half of these go on to develop

an overt MPN during follow up (*8,20,21*). Patients with EHPVO should be screened for the JAK2V617F mutation to avoid overlooking a potential underlying persistent thrombophilia due to MPN (*9*).

We reported that hypersplenic hematologic changes of thrombo-leukocytopenia in EHPVO patients might be veiled by MPN-induced proliferation of blood cells (*9*). In this finding, we suspected that hypersplenism as a comorbidity with MPN might be associated with latent MPN, and that the spleen may have contributed to the normalized blood counts in latent MPN. These splenectomies with MPN led to the strong suspicion that extreme thrombocytosis in postoperative asplenia was veiled by preoperative EHPVO-induced hypersplenism. This phenomenon may also explain the spleen function's contribution to a condition of latent MPN including masked polycythemia vera.

Thrombotic complication is very serious because EHPVO patients often suffer from vascular disease, including myocardial or cerebral infarction. Furthermore, we have experienced cases of myocardial and cerebral infarction in EHPVO patients in their 30s (*9*). Owing to the thrombotic tendency, prophylactic thrombotic agents should be considered in EHPVO; however, the majority of symptomatic EHPVO patients experience intestinal variceal rupture. The dilemma between thrombosis and hemorrhage in EHPVO is the most difficult for general gastroenterologists (*9,22*). Prognosis in EHPVO patients is strongly associated with the control of esophagogastric variceal bleeding. EHPVO patients that are appropriately controlled for portal hypertensive complication showed favorable prognosis with a survival rate of 69-86% at 10 years (*23*). For eradication of the refractory esophagogastric varices of EHPVO with MPN, we experienced successful cases of combination therapy of Hassab's operation and subsequent endoscopic variceal ligation (*20,23*). Hassab's operation includes splenectomy with devascularization of the upper half of the stomach and distal esophagus (*24,25*). However, portal thrombus after splenectomy was a major postoperative complication in portal hypertension. In MPN cases, splenectomy induces excessive thrombocytosis, and thrombocytosis with reduced portal vein velocity is associated with the formation of further portal vein thrombosis causing hepatic failure or disappointing portal hypertension. Splenectomy in cases of hypersplenism with large splenic vein diameter is thought to further promote the progression of portal vein thrombus, and early antithrombotic administration should be considered in these situations (*23,26*). Contrary to thrombotic complications, extreme thrombocytosis is sometimes characterized by severe bleeding tendency with decrease or abnormality of von Willebrand factor as a consequence of the precipitous increase in platelets, referred to as acquired von Willebrand factor syndrome (*27-29*). All 3 MPN cases with splenectomy demonstrated excessive thrombocytosis and required strict cytoreduction treatment for platelet count to address concern about subsequent coagulopathy by acquired von Willebrand factor syndrome. In our cases, the timely introduction of cytoreduction therapy for extreme thrombocytosis by hematologists was thought to be possible because of a preoperative diagnosis of MPN and constant perioperative collaboration with hematologists. If hematological disease including MPN is associated with EHPVO, collaboration between gastroenterologists and hematologists is essential for better treatment and prognosis.

EMH is reportedly associated with many diseases, including chronic anemia, sickle cell disease, thalassemia, spherocytosis, and hematological neoplasms (*27*). EMH is generally a compensatory response occurring secondary to inadequate bone marrow function. Few papers on splenic EMH with MPN have been published, and less frequent cases of splenic EMH associated with MPN under hypersplenic condition of EHPVO have rarely been reported.

Further detailed data on EHPVO with MPN in a large series must be accumulated; however, we hope that our results will contribute to the clarification of pathogenesis and better treatment for the intractable conditions.

Gastroenterologists sometimes experience EHPVO patients requiring splenectomy for complications of portal hypertension, including hemorrhagic esophagogastric varices. It is important to consider underlying MPN based on perioperative hematological change and histological character of resected spleen. Otherwise, the gastroenterologist may overlook the underlying MPN causing EHPVO due to feigned normalization of platelet counts by the conflicting effects of hypersplenic hematopenia by EHPVO and blood cell proliferation by MPN. Based on cases of EHPVO with MPN, our team considers screening for JAK2V617F mutation to be essential for all patients with suspected EHPVO.

In conclusion, in EHPVO patients with MPN, hypersplenism causes feigned normalization of platelet count by masking MPN-induced thrombocytosis; however, splenectomy unveils postoperative thrombocytosis. Spleen in EHPVO with MPN also participates in extramedullary hematopoiesis.

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Functional impairments in NBIA patients: Preliminary results

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SUMMARY Neurodegeneration with brain iron accumulation (NBIA) is a heterogeneous group (genetically and phenotypically) of genetically determined disorders. Up to date there is no cure for this disease, so the applied treatments focus on symptoms control and palliative care. The main problems are delayed motor development, gait deterioration, postural instability, cognitive dysfunctions, abnormal muscle tone and many others. As gait and balance deficits are predominant features of NBIA patients this study aimed at the use of the objective, instrumented functional tests as well as functional assessment scales to assess their functional impairments. Twenty three NBIA patients recruited for the study underwent objective, instrumented gait analysis, balance assessment, pedobarography and functional evaluation with Gross Motor Function Measure (GMFM-88). The results showed high variability and heterogeneity of NBIA functional status (GMFM from 27.5 to 100.0), but also showed some differences in gait pattern between their types ($p < 0.05$ at the pelvis, hip and knee). We think that these results could help design objective assessment protocols in future clinical studies.

Keywords NBIA, gait, balance, functional assessment

1. Introduction

Neurodegeneration with brain iron accumulation (NBIA) is a heterogeneous group (genetically and phenotypically) of ultra-rare genetically determined disorders, in which the main feature is accumulation of iron in the brain. Its heterogeneity of symptoms and rarity makes diagnosis very difficult and challenging (*1*). The diagnosis is usually first suspected when MRI features (hypointensity of the basal ganglia) are connected with progressive movement disorders (*2*). The main feature of NBIA is iron deposition in the brain, but some studies show, that this deposition may be secondary to a metabolic impairment of the neural cells, concerning such pathways as mitochondrial functions, lipid metabolism autophagy and iron homeostasis (*3*). Its assumed prevalence is from 0.1 to 0.3 per 100,000. Analysis of publicly available Genome Aggregation Database revealed, that prevalence is most probably much higher, reaching 0.92 per 100,000 (*4*).

Panthotenate kinase-associated neurodegeneration (PKAN, formerly Hallevorden-Spatz syndome) accounts for 50 % of all diagnosed NBIA patients. The carrier frequency is estimated as 1 per 275 to 500 people. In classic PKAN (75 % of cases) the disease onset appears in early childhood and the progression is fast. The first symptoms comprise clumsiness, motor development delay, dyspraxia, followed by gait pattern deterioration (in some cases in the form of toe walking), postural instability and visual impairment. Often abnormal muscle tone is present in the form of spasticity or rigidity. In severe cases of increased muscle tone bone fractures could appear, as well as osteoporosis due to reduced mobility. In atypical PKAN the onset appears later, patients have speech difficulties, milder gait pattern abnormalities, mild dystonia, and sometimes neuropsychiatric features are present. In time patients develop cognitive dysfunction (very variable in severity), which is negatively correlated with the onset age. Lost with age skills are not regained, and the decline shows a step-like pattern, with periods of relative stability (*5*). Some patients suffer additionally from "eye of a tiger" sign, dysarthia and behavioral disturbances (*6*).

The second most common type is PLA2G6-associated neurodegeneration (PLAN), approximately 20 % of NBIA cases, and the third MPAN (10%) (*7*). In PLAN development delay with visual impairment and facial dysmorphism are present. In some patients

epileptic seizures and neuroaxonal dystrophy occur (*7*). Other manifestations could also be present, such as dystonia, visual problems, and in some patients there is rapid progression of the disease, ending with death at an early age (*6*).

Mitochondrial membrane protein-associated neurodegeneration (MPAN) patients present juvenile-onset gait deterioration, rapid cognitive decline and suffer from neuropsychiatric problems (*6*). In some MPAN pediatric patients early signs of cardiac autonomic dysfunction symptoms were found, so monitoring of heart function in patients with this type of NBIA is recommended (*8*).

There are also other, less common, types of NBIA. In the fatty acid hydroxylase-associated neurodegeneration (FAHN) type the gait abnormality is the most characteristic feature, while in less than half of patients abnormal cognition is present. In some patients there is late onset associated with rapid progression. The majority of patients have dystonia and ataxia (*6*). The beta-propeller protein-associated neurodegeneration (BPAN) type starts with early occurring psychomotor retardation, which remains stable until adulthood. In their twenties / thirties dystonia rapidly occurs with parkinsonism and dementia, accompanied with rapid eye movements, dysautonomia and sleep disorders (*9*). In Parkinson disease type 9 (PARK9) type the levodopa-resistant parkinsonism is present accompanied by visual abnormalities, autonomic and psychiatric dysfunctions and dementia (*9*).

Up to date there is no cure for NBIA, so the applied treatments focus on symptom control, and palliative care. In cases of severe increased muscle tone botulinum toxin or baclofen are used. In some cases also benzodiazepines, deep brain stimulation and transcranial magnetic stimulation of the premotor cortex are considered. In cases of parkinsonism features (tremor, rigidity, severe bradykinesia) levodopa treatment is implemented. An intensive rehabilitation approach is also part of treatment, with occupational therapy, speech and swallow therapy. Assistive devices and environment adaptation to the patients' needs are recommended (*5,10-13*).

In case of PKAN two experimental therapies are now considered: iron chelation and high-dose pantothenate therapy (*5,14*), although the efficacy of them is, so far, doubtful (*9*).

The European Treat Iron-Related Childhood-Onset Neurodegeneration (TIRCON) project carried under EU FP7 connected dispersed NBIA scientific, clinical and patient-oriented communities from different European and non-European countries, enabling the creation of the NBIA registry and biobank and scientific international collaboration. This project identified also several problems and shortcomings, among others: problems with defining the reasonable endpoints in the future clinical studies, lack of disease-specific clinical scales assessing current status of patients, and lack of defined markers of disease progression (*15*). All these conditions are indispensable for assessment of efficacy of applied treatments. Therefore this study aimed at the use of the objective, instrumented functional tests as well as functional assessment scales in NBIA patients.

2. Patients and Methods

2.1. Patients

Twenty three NBIA patients, aged from 4 to 21 year (14 patients with MPAN, 5 with PKAN, 4 with BPAN), participated in the study. This was an opportunistic study, but all parents/guardians were informed about the possible use of the data for research and the purpose of the study, the methods used, and gave their consent. Those patients who were able to understand the study, regardless of their age, were also informed and gave their consent. The study conformed to the provisions of the latest version of the Declaration of Helsinki. All patients underwent functional assessment performed by an experienced physiotherapist, and instrumented gait analysis. Due to balance and communication problems pedobarography and balance assessment were performed in less than half of the patients. Ten patients underwent the second evaluation with instrumented, objective tests approximately one year after the initial assessment. This was because of two reasons: first, decline in functional status and loss of walking ability in the case of 11 patients, and second, various times of patients' recruitment to the study. The study was performed from February 2021 until March 2023.

