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CONTENTS

Volume 13, Number 4, 2024

Correspondence

Letter

Editorial

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Updated information on neuro-prognosticative tools to predict outcomes for patients with hypoxic-ischemic encephalopathy induced by cardiac arrest

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SUMMARY Hypoxic-ischemic encephalopathy (HIE), caused by cardiac arrest (CA) is a refractory condition in clinical settings. The clinician and family members have to make a hard decision: continue expensive life-sustaining therapy or withdraw the expensive intervention. The core problem lies in "whether this patient can still be awakened and achieve neurological recovery". This study briefly summarizes the use of mainstream neuro-prognosticative tools thus far with the latest available evidence. To gain a better understanding of the pathophysiological state of patients with HIE, comprehensive use of these tools and repeated assessments are recommended. The final decision should be made cautiously and comprehensively in light of the patient's medical history, pathophysiological state, results of neuro-prognosticative evaluations, and the clinician's clinical experience *per se*. Novel computerized technologies such as artificial intelligence, big data, and machine learning should be used to develop neuro-prognosticative tools for refractory CA-induced HIE.

Keywords hypoxic-ischemic encephalopathy (HIE), cardiac arrest (CA), neuro-prognosticative tools, awaken, neurological recovery

1. Introduction

Hypoxic-ischemic encephalopathy (HIE) in adults is a refractory condition that is commonly caused by cardiac arrest (CA). Survivors usually fall into a coma or unresponsive wakefulness syndrome. Approximately 40–66% of HIE survivors cannot be awakened (*1*) and have to undergo expensive intensive care and life-sustaining therapy. This situation might compel the clinician and family members to make a hard decision: continue such expensive treatment or withdraw it. The core issue lies in whether the patient can be awakened and recover even after prolonged treatment. Neuro-prognosticative evaluations play a vital role in making this decision, which can differentiate a state of prolonged coma from a state of irreversible cerebral damage.

2. Available neuro-prognosticative tools

By far, the available neuro-prognosticative tools can be classified into four types: *i*) clinical assessments, *ii*) electrophysiological tools, *iii*) biomarkers, and *iv*) neuroimaging tools (Figure 1).

Clinical assessments should include Glasgow Coma Scale motor response (GCS-M) and brainstem reflexes, and corneal and pupillary reflexes (*2*). Scores of GCS-M < 2 (*3,4*) and absence of bilateral brainstem reflexes (4) may indicate a poor prognosis. Conversely, Kamps *et al*. pointed out that the response to pain stimulation and corneal reflex are not a reliable tool for the early prediction of poor outcomes in patients undergoing hypothermia therapy (*3*). However, a later study verified that quantitative pupillometry is an excellent tool to predict HIE with a poor prognosis on day one after CA (*5*).

Electrophysiological tools include somatosensory evoked potentials (SSEP) and electroencephalography (EEG). In terms of SSEP, the most commonly used index is the N20 response in SSEP assessments. The N20 response is measured as the response from the primary somatosensory cortex after 20 ms of stimulation of the median nerve at the wrist (*2*). Early in 2003, Robinson *et al*. reported less than 1% changes in awakening in coma of HIE patients with absent somatosensory evoked potential response (*6*). Oddo and Friberg also pointed out that the absence of a bilateral

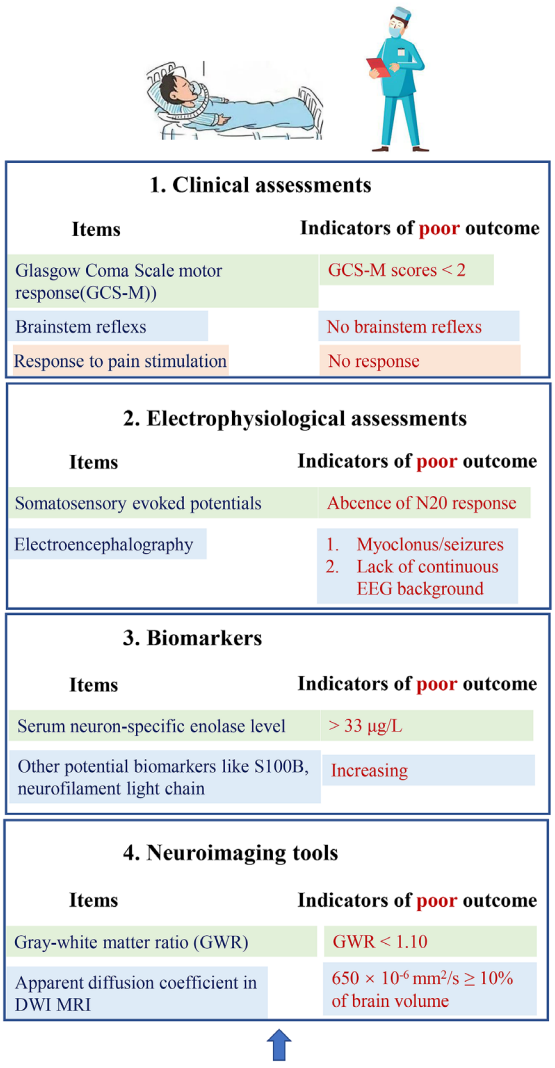

Figure 1. The recommended neuro-prognosticative tools for predicting the outcome HIE induced by CA

N20 response can predict 100% HIE with a poor prognosis (*2*). In addition, a later study found that the combined use of N60 and mismatch negativity achieved satisfactory sensitivity (82.7%) and specificity (82.0%) at predicting whether patients could be awakened (*7*). The limitations of the use of such SSEP indices lie in: *i*) they are easily affected by injuries to the cervical spinal cord and isolated lesions in the brain stem (*1*); *ii*) they have low sensitivity at predicting a good prognosis (*2*); and *iii*) interpretation of the evoked potentials may sometimes be subjective. Accordingly, EEG is, owing to its noninvasive and inexpensive nature, another commonly used electrophysiological tool to predict the clinical outcomes of HIE. In addition, EEG can be used in patients undergoing hypothermia therapy. However, there is still a lack of a "standard predictive model/pattern" of EEG in such patients with HIE. Generally, earlier recovery of continuous EEG background activity and later onset of myoclonus/seizures are indicators of a better outcome, whereas severe and frequent myoclonus/seizures indicate a worse outcome (*8*). Suppressed EEG, burst suppression, and generalized periodic discharges superimposed on a suppressed background have been observed in patients with severe HIE (*9*). A later study evaluated the changes in EEG patterns affected by pain stimulation. It found that awakening patients after pain stimuli had a higher γ, β, and α spectral power in the frontal and parietal lobes, a lower δ and θ spectral power in the bilateral temporal and occipital lobes, higher entropy in the frontal and parietal lobes, lower entropy in the temporal occipital lobes, and stronger γ and β connectivity in nearly the whole brain, but weaker θ and δ connectivity in some brain regions in comparison to unawakening patients (*1*). These patterns may be useful in predicting the prognosis for HIE.

The most important biomarker for predicting HIE is concentration of serum neuron-specific enolase (NSE). A serum level > 33 μg/L was identified as the cutoff value to indicate a poor outcome for HIE 24–72 h after CA (*10,11*). However, the thresholds of NSE levels to predict a poor outcome vary among different studies. Stammet *et al*. reported 50 μg/L 72 h after CA (*12*), and Streitberger *et al*. reported that 90 μg/L is better, considering specificity and sensitivity (*13*). Endisch *et al*. found that patients with serum NSE levels > 67 μg/L 48 h after CA had severe HIE (9). However, elevated serum NSE levels undoubtedly indicate a poorer outcome.

Computed tomography (CT) and magnetic resonance imaging (MRI) are commonly used neuroimaging tools to examine HIE. Considering potential confounding factors such as edema in the early stages, however, CT and MRI are more commonly used in patients with HIE > 7 days after CA (*2*). For better observation/quantification of brain injury following CA-induced HIE in the early stage, diffusion-weighted imaging (DWI) MRI is recommended to identify abnormalities in the brain structure and predict HIE prognosis. Hirsch *et al*. found that an apparent diffusion coefficient of 650 × 10^{-6} mm^2/s ≥ 10% of brain volume is a threshold indicating a poor prognosis in patients with HIE (*14*). However, the most widely accepted index is the gray-white matter ratio (GWR). A GWR < 1.10 in patients with HIE indicated a poor outcome because over 70% of patients with a GWR < 1.10 were found to have "near-complete cortical and hippocampal neuronal death" (*9*). Conversely, GWR > 1.3 might predict a good outcome even in patients with severe HIE (*9*).

In addition to the aforementioned tools, several non-mainstream tools have been mentioned in sporadic studies. For example, Preuß *et al*. evaluated the association between mean arterial blood pressure (MAP) and HIE severity after CA. They found that MAP was associated with CA survival but not with HIE severity. Patients with HIE who have fewer vasopressor requirements might have a higher chance of being awakened from a coma (*15*). Potential HIE-related biomarkers include S100B (*16*), neurofilament light

chains (*17,18*), and glial fibrillary acidic protein (*18*). However, the value of these promising predictive tools requires further investigation.

3. Insights and conclusion

The clinical outcomes of HIE caused by CA remain poor. Indeed, neurological recovery is rare in these patients (*11*) even they received prolonged intensive care and a spectrum of therapies, such as electrical stimulation, hyperbaric oxygen therapy, acupuncture, and electroacupuncture, have been attempted. Thus, selection of patients potentially having a good outcome as a result of further active treatment or selection of patients potentially having a hopeless outcome necessitating withdrawal of expensive interventions might be a knotty problem faced by all clinicians. Indeed, "withdrawal of expensive interventions" might lead to ethical/humanistic problems. Hence, the evaluation/prediction of HIE outcomes must be performed cautiously and rigorously. Several suggestions have been proposed for the future prediction of HIE outcomes.

i) Comprehensive evaluation using multimodal approaches. As described earlier, each evaluation tool has its particular advantages and disadvantages. To reach a robust conclusion, a battery of tools should be used to evaluate a given patient (Figure 1) to avoid possible bias and misjudgment. Reduplicative evaluations should be performed at different times. We should keep in mind that all the "assessment results" are for reference only, and the final decision should be made cautiously and comprehensively in light of the patient's medical history, pathophysiological state, results of the neuro-prognosticative evaluations, and the clinician's clinical experience *per se*.

ii) Owing to novel computerized technologies, such as artificial intelligence, big data, and machine learning, more precise and reliable evaluation is possible. Recently, Gramespacher *et al*. described a novel automated cerebral CT (CCT) analysis based on supervised machine learning to predict the clinical outcomes of patients with HIE caused by out-of-hospital CA (*19*). They found that machine learning-assisted gray matter analysis of CCT images might be a reliable and time-independent approach to predict outcomes along with conventional prognostic assessments (*19*). The development of such a novel assessment tool should be a future direction for predicting the clinical outcomes of HIE.

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References

1. Huang H, Su Y, Niu Z, Liu G, Li X, Jiang M. Comatose patients after cardiopulmonary resuscitation: An analysis based on quantitative methods of EEG reactivity. Front Neurol. 2022; 13:877406.
2. Oddo M, Friberg H. Neuroprognostication after cardiac arrest in the light of targeted temperature management. Curr Opin Crit Care. 2017; 23:244-250.
3. Kamps MJ, Horn J, Oddo M, Fugate JE, Storm C, Cronberg T, Wijman CA, Wu O, Binnekade JM, Hoedemaekers CW. Prognostication of neurologic outcome in cardiac arrest patients after mild therapeutic hypothermia: A meta-analysis of the current literature. Intensive Care Med. 2013; 39:1671-1682.
4. Dragancea I, Horn J, Kuiper M, Friberg H, Ullen S, Wetterslev J, Cranshaw J, Hassager C, Nielsen N, Cronberg T, Investigators TTMT. Neurological prognostication after cardiac arrest and targeted temperature management 33 degrees C versus 36 degrees C: Results from a randomised controlled clinical trial. Resuscitation. 2015; 93:164-170.
5. Oddo M, Sandroni C, Citerio G, Miroz JP, Horn J, Rundgren M, Cariou A, Payen JF, Storm C, Stammet P, Taccone FS. Quantitative versus standard pupillary light reflex for early prognostication in comatose cardiac arrest patients: an international prospective multicenter double-blinded study. Intensive Care Med. 2018; 44:2102-2111.
6. Robinson LR, Micklesen PJ, Tirschwell DL, Lew HL. Predictive value of somatosensory evoked potentials for awakening from coma. Crit Care Med. 2003; 31:960-967.
7. Liu Y, Huang H, Su Y, Wang M, Zhang Y, Chen W, Liu G, Jiang M. The combination of N60 with mismatch negativity improves the prediction of awakening from coma. Neurocrit Care. 2022; 36:727-737.
8. Petzinka VN, Endisch C, Streitberger KJ, Salih F, Ploner CJ, Storm C, Nee J, Leithner C. Unresponsive wakefulness or coma after cardiac arrest-A long-term follow-up study. Resuscitation. 2018; 131:121-127.
9. Endisch C, Westhall E, Kenda M, Streitberger KJ, Kirkegaard H, Stenzel W, Storm C, Ploner CJ, Cronberg T, Friberg H, Englund E, Leithner C. Hypoxic-ischemic encephalopathy evaluated by brain autopsy and neuroprognostication after cardiac arrest. JAMA Neurol. 2020; 77:1430-1439.
10. Zandbergen EG, Hijdra A, Koelman JH, Hart AA, Vos PE, Verbeek MM, de Haan RJ, Group PS. Prediction of poor outcome within the first 3 days of postanoxic coma. Neurology. 2006; 66:62-68.
11. Oddo M, Rossetti AO. Predicting neurological outcome after cardiac arrest. Curr Opin Crit Care. 2011; 17:254-259.
12. Stammet P, Collignon O, Hassager C, *et al*. Neuron-specific enolase as a predictor of death or poor neurological outcome after out-of-hospital Cardiac Arrest and Targeted Temperature Management at 33 degrees C and 36 degrees C. J Am Coll Cardiol. 2015; 65:2104-2114.
13. Streitberger KJ, Leithner C, Wattenberg M, Tonner PH, Hasslacher J, Joannidis M, Pellis T, Di Luca E, Fodisch M, Krannich A, Ploner CJ, Storm C. Neuron-specific enolase predicts poor outcome after cardiac rrest and Targeted Temperature Management: A Multicenter Study on 1,053

Patients. Crit Care Med. 2017; 45:1145-1151.
14. Hirsch KG, Mlynash M, Eyngorn I, Pirsaheli R, Okada A, Komshian S, Chen C, Mayer SA, Meschia JF, Bernstein RA, Wu O, Greer DM, Wijman CA, Albers GW. Multi-Center Study of Diffusion-Weighted Imaging in Coma After Cardiac Arrest. Neurocrit Care. 2016; 24:82-89.
15. Preuss S, Multmeier J, Stenzel W, Major S, Ploner CJ, Storm C, Nee J, Leithner C, Endisch C. Survival, but not the severity of hypoxic-ischemic encephalopathy, is associated with higher mean arterial blood pressure after cardiac arrest: A retrospective cohort study. Front Cardiovasc Med. 2024; 11:1337344.
16. Wang CH, Chang WT, Su KI, Huang CH, Tsai MS, Chou E, Lu TC, Chen WJ, Lee CC, Chen SC. Neuroprognostic accuracy of blood biomarkers for post-cardiac arrest patients: A systematic review and meta-analysis. Resuscitation. 2020; 148:108-117.
17. Moseby-Knappe M, Mattsson N, Nielsen N, *et al*. Serum Neurofilament Light chain for prognosis of outcome after cardiac arrest. JAMA Neurol. 2019; 76:64-71.
18. Klitholm M, Jeppesen AN, Christensen S, Parkner T, Tybirk L, Kirkegaard H, Sandfeld-Paulsen B, Grejs AM. Neurofilament Light Chain and Glial Fibrillary Acidic Protein as early prognostic biomarkers after out-of-hospital cardiac arrest. Resuscitation. 2023; 193:109983.
19. Gramespacher H, Schmieschek MHT, Warnke C, Adler C, Bittner S, Dronse J, Richter N, Zaeske C, Gietzen C, Schlamann M, Baldus S, Fink GR, Onur OA. Analysis of cerebral CT based on supervised machine learning as a predictor of outcome after out-of-hospital cardiac rrest. Neurology. 2024; 103:e209583.

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Policy Forum

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Protecting the socioeconomic rights and interests of patients with rare diseases based on an innovative payment mechanism

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SUMMARY The pathogenesis of diseases in the field of rare diseases is complex. Many rare diseases have yet to be conquered worldwide, and there are still no effective treatments for most rare diseases, resulting in limited accessibility to medications. Over the past few years, China has been committed to ensuring the availability of drugs for rare diseases, both at the national level and in all sectors of society. Through National Healthcare Insurance Negotiations (hereinafter referred to as "national negotiations"), the National Healthcare Security Administration has included several drugs for rare diseases in medical insurance coverage, addressing some of the issues with medications for rare diseases. National Negotiations have reduced the prices of drugs for rare diseases to a certain extent, but they remain expensive for many patients. By the end of 2023, out of the 165 drugs for rare diseases that had been launched, 53 were still not covered by medical insurance, leaving patients with a significant financial burden. Addressing payment issues remains a current challenge, and various regions in China are actively exploring innovative methods of paying for rare disease care to protect the socioeconomic rights and interests of patients with rare diseases.

Keywords rare diseases, payment methods, national negotiations, special funds

1. Introduction

There is no universally accepted definition of rare diseases globally. In China, according to the 2021 Research Report on the Definition of Rare Diseases in China, diseases with an incidence of less than 1/10,000 in newborns, a prevalence of less than 1/10,000, and affecting fewer than 140,000 individuals are classified as rare diseases (*1*). Although rare diseases have a low incidence, China has a large population, and the number of people with rare diseases exceeds 20 million, so rare diseases are not rare in China. The majority of patients with rare diseases have no access to medications or cannot afford the cost of treatment, placing a significant financial and emotional burden on their families. Many households fall into poverty due to illness or return to poverty as a result of it. There are currently over 7,000 known rare diseases worldwide. According to statistics, there are no effective treatments for 95% of these rare diseases (*2*). By the end of 2023, based on the First List of Rare Diseases and the Second List of Rare Diseases, China had 165 drugs for rare diseases on the market, covering 92 types of rare diseases. However, the number of known rare diseases in China is approximately 1,400 (*3*).