2.2. Gait analysis

The instrumented gait analysis was performed with VICON system with 12 MX cameras. The lower body Plug-In-Gait marker set and model were used. The patients were walking several times along the 10 m walkway with self-selected gait speed, and six technically correct trials were later averaged in Polygon, averaged data extracted and analyzed. Spatio-temporal data were expressed as per cent of the age and sex matched reference data, except for step width, which was normalized by ASIS-ASIS distance (distance between anterior superior iliac spines). The following kinematic data were extracted: pelvic tilt, pelvic range in transversal plane, hip range in sagittal plane, knee flexion at initial contact, midstance, and swing, foot range at push-off, and foot progression.

Additionally several gait indices, reflecting the patient's gait pathology, were calculated. In Nexus software the Gait Deviation Index (GDI) was calculated for each trial of each patient, separately for left and right leg, and later averaged for the patient's session.

GDI (*16*) is a single number, resulting from kinematic plots and principal component analysis. The methodology

uses three dimensional angles of the pelvis and hip, at the knee and ankle joints only angles in sagittal plane are used, and foot progression angle. This index is transformed and scaled in such a way, that its average for healthy subjects is 100, with a standard deviation of 10.

Another index is Gait Profile Score (GPS), which describes the overall gait pathology (*17*). It is composed of Gait Variable Scores (GVSs) calculated from 9 main kinematic gait variables, which can be presented as a Movement Analysis Profile (MAP). GVS is calculated as root mean square (RMS) difference between kinematic variable across gait cycle of the patient and reference variable representing healthy subjects. They are calculated for: pelvic tilt, hip flexion, knee flexion, ankle dorsiflexion, pelvic obliquity, hip abduction, pelvic rotation, and foot progression. From GVSs an overall index, GPS is calculated.

2.3. Pedobarography

Plantar loads during gait were registered on the Emed system (Novel Company) in 10 patients. Children were asked to walk barefoot several times on a pathway with a built-in pedobarography platform with their normal, self-selected speed. Data from three plantar loads of left and three plantar loads of right foot were averaged and taken for further analysis. Total load and loads on foot segments (feet were divided into segments automatically by the Novel software) were normalized to the patient's body weight.

2.4. Balance

The patients underwent balance test on Kistler force plates in two conditions: with eyes open (9 patients) and eyes closed (8 patients). Patients were asked to stand as quietly as possibly on the platform for 40 to 50 seconds with feet parallel, the distance between them equal to pelvis width, arms hanging freely along the torso. The data from the middle 30 seconds were used for the analysis. Matlab's own procedure was used to extract medio-lateral (separately for left and right) and antero-posterior (separately for anterior and posterior) displacements, the mean radius of sway and total sway path.

2.5. Functional evaluation

This evaluation was performed by one, experienced physiotherapist with Gross Motor Function Measure (GMFM-88), an assessment tool designed to measure the changes of gross motor functions in children with cerebral palsy, but can be used also for children with developmental problems. GMFM-88 assesses 88 activities in 5 dimensions: A. Lying and rolling, B. Sitting, C. Crawling and kneeling, D. Standing, E. Walking, Running and Jumping. These activities are ordered based on the levels of difficulty, and the scale has ratings from 0 to 100. This measure is validated on children from 5 months old to 16 years of age (*18-20*).

2.6. Statistics

Depending on the type of the distribution the data were summarized by means and standard deviation or median, minimum and maximum. The comparisons of the results between the three types of NBIA (MPAN, PKAN and BPAN) were done with ANOVA test or its non-parametric equivalent test. The comparisons between first and second evaluation was done using sign test for dependent samples. The dependence between the GMFM and gait indices was explored with a Spearman rank correlation test. The statistical significance was assumed at the level 0.05, and the STATISTICA 10.0 (TIBCO Software Inc.) was used.

3. Results and Discussion

3.1. Summary of the results

To our knowledge this is the first study which assessed in an objective way the functional status of children with NBIA by instrumented methods and functional tests, and therefore the comparisons of our results with the results concerning NBIA of others is impossible (confirmed by literature search in databases such as PubMed, World of Science, and ScienceDirect using combinations of the key words: NBIA, function, functional evaluation *etc.*).

The results of the objective and functional tests are summarized in Tables 1-5.

From the literature (Introduction) it is known that most patients with various types of NBIA suffer from gait and balance disorders. They are variable and their progression is not uniform. Our results confirm these statements. Despite the high variability it can be seen that the gait speed is reduced in comparison to healthy peers due to reduced cadence and step length. In the case of the balance study only 2 patients had all measured parameters within normal range during standing with eyes open, and one of these patients had also all parameters within normal range while standing with eyes closed, but in the case of a majority of evaluated patients at least one parameter exceeded the normal range, and in some patients nearly all of them.

The maximum number of parameters exceeding

Table 1. The spatio-temporal parameters of NBIA patients gait

Parameter	Mean	SD	Min	Max
Gait speed [%]	54.1	24.4	11.0	100.0
Cadence [%]	72.3	20.6	35.0	100.0
Step width / ASIS-ASIS	0.72	0.21	0.18	1.05
Step length [%]	73.2	21.4	21.0	100.0

Table 2. The kinematic data

Parameter	Median	Min	Max	Normal value (*21*)
Tilt [°]	14.0	-3.0	24.0	12 - 15
Pelvis range transverse [°]	14.0	4.0	35.0	8 - 10
Hip range sagittal [°]	33.5	14.0	50.0	43
Knee initial contact [°]	10.0	-22.0	36.0	0 - 4
Knee flexion in stance [°]	4.0	-22.0	35.0	0 - 4
Knee max in swing [°]	54.5	23.0	65.0	60 - 65
Push-off range [°]	24.5	0.0	53.0	25 - 30
Foot progression [°]	-5.0	-50.0	8.0	-12 - -15

Table 3. The Gait Variability Scores (GVS), Gait Profile Score (GPS) and Gait Deviation Index (GDI)

Parameter	Median	Min	Max
GVS pelvis sagittal	5.77	1.38	15.30
GVS hip sagittal	8.62	4.01	26.26
GVS knee sagittal	11.99	5.54	31.64
GVS ankle sagittal	6.96	2.96	28.45
GVS pelvis frontal	3.03	0.81	5.60
GVS hip frontal	5.17	1.43	14.62
GVS pelvis transverse	5.13	1.55	14.97
GVS hip transverse	11.12	4.71	45.42
GVS foot progression	10.66	2.35	35.94
GPS	8.63	5.40	17.69
GDI	73.70	48.70	97.20

Table 4. The loads normalized to the body weight, arch index and hallux angle

Parameter	Median	Minimum	Maximum	Normal value
Max force/BW [%]	131.9	110.3	164.9	130.0
MH1/BW [%]	19.7	7.1	33.2	20.0
MH2/BW [%]	23.5	10.9	30.9	30.0
MH3/BW [%]	23.2	13.5	33.1	35.0
MH4/BW [%]	16.9	10.4	36.8	30.0
MH5/BW [%]	10.4	3.4	28.7	20.0
Big toe/BW [%]	13.1	1.0	39.7	20.0
Second toe/BW [%]	3.6	0.4	10.2	10.0
Toes3-5/BW [%]	4.3	0.8	15.1	15.0
Hinfoot/BW [%]	75.9	31.1	115.8	70.0
Midfoot/BW [%]	32.3	6.4	54.0	20.0
Arch index	0.25	0.11	0.36	0.21 – 0.26
Hallux angle [°]	1.0	-5.0	15.0	< 15.0

Table 5. The results of Gross Motor Function Measure (GMFM)

	Median	Min	Max
GMFM_total	77.4	27.4	100.0
GMFM_A	96.1	72.5	100.0
GMFM_B	98.3	35.0	100.0
GMFM_C	85.7	2.4	100.0
GMFM_D	74.4	0.0	100.0
GMFM_E	45.8	4.2	100.0

normal range was 4 in the case of eyes open condition (1 patient) and 6 (all of parameters) in the case of eyes closed condition (1 patient).

3.2. Comparison between NBIA types

The differences between MPAN, PKAN and BPAN are presented in Figure 1 (gait), and Table 6. There were only four gait parameters, which were statistically significantly different between the NBIA types. In the case of balance, pedobarography and GMFM (total and in 5 dimensions) no statistically significant differences were found.

The three types in our NBIA group (MPAN, PKAN and BPAN) present slightly different types of gait disorders. During level walking the MPAN patients have increased knee flexion at initial contact, while PKAN patients start contact with ground with either straight or hyperextended knee. The position of the knee of BPAN patients is similar to their healthy peers. GVS of pelvis in transverse plane is the smallest in BPAN patients, which means the movement closest to healthy persons. All NBIA patients have high values of GVS hip in transverse plane: which means a lot of abnormal movement, but in the case of MPAN patients most of them have lower than the other two groups values, meaning more normal movement. Also there are some differences between the three types in the placement of the feet in relation to the line of progression, with high out toeing in the case of MPAN group. Surprisingly we

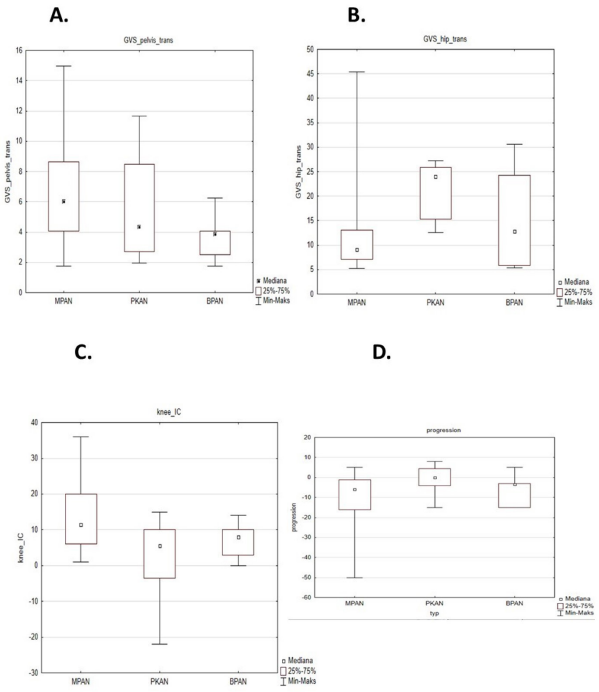

Figure 1. Differences between NBIA types during gait.

did not find any differences between the three groups in GMFM-88 assessment, neither in total nor in any of the 5 dimensions. Probably despite the fact that this evaluation is detailed and takes into consideration many aspects of activity in all of the functional dimensions its

Table 6. Summary statistics of statistically significant different parameters of gait for MPAN, PKAN and BPAN patients

Group	GVS pelvis transverse Median (Min – Max)	GVS hip transverse Median (Min – Max)	Knee initial contact [°] Median (Min – Max)	Foot progression [°] Median (Min – Max)
MPAN	6.78 (2.61 – 11.96)	8.42 (5.24 – 18.57)	10.0 (4.0 – 36.0)	-9.5 (-20.0 – 0.0)
PKAN	2.85 (1.55 – 11.66)	17.05 (5.46 – 26.32)	5.0 (-7.0 – 15.0)	3.0 (0.0 – 5.0)
BPAN	2.78 (1.74 – 4.07)	14.0 (10.77 – 30.62)	10.0 (0.0 – 15.0)	-4.0 (-15.0 – 0.0)

Table 7. Correlation coefficients (R) and determination coefficients (R^2) between GPS, GDI and GMFM

	GPS		GDI	
	R	R^2	R	R^2
GMFM total	-0.65	42.3	-0.65	42.3
GMFM A	-0.46	21.2	-0.46	21.2
GMFM B	-	-	-	-
GMFM C	-0.62	38.4	-0.62	38.4
GMFM D	-0.68	46.3	-0.68	46.3
GMFM E	-0.69	47.6	-0.69	47.6

sensitivity is much lower than parameters and indices from objective, instrumented gait analysis.