China's efforts to protect patients with rare diseases began relatively late, with only some economically developed regions initially exploring protections for those patients. Since 2018, China has increasingly prioritized the protection of patients with rare diseases, introducing a series of policies to ensure patients have access to medications (*4-14*), as shown in Figure 1. In 2018 and 2023, China issued the First List of Rare Diseases (covering 121 diseases) and the Second List of Rare Diseases (covering 86 diseases), respectively (*15-17*), and these lists cover a total of 207 rare diseases. In 2020, the Opinions of the Central Committee of the Communist Party of China and the State Council on Further Reform of the Medical Insurance System proposed a multi-level medical insurance system. This system is centered around basic medical insurance, supported by medical assistance, and supplemented by additional medical insurance, commercial health insurance, charitable donations, and medical mutual aid (*18*). In 2024, the National Healthcare Security Administration proposed the establishment of a comprehensive "1+3+N" multi-level medical insurance system (*19*). The "1" refers to a basic information platform, which includes features such as "one person, one file" and "one drug, one code";

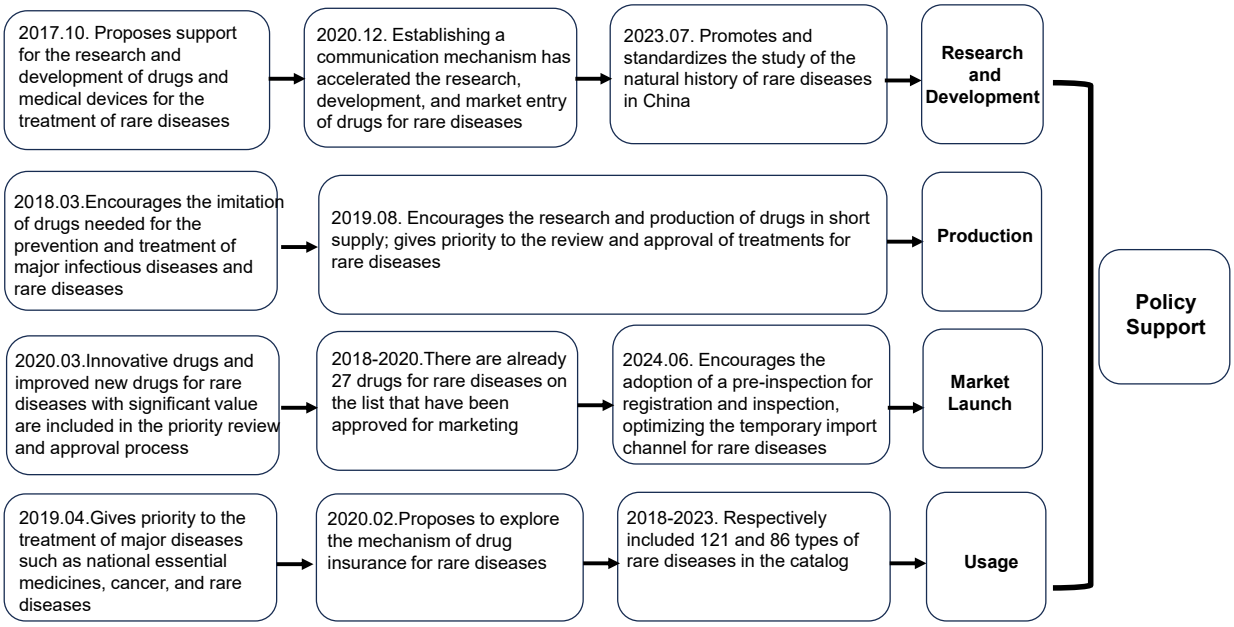

Figure 1. National policy documents on efforts to ensure the availability of drugs for rare diseases.

the "3" refers to the three levels within the basic medical insurance system, and the "N" represents commercial supplementary medical insurance, other forms of commercial health insurance, charities, funds, *etc*.

China has initially established a multi-level insurance system for rare diseases. However, the actual socioeconomic rights of patients with rare diseases are still in their infancy. From a health economics perspective, there are still many obstacles to overcome. Only by addressing issues related to funding and payment can the difficulties in diagnosis and treatment of patients with rare diseases be resolved, thereby protecting their socioeconomic rights.

2. Current status of the development of innovative payments for diagnosis and treatment of rare diseases in China

The small number of patients with rare diseases, the small market size, and the high research and development costs make drugs for rare diseases expensive. From an economic perspective, there is relatively insufficient motivation to research and develop drugs for rare diseases. Hospitals face pressure from assessments, and the extensive use of high-priced drugs may lead to rapid growth in indicators such as per capita costs, affecting their rating and thereby affecting the settlement of medical insurance funds. In order to address the issue of medications, various provinces and cities in China have explored multiple forms of payment methods. At the level of basic medical insurance, there are mainly one-way payment and outpatient management of special and chronic diseases. For outside medical insurance and supplementary medical insurance, there are forms such as special funds, commercial supplementary medical insurance, medical assistance, and serious illness medical insurance, as shown in Table 1.

2.1. Accelerating the inclusion of and payments for drugs for rare diseases in medical insurance through national negotiations

As economic growth has enhanced social security, China has begun the work of including drugs in national negotiations (*20*). While better meeting the basic needs of insured individuals for medications for common diseases, drugs for rare diseases have been included in the scope of national negotiations since 2019, and several exorbitantly priced drugs for rare diseases have been successfully negotiated down in price. From 2019 to 2023, prices for a total of 42 drugs for rare diseases were successfully negotiated, as shown in Table 2. The 2023 version of the National essential drug list includes 112 drugs for rare diseases, covering 64 types of rare diseases. The treatment of rare diseases is mainly medication-based, and insurance for rare diseases has significantly improved.

In order to better negotiate drug prices nationally, China has introduced a "dual-channel" policy. The "dual-channel" refers to meeting the reasonable needs of ensuring the supply and facilitating the clinical use of negotiated drugs through two channels – designated medical facilities and designated retail pharmacies – and simultaneously incorporating them into the mechanism of medical insurance payment. Although the prices of medications for rare diseases have been reduced through

Table 1. Payment methods related to rare diseases

Name	Policy Content	Executing Area
Health insurance one-way payment	A policy established by the government, it is used to separately reimburse specific medical expenses. These expenses usually do not fall under regular reimbursement by hospital medical insurance funds.	Jiangsu Province
special and chronic disease outpatient management	This is established for certain diseases that have costly medical expenses, a clear diagnosis, can be treated on an outpatient basis, and that fall within the affordable scope of the medical insurance pooling fund.	Shanxi Province; Guangxi Province
Rare disease special fund	A fund dedicated to the financing and payment of the costs of rare disease treatment, designed to provide some sort of transitional protection	Zhejiang Province
Commercial supplementary medical insurance	This is commercial supplementary medical insurance, guided by local governments and jointly established by insurance companies and third-party operating platforms.	Shanghai's commercial supplementary commercial medical insurance
Other supplementary payment methods	Some regions have serious illness medical insurance or medical assistance systems for rare diseases. Medical assistance is a system in which the government gives special help and support to citizens who cannot obtain basic medical insurance or afford medical expenses due to poverty.	Shandong Province; Foshan, Guangdong Province

Table 2. National negotiations regarding medical insurance over the years

Year of negotiations	2015	2017	2018	2019	2020	2021	2022	2023
Number of qualifying entries	3	36	17	97	119	94	111	121
Average reduction	58.7%	44%	56.7%	60.7%	50.64%	61.71%	60.1%	61.7%
Number of drugs for rare diseases	0	0	0	7	6	7	7	15

Note: National Healthcare Insurance Negotiations of the People's Republic of China were organized by the National Health and Family Planning Commission in 2015, by the Ministry of Human Resources and Social Security in 2017, and by the National Healthcare Security Administration from 2018 to 2023.

national negotiations, fundamentally, these drugs remain expensive. In response to national negotiations over high-priced drugs, many provinces and cities have also implemented policies such as one-way payment and outpatient management of special and chronic diseases. For example, Jiangsu Province prioritizes the inclusion of drugs with long usage cycles, high treatment costs, and innovative drugs in "dual-channel" management and implements separate payment policies for some high-priced drugs without a deductible, while ensuring that the original benefits are not reduced (*21*). Shanxi Province has included some negotiated drugs in the provincial special drug catalog. For drugs for rare diseases within the catalog, including pulmonary arterial hypertension, multiple sclerosis, spinal muscular atrophy, Fabry disease, and acromegaly, the reimbursement rate has been increased by 10% compared to the original special drug policy, further ensuring the affordability for patients (*22*). The Guangxi Zhuang Autonomous Region has included 36 drugs for rare diseases suitable for outpatient treatment in the separate outpatient overall payment, benefiting patients with 27 types of rare diseases such as multiple sclerosis, narcolepsy, myasthenia gravis, neuromyelitis optica, and Gaucher's disease. An annual maximum payment limit is set, with a cap of 40,000 RMB for resident medical insurance and 80,000 RMB for employee medical insurance (*23*).

2.2. Payment for rare diseases from a special fund

Drugs for rare diseases are expensive, hampering their full coverage by China's basic medical insurance . Many experts have suggested establishing a dedicated fund for raising and paying for the costs of diagnosing and treating rare diseases (*24*). Currently, there is no special fund for drugs for rare diseases at the national level. Only some economically developed provinces and cities, such as Zhejiang, Jiangsu, and Qingdao in Shandong, have established special funds for rare diseases. By capping personal out-of-pocket expenses, these funds effectively address the accessibility of medication for patients. In Zhejiang, a sub-account is set up under the provincial medical insurance fund's special fiscal account. In accordance with the standard of 2 RMB per person per year, funds are transferred in one lump sum from the major illness insurance fund of the overall planning area to the Zhejiang Provincial Insurance Fund for Drugs for Rare Diseases. The maximum out-of-pocket expense for patients is capped at 100,000 RMB (*25*). Jiangsu Province has implemented provincial-level coordination and separate financing for insurance funds for drugs for rare diseases. It has established a multi-channel fundraising mechanism led by the government, with participation from market entities and charitable organizations. The insurance funds for drugs

for rare diseases are managed within the provincial fiscal social security special account, ensuring that they are used exclusively for their intended purpose and are independently accounted for (*26*).

2.3. Payment of commercial supplementary medical insurance

Through the 2024 Observational Report on Trends in the Rare Disease Sector in China, as of the end of 2023, more than 620 "commercial supplementary medical insurance" products have been launched in various cities across China, becoming an important part of the government-led creation of a "multi-level medical insurance system." Many insurance plans include coverage for drugs for rare diseases. For example, Shenzhen's commercial supplementary medical insurance further reimburses 80% of the drugs for rare diseases listed in the medical insurance catalog after deducting the deductible from the payments made by basic medical insurance. The annual coverage limit is up to 1.2 million RMB (*27*). Shanghai's commercial supplementary medical insurance further reimburses 70% after the basic medical insurance payment and deduction of the deductible, with an annual coverage limit of up to 1 million RMB (*28*).

2.4. Current status of additional supplementary payments

In some regions, medical assistance models for drugs for rare diseases have been explored. For instance, Foshan in Guangdong has implemented policies where, after reimbursement by basic medical insurance, major illness insurance, and various supplementary medical insurance plans, further assistance with the remaining personal payment for medical expenses is provided at a level of 80%, with an annual limit of 300,000 RMB (*29*). Shandong has implemented a major illness medical insurance coverage model for certain rare diseases, such as Gaucher's disease, Pompe disease, and Fabry disease, with segmented reimbursement, and the annual maximum reimbursement is up to 900,000 RMB (*30*).

3. Challenges and Perspectives

The affordability of medical insurance funds varies across China, and the high cost of medications for rare diseases inevitably impacts these funds. The epidemiological data for the diseases treated with these drugs, especially in terms of baseline information, is not sufficient. The estimated number of patients based on existing data or foreign indicators is not accurate, hampering the ability to precisely predict their impact on the medical insurance budget. Therefore, covering all medications for rare diseases under medical insurance is less feasible.

The primary factor in establishing a dedicated fund for rare diseases is fundraising. However, with the numerous types of rare diseases and insufficient epidemiological data, calculating how much funding is required and finding sources for such funds has always been challenging. Setting up a dedicated fund requires the formulation of separate policies for rare diseases, which stokes controversy regarding fairness to patients with other diseases.

In conclusion, constructing a multi-party shared responsibility and diverse payment system has always been a key focus of China's. Regions are encouraged to calculate payments based on their own economic conditions and rare disease-related data and to innovate payment methods to protect patient rights, though this is a long and arduous task. As an important supplement to medical insurance, perfecting a diverse payment system is indispensable for commercial insurance. In the name of aiding patient in the information age, big data could be used to facilitate the exchange of disease data and reimbursement data by medical insurance departments of commercial insurance companies, enabling precise calculations. This, in turn, would allow for the development of appropriate payment mechanisms.

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References

1. He A, Xu Y, Song J, Chen M. Analysis on the supplies of orphan drugs in national negotiated drugs - Taking 20 orphan drugs as examples. China Health Insurance. 2023; 9:18-26. (in Chinese)
2. Zhang S, Zhang X. Recent polices and practice in rare diseases in China. J Rare Diseases. 2022; 1:1-6. (in Chinese)
3. Frost & Sullivan. 2024 Observational Report on Trends in the Rare Disease Sector in China. *https://www.frostchina.com/content/insight/detail/65defef3a2aa84f5d859534e* (accessed November 18, 2024). (in Chinese)
4. The General Office of the State Council. Opinions on further reform of the review and approval system to encourage innovation in pharmaceuticals and medical devices. *https://www.gov.cn/zhengce/2017-10/08/content_5230105.htm* (accessed November 18, 2024). (in Chinese)
5. National Medical Products Administration. Management approaches for drug research and development and communication of technical reviews. *https://www.nmpa.gov.cn/xxgk/ggtg/ypggtg/ypqtggtg/20201221172016170.html* (accessed November 21, 2024). (in Chinese)
6. Center for Drug Evaluation. Guidelines for the study of disease natural history in rare disease drug development. *https://www.cde.org.cn/main/news/viewInfoCommon/bee*

f37b41b0a2d10b72ba1465a7a19e1 (accessed November 18, 2024). (in Chinese)
7. The General Office of the State Council. Opinions on reforming and improving policies to ensure the supply and facilitate the use of generic drugs. *https://www.gov.cn/gongbao/content/2018/content_5283184.htm* (accessed November 18, 2024). (in Chinese)
8. Standing Committee of the National People's Congress. Drug Administration Law. *https://www.gov.cn/xinwen/2019-08/26/content_5424780.htm* (accessed November 18, 2024). (in Chinese)
9. State Administration for Market Regulation. Management of drug registration. *https://www.samr.gov.cn/zw/zfxxgk/fdzdgknr/fgs/art/2023/art_3275cb2a929d4c34ac8c0421b2a9c257.html* (accessed November 18, 2024). (in Chinese)
10. Center for Drug Evaluation. Notice on the list of new foreign drugs urgently needed in China. *https://www.cde.org.cn/main/news/viewInfoCommon/08818b168ccc85db9a42a0f6623b5688* (accessed November 21, 2024). (in Chinese)
11. National Medical Products Administration. Announcement on further optimization of the review and approval process for drugs marketed overseas that are urgently needed in China. *https://www.nmpa.gov.cn/xxgk/zhqyj/zhqyjyp/20240625142147136.html* (accessed November 18, 2024) (in Chinese)
12. National Healthcare Security Administration. Proposed adjustment of the 2019 list of covered by national health insurance. *https://www.nhsa.gov.cn/art/2019/4/17/art_53_1215.html* (accessed November 18, 2024). (in Chinese)
13. The National Health Commission. Second list of rare diseases. *http://www.nhc.gov.cn/yzygj/s7659/202309/19941f5eb0994615b34273bc27bf360d.shtml* (accessed November 18, 2024). (in Chinese)
14. Center for Drug Evaluation. Notice on the publication of the list of new foreign drugs urgently needed in China. *https://www.cde.org.cn/main/news/viewInfoCommon/08818b168ccc85db9a42a0f6623b5688* (accessed November 18, 2024). (in Chinese)
15. He J, Song P, Kang Q, Zhang X, Hu J, Yang Y, Tang M, Chen D, Hu S, Jin C. Overview on social security system of rare diseases in China. Biosci Trends. 2019; 13:314-323.
16. Tang M, Yang Y, Ye Z, Song P, Jin C, Kang Q, He J. Release and impact of China's" Second List of Rare Diseases." Intractable Rare Dis Res. 2023; 12: 251-256.
17. Ying Z, Gong L, Li C. An update on China's national policies regarding rare diseases. Intractable Rare Dis Res. 2021; 10:148-153.
18. The State Council of the People's Republic of China. Opinions of the Central Committee of the Communist Party of China and the State Council on further reform of the medical insurance system. *https://www.gov.cn/gongbao/content/2020/content_5496762.htm* (accessed November 18, 2024). (in Chinese)
19. The National Healthcare Security Administration of the People's Republic of China. The Party Group of the National Healthcare Security Administration held a meeting to specifically convey and study the spirit of the Third Plenary Session of the 20th Central Committee of the Communist Party. *https://www.nhsa.gov.cn/art/2024/7/19/art_14_13292.html* (accessed November 18, 2024). (in Chinese)
20. Tang M, Song P, He J. Progress on drug pricing negotiations in China. Biosci Trends. 2019; 13:464-468.
21. Yuan X. Shang P. Zhang D. Tang W, Li X. Budget impact analysis of different payment methods on the medical insurance under the "Dual-Channel" mode taking the treatment of multi-drug resistant tuberculosis as an example. Chinese Health Economics. 2022; 41:32-35.
22. Shanxisheng Yiliao Baozhangju. Notice on further improving the management of national negotiated drug use. *http://ybj.shanxi.gov.cn/xxfb/xxgkml/202104/t20210423_2736996.html* (accessed November 18, 2024). (in Chinese)
23. Guangxi Zhuangzu Zizhiqu Yiliao Baozhangju. Notice on adjusting the separate outpatient overall payment and the list of special drugs for outpatient care of chronic diseases. *http://ybj.gxzf.gov.cn/zfxxgkzl/zcfg/zcfg_9912/zcfggybf/t18662334.shtml* (accessed November 18, 2024). (in Chinese)
24. Tao L, Wang F, Li H, Zheng J. Discussion on the construction and design of the operation mechanism of the special fund for rare diseases in China. China Health Insurance. 2024; 4:57-63. (in Chinese)
25. Zhejiang Healthcare Security Administration. Notice on establishing a drug insurance mechanism for rare diseases in Zhejiang Province. *http://ybj.zj.gov.cn/art/2019/12/31/art_1229262886_55746768.html* (accessed November 18, 2024). (in Chinese)
26. Jiangsu Healthcare Security Administration. Regulations on medical insurance in Jiangsu Province. *https://mp.weixin.qq.com/s/OVlOOsjSdv3y4ogOvNMb6w* (accessed November 19, 2024). (in Chinese)
27. Shenzhen Healthcare Security Administration. Shenzhen's commercial supplementary medical insurance coverage benefits. *https://hsa.sz.gov.cn/ztzl/szhmb/cbdy/content/post_10593003.html* (accessed November 19, 2024). (in Chinese)
28. Shanghai Healthcare Security Administration. Enrollment in Shanghai's commercial supplementary medical insurance begins on May 9, 2023. *https://ybj.sh.gov.cn/ybdt/20230515/1696f0fceaae4161b2b42efeaee0770f.html* (accessed November 19, 2024). (in Chinese)
29. The People's Government of Foshan City. Notice from the Foshan Healthcare Security Administration on the issuance of the detailed implementation rules for medical assistance in the City of Foshan. *https://www.foshan.gov.cn/zwgk/zfgb/szfgfxwj/content/post_4381320.html* (accessed November 19, 2024). (in Chinese)
30. Health Care Security Administration of Shandong Province. Notice on including some specialty drugs for rare diseases in our major illness insurance coverage. *http://ybj.shandong.gov.cn/art/2020/12/31/art_113602_10270748.html* (accessed November 19, 2024). (in Chinese)

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Mini-Review

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Cervicofacial emphysema: A systematic review

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SUMMARY Cervicofacial Emphysema (CFE) is a self-limiting condition, defined by the presence of air in face and neck. The purpose of the manuscript is to systematically review the existing literature on CFE evaluation and management for updated clinical understanding of this condition. A literature search was conducted of publications about CFE on PubMed and Google Scholar by identifying all the articles with key search terms "Cervicofacial Emphysema" and "Sub Cutaneous Emphysema". Inclusion criteria were case series published in English between 1980 and 2024. In total, 241 case series were selected and reviewed to determine presenting symptoms, clinical signs and predisposing factors associated with CFE. Average age at diagnosis was 38.1 years, male and female are almost equally affected. The most common presenting symptoms were face and neck swelling. The most common finding was crepitus. The condition was most commonly reported in patients undergoing dental procedures, otorhinolaryngology procedures, or in patients who experienced transient change in intra nasal/thoracic pressure. The management includes clinical monitoring, reassurance of the patient, antibiotic prophylaxis and monitoring to rule out pneumomediastinum. The odds of concurrent pneumomediastinum is highest in patients with abdominal procedures as an etiology of CFE.

Keywords cervicofacial emphysema, crepitus, face, neck swelling

1. Introduction

Cervicofacial Emphysema (CFE) is defined as the presence of air within the facial planes (*1*). It often occurs in the setting of trauma such as injury to face, dental procedures, recent surgery, or conditions causing changes in intranasal/intrathoracic pressure (*2*). The most common reported presenting symptom is face and neck swelling (*3*). The most common clinical exam finding is crepitus (*4*). Clasically, for patients with cervicofacial emphysema, workup includes evaluation of mediastinal involvement (pneumomediastinum/pneumothorax) (*5*) The condition is non-fatal and self-limited. Therefore management is conservative and includes reassurance, clinical observation, and antibiotics. The majority of patients with cervicofacial emphysema make an uneventful recovery (*3*).

While previous studies enumerate the presenting clinical characteristics of CFE, there is a paucity of work describing the various triggering events / predisposing risk factors of this interesting clinical entity as well as risk factors for progression to pneumomediastinum. Thus, the purpose of the present manuscript is to systematically review the existing literature on various causes of CFE, evaluation, and management to create an up-to-date understanding of this condition with a relevant review of the literature.

2. Methodology and Literature Search Strategy

We systematically searched clinical literature databases including pub Med and Google Scholar for case series on CFE published between 1980 and 2024 following PRISMA guidelines. Key search terms included "cervicofacial emphysema" and "subcutaneous emphysema". Inclusion criteria were articles that described clinicodemographic information for patients with CFE, articles that detailed patients older than 18 years of age, and articles published in the English language. Abstracts of all articles were independently screened by authors to assess eligibility. Articles with insufficient clinical details (on symptomatology, workup, and treatment) were excluded from review with discrepancies resolved by senior author. Clinical

information of interest included patient age, sex, presenting symptoms, clinical exam findings, triggering events, comorbidities, and management. The full text of selected articles was then reviewed, and reference lists were examined for additional relevant studies. All data was accessed between May to June 2024.

3. Results

Our initial search strategy led to abstracts of 363 publications which were screened for inclusion criteria eligibility (Figure 1). 122 records were excluded as they were not relevant to our current study. It was determined that 241 articles (detailing a total of 267 cases) had sufficient detail for full text review.

3.1. Demographics

Among 241 articles from 1980-2024, we found 267 reported cases of CFE. This population contained a similar number of male (136, 50. 9%) and female (131, 49%) patients. The mean age of this cohort was 38.1 years, with an age range spanning from 19 to 85 years. The age distribution was as follows: 3.7% (n = 10) age < 20, 24.7% (n = 66) age 20-30, 15.7% (n = 42) age 31-40, 18% (n = 49) age 41-50, 13% (n = 36) age 51-60, 9.3% (n = 25) age 61-70, 13%(n = 35) age > 70. Age was not mentioned for 4 patients.

3.2. Features of presentation

The most common presenting symptom in the analyzed cohort of patients was face swelling, which occurred in 47% of patients (n = 125). Other common symptoms included neck swelling (18%, n = 48), and eyelid swelling (14%, n = 37). There were also sporadic complaints of facial pain (9%, n = 24), dyspnea (5%, n = 15), chest pain (4.8%, n = 13), dysphagia (2.6%, n = 7), and dysphonia (2%, n = 6). Clinical examination revealed the presence of facial crepitus in 55% (n = 146) of the patients. Pneumomediastinum was observed on radiological investigation in 28% (n = 74) of the patients. Pneumothorax was present in 8% (n = 22) of patients. Other less commonly reported findings were pneumoperitoneum (1.4%, n = 4) and pneumopericardium (1%, n = 3).

3.3. Predisposing factors

The most common triggering event / predisposing risk factor for development of cervicofacial emphysema was sudden nasal/intrathoracic pressure changes, which was identified in 31% (n = 83) of patients. This was followed by dental procedures (*i.e.* extraction (*6*), endodontic/restorative procedures (*7*)), which triggered CFE in 27.7% (n = 74) of the patients. Otherwise, the etiology of CFE was facial injuries in 15% (n = 41) of patients, otorhinolaryngology procedures in 8.2% (n = 22) of patients, thoracic in 5.6% (n = 15) and abdominal procedures in 2.9% (n = 8). Interestingly, spontaneous occurrence was found in 5% (n = 14) of patients. These triggering factors are detailed in Figure 2. Only 8.6% (n = 23) of patients reported having a smoking history.

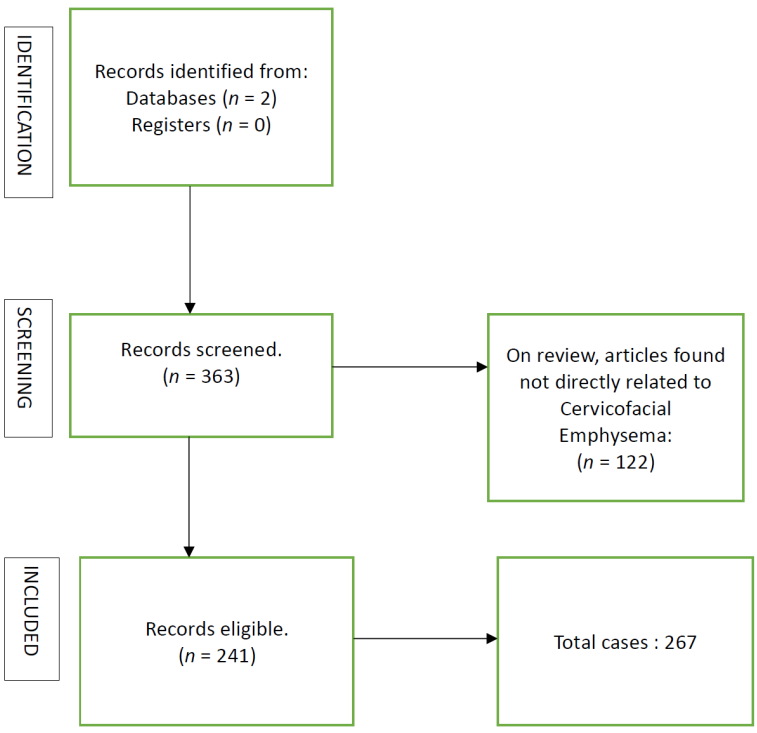


Figure 1. Identification of studies *via* databases and registers.

3.4. Clinical outcomes

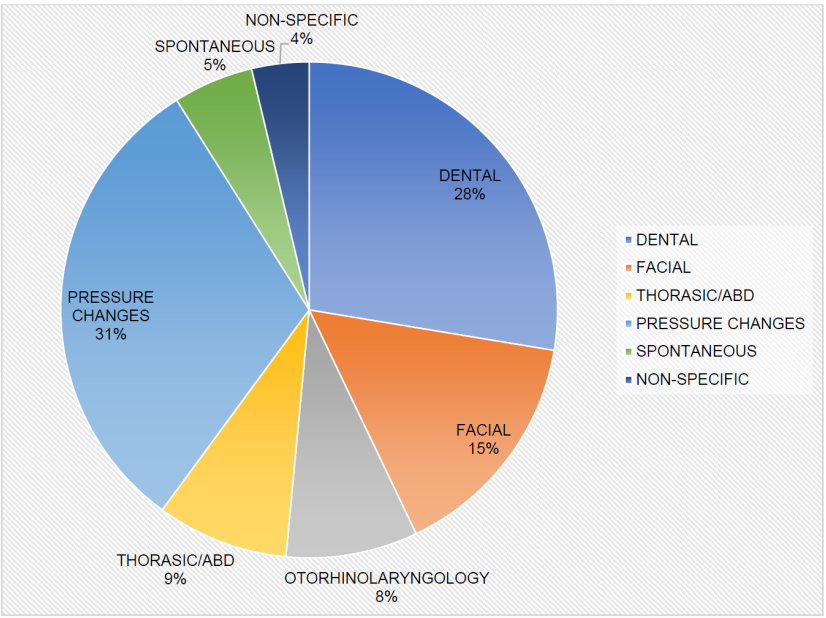
In our review, 74 cases developed pneumomediastinum either after or concurrently with CFE. The most common triggering events for CFE in this population were dental procedures (32%), abdominal procedures (20%), and facial injuries (20%). The odds of pneumomediastinum developing with CFE were 1.37 (95% CI: 0.77 – 2.46) for patients with dental procedures, 5.88 (95% CI: 2.37 – 14.56) for patients with abdominal procedures, and 1.63 (95% CI: 0.81 – 3.29) for patients with facial injuries.

In our review, the average time from presentation to resolution of CFE was noticed to be around 1 week. 208 (77.9%) cases resolved completely in 7 days and 44 (16.4%) cases resolved completely in 8-14 days. There was no follow-up reported in 15 (5.6%) cases. The average number of days from presentation to CFE resolution was 2.4 days for dental procedures, 2.3 days for otorhinolaryngology procedures ,3.1 days for thoracic procedures, and 4.4 days for abdominal procedures.

In our review of CFE, the most common treatment was antibiotics, which was given in 165 (61.7%) cases. Surgical aspiration was required due to eyelid closure in 6 (2.2%) patients, and canthotomy was required for 2 (0.7%) patients. Oxygen was given in 10 (3.7%) patients due to desaturation. 2.2% (n = 6) of patients passed away due to other pre-existing conditions. 10 out of 22 (45%) cases of pneumothorax required placement of chest tube.

4. Discussion

Cervicofacial emphysema is an uncommon clinical entity which is often unrecognized and may be misdiagnosed as anaphylaxis, angioedema, or internal hemorrhage(*8*). The pathophysiology of CFE is inclusion of air, typically under pressure, into subcutaneous tissues. This air has the potential to spread along fascial planes in the neck and mediastinum(*9*). Air typically gains access into these subcutaneous planes when the integrity of the oral mucosa is interrupted or and intraoral pressure is increased (*1*). The clinical presentation is characterized by the sudden onset of facial swelling. Crepitus, pain, and tenderness may be noted as well (*10*). Retrosternal pain, dyspnea and Hamman sign may indicate concurrent pneumomediastinum. Diagnosis is suspected on clinical examination and may be confirmed/monitored with



SURGICAL

- Dental – 74 (27.7%)
- Facial Injuries – 41 (15.3%)
- Otorhinolaryngology – 22 (8.2%)
- Thoracic-15 (5.6%)
- Abdominal– 8 (2.9%)

NON-SURGICAL

- Pressure changes- 83 (31%)
  - Nose Blowing – 31 (11.6%)
  - Vigorous Cough – 18 (6.7%)
  - Valsalva – 11 (4.1%)
  - Sneezing – 8 (2.9%)
  - Labor – 8 (2.9%)
  - Barotrauma – 3 (1.1%)
  - Cocaine Insufflation – 2 (0.7%)
  - Retching – 2 (0.7%)
- Spontaneous – 14 (5.2%)
- Non-Specific – 10 (4%)
  - Self-inflicted -7 (2.6%)
  - Cryotherapy-2 (0.7%)
  - Trigeminal Neuralgia correction 1 (0.3%)

Figure 2. Causes of cervicofacial emphysema (CFE).

radiologic imaging (X-Ray/CT) which will demonstrate the presence of subcutaneous air (*11*).

Dental procedures are considered to be the most common source of CFE (*9*). High speed dental air turbine drills(*12,13*) are used in endodontic treatment(*14*) and surgical tooth extraction(*7*). During these interventions, compressed air may be forced into subcutaneous layers at a high pressure. Hydrogen peroxide usage has also been implicated in the development of CFE during endodontic treatment(*7,15*). Our study, confirms these findings and reports on relative incidences of other triggering events within the literature – the most common etiologies of CFE in our study were dental procedures (27.7%) as well as activities leading to sudden change in intrathoracic pressure (*i.e.* from repeated nose blowing, sneezing, vigorous coughing, retching, or valsalva) (31%).

Treatment of cervicofacial emphysema is usually conservative which includes bed rest, observation and reassurance, as the condition is benign and self-limiting (*11*). In fact, most patients with cervicofacial emphysema recover within 7 days (*16*). This was true for patients in our cohort, as most recovered within 1 week on average. It has been reported that supplemental oxygen can hasten the resolution of subcutaneous emphysema because oxygen, which replaces the air, is more readily absorbed (*14*). Surprisingly, oxygen was given to only 10 (3.7%) patients included in our study. The use of supplemental oxygen to improve time to recovery in patients with CFE needs to be the subject of future studies.

Complications of CFE, such as sepsis, can develop when microorganisms from oral cavity migrate to the mediastinum. Generally, broad-spectrum antibiotics are administered to avoid development of such severe infections (*17*). In our review, antibiotics were given in 165(61.7%) patients. The data collected in this study were insufficiently granular to understand whether or not the administration of antibiotics helped decrease incidence of sepsis. However, we support this treatment approach, as there is a potential for spread of bacterial organisms from subcutaneous tissue to mediastinum which may lead to significant adverse events. The authors also feel that most patients with CFE can be managed conservatively at home with supportive measures. In our review, supportive measures provided to patients included techniques to decrease airway pressure (*i.e.* telling patients to avoid blowing their nose or bearing down for a few days) (*18*).

Life threatening complications of CFE include pneumothorax(*5*), pneumomediastinum(*5,8,19*), and pneumopericardium(*20*). Urgent surgical decompression may be required if cardiovascular collapse or large airway obstruction occurs (*8*). Patients may be observed in hospital if there is any suspicion of impending respiratory compromise. In our review, a majority of the cases of pneumothorax cases were mild and self-limited, however 10 out of 22 cases of pneumothorax required chest tube drainage. Pneumomediastinum was seen in 74 patients included in our study (28%). The odds of pneumomediastinum in patients with CFE were significantly higher in those who had an abdominal procedure as a trigger for CFE (OR 5.88, 95% CI: 2.37 – 14.56). These finding is easily conceivable as subcutaneous air in the head and neck originating from the abdomen must pass through the mediastinum. Still, this is the first publication to indicate that CFE secondary to recent abdominal procedure is significantly associated with comorbid pneumomediastinum, and should therefore prompt chest imaging for further interrogation. The importance of chest imaging in these patients is underscored by the fact that resolution of CFE for patients with recent abdominal procedures takes a longer period of time (4.4 days) as compared to CFE from other etiologies (~2 days).

CFE from dental procedures is known to sometimes cause pneumomediastinum/pneumothorax secondary to alveolar rupture and escape of air into perivascular planes (*5,6*). Additionally, the roots of the lower posterior molar teeth communicate directly with sublingual and submandibular space. The sublingual space communicates with the pterygomandibular, parapharyngeal and retropharyngeal spaces, a main route of communication from oral cavity to mediastinum(*16,21*). However, interestingly, while a majority of patients with pneumomediastinum in our review had dental procedures as an etiology of CFE (32%), CFE from dental procedure was not significantly associated with the development of pneumomediastinum.

In the present analysis, 6 out of 267 patients passed away, all due to complications of underlying comorbidities such as tension pnuemomediastinum(*22*), massive cerebrovascular accident(*23*), or acute respiratory distress syndrome (*24*). Most of these patients were also within the elderly age group (mean 56 years + 28.2 years).

5. Conclusion

In conclusion CFE is generally a self-limiting condition that occurs due to dental, thoracic, abdominal, or otorhinolaryngology procedures. It may also occur sporadically where there are transient changes in intranasal/ thoracic pressure. Diagnosis is clinical and may be confirmed with radiological examination to determine the extent of gas dissection into the subcutaneous space. CFE from abdominal procedures have a significant association with comorbid pneumomediastinum. Management is conservative and includes reassurance, supplemental oxygen, clinical observation and antibiotics. The condition is non-fatal and self-limiting. The majority make an uneventful recovery.