The dependence between overall gait indices GDI and GPS and GMFM and its dimensions is presented in Table 7. The only functional domain, which did not show any relation with gait indices is Sitting, which is not surprising. The correlations are medium, and calculated from the correlation coefficients the determination coefficients (R^2) are all below 50.0. The determination coefficient is a measure, which shows how well the change of one parameter can be explained by the change occurring in the second one. The obtained values were between 21.2 and 47.6, showing that there are other factors not accounted for. These values prove that functional assessment measures and gait are interrelated, but they are complementary: they assess different aspects of motor performance of NBIA patients.

3.3. First *vs.* second assessment

In the case of balance assessment and pedobarography there were no statistically significant differences between the first and second evaluation, in the case of gait analysis only two parameters differed statistically significant, both reflecting pelvic movement. GVS pelvis in sagittal plane changed from median value 6.73 to 5.29 ($p = 0.032$), and GVS pelvis in transverse plane from median value 4.96 to 6.41 ($p = 0.041$).

Eleven of our patients could not come to the second evaluation after one year from the first one, confirming the fact, that many NBIA patients suffer from rapid decline of their functional status and could within a very short time lose their ability to walk. But in those who participated in their second evaluation there was nearly no change in their gait patterns. The only two parameters were indices assessing the pelvic movements: GVS in sagittal plane decreased, and GVS in transverse plane increased. Both changes are positive. Decreased GVS pelvis in sagittal plane means change of pelvis movement toward the more normal pattern. In many patients with locomotor problems the compensatory movements are present in pelvis in transverse plane, so paradoxically the increase of GVS pelvis in transverse plane could indicate that they gained greater opportunity to use the pelvis to compensate for their deficits. The main conclusion from these findings is, that NBIA patients who do not rapidly lose their walking ability could maintain their gait pattern on a stable level for quite a long period of time.

There are many shortcomings connected with this study. Gait and balance objective assessments require the cooperation and understanding of the instructions by the patients. Due to communication problems, developmental delays, *etc*. some patients were unable to undergo pedobarography or balance evaluations. Some patients could not stand still for the required 40-50 sec, so balance evaluation was impossible. The number of evaluated patients is small, but such is the nature of NBIA as a rare disease. The majority of the group were MPAN patients, as this type dominates in Poland. This small number together with heterogeneity, and individual variability of NBIA makes statistical analysis and drawing meaningful conclusions difficult.

4. Conclusions

Despite the small number of patients and limitations of this study we think that these results could help design objective protocols in the future. Especially instrumented gait analysis turned out to be the easiest objective test to perform, even in those patients who had problems with understanding and obeying instructions, mainly because of the high engagement of the parents, and together with GMFM-88 it can constitute the basis for future clinical trials.

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Brief Report

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Genetic analysis of a novel *FBN1* mutation in a pediatric Marfan syndrome patient

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SUMMARY The aim of this study was to investigate a novel *FBN1* gene mutation in a pediatric patient with Marfan syndrome (MFS) to provide a theoretical basis for genetic counseling. The subject was a 5-month-old male infant. With informed consent from the proband and his family, 2 mL of peripheral venous blood was collected from the patient, his father, mother, and sister. DNA was extracted using a DNA extraction kit with EDTA-K as an anticoagulant. The extracted DNA was subjected to minigene transcription and bioinformatics analysis. For minigene construction, wild-type and mutant minigenes were inserted into pcMINI and pcMINI-C vectors, respectively. Four recombinant vectors were transfected into the HeLa and 293T cell lines. After transfection for 48 hours, RNA was extracted from eight samples. DNA was also extracted from the family members' samples to construct a library. Target regions were captured using the SureSelect Human All Exon V6 (Agilent) kit and were sequenced with Illumina NovaSeq (sequencing read length 2×150 bp). Bioinformatic analysis identified the c.8226+5del mutation as a variant of uncertain clinical significance (VOUS). Literature and database reviews confirmed that this mutation had not been previously reported, identifying it as a novel mutation. The study identified a novel *FBN1* mutation, c.8226+5del, that may be associated with clinical features such as low-set ears and distinctive facial characteristics in the proband. This mutation likely affects normal mRNA splicing, altering the structure and function of Exon 64 and potentially contributing to the development of autosomal dominant MFS.

Keywords Marfan syndrome (MFS), *FBN1*, mutation, novel, gene mutation

1. Introduction

Marfan syndrome (MFS) is a hereditary connective tissue disorder that is inherited in an autosomal dominant manner. The overall incidence of this disease is relatively low, approximately 0.01% to 0.02%, with 20% to 30% of cases arising from de novo mutations. Incidence among live-born infants is about 0.01% (*1,2*). Although the incidence in live-born infants is low, the clinical symptoms are often severe, leading to a poor prognosis, and most affected children do not survive beyond 17 months. The primary characteristics of MFS include skeletal, ocular, and cardiovascular abnormalities. This disease is closely associated with age, and patients typically exhibit features such as a tall and slender stature, elongated limbs, angina, arachnodactyly, arrhythmias, spinal abnormalities, and retinal detachment (*3*). If not promptly managed, the disease can also affect the skin, lungs, and central nervous system (*4*). Currently, the Ghent criteria are the gold standard for the diagnosis and treatment of MFS. However, the genetic, environmental, and physical characteristics of patients can vary significantly, resulting in diverse clinical presentations and diagnostic challenges (*5*).

In the 1990s, Dietz and colleagues proposed that mutations in the fibrillin-1 (*FBN1*) are a significant factor in the development of MFS (*6*). More recent research has confirmed that *FBN1* is the causative gene for MFS (*7*). The current study analyzed the *FBN1* gene in a pediatric MFS patient using high-throughput sequencing. Results revealed a novel mutation site, further expanding the genetic data associated with this disease.

2. Materials and Methods

2.1. Materials

The subject was a 5-month-old male infant who was delivered *via* cesarean section at 35 weeks and 4 days of gestation due to oligohydramnios. This study adhered to the principles of the Helsinki Declaration and was approved by the Ethics Committee of the Medical School (NO. 2020034). Informed consent was obtained from all participants, with consent for those under 18 years of age being provided by their guardians.

2.2 Methods

2.2.1. Specimen collection

After obtaining informed consent from the proband's family, 2 mL of peripheral venous blood was collected from the patient, his father, mother, and sister. The blood samples were collected in EDTA-K tubes to prevent coagulation. DNA was extracted from the samples using a DNA extraction kit (QIAGEN, Germany) following standard procedures.

2.2.2. Methods of detection

2.2.2.1. Introduction of restriction sites into minigenes

Leukocyte gDNA was extracted using a DNA extraction kit, and DNA was amplified using a PCR machine. The primers used for the reaction are listed in Table 1. Each PCR tube contained 22 μL of 1.1× Mix, 1 μL of upstream primer (Primer-F), 1 μL of downstream primer (Primer-R), and 1 μL (0.5 μg) of gDNA, with RNase-free water added to reach a final volume of 30 μL. PCR amplification was performed with an annealing temperature of 57℃ for 30 cycles. The PCR products were then separated by gel electrophoresis, and the desired bands were excised and purified.

2.2.2.2. Construction of recombinant vectors

The construction of recombinant vectors involved restriction digestion, ligation, transformation, and verification of recombinant clones.

Restriction digestion of recombinant vectors: To the DNA fragment (25 μL, 500 ng), 3 μL of 10× NEB buffer, 0.6 μL each of Enzyme 1 and Enzyme 2, and ddH_2O were added to reach a final volume of 30 μL. The mixture was incubated at 37℃ for 2 hours. After digestion, the recombinant vector was verified with gel electrophoresis and the desired bands were excised for purification.

Construction of recombinant vectors: Seven μL of the digested DNA fragment (wild-type/mutant), 1 μL of 10× ligase buffer, 1 μL of digested vector, and 1 μL of ligase were combined. The mixture was incubated at 4℃ overnight for ligation. The ligated product was transformed into E. coli DH5α competent cells and incubated at 37℃ overnight. Several single colonies were randomly selected for identification. Verification methods included colony/liquid PCR and Sanger sequencing.

2.2.2.3. Cell transfection

The recombinant vectors were transiently transfected into the HeLa and 293T cell lines following the instructions provided by the lipofection reagent manufacturer. Samples were collected 48 hours post-transfection.

2.2.2.4. Minigene transcription analysis

Total RNA was extracted from the transfected cell samples according to the kit instructions. After determining the RNA concentration, equal amounts of RNA were reverse transcribed into cDNA. PCR amplification was performed using flanking primers specific to the minigene vector. The resulting gene transcription bands were detected with agarose gel electrophoresis. Each band was then excised and subjected to Sanger sequencing for further analysis.

2.2.3. Bioinformatic analysis

Quality control of the raw data was performed using the software FastQC. The filtered sequencing reads were

Table 1. Experimental primers

Primer name	Primer sequence (5′-3′)
231081-*FBN1*-F	TGGCCTCTCCGAATCACTAG
231361-*FBN1*-F	GGCCCTAGTGGTTTTGAATG
234049-*FBN1*-R	CCAATGGAAATACACGTCCC
234456-*FBN1*-R	GCACCATTACAAACCCTCAC
pcMINI-*FBN1*-KpnI-F	GGTAGGTACCTACATGGCCTCCCTCATCTA
pcMINI-*FBN1*-EcoRI-R	TGCAGAATTCCTCCACGTTATTTTTGTCTA
FBN1-mut-R	GAAACTAACTTCTGACCACCTCGATATTGG
FBN1-mut-F	CCAATATCGAGGTGGTCAGAAGTTAGTTTC
pcMINI-C-*FBN1*-KpnI-F	GGTAGGTACCAGGGAACTGGGAATTAGAGG
pcMINI-C-*FBN1*-EcoRI-R	TGCAGAATTCTTAATGAAGCAAAACCTGGA

aligned to the GRCh37/hg19 reference genome using the software BWA (version 0.7.15-hg19). Duplicate sequences were removed with the software Picard. Single nucleotide variants (SNVs) and insertion-deletion variants (Indels) were detected using the GATK toolkit (version 3.7-0). Copy number variations (CNVs) were identified using XHMM (version 1.0) and CNVkit (version 0.8.4). Annotation of the variants was carried out using the software Annovar and VEP.

2.2.4. Quality control analysis

2.2.4.1. Data quality control

Throughout the detection and analysis process, quality control analysis of the samples was performed. Unqualified samples were excluded. Key metrics recorded included Q20, Q30, GC content, average sequencing depth, and coverage of the targeted regions.

2.2.4.2. Sample identification

In this study, high-frequency SNP loci (8–10) were used as sample identification markers to prevent sample damage and cross-contamination during testing. NGS experiments were conducted and SNP locus analysis was performed simultaneously to ensure that the test samples were free from cross-contamination.

2.2.5. Pathogenic variant filtering and selection

2.2.5.1. Filtering of high-frequency SNVs/Indels

High-frequency mutations (MAF > 0.01, MAF > 0.05) were filtered out using population genetic variant databases such as dbSNP, 1000 Genomes, ExAC, and gnomAD, along with a local database. For unique cases, personalized threshold screening was used.