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

References

1. Brasileiro BF, Cortez AL, Asprino L, Passeri LA, De Moraes M, Mazzonetto R, Moreira RW. Traumatic subcutaneous emphysema of the face associated with paranasal sinus fractures: a prospective study. J Oral Maxillofac Surg. 2005; 63:1080-1087.
2. Celebioglu S, Keser A, Ortak T. An unusual complication of rhinoplasty: subcutaneous emphysema. Br J Plast Surg. 1998; 51:266-267.
3. Pynn BR, Amato D, Walker DA. Subcutaneous emphysema following dental treatment: A report of two cases and review of the literature. J Can Dent Assoc. 1992; 58:496-499.
4. Bekiroglu F, Rout PG. Surgical emphysema following dental treatment: two cases. Dent Update. 1997; 24:412-414.
5. Choi PW. Pneumomediastinum, pneumothorax, and subcutaneous emphysema caused by colonoscopic perforation: A report of two cases. J Emerg Med. 2017; 52:e117-e22.
6. Pousios D, Panagiotopoulos N, Sioutis N, Piyis A, Gourgiotis S. Iatrogenic pneumomediastinum and facial emphysema after surgical tooth extraction. Ann Thorac Surg. 2010; 89:640.
7. An GK, Zats B, Kunin M. Orbital, mediastinal, and cervicofacial subcutaneous emphysema after endodontic retreatment of a mandibular premolar: a case report. J Endod. 2014; 40:880-883.
8. Karki AJ, Stokes MM, Fraser JS, Adlam DM. Surgical emphysema following a restorative procedure: A case report. Dent Update. 2006; 33:171-172, 174.
9. Bohnenkamp DM. Subcutaneous facial emphysema resulting from routine tooth preparation: A clinical report. J Prosthet Dent. 1996; 76:1-3.
10. Azenha MR, Yamaji MA, Avelar RL, de Freitas QE, Laureano Filho JR, de Oliveira Neto PJ. Retropharyngeal and cervicofacial subcutaneous emphysema after maxillofacial trauma. Oral Maxillofac Surg. 2011; 15:245-249.
11. Alonso V, Garcia-Caballero L, Couto I, Diniz M, Diz P, Limeres J. Subcutaneous emphysema related to air-powder tooth polishing: a report of three cases. Aust Dent J. 2017; 62:510-515.
12. Tamir S, Backleh G, Hamdan K, Eliashar R. Cervico-facial emphysema and pneumomediastinum complicating a high-speed drill dental procedure. Isr Med Assoc J. 2005; 7:124-125.
13. Penna KJ, Neshat K. Cervicofacial subcutaneous emphysema after lower root canal therapy. N Y State Dent J. 2001; 67:28-29.
14. Durukan P, Salt O, Ozkan S, Durukan B, Kavalci C. Cervicofacial emphysema and pneumomediastinum after a high-speed air drill endodontic treatment procedure. Am J Emerg Med. 2012; 30:2095.e3-6.
15. Bavinger JV. Subcutaneous and retropharyngeal emphysema following dental restoration: an uncommon complication. Ann Emerg Med. 1982; 11:371-374.
16. Finlayson RS, Stevens FD. Subcutaneous facial emphysema secondary to use of the Cavi-Jet. J Periodontol. 1988; 59:315-317.
17. Abrahams JM, Jakubowski J, Liang D, McClure S. Subcutaneous emphysema to the head and neck resulting from a dental crown preparation. J Mich Dent Assoc. 2013; 95:54-56.
18. Jawaid MS. Orbital emphysema: nose blowing leading to a blown orbit. BMJ Case Rep. 2015; 2015: bcr2015212554..
19. Elia F, Laface B, Pagnozzi F, Boccuzzi A, Ferrari G, Perna M, *et al*. Cervicofacial emphysema and pneumomediastinum complicating a dental procedure. J Emerg Med. 2013; 45:e179-81.
20. Marchon KA, Nunn MO, Chakera AJ. Images of the month: An incidental finding of spontaneous pneumomediastinum (Hamman's syndrome) secondary to diabetic ketoacidosis during the coronavirus pandemic. Clin Med (Lond). 2020; 20:e275-e7.
21. Ashley M, Jones C. Pneumomediastinum: an unusual radiographic finding following mid-facial trauma injury. Injury. 1997; 28:229-230.
22. Nachi S, Okada H, Kato H, Suzuki K, Nakano S, Yoshida T, Yoshida S, Ushikoshi H, Toyoda I, Ogura S. Simple pneumopericardium due to blunt trauma progressing to tension pneumopericardium during transportation. Am J Emerg Med. 2016; 34:933.e3-5.
23. Richardson MG, Dooley JW. Acute facial, cervical, and thoracic subcutaneous emphysema: a complication of fiberoptic laryngoscopy. Anesth Analg. 1996; 82:878-880.
24. Powell C, Kendall B, Wernick R, Heffner JE. A 34-year-old man with amyopathic dermatomyositis and rapidly progressive dyspnea with facial swelling. Diagnosis: pneumomediastinum and subcutaneous emphysema secondary to amyopathic dermatomyositis-associated interstitial lung disease. Chest. 2007; 132:1710-1713.

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Original Article

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Classification and epidemiologic analysis of 86 diseases in *China's Second List of Rare Diseases*

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SUMMARY Following the release of *China's First List of Rare Diseases* in May 2018, the Chinese government officially published *China's Second List of Rare Diseases* in September 2023. To date, there is no unified standard and international consensus for rare diseases, and epidemiologic data for most rare diseases in China are lacking. We investigated 86 rare diseases on the second list using Orphanet and other databases to clarify the classification, nomenclature, and epidemiologic data for these diseases, and we summarized the genotype and phenotype of hereditary diseases. The results showed that most of 86 rare diseases were coded in the database of Unified Medical Language System (UMLS), Orphanet, Medical Subject Headings (MeSH) and International Classification of Diseases, Eleventh Revision (ICD-11). Some rare diseases are composed by group of different disorders, in which multiple identifiers existed. Meanwhile, some rare diseases have different subtypes, which correspond to different identifiers. This increases the actual number of rare diseases in the second list. Over 50% of rare diseases are genetic rare diseases and they are mainly classified into neoplastic diseases, transplant-related disorders and neurological diseases. Epidemiologic data indicated that these rare diseases had a broad prevalence spectrum and over 20 rare diseases had a prevalence of over 1/10,000, these rare diseases in the *China's Second List of Rare Diseases* expanded the number and scope of rare diseases according to the China's official definition of rare diseases.

Keywords *China's Second List of Rare Diseases*, classification, nomenclature, epidemiology, incidence, prevalence

1. Introduction

A rare disease is a health condition with low prevalence and incidence compared with other more prevalent diseases in the general population. Although these diseases are individually rare, over 7,000 conditions have been identified, which affect 3.5%-5.9% of individuals worldwide or an estimated 263-446 million individuals collectively (*1*). Rare diseases are a global public health issue. Patients with rare diseases face challenges regarding diagnosis, treatment, and care. Great advances have been made in the diagnosis, treatment, care, and epidemiology of rare diseases in China, and several polices have been enacted to ensure progress in the area of rare diseases (*2,3*).

Before China's official definition of rare diseases in 2021, five bodies including the National Health Commission of the People's Republic of China and the National Medical Products Administration issued *China's First List of Rare Diseases* in May 2018 (*4,5*). Five years later, *China's Second List of Rare Diseases* was officially released on 21 September 2023 by six bodies. Until now, 207 rare diseases have been included on this list. In September 2021, China defined rare diseases as a condition with at least one of the following three criteria: an incidence among newborns of less than 1/10,000, a prevalence of less than 1/10,000, and an affected population of less than 140,000 (*6*). Thus, the rare diseases list and this definition have now been simultaneously adopted in China.

There is no unified international standard and consensus to date on how rare diseases should generally be named and classified. The nomenclature of rare diseases is recorded using international terminologies in different reference databases. In this study, we investigated the classification, genetic information, incidence, and prevalence of 86 conditions in *China's Second List of Rare Diseases* using Orphanet, OMIM, and other databases.

2. Methods

The nomenclature of 86 conditions in *China's Second List of Rare Diseases* was retrieved using international terminologies in different reference databases, including ICD-10 (*7*) and ICD-11 (*8*) (*https://icd.who.int/en*), Online Mendelian Inheritance in Man (OMIM) (*www.omim.org*), Orphanet (*https://www.orpha.net/consor/cgi-bin/index.php*), UMLS (*9*) (*https://www.nlm.nih.gov/research/umls/index.html*), MeSH (*https://www.nlm.nih.gov/mesh/meshhome.html*), Medical Dictionary for Regulatory Activities Terminology (MedDRA) (*10*) (*https://www.meddra.org/*), and Genetic and Rare Diseases (GARD) (*https://rarediseases.info.nih.gov/*). The classification and epidemiology of 86 rare diseases were analyzed through Orphanet database. The genetic information was summarized using OMIM database.

3. Results

3.1. Nomenclature of 86 diseases in *China's Second List of Rare Diseases*

Rare diseases in *China's Second List of Rare Diseases* were mapped to seven different reference databases (Table 1). UMLS coded the most rare diseases (n = 78, 90.70%) with a unique identifier, followed by 77 diseases (89.53%) in ORPHAcode, 74 diseases (86.05%) in MeSH, 66 diseases (76.74%) in ICD-11, and 64 diseases (74.42%) in ICD-10, 59 diseases (68.60%) in GARD and 57 diseases (66.28%) in MedDRA (Supplemental Table S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=220*). In ICD-10, identifier C49.9 encodes both dermatofibrosarcoma protuberans and epithelioid sarcoma, Lennox-Gastaut syndrome and West syndrome were classified into other generalized epilepsy and epileptic syndromes (G40.4). Bardet-Biedl syndrome and IGF1 deficiency were classified into hypopituitarism in ICD-11 (5A61.0). Except MeSH, melanoma and pemphigus were coded by at least two different codes in all other 6 nomenclature systems. PIK3CA related overgrowth syndrome and was multiple coded in 5 different nomenclature systems.

In Orphanet database, acquired hemophilia, congenital biliary atresia, malignant hyperthermia, melanoma, pemphigus and thalassemia major were coded using more than two different codes. ORPHA163596 and 231214 coded alpha-thalassemia major and beta-thalassemia major, respectively. Syndrome with alpha-thalassemia as a major feature (ORPHA 232288) was a group of disorders consisting of alpha-thalassemia-intellectual disability syndrome linked to chromosome 16 (ORPHA 98791), alpha-thalassemia-myelodysplastic syndrome (ORPHA 231401) and X-linked alpha-thalassemia-intellectual disability syndrome (ORPHA 847). Code 182095 represents a group of disorders of interstitial lung disease (ILD), including interstitial lung disease in childhood and adulthood ORPHA (264757), interstitial lung disease specific to adulthood (ORPHA 264735) and interstitial lung disease specific to childhood (ORPHA 264656).

In ICD-10, nine different diseases were non-coded. Cutaneous neuroendocrine carcinoma, melanoma, pemphigus, pheochromocytoma, PIK3CA related overgrowth syndrome and thalassemia major were coded using more than three different codes. In ICD-11, five diseases were non-coded. Thirteen types of rare tumor were coded using at least two different identifiers. A total of 26 diseases were coded using more than two different identifiers and 13 of them were tumor diseases. In UMLS, gastroenteropancreatic neuroendocrine neoplasm was non-coded. In the databases of MeSH, GARD and MeDRA, a total of 11, 24 and 26 rare diseases were non-coded, respectively (Table 1, Supplemental Table S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=220*).

3.2. Classification of 86 diseases in *China's Second List of Rare Diseases*

In the Orphanet classification system of rare diseases, 83 diseases with ORPHAcodes in *China's Second List of Rare Diseases* were mapped (Supplemental Table S2, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=221*). Of these, 45 are genetic rare diseases, accounting for 53.49%. These were followed by 28 (32.56%) neoplastic diseases and transplant-related disorders, respectively; 24 neurological diseases (27.91%), 15 skin diseases (17.44%), 13 renal diseases (15.12%), 12 developmental anomalies during embryogenesis, hepatic diseases, hematological diseases, endocrine diseases, ophthalmic disorders and systemic and rheumatological diseases, each accounting for 13.95% (Table 2).

3.3. Group of rare disorders and genotypic heterozygosity of rare diseases

Some rare diseases are groups of disorders and others have different subtypes according to classification, leading to a higher number of rare diseases in *China's Second List of Rare Diseases* than the actual number (Supplemental Table S3, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=222*). As an

Table 1. Identifiers of 86 rare diseases in *China's Second List of Rare Diseases* encoded by seven different reference databases

#	Rare disease	ORPHA	ICD-10	ICD-11	UMLS	MeSH	GARD	MedDRA
1	Achondroplasia	15	Q77.4	LD24.00	C0001080	D000130	8173	C0001080
2	Acquired hemophilia	599480	D66.	3B22	C0272325	C536392	6405	10082745
		955485	X01		C0398609			10082747
3	Acromegaly	963	E22.0	5A60.0	C0001206	D000172	5725	10000599
4	Adult-onset Still disease	829	M06.1	FA23	C0085253	D016706	436	10064056
5	Alagille syndrome	52	Q44.7	LB20.0Y	C0085280	D016738	804	10053870
6	Alpha-1-antitrypsin deficiency	60	E88.0	5C5A	C0221757	D019896	5784	10001806
7	ANCA-associated vasculitis	156152	-	4A44.A	C2717865	D056648	-	-
8	Bardet-Biedl syndrome	110	Q87.8	5A61.0	C0752166	D020788	6866	10056715
9	Behçet's disease	117	M35.2	4A62	C0004943	D001528	848	10004213
10	Blue rubber bleb nevus	1059	Q27.8	LC51	C0346072	C536240	5940	-
11	CDKL5-deficiency disorder	505652	-	8A62.Y	C4750718	C564064	-	10083005
12	Choroideremia	180	H31.2	9B61	C0008525	D015794	6061	10008791
13	Chronic inflammatory demyelinating polyneuropathy	2932	G61.8	8C01.3	C0393819	D020277	6102	10057645
14	Clear cell sarcoma of kidney	457246	C64	XH0765	C0334488	-	-	10009253
15	Cold agglutinin disease	56425	D59.1	3A20.1	C0175816	D000744	6130	-
16	Congenital biliary atresia	498345	Q44.2	LB20.21	C5680082	D001656	-	-
		30391						
17	Congenital factor VII deficiency	327	D68.2	3B14.7	C0015503	D005168	2238	10016079
18	Cryopyrin associated periodic syndrome/ NLRP3-associated systemic autoinflammatory disease	208650	-	4A60.1	C2316212	D056587	10927	10068850
19	Cutaneous neuroendocrine carcinoma (Merkel cell carcinoma)	79140	C44.3	2C34	C0007129	D015266	9266	-
			C44.6	XH8IN8				
			C44.7					
20	Cutaneous T-cell lymphomas	178551	C84.8	XH1951	C5680497	D016410	-	-
			C86.3	2B0Y				
			C86.6	2B0Z				
				2B03.0				
				XH84A5				
				XH5SC3				
				XH7S84				
				XH7EL2				
				XH2513				
21	Cystinosis	213	E72.0	5C60.1	C4316899	D003554	6236	10011777
22	Dermatofibrosarcoma protuberans	31112	C49.9	2B53.Y	C0392784	D018223	9569	10057070
				XH4QZ8				
				XH5CT4				
				XH9V92				
23	Eosinophilic gastroenteritis	2070	K52.8	DA94.21	C1262481	C535952	-	10017902

Note: "- "indicates no records are available.

Table 1. Identifiers of 86 rare diseases in *China's Second List of Rare Diseases* encoded by seven different reference databases (continued)

#	Rare disease	ORPHA	ICD-10	ICD-11	UMLS	MeSH	GARD	MedDRA
24	Epithelioid sarcoma	293202	C49.9	2B5F.2 XH4F96 XH92Y0 XH4BT2 XH13Z5	C0205944	D012509	10181	10015099
25	Facioscapulohumeral muscular dystrophy	269	G71.0	8C70.3	C0238288	D020391	9941	10064087
26	Familial hemophagocytic lymphohistiocytosis	540	D76.1	4A01.23	C0272199	-	6589	10070904
27	Familial adenomatous polyposis	733	D12.6	2B90.Y	C0032580	D011125	6408	10056981
28	Fibrodysplasia ossificans progressiva	337	M61.1	FB31.1	C0016037	D009221	6445	10068715
29	Fragile X syndrome	908	Q99.2	LD55	C0016667	D005600	6464	10017324
30	Gangliosidosis	309144	E75.1 E75.0	5C56.00	C0017083	D005733	12510	-
31	Gastroenteropancreatic neuroendocrine neoplasm	100092	-	-	-	-	2437	-
32	Gastrointestinal stromal tumor	44890	C26.9	2B5B 2B5B.0 2B5B.1 2B5B.Y 2B5B.Z 2E87 XH9HQ1	C0238198	D046152	8598	10051066
33	Generalized pustular psoriasis	247353	L40.1	EA90.40	C0343055	-	12819	-
34	Genetic hypoparathyroidism	208593	-	-	C5680825	-	-	-
35	Giant cell arteritis	397	M31.6	4A44.2	C0039483	D013700	9615	10018250
36	Giant cell tumor of bone	363976	D48.0	2F7B 2F9B XH0492 XH4TC2	C0206638	D018212	-	-
37	Glanzmann thrombasthenia	849	D69.1	3B62.0Y	C0040015	D013915	2478	-
38	Glioblastoma	360	C71.9	2A00.00 XH0MB1 XH17J4 XH2BA5 XH49K9 XH4FN3 XH5571 XH7F82 XH8UC5 2A02.00	C1621958	D005909	2491	10018336
39	Gorlin syndrome	377	C44.9	LD2D.4	C0004779	D001478	7166	10062804
40	Hidradenitis suppurativa	-	L73.2	ED92.0	C0162836	D017497	-	-
41	Hutchinson-Gilford progeria syndrome	740	E34.8	LD2B	C0033300	D011371	7467	10036794

Note: "- "indicates no records are available.

Table 1. Identifiers of 86 rare diseases in *China's Second List of Rare Diseases* encoded by seven different reference databases (continued)

#	Rare disease	ORPHA	ICD-10	ICD-11	UMLS	MeSH	GARD	MedDRA
42	Inflammatory myofibroblastic tumor	178342	D48.7	2E92.1 2F30.Y XH66Z0	C0334121	-	7146	10067917
43	Leber congenital amaurosis	65	H35.5	9B70	C0339527	D057130	634	10070667
44	Lennox-Gastaut syndrome	2382	G40.4	8A62.1	C0238111	D065768	9912	10048816
45	Limbal stem cell deficiency	171673	H18.7	-	C1561989	D000092423	-	10072138
46	Malignant hyperthermia	423 46650	T88.3	8C78 NE86	C0024591 C2930828	D008305	-	-
47	Malignant pleural mesothelioma	50251	C45.0	2C26.0	C1377913	D000086002	7026	10059518
48	Melanoma	617910 252031 404560 618 293822 168999 97338 252050 39044	C43.001 C43.101 C43.151 C43.201 C43.251 C43.301 C43.302 C43.351 C43.352 C43.401 C43.402 C43.501 C43.551 C43.552 C43.553 C43.601 C43.602 C43.651 C43.701 C43.751 C43.752 C43.851 C43.901 C43.902	XH4846 XH25M1 2C00.1 2C22.3 2C30 2C30.0 2C30.1 2C30.2 2C30.3 2C30.Y 2C30.Z 2C70.1 2C71.1 2C81.1 2D00.0 2D01.0 2E63 2E63.0 2E63.0Z 2E63.1 2E63.Y 2E63.Z 9B71.40	C0206651 C0220633 C0346360 C1512419 C2314896 C4749348 C4749577 C5191057	D008545	8621 120161	10061252 10066384
49	Metachromatic leukodystrophy	512	E75.2	5C56.02	C0023522	D007966	3230	10067609
50	Monogenic non-syndromic obesity-Genetic non-syndromic obesity	98267	E66.8	5B81.Y	C5680229	-	-	-

Note: "- "indicates no records are available.