2.2.5.2. Classification of SNVs/Indels and selection of candidate gene variants

According to ACMG standards, variants are classified into five categories: pathogenic (P), likely pathogenic (LP), variants of uncertain significance (VOUS), likely benign (LB), and benign (B). Using these guidelines, in-depth studies of suspected pathogenic mutations that matched the expression profile of the proband we conducted using Sanger sequencing. However, three types of SNVs/Indels were excluded from the final report:

(1) Mutations likely to be benign (as per ACMG guidelines).

(2) VOUS gene mutations inherited in a dominant manner from asymptomatic parents.

(3) Potentially pathogenic mutations that did not meet the patient's clinical criteria (as per ACMG guidelines).

2.2.6. Filtering and interpretation of known or potentially clinically significant CNVs

CNVs were interpreted according to ACMG guidelines. CNVs classified as benign or likely benign were not included in the report. With the assent of the testing laboratory, submitting personnel can retrieve information on the CNVs from the list of excluded variants.

3. Results and Discussion

3.1. Physical examination results

The patient was a 5-month-old male, delivered *via* cesarean section at 35 weeks and 4 days of gestation due to oligohydramnios. Physical examination findings included: distinct facial features, prominent eyes, downward slanting palpebral fissures, a broad forehead, low-set ears, visible veins on the skin, and a normal height. A fundus examination revealed retinal white spots and pigment deposition in the left lens.

The patient's mother has had a visual acuity of 0.2–0.4 since childhood without correction, a broad forehead, prominent eyes, low-set ears, abnormally shaped auricles, pectus carinatum, pelvic abnormalities, long fingers with visible surface veins, and irregular teeth alignment. A fundus examination revealed partial lens dislocation and surface pigment deposition.

The patient's sister, born to the same parents, has amblyopia in one eye but otherwise normal facial features.

3.2. Analysis of transcription results

The minigenes, both wild-type and mutant, were inserted into the pcMINI and pcMINI-C vectors. These four recombinant vectors were then transfected into the HeLa and 293T cell lines. After transfection for 48 hours, RNA was extracted from a total of eight samples.

3.2.1. Analysis of pcMINI series results

The minigene construction strategy for pcMINI-*FBN1*-wt/mut involved inserting part of Intron 63 (346 bp), Exon 64 (175 bp), and part of Intron 64 (439 bp) into the pcMINI vector. This vector contains the universal ExonA-intronA-MCS-intronB-ExonB sequence. After transfecting the cells, the splicing of ExonA-Exon64-ExonB was observed for abnormalities. The results are shown in Figure 1.

RT-PCR results indicated that in both HeLa and 293T cells, the wild-type minigene produced a single band of the expected length, 564 bp, named band a. The mutant minigene produced two smaller bands than the wild-type, named band b and band c. Sanger sequencing was

performed on band a from the wild-type and bands b and c from the mutant minigenes in both cell lines.

Sequencing results revealed that wild-type band a represented normal splicing, with the structure ExonA (192 bp) - Exon64 (175 bp) - ExonB (57 bp). Mutant band b exhibited abnormal splicing, with the structure ExonA (192 bp) - ΔExon64 (101 bp) - ExonB (57 bp). Mutant band c also exhibited abnormal splicing, with Exon64 skipped, resulting in the structure ExonA (192 bp) - ExonB (57 bp).

3.2.2. Analysis of pcMINI-C series results

The minigene construction strategy for pcMINI-C-*FBN1*-wt/mut involved inserting part of Intron 63 (186 bp), Exon 64 (175 bp), Intron 64 (1,189 bp), and Exon 65 (390 bp) into the pcMINI-C vector, which contains the universal ExonA-IntronA-MCS sequence. After transfecting the cells, the splicing of ExonA-Exon64-Exon65 was observed for abnormalities. The results are shown in Figure 2.

RT-PCR results indicated that in both HeLa and 293T cells, the wild-type minigene produced a single band of the expected length, 936 bp, named band a. The mutant minigene produced two smaller bands than the wild-

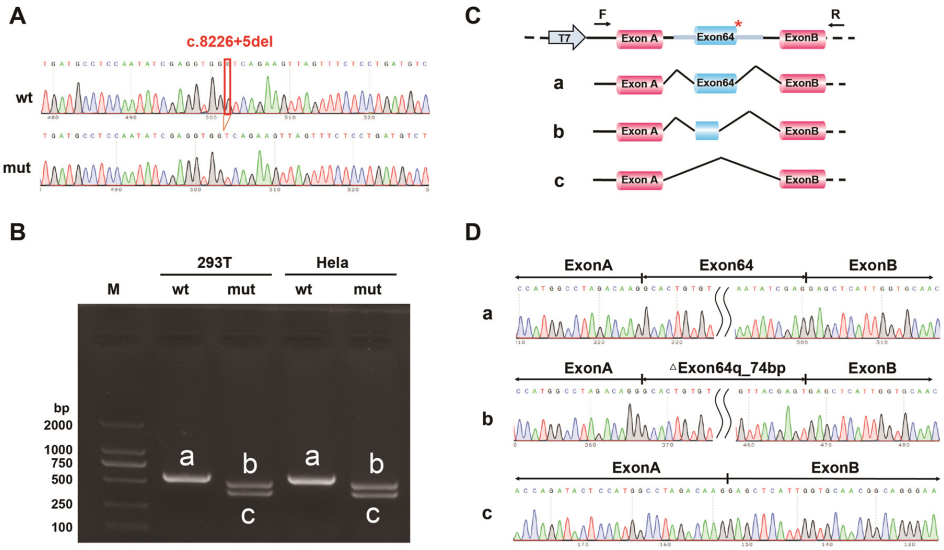

Figure 1. Detection of the pcMINI vector. (A) Sequencing diagram of minigene construction, with wt (wild type) shown on top and mut (mutation) shown below; **(B)** Gel electrophoresis of the results of RT-PCR transcription analysis, with bands labeled as a, b, c in HeLa and 293T cells; **(C)** Schematic diagram illustrating the minigene construction strategy and splicing pattern; **(D)** Sequencing results corresponding to splicing bands. The red asterisk (*) indicates the mutation site.

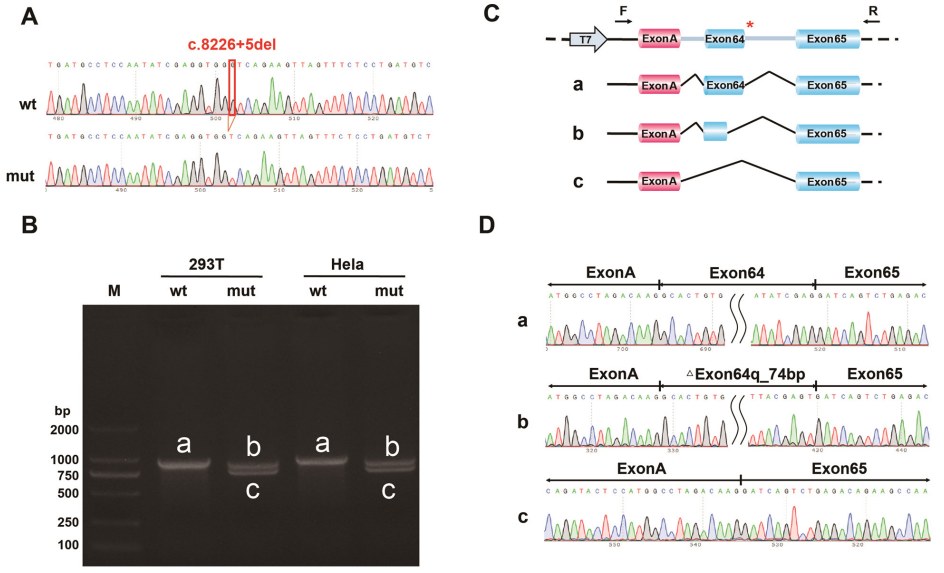

Figure 2. Detection of the pcMINI-C vector. (A) Sequencing diagram of minigene construction, with wt (wild type) shown on top and mut (mutation) shown below; **(B)** Gel electrophoresis of the results of RT-PCR transcription analysis, with bands labeled as a, b, c in HeLa and 293T cells; **(C)** Schematic diagram illustrating the minigene construction strategy and splicing pattern; **(D)** Sequencing results corresponding to splicing bands. The red asterisk (*) indicates the mutation site.

type, named band b and band c. Sanger sequencing was performed on wild-type band a and mutant bands b and c.

Sequencing results revealed that wild-type band a represented normal splicing, with the structure ExonA (192 bp) - Exon64 (175 bp) - Exon65 (390 bp). Mutant band b exhibited abnormal splicing, with the structure ExonA (192 bp) - ΔExon64 (101 bp) - Exon65 (390 bp). Mutant band c also exhibited abnormal splicing, with Exon64 skipped, resulting in the structure ExonA (192 bp) - Exon65 (390 bp).

The *in vitro* minigene assay results indicated that the c.8226+5del mutation affects normal mRNA splicing. Both pcMINI and pcMINI-C vector assays yielded consistent results. The c.8226+5del mutation led to the deletion of 74 bp on the right side of Exon64, resulting in the cDNA and protein sequence c.8153_8226del p.Cys2718*, where a premature termination codon (PTC) was introduced in Exon64 and a truncated protein of 2,717 aa was produced. In addition, the c.8226+5del mutation caused the skipping of Exon64, resulting in the cDNA and protein sequence c.8052_8226del p.His2685Ile fs*9, where a PTC was introduced in Exon65 and a truncated protein of 2,692 aa was produced.

3.3. Sequencing results of *FBN1* gene variant c.8226+5del

DNA was extracted from samples of the tested family, and a library was constructed. Target regions were captured using the SureSelect Human All Exon V6 (Agilent) hybridization capture kit, followed by high-throughput sequencing using Illumina NovaSeq (sequencing read length: 2×150 bp). Through bioinformatic analysis, the variant c.8226+5del that was detected in genetic testing was classified as a VOUS (as shown in Table 2, Figures 3 and 4).

A significant characteristic of MFS is multiple systemic developmental mutations in the human body. Approximately 80% of patients clinically manifest with lens dislocation or subluxation, and in severe cases, they may also develop complications such as aortic aneurysm, contributing to a high mortality rate among patients. *FBN1* is located on 15q21.1, spans 235 kb, and encodes fibrillin-1, consisting of 65 exons. Fibrillin-1, a glycoprotein with a molecular weight of 350,000 that consists of 2871 aa, forms the extracellular matrix primarily in elastic or inelastic tissues (*8, 9*). The protein is predominantly distributed in systemic elastic tissues, including the skin, tendons, lungs, cardiovascular system, and the suspensory ligament of the lens. According to the HGMD database, there were approximately 2,000 confirmed *FBN1* gene mutation sites prior to 2018, with 67% being missense mutations (*10*). Clinical manifestations of MFS are influenced by the location and type of *FBN1* gene mutations (*11*).

MFS exhibits significant clinical heterogeneity, primarily affecting the skeletal, ocular, and

Table 2. Sequencing results for *FBN1* gene variant c.8226+5del

Sequencer	Volume of sequencing data	Average depth of target region	20× coverage (%)	Q30(%)	Sequencing platform
Patient	15.51G	159.7X	96.91	92.43	Illumina NovaSeq (2×150bp)
Mother of the patient	16.33G	175.13X	97.22	94.45	
Father of the patient	18.37G	205.54X	97.90	94.46	

Figure 3. IGV schematic diagram of sequencing results for *FBN1* gene variant c.8226+5del. (A), Patient; **(B),** Mother of the patient; **(C),** Father of the patient.

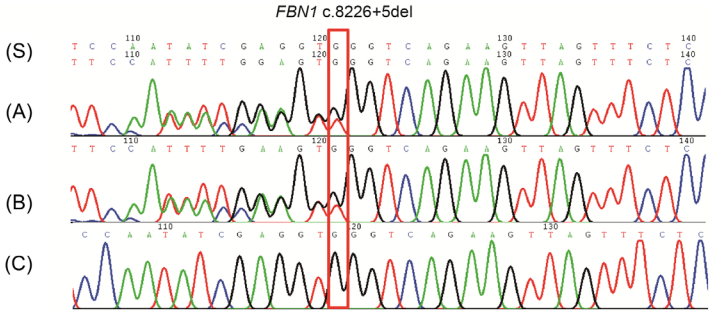

Figure 4. Schematic representation of Sanger sequencing validation results for *FBN1* gene variant c.8226+5del in the patient and parents. (S), Sequencer; **(A),** Patient; **(B),** Mother of the patient; **(C),** Father of the patient.

cardiovascular systems. Major clinical manifestations include dolichocephaly, underdeveloped cheekbones, micrognathia, distinctive facial features, aortic aneurysm-like dilation, aortic valve regurgitation, aortic dissection, mitral valve prolapse, arachnodactyly, spider-like fingers and toes, chest deformities, pes planus, ectopia lentis, myopia, and retinal detachment (*12*). In addition, patients may also present with abnormalities in the lungs, skin, and central nervous system. Due to the lack of uniform clinical features of MFS, there is currently limited in-depth research on expression of the disease genotype (*13*). However, studies have confirmed that the pathogenesis of MFS is associated with *FBN1* mutations, leading to systemic connective tissue diseases that significantly impact the quality of life and daily activities of patients. Moreover, the disease is hereditary, and some patients may experience respiratory or circulatory failure crises or even death, causing serious physical and psychological harm to the affected children and their families (*14*). Therefore, genetic screening for patients (those with hereditary MFS, carriers, and infants with cardiovascular malformations) is of great significance. A positive screening result requires special attention, timely notification of the patient and their family, explanation of disease development and prognosis, enhanced cooperation between patients and healthcare providers, and prevention of the further progression of the disease and complications. There may be two causes of *FBN1* gene mutations: mutations occurring during embryonic development and parental germline mosaicism (*15*). Germline mosaicism refers to mutations occurring only during early embryonic germ cell growth. This cell lineage accounts for a small proportion of the peripheral blood genome, and most of the other body cells, except for a small part of the germ cells, do not carry mutations, resulting in a low detection efficiency. Therefore, families of patients with this feature require prenatal genetic diagnosis (*16, 17*). The *FBN1* gene mutation c.8226+5del that was detected in this study has not been reported in the literature and is classified as a VOUS. MFS caused by this gene is often inherited in an autosomal dominant manner. Family testing results confirmed that the mother of the patient also carried this mutation, and the clinical phenotype of the mother was similar to that of the patient. However, whether c.8226+5del is the key gene for the onset of MFS, and the changes in Exon64 caused by this gene mutation and the resulting alterations in protein function, is currently unknown and needs to be studied further for confirmation.

In summary, this study identified a novel variant site, c.8226+5del, in the *FBN1* gene that may be associated with clinical features such as "low-set ears" and a "distinctive facial appearance" in MFS patients. This mutation may affect the normal splicing of gene mRNA, leading to changes in the structure and function of Exon64, thereby causing autosomal dominant inherited MFS. These findings could provide insights for subsequent clinical research and genetic testing.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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Brief Report

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Skeletal computed tomography findings of upper extremities in middle-aged persons with thalidomide embryopathy

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SUMMARY Individuals with thalidomide embryopathy are now approximately 60 years old. For years, they have been compensating for their hypoplastic limbs in various aspects of daily living, and they face secondary problems such as limb and back pain. Imaging analysis is beneficial for understanding the pathogenesis of these problems. However, previous studies on skeletal imaging were mainly radiographic studies conducted at young ages, and there are few studies on skeletal imaging after aging, with most of them being case reports. In this study, detailed analyses of the skeletons of the upper extremities were performed using three-dimensional computed tomography and multiplanar reconstruction images in five individuals with thalidomide embryopathy aged approximately 60 years. Each individual frequently complained of neck, shoulder, and/or back pain. Dislocation, subluxation, and osteoarthritis were observed in the shoulder joints in some individuals. Hypoplasia of the trochlea and/or capitulum of the humerus, coronoid fossa, olecranon, and coronoid processes was observed in the elbow joints. Fusion and hypoplasia of the carpal bones were frequently observed in wrist joints. Radiocarpal and ulnocarpal synostoses were also observed. The joint instability and osteoarthritis found in this study may have contribute to upper limb pain in individuals with thalidomide embryopathy.

Keywords skeletal imaging, 3D-CT, phocomelia

1. Introduction

Thalidomide embryopathy (TE) is a well-known drug-induced tragedy affecting over 10,000 infants worldwide. It is characterized by congenital limb malformations, auditory hypoplasia, and internal organ malformations. Phocomelia or radial longitudinal deficiency in the upper extremities is characteristic of TE.

Most individuals with TE are now approximately 60 years of age. In addition to symptoms associated with congenital malformations, they frequently complain of age-related secondary problems such as limb and back pain (*1*). The pathophysiology of TE needs to be understood through imaging analyses to address these secondary problems. However, previous reports on skeletal imaging were mostly based on radiographic findings at young ages, and there are few studies on skeletal imaging after the individuals had aged (*2-5*).

In this study, the computed tomography (CT) findings of the upper limbs in five individuals with TE after aging were analyzed using three-dimensional-CT (3D-CT) and multiplanar reconstruction (MPR).

2. Materials and Methods

From October 2022 to February 2023, five individuals with TE (age: 59–61 years, two men and three women) underwent CT from the head to the pelvis for internal medicine checkup, and we analyzed 3D-CT and MPR findings of the upper extremity skeletons. 3D medical imaging workstation "Ziostation2" (Ziosoft, Japan) was used in 3D-CT analysis.

This study was approved by the National Center for Global Health and Medicine Ethics Committee (NCGM-S-004260-02), and it conformed to the provisions of the Declaration of Helsinki. Written informed consent concerning this research was obtained from all participants.

3. Results and Discussion

Detailed findings and clinical manifestations of each upper limb in the five individuals (CaseA–E) are shown in Table 1. Regarding the clinical phenotypes, malformations were limited to the forearms and hands of all individuals. Clubhands with short forearms were present in seven limbs of four individuals. The degree of digit malformation differed among the limbs. Concerning clinical symptoms, three out of five individuals complained of a forward-leaning posture to compensate for their short upper limbs during daily activities, and they also suffered from neck and/or shoulder pain.

Regarding the CT findings of the shoulder joints, five of the ten upper limbs showed normal morphology. The other five limbs are shown in Figure 1. Abnormal humeral head position and/or osteoarthritis was seen in some limbs. In A-right, the humeral head was slightly elevated, and A-left showed downward subluxation of the humeral head and osteoarthritis. In A-left, the clavicle and acromion were long, and the acromioclavicular joint projected outward, exhibiting the so-called pointed shoulder. D-right and D-left show hypoplastic humeral heads. In D-right, the humeral head was dislocated anteroinferiorly. D-left and E-left also presented with osteoarthritis.

The characteristic CT findings of the elbow joints are shown in Figure 2. B-right and D-left, which did not have radial defects or proximal radioulnar synostosis, show normal elbow joint morphology. The other eight limbs showed hypoplasia of the trochlea and/or capitulum of the humerus, coronoid fossa, olecranon, and coronoid process: B-left showed typical features. D-right showed proximal radioulnar synostosis and the corresponding deformity of the humerus. E-left shows a radial defect, with a radius-remnant-like object attached to the coronoid process.

In the wrist joints, nine of the 10 upper limbs showed hypoplasia and fusion of the carpal bones. The wrist joint showed various morphologies, and Figure 3 shows the typical deformities. In A-right, the distal end of the ulna showed a bowl-shaped deformation. A-left showed ulnocarpal synostosis. In B-left and C-right, the carpal bones contacted the ulna only in a limited area. D-right showed radiocarpal synostosis.

Thalidomide was marketed as a sleeping pill and morning sickness medicine in the late 1950s and the early 1960s. Congenital malformations have been reported in children born to mothers receiving thalidomide; this condition was called TE. The most common malformations are congenital limb malformations, auditory hypoplasia, and internal organ malformations. In Japan, lower extremity malformations are rare and almost all extremity malformations are limited to the upper limbs (*6*).

People with TE are now approximately 60 years of age, and in addition to the symptoms associated with the original malformations, they complain of age-related secondary problems. In a scoping review of 25 relevant articles on age-related changes in TE, Newbronner (*1*) found that individuals with TE frequently reported joint pain, especially in the neck, shoulder, and back. Approximately 30–70% of individuals with TE experienced joint pain (*1,7-9*). Merkle (*2*) reported that 58% of individuals with TE experienced shoulder joint pain, and approximately one-third had osteoarthritis in the shoulder joints.

Imaging analysis is beneficial for investigating the pathogenesis of these secondary problems. However, previous reports on radiographic findings of TE mainly comprise reports of when the disease was first reported (*10-12*), and there are few studies on skeletal imaging after aging. Previous studies have included a few surgical reports of joint replacement for shoulder osteoarthritis (*2,3*), 31 cases of lower extremity CT analysis (*4*), and one case report on shoulder osteoarthritis using radiography, 3D-CT, and Magnetic Resonance Imaging (MRI) (*5*).

Henkel (*13*) classified congenital malformations of the upper extremities as 1. mildest to 4. the most severe; 1. distal ectromelia: malformation involving the radial ray of the hand and the radius; 2. axial ectromelia: malformation involving the radial ray of the hand, the radius, and the humerus; 3. phocomelia: absent humerus, radius, and ulna, and several ulnar hands connected to the shoulder girdle, 4. amelia: arm completely absent. In the present study, only case D showed humeral hypoplasia (axial ectromelia), while the other four cases exhibited malformations localized in the forearms and hands. (distal ectromelia)

According to Mansour (*14*), a pointed shoulder with long clavicle and acromion, and a prominent acromioclavicular joint is characteristic. Shoulder joint dislocation can also be caused by marked hypoplasia of the superior girdle muscles (*15*). In the present study, one of the 10 limbs had a pointed shoulder, and three limbs showed an abnormal position of the head of the humerus, which was consistent with previous studies. All three limbs also showed osteoarthritis, which is consistent with a previous study that reported shoulder osteoarthritis in TE after aging (*2*).

In the elbow joints, radial defects and proximal radioulnar synostosis were found in eight of the ten limbs. This result is consistent with a previous study that showed hypoplasia of the elbow joint, proximal radioulnar synostosis, and ulnohumeral synostosis as characteristic features (*15*). In this study, articular surface deformity was revealed by separating the humerus from the radius and/or ulna using 3D-CT imaging techniques. Hypoplasia of the trochlea and/or capitulum of the humerus, coronoid fossa, olecranon, and coronoid processes was observed. Articular surface deformation can be an age-related change corresponding to congenital bone loss or bone fusion.