Table 1. Identifiers of 86 rare diseases in *China's Second List of Rare Diseases* encoded by seven different reference databases (continued)

#	Rare disease	ORPHA	ICD-10	ICD-11	UMLS	MeSH	GARD	MedDRA
51	Multiple endocrine neoplasia	276161	D44.8	2F7A.Y	C0027662	D009377	-	10061299
52	Narcolepsy	619284	-	7A20	C0027404	D009290	-	10028713
				7A20.0				
				7A20.1				
				7A20.Z				
				VV01				
53	Neuroblastoma	635	C74.9	2A00.11	C0027819	D009447	7185	10029260
				XH85Z0				
54	Neurofibromatosis	634518	Q85.001	LD2D.1	C5816781	D017253	-	-
55	Neuronal ceroid lipofuscinosis	216	E75.4	5C56.1	C0027877	D009472	10739	10074607
56	Neurotrophic keratitis	137596	H16.2	1F00.10	C0339296	-	-	10069732
57	Osteosarcoma	668	C41.9	2B50	C0029463	D012516	7284	10031291
				2B50.0				
				2B50.1				
				2B50.2				
				2B50.Y				
				2B50.Z				
				XH06W9				
				XH0Y34				
				XH1S32				
				XH1XF3				
				XH1Y90				
				XH23T4				
				XH29N8				
				XH2CD6				
				XH3T03				
				XH48A9				
				XH4EZ4				
				XH5CL5				
				XH5FH4				
				XH6E77				
				XH6LT5				
				XH6TL0				
				XH7N84				
				XH7XB9				
				XH8HG5				
				XH8J23				
				XH8X47				
				XH9119				
				XH9344				
				XH9LS2				

Note: "- "indicates no records are available.

Table 1. Identifiers of 86 rare diseases in *China's Second List of Rare Diseases* encoded by seven different reference databases (continued)

#	Rare disease	ORPHA	ICD-10	ICD-11	UMLS	MeSH	GARD	MedDRA
58	Pemphigus	704	L10.0	EB40.0	C0030809	D010392	6559	10052802
		2841	L10.1	EB40.0Y	C0085106		7354	10057053
		46485	L10.2	EB40.1	C0263312		7355	10057056
		63455	L10.3	EB40.1	C0263313			10057069
		79479	L10.4	EB40.1	C0263314			10058917
		79480	L10.8	EB40.2	C0263316			
		79481	L10.8	EB40.Y	C1112570			
		208524	L10.8	EC20.2	C1274167			
		555905	Q82.8		C4749730			
		636955			C5681323			
59	Persistent pulmonary hypertension of the newborn	-	P29.3	KB42	C0031190	D010547	-	-
60	Pheochromocytoma	-	C74.101	5A75	C0031511	D010673	-	-
			D35.051	XH3854	C0334419			
				XH9K97	C1302282			
				2D11.1				
61	PIK3CA related overgrowth syndrome	530313	Q04.5	EF02.1	C0431391	C536142	6950	10081236
			Q74.0	LA05.1	C1865285	C567763	10939	
			Q74.2	LB97.1	C2751313	C567863	2637	
			Q87.3	LD2C	C2752042	D065705		
				LD2F.1Y	C4749904			
				Q74.0	C5192432			
					C5679987			
					C5679988			
					C5680341			
62	Polycythaemia vera	729	D45	2A20.4	C0032463	D011087	7422	10036057
63	Primary biliary cholangitis	186	K74.3	DB96.1	C0008312	D008105	7459	10080429
				DB96.10				
				DB96.1Y				
				DB96.1Z				
64	Primary ciliary dyskinesia	244	Q34.8	LA75.Y	C4551720	D002925	4484	10069713
65	Primary IGF1 deficiency	73272	E34.3	5A61.0	C1837475	C563867	10627	-
66	Primary immunodeficiency	101997	-	L1-4A0	C0398686	D000081207	-	10064859
67	Primary myelofibrosis	824	D47.4	2A20.2	C0001815	D055728	8618	10077161
				XH7GG7				
68	Primary sclerosing cholangitis	171	K83.0	DB96.20	C0566602	D015209	1280	10036732
				DB96.2Y				
				DB96.2Z				

Note: "- "indicates no records are available.

Table 1. Identifiers of 86 rare diseases in *China's Second List of Rare Diseases* encoded by seven different reference databases (continued)

#	Rare disease	ORPHA	ICD-10	ICD-11	UMLS	MeSH	GARD	MedDRA
69	Progressive fibrosing interstitial lung disease	182095	J84.9 J84.1	CB03.6 CB04.Y CB04.Z CB05.1 CB05.2 CB05.3 CB05.4 CB05.Y CB05.Z	C0206062	D017563	-	10022611
70	Recurrent pericarditis	251307	I09.2	-	C4707790	-	-	-
71	Retinopathy of prematurity	90050	H35.1	9B71.3	C0035344	D012178	5695	10038933
72	Rett syndrome	778	F84.2	LD90.4	C0035372	D015518	5696	10039000
73	Short bowel syndrome	104008	-	DA96.04 KB89.1	C0036992	D012778	1502	10049416
74	Systemic juvenile idiopathic arthritis	85414	M08.2	FA24.4	C0087031	-	10966	10042061
75	Systemic mastocytosis	2467	C96.2	2A21.0 2A21.0Y 2A21.0Z XH10N1 XH1H01 XH2Y59 XH5191	C0221013	D034721	8616	10042949
76	Takayasu arteritis	3287	M31.4	4A44.1	C0039263	D013625	7730	10043097
77	Tenosynovial giant cell tumor/Pigmented villonodular synovitis	66627	M12.2	XH6911 XH0HZ1 XH52J9 XH5AQ9	C1318543	D000070779	7396	-
78	Thalassemia major	163596 231214 98791 231401 847	D46.7 D56.0 D56.1	3A50.03 3A50.0Y 3A50.1 3A50.2 D56.0	C5680928 C0002875 C0272005 C0585216 C0795917 C1845055	D017086	5864	-
79	Thrombotic thrombocytopenic purpura	54057	M31.1	3B64.14	C0034155	D011697	-	10043648
80	Transthyretin amyloidosis	271861	-	5D00.20 BC43.20	C5679761	C567782	-	-
81	Tumor necrosis factor receptor associated periodic syndrome	32960	E85.0	4A60.2	C1275126	-	8457	-
82	Tumor-induced osteomalacia	352540	M83.8	-	C1274103	C537751	9652	-
83	Von Hippel-Lindau syndrome	892	Q85.8	5A75	C0019562	D006623	7855	10047716
84	Von Willebrand disease type3	166096	D68.0	3B12	C1264041	D056729	-	-

Note: "- "indicates no records are available.

Table 1. Identifiers of 86 rare diseases in *China's Second List of Rare Diseases* encoded by seven different reference databases (continued)

#	Rare disease	ORPHA	ICD-10	ICD-11	UMLS	MeSH	GARD	MedDRA
85	Waldenström macroglobulinemia/ Lymphoplasmacytic lymphoma	33226	C88.0	XH8GW4 2A85.4 XH0QZ9	C0024419	D008258	7872	10047801
86	West syndrome/Infantile spasms syndrome	3451	G40.4	8A62.0	C0037769	D013036	7887	10021750

Note: "-" indicates no records are available.

Table 2. The number and percentage of 86 rare diseases in *China's Second List of Rare* according to classification system in Orphanet database

Orphanet classification	number	Percentage (%)
genetic diseases	45	52.35
neoplastic diseases	28	32.56
transplant-related disorders	28	32.56
neurological diseases	24	27.91
skin diseases	15	17.44
renal diseases	13	15.12
developmental anomalies during embryogenesis	12	13.95
hematological diseases	12	13.95
endocrine diseases	12	13.95
ophthalmic disorders	12	13.95
systemic and rheumatological diseases	12	13.95
bone diseases	7	8.14
respiratory diseases	7	8.14
systemic or rheumatologic diseases of childhood	7	8.14
hepatic diseases	5	5.81
circulatory system diseases	5	5.81
inborn errors of metabolism	5	5.81
gastroenterological diseases	5	5.81
immunological diseases	4	4.65
infertility disorders	3	3.45
abdominal surgical diseases	2	2.33
cardiac malformations	1	1.16
gynecological and obstetric diseases	1	1.16

example, primary immunodeficiency (ORPHA 101997) includes primary immunodeficiency owing to a defect in adaptive immunity (ORPHA 179006) and primary immunodeficiency owing to a defect in innate immunity (ORPHA 101988). The former group includes combined T- and B-cell immunodeficiency (ORPHA 101972), immune dysregulation disease with immunodeficiency (ORPHA 169361), immunodeficiency predominantly affecting antibody production (ORPHA 101977), and combined immunodeficiency (ORPHA 331217). The latter group includes autoinflammatory syndrome with immune deficiency (ORPHA 290839), genetic susceptibility to infections owing to particular pathogens (ORPHA 183710) , immunodeficiency owing to a complement cascade protein anomaly (ORPHA 101992), other immunodeficiency syndromes owing to defects in innate immunity (ORPHA 331193), primary immunodeficiency with predisposition to severe viral infection (ORPHA 431156), and quantitative and/ or qualitative congenital phagocyte defect (ORPHA 101985).

Genetic rare diseases in the second list are summarized in Supplemental Table S4 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=223*). Inheritance, phenotype, and pathogenic genes are included in the table as data collected from the OMIM database. Some rare diseases are caused by mutation of single genes and others are caused by mutation in multiple genes. A total of 53 different phenotypes with 49 pathogenic genes exist in primary ciliary dyskinesia, in both the autosomal recessive

(AR), autosomal dominant (AD) or X-linked recessive inheritance format. Bardet–Biedl syndrome (BBS) is inherited mainly in the form of AR with 22 different phenotypes and 24 genes. Phenotype BBS1 corresponds to three different pathogenic genes *CCDC28B*, *ARL6*, and *BBS1*, with AD or AR inheritance patterns. The phenotype BBS14 corresponds to the *TMEM67* and *CEP290* genes inherited in AR form. The *ARL6* gene corresponds to phenotypes of both BBS1 and BBS3. There are 20 different genes and a corresponding 18 phenotypes in Leber congenital amaurosis. For neuronal ceroid lipofuscinosis, a total of 12 genes corresponds to 14 different subtypes and phenotypes. For Alagille syndrome, three subtypes are classified according to different pathogenic genes with microdeletion in 20p12, *JAG1*, and *NOTCH2*. In summary, genotypic and phenotypic heterozygosity exist among these rare diseases.

3.4. Epidemiology of diseases i*n China's Second List of Rare Diseases*

According to data from Orphanet, the epidemiology of the 86 rare diseases is described in Table 3. Data on the newborn incidence, incidence, and prevalence of 71 rare diseases (82.56%) in *China's Second List of Rare Diseases* are available, including data on the newborn incidence of 7 (8.14%), incidence of 20 rare diseases (23.26%) and the prevalence of 64 (74.42%) rare diseases. As we mentioned above (also see Supplementarry Table S3 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=222*), some rare disorders are groups composed of different disoders with some correlations, hence, only disorders in the group with epidemiological data were collected. Newborn incidence of 0.6/100,000 in the pediatric population and incidence of 1.6-23/100,000 per year in children were reported in systemic juvenile idiopathic arthritis. The birth incidence of familial adenomatous polyposis was as high as 12.05/100,000. In all, an annual incidence of 20 rare diseases available in Orphanet, a relatively broad newborn incidence was observed in malignant hyperthermia of anesthesia, systemic juvenile idiopathic arthritis, primary biliary cholangitis, polycythaemia vera, Lennox-Gastaut syndrome and cutaneous neuroendocrine carcinoma (Merkel cell carcinoma). The annual incidence of beta-thalassemia major is estimated at 1/100,000 worldwide and 1/10,000 in the European population.

A total of 23 rare diseases or its subtypes showed a prevalence of higher than 1/10,000 including alpha-1-antitrypsin deficiency, ANCA-associated vasculitis, dermatofibrosarcoma protuberans, Fragile X syndrome, neuroendocrine tumor of pancreas, gastrointestinal stromal tumor, giant cell arteritis, Lennox-Gastaut syndrome, Limbal stem cell deficiency, narcolepsy type 1, neuroblastoma, neurotrophic keratitis, pemphigus vulgaris, polycythaemia vera, primary biliary cholangitis, retinopathy of prematurity, gastroschisis, systemic mastocytosis, tenosynovial giant cell tumor (pigmented villonodular synovitis), Hb Bart's hydrops fetalis and thrombotic thrombocytopenic purpura. For thrombotic thrombocytopenic purpura (TTP), the prevalence of iTTP was 14.29/100,000, which is higher than that of the cTTP type. Thirty-five different rare diseases had a prevalence of less than 10/100,000 and greater than or equal to 1/100,000, these included acromegaly, adult-onset Still disease, Bardet-Biedl syndrome, Behçet's disease, CDKL5-deficiency disorder, choroideremia, chronic inflammatory demyelinating polyneuropathy, biliary atresia, cutaneous neuroendocrine carcinoma, cystinosis, eosinophilic gastroenteritis, facioscapulohumeral muscular dystrophy, familial adenomatous polyposis, glioblastoma, Gorlin syndrome, Leber congenital amaurosis, malignant pleural mesothelioma, multiple endocrine neoplasia, and so on. Epithelioid sarcoma, Hutchinson-Gilford progeria syndrome, primary IGF1 deficiency, fibrodysplasia ossificans progressiva, hypoplastic pancreas-intestinal atresia-hypoplastic gallbladder syndrome, cold agglutinin disease, congenital factor VII deficiency and another 13 rare diseases had relatively low prevalence at less than 1/100,000.

4. Discussion

The study nominated the 86 diseases in *China's Second List of Rare Diseases* using different reference systems. The results showed that most rare diseases have unique identifiers, especially in the Orphanet database. Orphanet nomenclature is a powerful classification tool based on the multidimensional nature of rare diseases, which provides a specific terminology for rare diseases. Each clinical entity is assigned a unique and time-stable ORPHAcode. This includes all disorders, subtypes of disorders, and groups of disorders. Over 6000 rare diseases are coded using ORPHAcodes (*11*). ORPHAcodes have high sensitivity and accuracy in describing rare diseases. More than half of ORPHAcodes identify diseases with very low prevalence (less than 1 case per million) (*12*). The International Classification of Diseases is a medical classification list produced by the World Health Organization that is predominantly used in health care systems worldwide. However, less rare diseases are listed in versions older than ICD-10. One ICD code can correspond to different rare entities or to both rare and non-rare entities. This is often not clearly distinguished in health information systems (*13,14*). ICD-11 was adopted by the World Health Assembly in May 2019 and came into effect in January 2022. This version includes nearly 5,500 rare diseases and each has a unique identifier. Rare diseases in ICD-11 are easily available in health information systems and are continually updated (*5,14*). It has a unique identifier for different subtypes of one disease and different conditions

Table 3. Incidence and prevalence of 86 rare diseases in *China's Second List of Rare Diseases* recorded in Orphanet database

#	Rare diseases	Newborn Incidence / 100,000 persons	Incidence / 100,000 persons	Prevalence / 100,000 persons
1	Achondroplasia	4	-	-
2	Acquired hemophilia A	-	-	-
	Acquired hemophilia B	-	-	-
	hemophilia C	-	-	0.1-0.9
3	Acromegaly	-	0.19-1.1 (annual)	1-9
4	Adult-onset Still disease	-	-	1-9
5	Alagille syndrome	-	-	1.43
6	Alpha-1-antitrypsin deficiency		-	10-50
7	ANCA-associated vasculitis	-	-	10-50
	Eosinophilic granulomatosis with polyangiitis	-	-	1-9
	Granulomatosis with polyangiitis	-	0.21-1.19	1-9
	Microscopic polyangiitis	-	-	1-9
8	Bardet-Biedl syndrome	-	-	1 (USA) 1.69 (Denmark) 1.52-2.22 (France)
9	Behçet's disease	-	-	1-9
10	Blue rubber bleb nevus	-	-	-
11	CDKL5-deficiency disorder		-	2.36 (UK (Scotland) birth prevalence
12	Choroideremia	-	-	1-9
13	Chronic inflammatory demyelinating polyneuropathy	-	-	1-9
14	Clear cell sarcoma of kidney	-	-	-
15	Cold agglutinin disease	-	-	0.1-0.9
16	biliary atresia		-	-
	Isolated biliary atresia		-	1-9
	Biliary atresia with splenic malformation syndrome	-	-	-
	Hypoplastic pancreas-intestinal atresia-hypoplastic gallbladder syndrome	-	-	< 0.1
17	Congenital factor VII deficiency	-	-	0.1-0.9
18	Cryopyrin associated periodic syndrome- NLRP3-associated systemic autoinflammatory disease	-	-	0.28 (France)
	CINCA syndrome	-	-	0.28 (the whole spectrum of CAPS)
	Familial cold urticaria	-	-	-
	Muckle-Wells syndrome	-	-	0.28 (France)
19	Cutaneous neuroendocrine carcinoma (Merkel cell carcinoma)	-	0.2-0.4 (annual, white population)	1-9
20	Cutaneous T-cell lymphomas	-	-	-
	Mycosis fungoides and variants		0.29-0.91	-
	Primary cutaneous CD30+ T-cell lymphoproliferative disease	-	-	-
	Adult T-cell leukemia-lymphoma	-	-	1-9
21	Cystinosis		-	1-9
22	Dermatofibrosarcoma protuberans	-	0.5	10-50
23	Eosinophilic gastroenteritis	-	-	-
24	Epithelioid sarcoma	-	-	< 0.1
25	Facioscapulohumeral muscular dystrophy	-	-	1-9
26	Familial hemophagocytic lymphohistiocytosis	-	-	-
27	Familial adenomatous polyposis	12.05	-	1-9
28	Fibrodysplasia ossificans progressiva	-	-	< 0.1
29	Fragile X syndrome	-	-	10-50
30	Gangliosidosis	-	-	-
	GM1 gangliosidosis		-	0.5-1 (live births)
	GM1 gangliosidosis type 1	-	-	0.5-1(live births)
	GM1 gangliosidosis type 2	-	-	< 0.1
	GM1 gangliosidosis type 3	-	-	< 0.1
	GM2 gangliosidosis	-	-	1-9
	GM2 gangliosidosis, AB variant	-	-	< 0.1
	Sandhoff disease	-	-	0.1-0.9
	Tay-Sachs disease	-	-	-
31	Gastroenteropancreatic neuroendocrine neoplasm	-	-	-
	Neuroendocrine tumor of stomach	-	-	1-9
	Neuroendocrine carcinoma of pancreas	-	-	-
	Neuroendocrine tumor of pancreas	-	-	10-50
32	Gastrointestinal stromal tumor	-	-	10-50
33	Generalized pustular psoriasis	-	-	0.1-0.9