Table1. Summary of the clinical phenotypes/symptoms and CT findings

	Case A		Case B		Case C		Case D		Case E	
	right	left	right	left	right	left	right	left	right	left
age(years),gender	60, female		60, female		61, male		59, female		60, male	
clinical phenotypes	short and bowed forearm, clubhand, deficient thumb	short and bowed forearm, clubhand, deficient thumb	hypoplastic thenar muscle	short and bowed forearm, clubhand, deficient thumb & digit II, hypoplastic digit III-V	short and bowed forearm, clubhand, hypoplastic thumb	short and bowed forearm, clubhand, hypoplastic thumb	deficient thumb	rudiment thumb	short and bowed forearm, clubhand, rudiment thumb	short and bowed forearm, clubhand, deficient thumb
clinical symptoms	pain in shoulders and upper limbs forward-leaning posture		pain in neck and shoulders forward-leaning posture poor posture balance		pain in neck, shoulders, back and knees forward-leaning posture		pain in both upper arms (left dominant)		pain in fingers	
CT findings shoulder joint	slightly elevated humeral head	osteoarthritis, downward subluxation, pointed shoulder	normal	normal	normal	normal	anteroinferior dislocation, hypoplastic humeral head	osteoarthritis, hypoplastic humeral head	normal	osteoarthritis
upper arm	normal	normal	normal	normal	normal	normal	short humerus	normal	normal	normal
elbow joint	hypoplasia of joint components*	hypoplasia of joint components*	normal	hypoplasia of joint components*	hypoplasia of joint components*	hypoplasia of joint components*	deformity of the trochlea and capitulum of humerus	normal	hypoplasia of joint components*	hypoplasia of joint components*
forearm	radial deficiency	radial deficiency	normal	radial deficiency	radial deficiency	radial deficiency	proximal radioulnar synostosis	normal	radial deficiency	rudiment radius
wrist joint	hypoplastic and fused carpus	hypoplastic and fused carpus ulnocarpal fusion	normal	hypoplastic and fused, carpus	hypoplastic and fused carpus	hypoplastic and fused carpus	hypoplastic and fused carpus, radiocarpal fusion	hypoplastic and fused carpus, radiocarpal fusion	hypoplastic and fused carpus	hypoplastic and fused carpus

*hypoplasia of the trochlea and capitulum of the humerus, coronoid fossa, olecranon, and coronoid process.

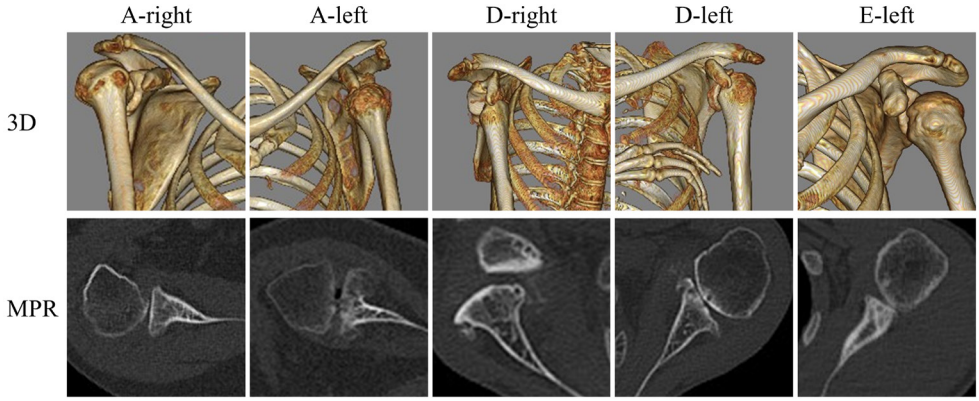

Figure 1. Abnormal findings of the shoulder joints. The humeral head is abnormally positioned in A-right (slightly elevated), A-left (downward subluxation), and D-right (anteroinferior dislocation). Osteoarthritis can be observed on A-left, D-left, and E-left. A-left also shows a pointed shoulder. D-right and D-left show hypoplastic humeral heads.

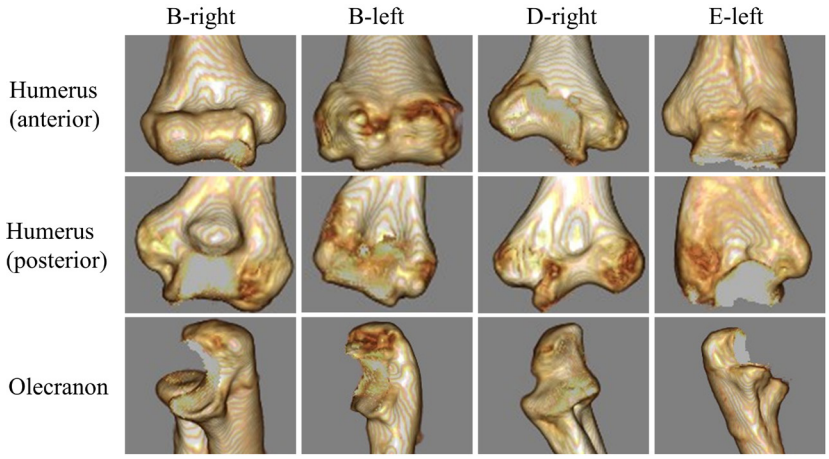

Figure 2. Characteristic findings of the elbow joints. B-right shows normal morphology. B-left shows hypoplasia of the trochlea and capitulum of the humerus, coronoid fossa, olecranon, and coronoid processes. D-right shows the proximal radioulnar synostosis and the corresponding deformity of the humerus. E-left shows a radial defect with a radius-remnant-like object attached to the coronoid process.

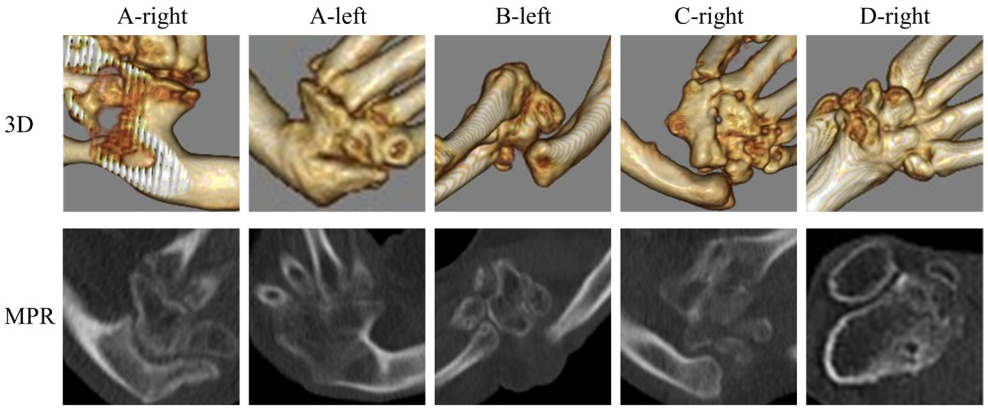

Figure 3. Typical deformities in the wrist joints. A-right shows a bowl-shaped deformation of the ulnar head. A-left shows ulnocarpal synostosis. B-left and C-right show club hands with carpal bones in contact with the ulna in only a limited area. D-right indicates radiocarpal synostosis.

In the wrist joints, 3D-CT elucidated the positional relationship between the carpal bones and the radius and/or ulna. In some cases, the carpal bones establish contact with the radius or ulna only in a limited area, suggesting joint instability. Previous studies on the carpal bones have shown radially predominant carpal bone defects or fusion as characteristic features. Longitudinal fusion is unique and is usually not seen in other diseases that show upper extremity malformations similar to TE (*14*). In this study, carpal bone hypoplasia and fusion were observed in nine of 10 limbs, which is consistent with previous studies.

In individuals with TE reported in this study, hypoplasia of the wrist and elbow joints was frequently observed. 3D-CT is particularly useful for analyzing the detailed morphology and 3D structure of articular surfaces. To the best of our knowledge, this is the first report on the morphology of the articular surface of the upper extremities in individuals with TE after aging. In addition, using 3D-CT, the three-dimensional relationship between the bones was clarified, suggesting joint hypoplasia and instability in each joint.

In this study, neck and shoulder pain were common complaints among individuals with TE. In addition to the pain caused by daily activities with a forward-leaning posture to compensate for the short upper limbs, joint instability and osteoarthritis observed in this study may have also contributed to pain.

One limitation of this study was the lack of documentation of imaging findings at young ages, which made it impossible to distinguish between congenital and post-aging findings. As individuals with TE age, the incidence of age-related secondary problems is expected to increase. The accumulation of imaging analysis studies in more cases and further understanding of the pathogenesis would be beneficial for improving joint protection and pain control. Although this study focused on skeletal CT findings, muscle hypoplasia is also known to occur in patients with TE. Studies involving the analysis of muscle mass can also be considered in future research.

In conclusion, skeletal CT analysis of the upper extremities of middle-aged individuals with TE revealed various bone deformities, including bone fusion and hypoplasia. Osteoarthritis was also frequently observed.

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Epidemiological estimates of paroxysmal nocturnal hemoglobinuria in Bulgaria

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SUMMARY Paroxysmal nocturnal hemoglobinuria is a rare clonal hematopoietic stem cell disorder with debilitating health consequences if untreated. Although cases have been described globally, precise epidemiological distribution is difficult to assess due to geographical underrepresentation in disease reporting. Evaluation of the burden of paroxysmal nocturnal hemoglobinuria in Bulgaria is currently missing. To provide epidemiological estimates, a systematic literature search for publications in the Bulgarian language or by Bulgarian authors was performed for a ten-year period (2013-2022), and clinically relevant information on case presentation was collected. Additionally, data was retrieved from the National Health Insurance Fund and National Statistical Institute on the count of registered cases with ICD-10 code "D59.5" and census for the same period. The estimated prevalence of paroxysmal nocturnal hemoglobinuria is relatively lower in the Bulgarian population than in other countries, and it is estimated to be 2.77 cases per 1,000,000 patient years. The treatment pattern mainly shows conventional blood product support use and is consistent with the pre-complement inhibition era. Underdiagnosis, lack of a reliable disease reporting system, and, until recently, restricted access to complement inhibitor therapy are significant impediments to the management of paroxysmal nocturnal hemoglobinuria in Bulgaria.

Keywords anemia, intravascular hemolysis, complement inactivating agents, health resources

1. Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a chronic multi-system, progressive, and life-threatening disorder characterized by intravascular hemolysis, thrombotic incidents, severe infections, and bone marrow failure (*1*). PNH arises due to clonal expansion of hematopoietic stem cells deficient in membrane expression of glycosyl-phosphatidyl inositol (GPI) linked proteins (*2*). GPI deficiency is caused by somatic mutations in the PIG-A gene (phosphatidyl-inositol glycan class A gene) of stem cells, as this gene codes for an enzyme necessary for GPI anchor biosynthesis (*2*). The GPI deficiency affects the membrane expression of two complement regulatory proteins: CD55 – decay accelerating factor, and CD59 – an inhibitor of the membrane attacking complex (*3*). Decreased or lacking membrane expression of CD55 and CD59 renders PNH blood cells increasingly susceptible to complement-mediated lysis (*4*). As a result of enhanced complement activation, the hallmark clinical features are intravascular hemolysis, cytopenia, and thrombosis, particularly unusual site thrombosis.

There is variation in reporting of PNH occurrence globally. According to an updated analysis from the International PNH registry, 68% of cases are reported from Europe, 14% - from the US, and only 18% from the rest of the world (*5*). Inconsistency of representation is most probably related to poor registration and disease reporting, either due to a lack of accessible diagnostic facilities or the inability to maintain data repositories. Previously, PNH distribution in Bulgaria has not been assessed and epidemiological estimates are currently unknown. Therefore, this report aims to summarize data on PNH cases in Bulgaria from publicly available sources and provide estimates of its occurrence in the country.

2. Systematic review of published cases

Literature was searched in a systematic manner for publications in Bulgarian language or by Bulgarian authors from 2013-2022. The following search strategy was applied: for publications in Bulgarian language the repository of the Central Medical Library, Medical

University-Sofia (*www.cml.mu-sofia.bg*) and Google Scholar were searched with text "пароксизмална нощна хемоглобинурия" (Bulgarian translation of "paroxysmal nocturnal hemoglobinuria"); Scopus was searched with item ["paroxysmal" AND "nocturnal" AND "hemoglobinuria"] within All fields with subsequent language filter for Bulgarian. For publications by Bulgarian authors, PubMed was searched with strategy ["paroxysmal nocturnal hemoglobinuria"] AND "Bulgaria" as all fields query and Scopus with "paroxysmal nocturnal hemoglobinuria" with filter "Bulgaria" by Country/Territory.

In total, 19 records were identified through database search. After removing duplicates, seven publications (five case reports and two original articles, 9 PNH cases; Supplemental Figure S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=204*) were selected as containing relevant data on PNH cases in Bulgaria (*6-12*). Items were excluded if they were reviews, not by Bulgarian authors, or other documents that did not report relevant information. Clinical characteristics of reported cases and sources are presented in Table 1.

Although some cases lack full demographic description, it is notable to mention that female cases were more prevalent (5/9 cases), and presenting features of the majority of them were late pregnancy complications. Additionally, two-thirds of the cases with available age data were younger than 40 years (4/6 cases), and nearly half of the cases (4/9 cases) were PNH clones in association with other hematologic disorders. Generally for all reported cases is the severe morbidity associated with the condition: transfusion dependency, pregnancy complications, intrauterine growth retardation, cardio-vascular co-morbidity, hemorrhagic diathesis, and severe Grade 4 thrombocytopenia. All required prolonged treatment and intensive blood product support.

3. Epidemiological data

Data was retrieved from the National Health Insurance Fund (NHIF) through an official query (№19-243/01.08.2023) under the Access to Public Information Act. Information was gathered on the number of registered patients with ICD-10 code "D59.5" among all hospitalized patients nationwide for the period 2013-2022 and on the number of patients registered with ICD-10 "D59.5" in the outpatient setting. Demographic statistics from the Bulgarian population census for 2013-2022 were downloaded from the National Statistical Institute (*www.nsi.bg*), and patient years were calculated. Count data on the distribution of hospitalized and outpatient cases with ICD code "D59.5" is presented in Table 2. The prevalence of PNH based on the number of patient-years for the ten years 2013-2022 is estimated at 2.77 cases per 1,000,000 patient-years (194 cases reported by NHIF for the studied period divided by 69,908,268 patient-years). However, these are only registered cases by ICD-10 code. As there is no available associated clinical data, it is difficult to clarify what proportion of these cases are strictly diagnosed based on flow cytometry testing and what proportion have been registered with the PNH ICD-10 code based on clinical features only. A prerequisite to this discrepancy is that the hospital registration system does not explicitly require the presence of flow cytometry results for the ICD-10 code "D59.5" but depends on the treating physician's discretion. Thus, this number may be an overestimation, and the PNH cases diagnosed by the gold standard with flow cytometry may be lower.

4. Discussion

Although PNH is a condition reported globally, precise epidemiological data on incidence and prevalence are still scarce. According to data from the 2022 Orphanet report on the prevalence of rare diseases, the prevalence of PNH in the European population is estimated at 2/100,000 (*13*). The report does not present data on incidence. A retrospective population-based study from the UK estimates yearly PNH incidence at 3.5 new cases per 1,000,000 population or around 220 newly diagnosed cases (*14*). In the same survey, the estimated annual prevalence is calculated at 3.81 /100,000 or 2,400 prevalent cases. Another retrospective study on Medicare data estimated the incidence to be 5.7/1,000,000 patient-years for three years (2015-2018) or 257 new cases yearly, with a prevalence of 12-13/1,000,000 patient-years (*15*). Both sexes are nearly equally affected (*16*). Some studies report a median age at diagnosis of around 50 years and a range of 5-91 years, while other studies reported a median age of around 30 years with an age range of 9-80 years (*17*).

The estimated prevalence of PNH in Bulgaria based on NHIF data seems lower than that reported for other countries. As NHIF data does not contain demographic characteristics such as sex and age, distribution by those parameters cannot be estimated. Assumptions could only be made from the systematic literature search that predominantly females are affected and subjects are of a younger age. The estimation of lower PNH prevalence in Bulgaria may be due to several reasons. On the one hand, this may indicate a lower predisposition of the Bulgarian population to PNH. It is more likely, however, to mirror underdiagnosis due to lower clinical suspicion for PNH and a lower referral rate for PNH screening by flow cytometry.

The treatment pattern that emerges from the case reports in Bulgaria reflects the difficulties in accessing complement inhibitor therapy in countries with limited resources (*18*). The current treatment strategy of PNH relies on complement inhibition by anti-C5 monoclonal antibodies (terminal complement inhibition) or small molecule C3 inhibitors (proximal complement inhibition) (*19*). Complement blockade is the only pathogenetically oriented treatment that achieves

Table 1. Clinical characteristics of published PNH cases in Bulgaria

Case No.	*Reference*	Publication year	Sex	Age, years	Main diagnosis/condition	Complications	Therapy	Outcome
1	Trayanov *et al.* (*6*)	2014	F	37	Pregnancy 31 g. w.	Grade 4 thrombocytopenia, Preeclampsia, IUGR, Oligohydramnion	PRBC, platelet transfusion, corticosteroids	Labor induction in 31 g.w., alive newborn baby, second grade prematurity
2	Popov *et al.* (*7*)	2015	F	73	Waldenstrom Macroglobulinemia	Hemolytic anemia	PRBC, Rituximab	Clinical improvement, control of hemolysis
3	Kosterizova *et al.* (*8*)	2015	F	37	Pregnancy, 34 g.w.	Hemolytic anemia, Bleeding diathesis, Grade 3 thrombocytopenia, IUGR, Oligohydramnion	Corticosteroids, Dycinone, Folic acid, Mg supplements, Enoxaparin, platelet transfusions (72 units in total)	Labor induction in 35 g.w., alive newborn baby, second grade prematurity
4	Nedkova *et al.* (*9*)	2019	M	49	Coronary artery bypass surgery	Hemolytic anemia, Atrial fibrillation, history of multiple myocardial infarctions	Corticosteroids, Rivaroxaban, iron supplements	Hemoglobin normalization, control of hemolysis
5	Atanasoska *et al.* (*10*)	2022	M	38	Hereditary spherocytosis, type 4	Hemolytic crisis with Hgb drop to 60 g/l, PNH clones in RBC, granulocytes and monocytes	NA	NA
6	Atanasoska *et al.* (*10*)	2022	F	-	Mother of the proband (case №5)	PNH clones in RBC, granulocytes and monocytes	NA	NA
7	Jordanova *et al.* (*11*)	2022	F	34	Aplastic anemia with PNH clones	HELLP syndrome during second pregnancy	Corticosteroid, Eculizumab	NA
8	Wong *et al.* (Ignatova, K) (*12*)	2022	-	-	PALOMINO clinical trial	-	Pegcetacoplan	Transfusion independency and no thrombotic complication in none of the participants
9	Wong *et al.* (Amine, I) (*12*)	2022	-	-	PALOMINO clinical trial	-	Pegcetacoplan	

g.w. – gestational week, IUGR – intrauterine growth retardation, PRBC -packed red blood cells, NA – not applicable

Table 2. Count data from NHIF on the distribution of PNH in Bulgaria*

Year	Diagnosis	Inpatient	Outpatient	Census by Infostat
2013	D59.5	17	8	7,245,677
2014	D59.5	29	4	7,202,198
2015	D59.5	23	8	7,153,784
2016	D59.5	12	8	7,101,859
2017	D59.5	12	7	7,050,034
2018	D59.5	27	7	7,000,039
2019	D59.5	4	9	6,951,482
2020	D59.5	1	5	6,916,548
2021	D59.5	2	6	6,838,937
2022	D59.5	0	5	6,447,710
Total		127	67	69,908,268

*Counts are uniquely identified cases by ID, not the number of hospital admissions or outpatient registrations.

hemolysis control, decreases thrombotic complications, improves the quality of life, and prolongs the survival of PNH patients (*20*). Regulatory approved complement inhibitors are the anti-C5 monoclonal antibodies eculizumab and ravulizumab and the C3 inhibitor, the cyclic peptide, Pegcetacoplan. However, due to their high costs, complement inhibitors are not available in many countries worldwide. In countries without access to complement inhibitor therapy, PNH treatment is based on conventional blood product support. Only one case (№7) was treated with a C5 complement inhibitor – eculizumab. Two other cases (№8 and №9) were treated with a C3 inhibitor – pegcetacoplan within the PALOMINO clinical trial. Although the majority of reported cases would have been indicated for complement inhibition therapy, the fact that only one case was treated with eculizumab outside of a clinical trial is indicative of limited access to the specific treatment due to the associated catastrophic health expenditure (*18*). The European Medicines Agency approved eculizumab for the treatment of PNH in 2007. However, in Bulgaria, it was granted reimbursement approval for the first time in April 2021 and only within the framework of a specific governmental regulation that would fund costs for eculizumab treatment only for patients younger than 18. It was not until the beginning of 2024 that both C5 inhibitors (eculizumab and ravulizumab) and the C3 inhibitor pegcetacoplan were incorporated into the public healthcare system and received full reimbursement status after undergoing health technology assessment procedures several times.

The rarity of PNH makes its epidemiological estimation difficult in real-world settings, particularly in the absence of reliable registration systems. Lack of reliable epidemiological data and insufficient clinical information are obstacles to assessing the exact disease burden in the Bulgarian population. Correct diagnosis highly relies on the access and referral rate to diagnostic testing, the latter being dependent on subjective factors such as clinical expertise and reluctance to screen potential cases. Further, the lack of public funding for the specific treatment until recently has additionally impeded the management of PNH cases. Gaps in epidemiological knowledge may be overcome through collaboration between centers and legislative efforts to support reliable disease reporting.

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Letter

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A patient treated with ofatumumab for myasthenia gravis in conjunction with systemic lupus erythematosus and thyroid carcinoma

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SUMMARY Myasthenia gravis (MG) is an autoimmune disease mediated by B cells and is associated with acetylcholine receptor (AChR) and muscle-specific receptor tyrosine kinase (MuSK) antibodies in the postsynaptic membrane at the neuromuscular junction. Anti-CD20 monoclonal antibodies, such as ofatumumab demonstrated promising disease control in MG patients. We presented the rare case of a 34-year-old female with acetylcholine receptor-positive myasthenia gravis (AChR-MG), concomitant with systemic lupus erythematosus (SLE) and metastatic thyroid carcinoma, who was treated with ofatumumab and exhibited improvements during follow-up.