Table 3. Incidence and prevalence of 86 rare diseases in *China's Second List of Rare Diseases* recorded in Orphanet database (continued)

#	Rare diseases	Newborn Incidence / 100,000 persons	Incidence / 100,000 persons	Prevalence / 100,000 persons
34	Genetic hypoparathyroidism	-	-	-
	Autoimmune polyendocrinopathy type 1	-	-	0.1-0.9
	Familial isolated hypoparathyroidism	-	-	< 0.1
	Pseudohypoparathyroidism	-	-	0.1-0.9
35	Giant cell arteritis	-	5.88-20 (annual, adults over 50 years old)	10-50
36	Giant cell tumor of bone	-	-	-
37	Glanzmann thrombasthenia	-	-	-
38	Glioblastoma	-	3	1-9
39	Gorlin syndrome	-	-	1-9
40	Hidradenitis suppurativa	-	-	-
41	Hutchinson-Gilford progeria syndrome	-	-	< 0.1
42	Inflammatory myofibroblastic tumor	-	-	-
43	Leber congenital amaurosis		-	1-9
44	Lennox-Gastaut syndrome	-	0.1-0.28	10-50
45	Limbal stem cell deficiency	-	-	10-50
46	Malignant hyperthermia	-	-	-
	Malignant hyperthermia of anesthesia	-	45342	-
47	Malignant pleural mesothelioma	-	-	-
48	Melanoma	-	-	-
	Conjunctival malignant melanoma	-	-	< 0.1
	Malignant melanoma of the mucosa	-	-	-
	Uveal melanoma	-	-	1-9
49	Metachromatic leukodystrophy	-	-	0.1-0.9
50	Monogenic non-syndromic obesity-Genetic non-syndromic obesity	-	-	-
51	Multiple endocrine neoplasia	-	-	3.33-10 (MEN1) 2.86 (MEN2)
52	Narcolepsy	-	-	-
	Narcolepsy type 1	-	-	10-50
	Narcolepsy type 2	-	-	-
53	Neuroblastoma	-	1.43 (annual, 15 years old)	10-50
54	Neurofibromatosis	-	-	-
	Full NF2-related schwannomatosis	-	-	1-9
	Full schwannomatosis	-	-	-
	Neurofibromatosis type 1		-	10-50
55	Neuronal ceroid lipofuscinosis	-	-	1-9
	ATP13A2-related juvenile neuronal ceroid lipofuscinosis	-	-	< 0.1
	Congenital neuronal ceroid lipofuscinosis	-	-	-
	Infantile neuronal ceroid lipofuscinosis	5 (Finland)	-	-
	Juvenile neuronal ceroid lipofuscinosis	2.22 (Sweden) 6.99 (Germany)		0.46 (Sweden)
	Late infantile neuronal ceroid lipofuscinosis	-		0.3 (Sweden)
56	Neurotrophic keratitis	-	-	42.02 (Europe)
57	Osteosarcoma	-	0.3 (annual)	1-9
58	Pemphigus	-	-	/
	Endemic pemphigus foliaceus	-	-	-
	Pemphigus foliaceus	-	-	-
	Pemphigus vulgaris	-	0.07-0.7 (annual)	10-50
59	Persistent pulmonary hypertension of the newborn	-	-	-
60	Pheochromocytoma	-	-	-
61	PIK3CA related overgrowth syndrome	-	-	-
	CLAPO syndrome	-	-	< 0.1
	CLOVES syndrome	-	-	< 0.1
	Congenital infiltrating lipomatosis of the face	-	-	< 0.1
	Hemihyperplasia-multiple lipomatosis syndrome	-	-	< 0.1
	Megalencephaly-capillary malformation-polymicrogyria syndrome	-	-	< 0.1
	Segmental progressive overgrowth syndrome with fibroadipose hyperplasia	-	-	< 0.1
62	Polycythaemia vera	-	1-2.78	10-50
63	Primary biliary cholangitis	-	0.33-5.8(annual)	10-50
64	Primary ciliary dyskinesia	3.33-6.67	-	-

Table 3. Incidence and prevalence of 86 rare diseases in *China's Second List of Rare Diseases* recorded in Orphanet database (continued)

#	Rare diseases	Newborn Incidence / 100,000 persons	Incidence / 100,000 persons	Prevalence / 100,000 persons
65	Primary IGF1 deficiency	-	-	< 0.1
66	Primary immunodeficiency	-	-	1-9
67	Primary myelofibrosis	-	1 (annual)	1-9
68	Primary sclerosing cholangitis	-	-	1-9
69	Interstitial lung disease	-	-	-
70	Recurrent pericarditis	-	-	-
71	Retinopathy of prematurity	-	-	10-50
72	Rett syndrome	-	-	1-9
73	Short bowel syndrome	-	-	1-9
	Gastroschisis		-	10-50
	Small bowel atresia		-	4-14.29 (live births, Europe)
74	Systemic juvenile idiopathic arthritis	0.6 (prediatric population)	1.6-23 (annual)	1-9
75	Systemic mastocytosis	-	-	10-50
	Aggressive systemic mastocytosis	-	-	0.1-0.9
	Indolent systemic mastocytosis	-	-	10-50
	Systemic mastocytosis with associated hematologic neoplasm	-	-	1-9
	Mast cell leukemia	-	-	< 0.1
	Acute mast cell leukemia	-	-	-
76	Takayasu arteritis	-	-	1-9
77	Tenosynovial giant cell tumor-Pigmented villonodular synovitis	-	-	10-50
78	Thalassemia major	-	-	-
	Beta-thalassemia major	-	1 (worldwide) 10 (EU)	-
	Hb Bart's hydrops fetalis		-	50-500 (live births, Southeast Asia)
79	Thrombotic thrombocytopenic purpura	0.1-0.61(iTTP)	-	1.29 (iTTP, France) 0.04-1.67 (cTTP)
80	Transthyretin amyloidosis	-	-	0.1-0.9
	ATTRV30M amyloidosis	-	0.87 (annual, Portugal)	22.94 (adults)
81	Tumor necrosis factor receptor associated periodic syndrome	-	0.57 (annual, children under 16, Germany)	
82	Tumor-induced osteomalacia	-	-	-
83	Von Hippel-Lindau syndrome	2.78	-	1-9
84	Von Willebrand disease type3	-	-	0.1-0.9
85	Waldenström macroglobulinemia- Lymphoplasmacytic lymphoma	-	0.38 (annual, USA)	0.99 (Europe)
86	West syndrome-Infantile spasms syndrome		-	1-9

in a group. ICD-11, Orphanet, OMIM, MalaCards and other databases all cross-reference each other.

According to global epidemiologic data and the 2021 definition of rare diseases, conditions with a relatively high newborn incidence and/or prevalence (1/10,000) are recorded in *China's Second List of Rare Diseases*. Data on these registered rare diseases are readily available to advance research and development of orphan drugs and treatments for rare diseases.

In conclusion, in this study, we reviewed the classification, nomenclature, and epidemiology of 86 rare diseases in *China's Second List of Rare Diseases*. Including the 121 rare diseases in the first list, a total of 207 rare diseases have been described by the Chinese government. Administrative policies regarding rare diseases have been enacted, which refer to research, medical insurance, orphan drugs, and standards for diagnosis and treatment. These policies make important contributions to progress in the area of rare diseases in China. The diseases on China's second list are important research topics and focusing on these will help China become a healthcare model with respect to rare diseases. The inclusion of conditions with relatively higher prevalence than those included in the current definitions will benefit more patients with rare diseases. With further development of national registries, rare diseases with very low prevalence will likely emerge.

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References

1. Shourick J, Wack M, Jannot AS. Assessing rare diseases prevalence using literature quantification. Orphanet J Rare Dis. 2021; 16:139.
2. Zhi W, Liu M, Yang D, Zhang S, Lu Y, Han J. Analysis of marketed orphan drugs in China. Intractable Rare Dis Res. 2023; 12:132-140.
3. Li X, Wu L, Yu L, He Y, Wang M, Mu Y. Policy analysis in the field of rare diseases in China: A combined study of content analysis and Bibliometrics analysis. Front Med (Lausanne). 2023; 10:1180550.
4. He J, Kang Q, Hu J, Song P, Jin C. China has officially released its first national list of rare diseases. Intractable Rare Dis Res. 2018; 7:145-147.
5. World Health Organization. Rare diseases. *https://www.who.int/standards/classifications/frequently-asked-questions/rare-diseases* (accessed October 2, 2024).
6. Lu Y, Han J. The definition of rare disease in China and its prospects. Intractable Rare Dis Res. 2022; 11:29-30.
7. World Health Organization. ICD-10 Version:2019. *https://icd.who.int/browse10/2019/en* (accessed October 4, 2024).
8. World Health Organization. International Statistical Classification of Diseases and Related Health Problems (ICD). *https://www.who.int/standards/classifications/classification-of-diseases* (accessed October 4, 2024).
9. Bodenreider O. The Unified Medical Language System (UMLS): Integrating biomedical terminology. Nucleic Acids Res. 2004; 32:D267-D270.
10. Brown EG, Wood L, Wood S. The medical dictionary for regulatory activities (MedDRA). Drug Saf. 1999; 20:109-117.
11. Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, Murphy D, Le Cam Y, Rath A. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. Eur J Hum Genet. 2020; 28:165-173.
12. Mazzucato M, Pozza LVD, Facchin P, *et al.* ORPHAcodes use for the coding of rare diseases: Comparison of the accuracy and cross country comparability. Orphanet J Rare Dis. 2023; 18:267.
13. Rath A, Aymé S, Bellet B. Classification of rare diseases: A worldwide effort to contribute to the International Classification of Diseases. Orphanet J Rare Dis. 2010; 5:O21.
14. Aymé S, Bellet B, Rath A. Rare diseases in ICD11: Making rare diseases visible in health information systems through appropriate coding. Orphanet J Rare Dis. 2015; 10:35.
15. Jia J, An Z, Ming Y, Guo Y, Li W, Liang Y, Guo D, Li X, Tai J, Chen G, Jin Y, Liu Z, Ni X, Shi T. eRAM: Encyclopedia of rare disease annotations for precision medicine. Nucleic Acids Res. 2018; 46:D937-D943.

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Original Article

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Evaluation of the safety and efficacy of miglustat for the treatment of Chinese patients with Niemann-Pick disease type C: A prospective, open-label, single-arm, phase IV trial

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SUMMARY Niemann-Pick disease type C (NPC) is a rare, autosomal recessive, neurodegenerative disease associated with a wide variety of progressive neurological manifestations. Miglustat has demonstrated efficacy to delay progressive neurological deterioration in patients with NPC. We conducted a multicenter, open-label, single-arm, phase IV, post-approval commitment study to evaluate the efficacy and safety of miglustat among Chinese patients with NPC. Eligible patients were aged ≥ 4 years with an established diagnosis of NPC with two type C1 or C2 pathogenic markers or one marker with a positive biomarker (oxysterol, lysosphingolipids, or bile acids) and high clinical suspicion of NPC. Patients received oral miglustat ranging from 100 mg twice daily to 200 mg three times daily. The primary outcome was change in horizontal saccadic eye movement parameters from baseline to week 52. Seventeen patients were enrolled (median age: 14.0 years). From baseline to week 52, mean saccadic peak acceleration and velocity increased by 19.2% and 12.5%, respectively, while mean peak duration and linear regression decreased by 6.5% and 15.6%, respectively. By week 52, ambulation, manipulation, language, swallowing, and ocular movements had improved or stabilized versus baseline. All patients experienced treatment-emergent adverse events (TEAEs). Treatment-related TEAEs were reported in 12 patients with the most common being diarrhea (n = 12). Two patients died due to accidental death and asphyxia unrelated to miglustat treatment. This study demonstrated disease stabilization in Chinese patients with NPC receiving miglustat. Safety findings were consistent with miglustat's known safety profile. The study was registered at *ClinicalTrials.gov* (NCT03910621).

Keywords horizontal saccadic eye movement, lysosomal lipid storage disorder, China

1. Introduction

Niemann-Pick disease is an autosomal recessive, genetic, lysosomal lipid storage disorder caused by the deposition of a group of sphingomyelins (*1*). Niemann-Pick disease type C (NPC) is characterized by intracellular lipid transport defects and secondary pathological accumulation of free cholesterol, sphingomyelin, and glycosphingolipids within lysosomes/endosomes in various tissues and organs, but most widely in the brain (*1,2*). The clinical presentation of NPC is heterogenous; the liver, spleen, lungs, and nervous system are often involved in NPC, and the disease symptoms commonly include mental and motor regression, ataxia, and cataplexy (*1*). Clinical neurological manifestations of NPC include vertical supranuclear gaze palsy, ataxia, dysarthria, dysphagia, dystonia, epileptic seizures, progressive dementia, psychotic symptoms, and cataplexy (*1*). The clinical manifestations of the disease in the nervous system are continuously progressive without interruption (*1*).

Individuals with NPC can be categorized according to the type and age of onset of first neurological symptoms, *i.e.*, neonatal (≤ 2 months), early infantile (> 2 months to 2 years old), late infantile (> 2-6 years old), juvenile (> 6-15 years old), and adolescent/adult

(> 15 years old) (*1*). In newborns, the disease will often result in death within a short period of time (*1*). Compared with the late-infantile neurologic onset form, pediatric individuals with the severe neurologic early-infantile form experience rapid disease progression (*1*). Individuals with juvenile neurologic onset typically survive until adolescence or later (*1*), but individuals with advanced-stage disease are often disabled, suffer from dementia, and require tube feeding. This form of the disease is associated with a significant mental and economic burden to the individuals themselves, and their caregivers, families, and society (*1,3,4*). In Europe, NPC has an estimated incidence of between 1:100,000-120,000 live births (*1,5-7*), while in the United States, the prevalence is approximately one case per million people (*8*). However, currently there are no reports on the incidence rate of NPC in the Chinese population (*9*).

Miglustat is currently the only approved therapy in the European Union (EU)/European Economic Area (EEA) and China for the management of progressive neurological deterioration in adults and children with NPC (*10-12*). There are no alternative treatments available for NPC in China beyond those which provide symptomatic relief. Miglustat is a competitive and reversible inhibitor of the glucosylceramide synthase enzyme (involved in the synthesis of most glycosphingolipids) (*11,13*), α-glucosidases I and II (key enzymes involved in intracellular processing of glycoproteins), non-lysosomal β-glucocerebrosidase, and intestinal disaccharidases (*11,14*). Moreover, miglustat can pass through the blood-brain barrier, reversibly inhibiting glucosylceramide synthetase, thus preventing the accumulation of glycosphingolipids in lysosomes (*11,13*). Miglustat received approval based on the data of an international, non-blinded, randomized controlled phase III clinical trial involving 29 patients with NPC. This study showed that miglustat could improve the horizontal saccadic eye movement (HSEM) velocity, and improve or delay deterioration of neurological symptoms in cognitive function, swallowing function, and walking ability (*13*). A retrospective observational cohort study conducted in 12 countries (excluding China) also showed an improvement or stabilization of neurological symptoms in 66 individuals with NPC who were treated with miglustat (*15*). In addition, clinical studies and case reports have shown that some individuals with early neurological signs of NPC who were treated with miglustat achieved either stabilized or delayed progressive neurological symptoms (*16-19*).

This post-approval commitment study was conducted to further evaluate the efficacy and safety of miglustat among Chinese individuals with NPC over a 12-month treatment period.

2. Patients and Methods

2.1. Study design and patients

This was a prospective, multicenter, open-label, single-arm, 52-week, phase IV confirmatory study (Figure 1A). The primary objective was to evaluate the safety and effectiveness of miglustat on the rate of disease progression and disease stabilization, by measuring changes in HSEM parameters that are highly correlated with disease progression in patients with NPC.

The inclusion criteria for patients included: aged ≥ 4 years; an established diagnosis of NPC (with two type C1 or C2 pathogenic markers or one marker with a positive biomarker [oxysterol, lysosphingolipids, or bile acids]) and high clinical suspicion of NPC; ability to perform the tests for the HSEM and vertical saccadic eye movements; and ability to swallow the study drug. Patients were permitted to receive any prior concomitant therapies, with the exception of concomitant eliglustat, benzodiazepines, any other drugs potentially influencing eye movements or any of the secondary outcome measures, or any other potentially disease-modifying investigational drug. A full list of inclusion and exclusion criteria are in the Supplemental Material (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=224*). Patients received oral miglustat ranging from 100 mg twice daily to 200 mg three times daily. The recommended dose was 200 mg three times daily based on previous studies of individuals with NPC and other neuronopathic glycosphingolipid storage disorders (*13*). The starting dose for patients with mild or moderate renal impairment was 200 mg twice daily or 200 mg once daily, respectively. For patients aged < 12 years, the starting dose was calculated according to body surface area.

This study was conducted in accordance with the ethical principles that originate from the Declaration of Helsinki, consistent with the International Council for Harmonization Good Clinical Practice guidelines (*20*), and all applicable local laws and regulations. The study protocol and amendments were reviewed by an Independent Ethics Committee at each study center (Supplemental Table S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=224*). All adult patients and the parents or legally designated representatives of pediatric patients (and assent from developmentally capable children) provided written informed consent to participate in the study.

2.2. Study endpoints

The primary outcome measure was the change in HSEM parameters from baseline to the end of treatment visit (week 52). Secondary outcome measures included the change in Pineda Disability Scale scores from baseline to week 52, the safety and tolerability of miglustat, and changes in height and body weight for pediatric patients.

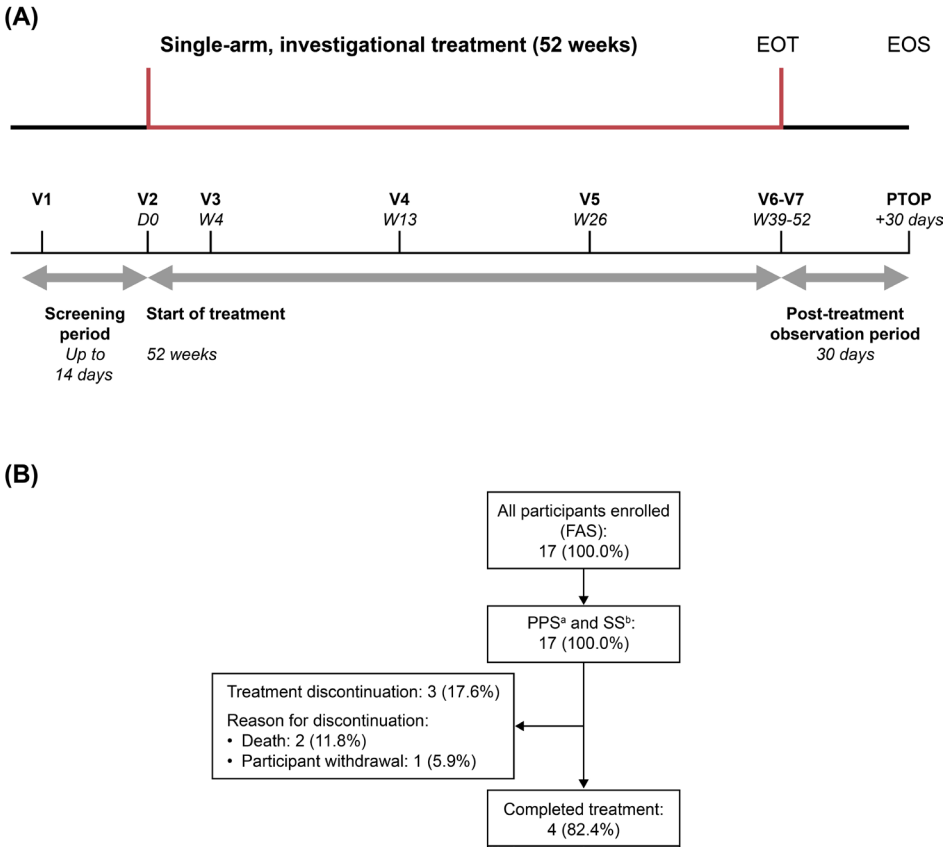

Figure 1. (A), Study design; (B), CONSORT flow diagram. Patients who prematurely discontinued the study treatment entered a PTOP, which lasted for at least 30 days after the last study drug intake. Expected duration of participation of each participant was up to 56 weeks (52 weeks treatment period + 30 days of PTOP). [a]All patients who received study treatment and had not presented with a protocol deviation that could affect the assessment of the primary endpoint. [b]All patients who received at least one dose of study medication. D: day; EOS: end of study; EOT: end of treatment; FAS: full analysis set; PPS: per protocol analysis set; PTOP: post-treatment observation period; SS: safety analysis set; V: visit; W: week.

2.3. Assessments

All efficacy parameters were measured from baseline to each visit (to weeks 13, 26, 39, and 52). A visit window of ± 7 days was allowed for all visits and a follow-up safety visit was performed 30 ± 2 days after the date of the last treatment dose. In case of premature discontinuation of treatment, the end of treatment visit was scheduled at the earliest opportunity, but no later than 7 days after the last dose of treatment. The absolute change and percentage change in HSEM parameters were assessed from baseline to each visit, including saccadic peak acceleration, mean velocity, peak duration, linear regression slopes, and line slopes of the ocular motor parameters. The HSEM parameter values were calculated by EyeSeeCam software (EyeSeeTec GmbH, Germany) and were then entered into the electronic data capture system. The change in Pineda Disability Scale score was a total additive score of six items of ambulation (scored from 0 to 5), manipulation (scored from 0 to 4), language (scored from 0 to 5), swallowing (scored from 0 to 4), seizures (scored from 0 to 3), and ocular movements (scored from 0 to 3). The total score ranged from 0 to 24, with a higher score indicating poorer condition. An item score of zero indicated no symptom or an absence of abnormalities. Body weight was measured at Screening and at each visit, and height was measured at Screening, week 26, and week 52.

Adverse events (AEs) were monitored and treatment-emergent AEs (TEAEs) were defined as AEs with onset date/time ≥ start date/time of study medication and ≤ 30 days after end of treatment, whether or not considered by the investigator as related to study medication. In cases where it could not be determined whether an AE was treatment-emergent, the AE was categorized as treatment-emergent. Other safety assessments included physical examinations, vital signs, and laboratory parameters.

2.4. Statistical methods

Approximately 19 patients with NPC were planned for enrollment in this study to ensure that 15 patients were treated with miglustat for a duration of 12 months (considering a possible 20% drop-out rate), as requested by the Chinese National Medical Products Administration. This single-arm study was descriptive in nature, and no formal statistical hypothesis or inference was made. The 95% confidence intervals (CIs) were estimated for the absolute mean change from baseline

and the percentage change mean from baseline on all efficacy variables. No imputation was made for the missing efficacy data.

The efficacy analysis was based on the full analysis set (FAS) that was defined as all enrolled patients who completed the Screening period. The per protocol analysis set (PPS), that comprised all patients who received study treatment who did not present a protocol deviation that could affect the assessment of the primary endpoint, supported the efficacy analysis. The safety analysis set (SS) included all patients who received at least one dose of study treatment.

3. Results

3.1. Patients

A total of 17 patients were enrolled between April 3, 2020, and March 29, 2022, at two centers in China. The FAS, PPS, and SS in this analysis comprised 17 patients with NPC who received at least one dose of miglustat, as none of these patients had any protocol deviations that affected the primary endpoint assessment. The majority of patients (n = 12, 70.6%) had one pathogenic mutation in *NPC1* with a positive biomarker test and high clinical suspicion of NPC, while the remaining five patients (29.4%) had two pathogenic mutations in *NPC1*. Median age was 14.0 (range, 6.0-33.0) years, 47.1% of patients were female, and all patients were from China (Table 1). At Screening, four patients (23.5%) reported a history of medical conditions and three (17.6%) patients had received prior therapy.

Table 1. Participant demographics and baseline characteristics (FAS)

Characteristics	Total (n = 17)
Age, years	
Mean (SD)	15.1 (6.6)
≤ 6 years	1 (5.9)
> 6 and ≤ 15 years	9 (52.9)
> 15 years	7 (41.2)
Sex	
Male	9 (52.9)
Female	8 (47.1)
Race	
Asian	17 (100.0)
Pathogenic mutation	
Two pathogenic mutations in *NPC1*	5 (29.4)
One pathogenic mutation in *NPC1* + a positive biomarker + high clinical suspicion of NPC	12 (70.6)
Prior medication	3 (17.7)
Concomitant medication	17 (100.0)
Any medical history	4 (23.5)
Hyperuricemia	2 (11.8)
Epilepsy	2 (11.8)
Hypothyroidism	1 (5.9)
Medical history of special interest	
Epilepsy	2 (11.8)

Data are reported as n (%) unless otherwise specified. FAS: full analysis set; NPC: Niemann-Pick disease type C; *NPC1*: Niemann-Pick disease type C1 gene; SD: standard deviation.

3.2. Treatment exposure

In total, 14 (82.4%) patients completed the 52-week treatment period and three patients (17.7%) discontinued miglustat early. The primary reasons for early discontinuation were death (n = 2, 11.8%) and participant withdrawal (n = 1, 5.9%; Figure 1B). During the study, 70.6% (n = 12) of the patients received miglustat 200 mg three times daily, and the remainder received 100 mg three times daily (n = 4, 23.5%), 200 mg twice daily (n = 2, 11.8%), or 100 mg twice daily (n = 1, 5.9%). Overall, 82.4% (n = 14) of patients received 80-120% of the planned dose. The overall median duration of miglustat exposure was 370 (range, 110-373) days, with most patients (n = 14, 82.4%) having > 360 days of exposure.

3.3. Efficacy

3.3.1. HSEM parameter analysis

The following HSEM parameter measures include those from the FAS population while the PPS population showed a similar trend to the FAS population (Supplemental Table S2, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=224*).

3.3.1.1. Saccadic peak acceleration

An increase in mean saccadic peak acceleration was observed from baseline in the patients completing 52 weeks of miglustat therapy (Figure 2A and 2B). The mean saccadic peak acceleration increased by 20.7% across the treatment period (95% CI: 7.2 to 34.3%; absolute mean change: 2,594.1 [standard deviation (SD): 2,943.7] deg/sec^2) at week 13 (n = 16); 6.9% (95% CI: -15.2 to 29.0%; absolute mean change: 1,029.2 [SD: 4,528.5] deg/sec^2) at week 26 (n = 14); 10.6% (95% CI: -7.1 to 28.4%; absolute mean change: 1,748.1 [SD: 4,415.3] deg/sec^2) at week 39 (n = 14); and 19.2% (95% CI: 11.7 to 26.74%; absolute mean change: 2,900.4 [SD: 1,923.4] deg/sec^2) at week 52 (n = 13) from the mean baseline value of 14,555.7 (95% CI: 11,786.2 to 17,325.3) deg/sec^2 (n = 17).

3.3.1.2. Mean velocity

The mean velocity values increased from baseline during the 52-week treatment period (Figure 2C and 2D). The mean velocity increased by 17.88% across the treatment period (95% CI: -5.3 to 41.0%; absolute mean change: 9.0 [SD: 30.0] deg/sec) at week 13 (n = 16); 11.3% (95% CI: -17.0 to 39.7%; absolute mean change: 3.7 [SD: 33.3] deg/sec) at week 26 (n = 14); 14.3% (95% CI: -6.0 to 34.6%; absolute mean change: 7.9 [SD: 25.0] deg/sec)

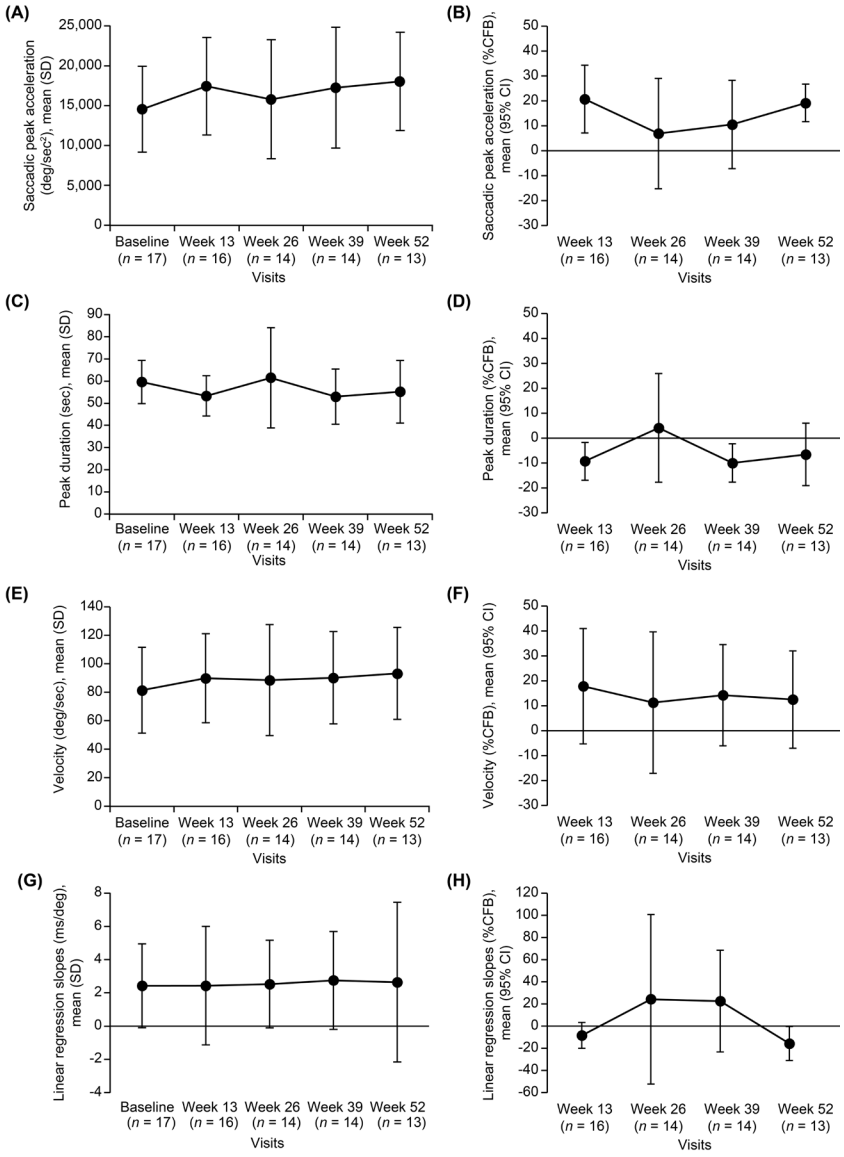

Figure 2. Mean HSEM parameters (left panels) and percentage change (right panels) from baseline to week 52 (including all time points). (A,B), Saccadic peak acceleration; **(C,D)**, velocity; **(E,F)**, Peak duration; **(G,H)**, Linear regression slope. %CFB: percentage change from baseline (FAS); CI: confidence interval; FAS: full analysis set; HSEM: horizontal saccadic eye movement; SD: standard deviation.

at week 39 (*n* = 14); and 12.5% (95% CI: -7.0 to 32.0%; absolute mean change: 8.8 [SD: 21.4] deg/sec) at week 52 (*n* = 13) in comparison with the mean baseline value of 81.5 (95% CI: 66.0 to 97.0) deg/sec (*n* = 17).

3.3.1.3. Peak duration

A decrease in mean peak duration parameter was observed from baseline to all visits (except at week 26) across the 52-week treatment period (Figure 2E and 2F). The mean change in peak duration was -9.3% (95% CI: -16.8% to -1.7%; absolute mean change: -6.1 [SD: 9.0] sec) at week 13 (*n* = 16); 4.1% (95% CI: -17.7 to 25.9%; absolute mean change: 2.0 [SD: 21.6] sec) at week 26 (*n* = 14); -9.9% (95% CI: -17.6% to -2.2%; absolute mean change: -5.8 [SD: 8.2] sec) at week 39 (*n* = 14); and -6.5% (95% CI: -19.0 to 6.0%; absolute mean change: -4.1 [SD: 11.3] sec) at week 52 (*n* = 13) from a mean baseline value of 59.6 (95% CI: 54.6 to 64.6) sec (*n* = 17).

3.3.1.4. Linear regression slopes

A reduction in mean linear regression slopes was observed from post-baseline during the 52-week treatment period; however, this was not consistent across all assessment visits (Figure 2G and 2H). The mean percentage change in linear regression slope was -8.2% (95% CI: -19.9 to 3.5%; absolute mean change: -0.01 [SD: 1.2] ms/deg) at week 13 (*n* = 16); 24.3% (95% CI: -52.2 to 100.82%; absolute mean change: 0.1 [SD: 3.2] ms/deg) at week 26 (*n* = 14); 22.7% (95% CI: -23.2 to 68.6%; absolute mean change: 0.4 [SD: 1.5] ms/deg) at week 39 (*n* = 14); and -15.6% (95% CI: -30.9% to -0.2%;

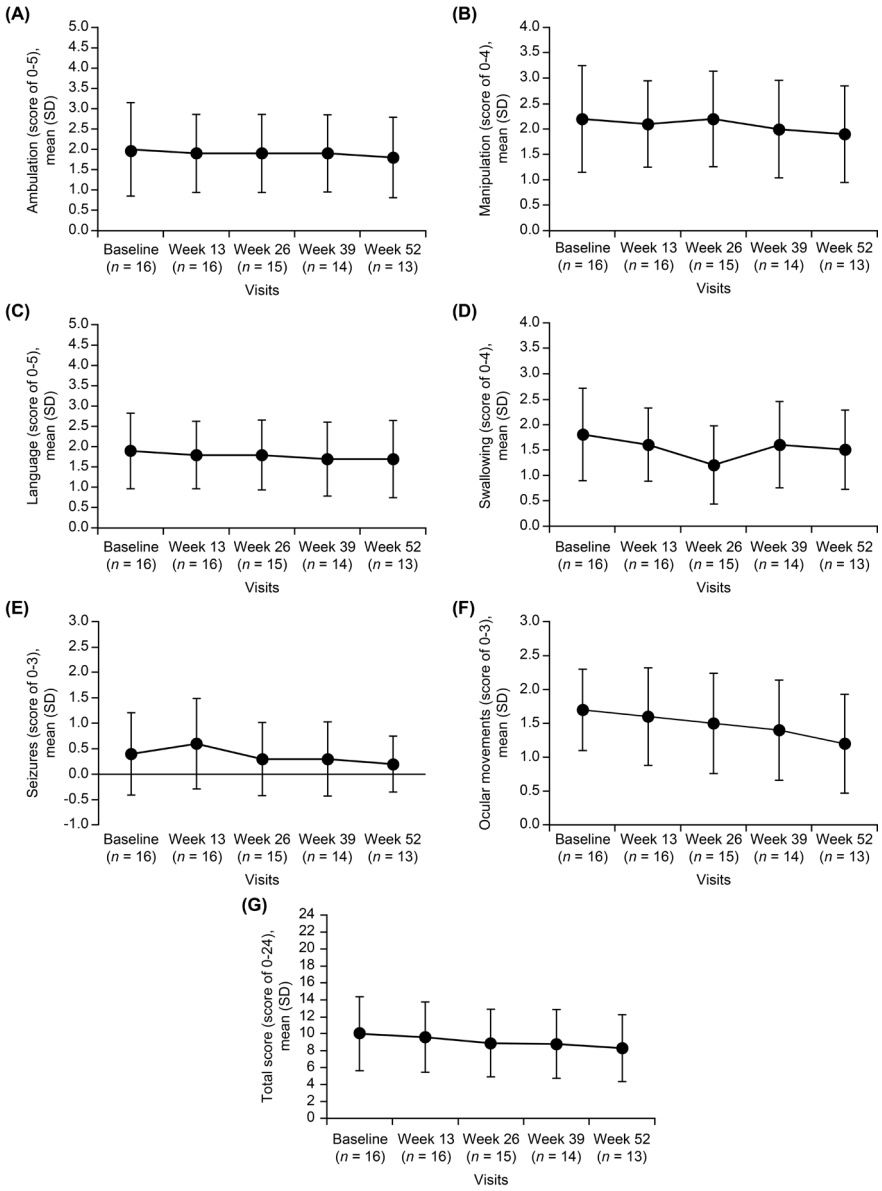

Figure 3. Mean of Pineda Disability Scale scores. (A), Ambulation; **(B)**, Manipulation; **(C)**, Language; **(D)**, Swallowing; **(E)**, Seizure; **(F)**, Ocular movements; **(G)**, Total score from baseline to week 52 (including all time points) (FAS). Mean (± SD) values are shown. Fourteen patients completed week 52 of treatment; however, Pineda Disability Scale data for one patient are missing. FAS: full analysis set; SD: standard deviation.

absolute mean change: 0.2 [SD: 2.0] ms/deg) at week 52 (n = 13), when compared with the mean baseline value of 2.4 (95% CI: 1.1 to 3.7) ms/deg (n = 17).