Keywords myasthenia gravis, ofatumumab, anti-CD20 monoclonal antibody

Myasthenia gravis (MG) — an antibody-mediated disorder — is characterized by muscle weakness and fatigue, where auto-antibodies target the nicotinic acetylcholine receptor (nACHR), the muscle-specific tyrosine kinase (MuSK), lipoprotein receptor-related protein 4 (LRP4), or agrin in the postsynaptic membrane at the neuromuscular junction (*1*). MG is commonly seen in younger females (< 40 years) with symptoms such as ocular symptoms (ptosis and fluctuating diplopia), dysarthria, hoarseness, dysphagia, facial weakness, limb weakness, and shortness of breath (*2*). Similar to other autoimmune diseases (ADs), MG patients have a higher risk of being affected by a second autoimmune disease, including autoimmune thyroid disease, followed by systemic lupus erythematosus (SLE) and rheumatoid arthritis (*3,4*). SLE is a systemic autoimmune disease characterized by antibodies to nuclear and cytoplasmic antigens with variable clinical features, disease course, and prognosis (*5*). Several studies have addressed the potentially increased risk of cancers in MG. Cancer development has been linked to a mutual immunological deficit and a simultaneous increased risk of autoimmune disease (*6,7*).

Several autoimmune conditions, including MG, multiple sclerosis, and B-cell proliferative disorders, exhibit promising responses to monoclonal antibodies that target CD20 and deplete B-cells. The first of these agents developed was rituximab, a murine-human chimeric anti-CD20 monoclonal antibody, which has been used successfully in MG patients refractory to conventional immunosuppressive therapy (*8*). Ofatumumab is a fully human anti-CD20 monoclonal antibody that binds to a distinct CD20 epitope from that of rituximab and increases cellular-dependent cytotoxicity and apoptosis. The off-label use of ofatumumab demonstrated excellent disease control in MG patients refractory to conventional therapy (*9*).

Here, we presented the rare case of a 34-year-old female with acetylcholine receptor-positive myasthenia gravis (AChR-MG), concomitant with SLE and metastatic thyroid carcinoma, who was treated with ofatumumab and exhibited improvements during follow-up.

A 34-year-old female was admitted to our hospital with manifestations of double vision, dysphagia, and limb weakness for 3 years. Her medical history was notable for Still's disease manifested as fever, arthritis for 5 years, and hypertension. She was diagnosed with papillary thyroid carcinoma with lymph node metastasis and underwent surgery twice (9 years and 3 years ago).

After her second thymectomy, the patient developed fluctuating double vision, slurred speech, and dysphagia. A year ago, her symptoms worsened significantly with limb fatigue, choking cough when drinking water, occasional chest tightness, and multiple falls. Four months ago, she was referred

Table 1. Laboratory and immunoserological analysis of the patient

Analysis	Finding	Normal values
Leukocytes , × 10^9/L	5.3	3.5-10
Hemoglobin (g/L)	125	122-157
Platelet (× 10^9/L)	115	100-300
Creatinine (μmol/L)	52	58-96
Alanine aminotransferase (U/L)	6	0-31
Albumin (g/L)	40	35-53
Lactate dehydrogenase (U/L)	174	0-247
TSH (μIU/mL)	0.722	0.27-0.42
FT4 (pmol/L)	17.4	12-22
TGAb (IU/mL)	13.8	0-115
TPOAb (IU/mL)	11.3	0-34
ANA Hep2 – IIF	1:3,200 homogenous type of staining	-
Anti-ds-DNA	negative	-
Anticardiolipin Ab IgG (ELISA) (GPL- U/mL)	51	0-10
Anticardiolipin Ab IgM (ELISA) (MPL- U/mL)	4.95	0-10
Anti-SSA	++	-
p-ANCA	+	-
Complement C3 (g/L)	0.87	0.9-1.8
Complement C4 (g/L)	0.171	0.1-0.4
IgG (g/L)	15.5	7-16
IgM (g/L)	1.36	0.4-2.3
IgE (IU/mL)	292.7	0-100
Coombs test (anti-IgG)	+	-

TSH thyroid-stimulating hormone, FT4 free thyroxin, TGAb antithyroglobulin antibody, TPOAb antithyroid peroxidase antibody.

to the Neurology Clinic, and her antibody tests for neuromuscular junction (NMJ) diseases were positive for acetylcholine receptor antibody (AChR; 14.7 nmol/L) and negative for VGCC, Musk, Titin, and Raynodine antibodies. She was diagnosed with AChR-antibody-positive, generalized MG and began taking 60 mg of pyridostigmine thrice daily. Her symptoms were not effectively controlled, so she was admitted to the Neurology department.

The physical examination revealed no hives, lymphadenopathy, synovitis, or edema. The cardiopulmonary and abdominal examinations were normal. The cranial nerves examination revealed limitations of abduction on both sides. The functional tests of trunk and extremity muscle groups exhibited mild muscle weakness and fatigue. Her QMG score was 15/39. The laboratory tests for blood, urine, liver enzymes, creatinine level, and thyroid function were normal. The immunoserological analysis revealed positive ANA on a substrate of HEp2 cells (indirect immunofluorescence) in a dilution higher than 1:3200 homogeneous type of staining, positive anti-cardiolipin antibodies (both IgG and IgM type), anti-SSA, and p-ANCA, with signs of complement activation. Her serum immunoglobulin levels were extremely high. The direct Coombs test was positive, but there were no other laboratory or clinical signs of active hemolysis (Table 1).

The thyroid ultrasonography exhibited postoperative changes. The chest CT demonstrated multiple pulmonary nodules, which radiologists interpreted as thyroid cancer metastases (Figure 1). The abdominal sonography and echocardiography were both normal.

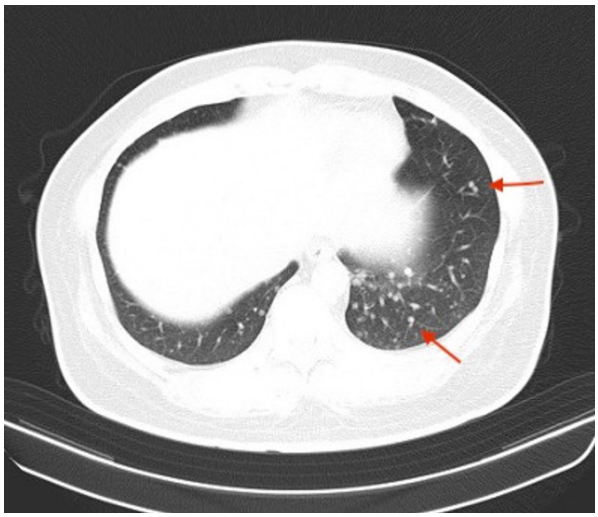

Figure 1. Chest CT. Multiple pulmonary nodules are shown by red arrows.ing intensity of HMW-MAA-positive cells in primary ALM lesions.

Five years ago, the patient was diagnosed with Still's disease, manifested as fever and arthritis. During this time, the rheumatologist diagnosed SLE based on the following criteria of the American College of Rheumatology: fever, autoimmune hemolysis, joint involvement, anti-cardiolipin antibodies, and low C3 (*10*). Following a multidisciplinary team consultation by Oncology, Radiology, Rheumatology, and Neurology departments, glucocorticoid and immunotherapy, such as anti-CD20 monoclonal antibodies, were considered reasonable treatments. Immunosuppressants, including

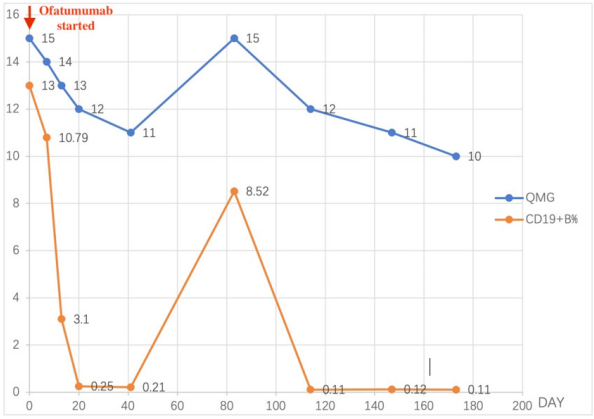

Figure 2. Curves of QMG score and CD19+ B Lymphocyte subsets versus time. The patient was started with ofatumumab on Day 0, then her QMG score (blue curve) and CD19+ B Lymphocyte subsets(orange curve, %) were recorded to Day 180.

Azathioprine, Mycophenolate mofetil (MMF), or Tacrolimus, were not recommended concerning metastasis of thyroid cancer. The young lady patient refused to take glucocorticoid, concerning its side effects as obesity and skin changes. We thus applied for off-label use of ofatumumab within our institution. She received three infusions of ofatumumab (20 mg) at weeks 0, 1, and 2, then 20 mg every month as a maintenance dose from week 4. During the six-month follow-up period, clinical signs of MG, including diplopia, dysphagia, and extremity weakness, improved substantially. Her QMG score and CD19+ B Lymphocyte subsets were recorded and are shown in Figure 2.

Rheumatic diseases, such as SLE, can affect the definitive diagnosis and treatment of MG patients and should be sought out. These diseases share a higher prevalence in young women, a relapsing-remitting course, and a positive autoimmune antibody (*5*). The majority of reported cases (61.5%) demonstrated that MG preceded SLE, but there have been cases where SLE preceded MG (*11*). This patient had fever and arthritis, which were suggestive of SLE, several years before the onset of MG symptoms, but SLE was not diagnosed until after the onset of MG. CXCL13, a chemokine that activates B and T lymphocytes, can further contribute to the pathogenesis of SLE and MG (*12*).

MG and SLE are both chronic autoimmune diseases requiring prolonged use of corticosteroids and immunosuppressants. For this patient with metastatic thyroid cancer, corticosteroid-sparing immunosuppressants (CSIS) was not a preferred choice, as studies have found a clear correlation between CSIS exposure and cancer risk in MG patients (*7,13*). Anti-CD20 monoclonal antibodies, such as ofatumumab, can reduce the concentration of serum auto-antibodies by eliminating CD20+ B cells, which has been demonstrated to be effective in some rituximab-intolerant SLE patients. It may be considered an organ-threatening, refractory autoimmune disease, including SLE and MG (*14*). Future controlled studies must evaluate the efficacy, doses, and appropriate re-treatment regimens for ofatumumab.

In this patient, we observed a parallel correlation between CD19+ B cell proportion and the QMG score, a scale of disease severity. CD19+ B cell proportion decreased from 13% to 0.25% within 20 days after ofatumumab injections, consistent with the improvement of her clinical symptoms. However, a transient increased QMG score (*15*) and CD19+ B lymphocyte subsets (8.52%) were observed at 80 days, probably due to her upper respiratory tract infection which was resolved after antibiotics treatment.

CD19 — a main surface antigen of plasma cells— plays an important role in secreting pathogenic antibodies, such as AChR-Ab. CD19+ B cell proportion strongly correlates with the severity of MG in a prediction model (*15*), which was consistent with the curve of this patient.

This case study demonstrated the effectiveness of ofatumumab in treating a patient with AChR-MG concomitant with SLE and metastatic thyroid carcinoma. Ofatumumab may be a future therapeutic option for MG, especially in those who cannot tolerate conventional immunosuppressants.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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