3.3.2. Pineda Disability Scale analysis

The Pineda Disability Scale score had a numerical improvement in ocular movements with a mean decrease of 0.4 (on a scale of 0-3) at week 52 from baseline (Figure 3). Other components of the Pineda Disability Scale also showed sustained response or minor numerical improvements in manipulation, language, swallowing, and seizures, although the changes in scores (range of changes from 0.1 to 0.4 on scales from 0-3 to 0-4) were less than those observed in ocular movements.

3.3.3. Height and body weight among pediatric patients

Among the 12 (70.6%) patients aged < 18 years, the mean values for height showed an increase during post-baseline periods when compared with the baseline period (Supplemental Table S3, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=224*). The mean height was 149.2 cm (SD: 22.7; absolute mean change: 1.2 [SD: 1.0] cm) at week 26 (n = 10) and 153.3 cm (SD: 21.3; absolute mean change: 2.6 [SD: 1.8] cm) at week 52 (n = 9), compared with the mean baseline value of 147.3 (SD: 21.8) cm (n = 12). For weight, the changes were minimal during post-baseline periods when compared with the baseline period (Supplemental Table S3, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=224*).

3.4. Safety

All 17 patients (100.0%) experienced at least one TEAE during the study. The most commonly reported TEAE was diarrhea (*n* = 12, 70.6%). Other commonly (≥ 2 patients) reported TEAEs were upper respiratory tract infection (*n* = 7, 41.2%), hyperuricemia (*n* = 4, 23.5%), and epistaxis (*n* = 3, 17.6%); abdominal pain, anal incontinence, large intestine infection, tremor, pyrexia, weight reduction, insomnia, leukocytosis, aggravation of NPC, and abnormal hepatic function were each reported in two patients [11.8%] (Table 2). Severe TEAEs occurred in four (23.5%) patients and these included pneumonia, asphyxia, accidental death, and Henoch-Schönlein purpura (each reported in one participant [5.9%]). Serious TEAEs were reported in five patients (29.4%); asphyxia, accidental death, malnutrition, pneumonia, and Henoch-Schönlein purpura were reported in one participant each and none were considered related to miglustat treatment. Two patients died due to accidental death and asphyxia, which were not considered to be related to miglustat treatment.

In total, 12 (70.6%) patients experienced TEAEs that were considered related to miglustat treatment, the most common of which were diarrhea (*n* = 12; 70.6%) and insomnia (*n* = 2, 11.8%; Table 2). Overall, five patients (29.4%) had TEAEs leading to interruption of miglustat treatment, of which four events were deemed treatment-related. The percentage of patients with TEAEs leading to discontinuation of miglustat treatment was 11.8% (*n* = 2); these two patients experienced fatal TEAEs (accidental death and asphyxia) that were not considered related to miglustat treatment per investigator's assessment.

There was a small mean change in hematological parameters between baseline and post-baseline periods (Supplemental Table S4, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=224*). During the study, no marked differences in the mean changes of chemistry parameters were observed.

Table 2. Summary of TEAEs by relationship to miglustat (preferred term) reported by at least two patients (SS)

TEAEs	Related to miglustat	Not related to miglustat
Any cause	12 (70.6)	17 (100.0)
Diarrhea	12 (70.6)	3 (17.6)
Anal incontinence	0	2 (11.8)
Upper respiratory tract infection	0	7 (41.2)
Hyperuricemia	1 (5.9)	4 (23.5)
Tremor	0	2 (11.8)
Pyrexia	0	2 (11.8)
Epistaxis	0	3 (17.6)
Insomnia	2 (11.8)	0
Leukocytosis	0	2 (11.8)
Niemann-Pick disease	0	2 (11.8)
Hepatic function abnormal	0	2 (11.8)

Data are reported as *n* (%). SS: safety analysis set; TEAE: treatment-emergent adverse events.

4. Discussion

This post-approval commitment study assessed the safety and efficacy of miglustat in 17 individuals with NPC. Without treatment, the manifestations of the disease continuously worsen without interruption (*1*). Results from the current study showed consistent findings with previous studies, suggesting that miglustat delays the progression of NPC and has manageable toxicity (*13,16,18*).

In neurometabolic disorders such as NPC, where multiple organ involvement is frequently seen, ocular motor problems can help demonstrate the severity or clinical progression (*13*). The choice of HSEM as the primary outcome measure in this study was dictated by knowledge of the disease evolution in NPC, in which, typically, vertical saccadic eye movements are affected earlier than HSEM (*21*). As demonstrated in previous studies, vertical saccadic eye movements are usually already severely affected at the time of diagnosis, and vertical gaze palsy is frequently present (*13*). In the present study, the mean saccadic peak acceleration and velocity increased by 19.2% and 12.5%, respectively, between baseline and week 52, while the mean peak duration and mean linear regression slope decreased by 6.5% and 15.6%, respectively, during the same period. Overall, this suggests that patients who received miglustat treatment experienced an improvement in HSEM, although this was not statistically evaluated. The mean ambulation, manipulation, language, swallowing, seizure, and ocular movement scores improved or stabilized between baseline and week 52. These data are consistent with the findings of the international, non-blinded, randomized controlled clinical study, which recruited 29 individuals with NPC and showed that miglustat could improve the individuals' HSEM velocity and improve or delay the deterioration of neurological symptoms in cognitive function, swallowing function, and walking ability (*13*). Data from an observational retrospective cohort study of 66 patients, across 25 expert centers, similarly demonstrated the stabilization and improvement of neurological symptoms as measured by four key parameters of neurological disease progression in NPC (ambulation, manipulation, language, and swallowing) following miglustat treatment (*12,13,15*). Similar findings were also observed in a prospective study of five children who received miglustat treatment for up to 6 years, although there was a trend towards deterioration after 5 years of treatment (*18*).

The TEAEs reported in this study were generally consistent with the known safety profile of miglustat (*13*), with the most prevalent TEAE of diarrhea being reported in 70.6% of patients. Sporadic occurrences of diarrhea were also observed in previous studies of miglustat and

it has been theorized that this occurs when sweets and/or milk-based foods are consumed (*12,18,19*). However, information on the diet of patients was not recorded in this study. Most patients could tolerate treatment (the majority of TEAEs were mild [100.0%] or moderate [76.5%] in intensity), with only 11.8% discontinuing miglustat due to TEAEs unrelated to miglustat treatment.

The safety profile of miglustat observed in the present study is consistent with that reported from 11 clinical trials of 247 patients, including 40 individuals with NPC, who received miglustat doses of 50-200 mg three times daily for an average duration of 2.1 years (*12*). Taken together, evidence from the current and previous studies shows that AEs following miglustat treatment are generally of mild-to-moderate severity (*12*). Weight loss, which is a frequent well-known side effect of miglustat treatment, was only reported in 11.8% of patients in the present study, whereas in the previous studies 55% of patients experienced TEAEs of weight loss 6–12 months after treatment initiation (*12*). The reason for this discrepancy is uncertain but it is likely that after more than a decade's worth of clinical experience, weight management practices for individuals receiving miglustat treatment have improved.

The selected study design was based on the best available knowledge and guidance from previously conducted studies, and the study evaluations used a broad approach with careful assessment of a large number of variables. Due to the progressive nature of NPC and its severity, together with the unblinding effect anticipated from the characteristic gastrointestinal side effects of miglustat and the use of objective endpoint measures, an open-label design was considered appropriate. These data should be considered in the context of the limited sample size and short duration of observation.

Currently, miglustat is the only approved disease-modifying treatment for NPC (*22,23*). No other approved therapies reverse the progressive deterioration of the nervous system that characterizes NPC (*22*), and health authority guidelines have not provided advice on suitable efficacy criteria or outcome measures to be tested in clinical studies. To the best of our knowledge, our study is the first clinical study to evaluate miglustat in patients with NPC in China. A search on both major English and Chinese medical literature databases for miglustat in Chinese patients with NPC only returned case studies. This further highlights that the results from our study provide more evidence for clinical practice in China by demonstrating that miglustat is efficacious and has a well-established and manageable safety profile.

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Conflict of Interest: Yufang Che and Xueyu Li are employees of Xi'an Janssen Pharmaceutical Ltd. and Jianmin Zhuo is an employee of, and owns stocks in, Janssen. Huiwen Zhang, Hui Xiong, Cuijie Wei, and Mengni Yi have no conflicts of interest to disclose.

References

1. Vanier MT. Niemann-Pick disease type C. Orphanet J Rare Dis. 2010; 5:16.
2. Mengel E, Klunemann HH, Lourenco CM, Hendriksz CJ, Sedel F, Walterfang M, Kolb SA. Niemann-Pick disease type C symptomatology: An expert-based clinical description. Orphanet J Rare Dis. 2013; 8:166.
3. Imrie J, Galani C, Gairy K, Lock K, Hunsche E. Cost of illness associated with Niemann-Pick disease type C in the UK. J Med Econ. 2009; 12:219-229.
4. Mengel E, Patterson MC, Chladek M, Guldberg C, Í Dali C, Symonds T, Lloyd-Price L, Mathieson T, Crowe J, Burbridge C. Impacts and burden of Niemann Pick type-C: A patient and caregiver perspective. Orphanet J Rare Dis. 2021; 16:493.
5. Patterson MC, Hendriksz CJ, Walterfang M, Sedel F, Vanier MT, Wijburg F, NP-C Guidelines Working Group. Recommendations for the diagnosis and management of Niemann-Pick disease type C: An update. Mol Genet Metab. 2012; 106:330-344.
6. Wassif CA, Cross JL, Iben J, Sanchez-Pulido L, Cougnoux A, Platt FM, Ory DS, Ponting CP, Bailey-Wilson JE, Biesecker LG, Porter FD. High incidence of unrecognized visceral/neurological late-onset Niemann-Pick disease, type C1, predicted by analysis of massively parallel sequencing data sets. Genet Med. 2016; 18:41-48.
7. Patterson MC, Mengel E, Vanier MT, Moneuse P, Rosenberg D, Pineda M. Treatment outcomes following continuous miglustat therapy in patients with Niemann-Pick disease type C: a final report of the NPC Registry. Orphanet J Rare Dis. 2020; 15:104.
8. Burton BK, Ellis AG, Orr B, Chatlani S, Yoon K, Shoaff JR, Gallo D. Estimating the prevalence of Niemann-Pick disease type C (NPC) in the United States. Mol Genet Metab. 2021; 134:182-187.
9. Liang H, Zhan X, Wang Y, Maegawa GHB, Zhang H. Development and validation of a new genotype-phenotype correlation for Niemann-Pick disease type C1. J Inherit Metab Dis. 2024; 47:317-326.
10. European Medicines Agency. Miglustat. Summary of product characteristics. *https://www.ema.europa.eu/en/documents/product-information/zavesca-epar-product-information_en.pdf* (accessed October 7, 2024).
11. Lyseng-Williamson KA. Miglustat: A review of its use in Niemann-Pick disease type C. Drugs. 2014; 74:61-74.
12. Actelion Pharmaceuticals. Investigator's Brochure JNJ-

66674140 Zavesca® (miglustat). 2020; pp. 123.
13. Patterson MC, Vecchio D, Prady H, Abel L, Wraith JE. Miglustat for treatment of Niemann-Pick C disease: A randomised controlled study. Lancet Neurol. 2007; 6:765-772.
14. Platt FM, Neises GR, Dwek RA, Butters TD. N-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. J Biol Chem. 1994; 269:8362-8365.
15. Pineda M, Wraith JE, Mengel E, Sedel F, Hwu WL, Rohrbach M, Bembi B, Walterfang M, Korenke GC, Marquardt T, Luzy C, Giorgino R, Patterson MC. Miglustat in patients with Niemann-Pick disease type C (NP-C): A multicenter observational retrospective cohort study. Mol Genet Metab. 2009; 98:243-249.
16. Karimzadeh P, Tonekaboni SH, Ashrafi MR, *et al.* Effects of miglustat on stabilization of neurological disorder in Niemann-Pick disease type C: Iranian pediatric case series. J Child Neurol. 2013; 28:1599-1606.
17. Fecarotta S, Amitrano M, Romano A, Della Casa R, Bruschini D, Astarita L, Parenti G, Andria G. The videofluoroscopic swallowing study shows a sustained improvement of dysphagia in children with Niemann-Pick disease type C after therapy with miglustat. Am J Med Genet A. 2011; 155A:540-547.
18. Chien YH, Peng SF, Yang CC, Lee NC, Tsai LK, Huang AC, Su SC, Tseng CC, Hwu WL. Long-term efficacy of miglustat in paediatric patients with Niemann-Pick disease type C. J Inherit Metab Dis. 2013; 36:129-137.
19. Heron B, Valayannopoulos V, Baruteau J, Chabrol B, Ogier H, Latour P, Dobbelaere D, Eyer D, Labarthe F, Maurey H, Cuisset JM, de Villemeur TB, Sedel F, Vanier MT. Miglustat therapy in the French cohort of paediatric patients with Niemann-Pick disease type C. Orphanet J Rare Dis. 2012; 7:36.
20. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice *https://database.ich.org/sites/default/files/ICH_E6%28R3%29_DraftGuideline_2023_0519.pdf* (accessed June 28, 2024).
21. Patterson MC, Di Bisceglie AM, Higgins JJ, Abel RB, Schiffmann R, Parker CC, Argoff CE, Grewal RP, Yu K, Pentchev PG, Brady RO, Barton NW. The effect of cholesterol-lowering agents on hepatic and plasma cholesterol in Niemann-Pick disease type C. Neurology. 1993; 43:61-64.
22. Cariati I, Masuelli L, Bei R, Tancredi V, Frank C, D'Arcangelo G. Neurodegeneration in Niemann-Pick type C disease: An updated review on pharmacological and non-pharmacological approaches to counteract brain and cognitive impairment. Int J Mol Sci. 2021; 22:6600.
23. Tirelli C, Rondinone O, Italia M, Mira S, Belmonte LA, De Grassi M, Guido G, Maggioni S, Mondoni M, Miozzo MR, Centanni S. The genetic basis, lung involvement, and therapeutic options in Niemann-Pick disease: A comprehensive review. Biomolecules. 2024; 14:211.

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Original Article

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Osteogenesis imperfecta in Peruvian children: Phenotypic and therapeutic insights from a pediatric hospital

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SUMMARY Osteogenesis imperfecta (OI) is a genetic disorder of the connective tissue that is characterized by high bone fragility. It has a worldwide incidence of 1 in 10,000. The diagnosis is mainly clinical-radiological. Treatment is based on the use of bisphosphonates and orthopedic surgeries. The objective of this study was to establish the clinical, radiological, and therapeutic characteristics of OI in pediatric patients of a national reference pediatrics institute. This was conducted through a descriptive and retrospective analysis. All patients under 18 years of age with a diagnosis of OI treated at the Instituto Nacional de Salud del Niño de Breña (INSN-Breña) between 2010 and 2021 were included. In total, 91 patients with OI were studied, more than half of whom were male. A total of 93.4% had a history of fractures, 72.5% had blue sclera, 39.6% had bowed legs and 20.9% had dentinogenesis imperfecta. The minimum-maximum value of fractures was 0-18. A total of 75.8% of patients started treatment with bisphosphonates and 41.8% used adjuvant medications. Less than 50% of patients required surgical treatment. Osteogenesis imperfecta is a genetic and chronic pathology. The use of the Van Dijk severity grade and the Aglan severity scale is simple to apply and therefore should be used to improve the classification of groups with the highest risk of fractures and response to treatment. Due to the low incidence of this disease, it is important to raise awareness and increase the research volume on this subject.

Keywords rare disease, bone fractures, collagen type I-II, antiresorptive therapy

1. Introduction

Osteogenesis imperfecta (OI), also known as brittle bone disease, is a genetic disorder of connective tissue characterized by increased bone fragility and reduced bone mass (*1*). It is a rare and underdiagnosed disease with a global incidence estimated to be 1 in 10,000 in the general population (*1*). In 90% of cases there is a variant in one of the genes that encode the alpha chains of type I collagen (*COL1A1* or *COL1A2*) (*1,2*). Currently, thanks to technological advances, more is known about the biological and genetic factors of OI, which has made it possible to establish a classification with up to 21 different genes (*3*). This condition predisposes patients to multiple fractures due to minimal trauma, bone deformities, as well as growth deficiencies. The classic extra-skeletal clinical manifestations are joint hypermobility, dentinogenesis imperfecta, hearing loss and blue sclerae, which can vary depending on the type of OI (*4,5*).

In Peru, OI is considered a rare disease due to its low prevalence (*6*). There are few epidemiological studies, and knowledge is based mainly on case reports, such as the one published on 11 cases at the Hospital Nacional Daniel Alcides Carrion (*6*). Another study whose objective was to identify genetic diseases in Peru, showed that between 2014 and 2018 at the Instituto Nacional de Salud del Niño-Breña (INSN-Breña), 82 patients with OI were observed (*7*). The diagnosis and treatment of this disease in Peru is made difficult by its complexity and the limited availability of specialized centers, which can lead to underdiagnosed and inadequate management of cases (*7*). Diagnosis is mainly based on clinical evaluation, physical examination and radiology, while molecular diagnosis is useful to determine the specific genetic cause and determine the risk of recurrence (*8*).

Treatment is based on three pillars: antiresorptive drugs (bisphosphonates) that increase bone mass and reduce the risk of fractures; orthopedic - surgical procedures to correct deformities and physical therapy.

Other drugs such as denosumab, which inhibits osteoclastic activity, are being studied to determine their benefit (*4,9*). The purpose of this study is to determine the clinical, radiological and therapeutic characteristics, such as the response to treatment with bisphosphonates, of patients with OI at the INSN-Breña between the years 2010-2021.

2. Patients and Methods

2.1. Study design

A descriptive and retrospective study was carried out. The study was conducted at INSN-Breña, a national reference center for pediatric diseases. It has a Genetics and Inborn Errors of Metabolism Service that has a database of patients with OI for more than 12 years. The present study collected information from patients with OI from 2010 to 2021.

Ethics approval and research approval were obtained from the Ethics Committees of the Universidad Científica del Sur (1048-2021-PRE15) and the INSN-Breña (PI-24/22) before observing the clinical history of the patients, and the study was conducted in accordance with the Declaration of Helsinki.

2.2. Inclusion and exclusion criteria

All patients under 18 years of age with a diagnosis of OI who met the following inclusion criteria were included: diagnosis of OI confirmed by clinical, imaging or molecular examination, bone densitometry with osteoporosis, family history of OI, history of pathological fractures and clinical symptoms compatible with OI. Medical records that were illegible, in poor condition, or lacked information on the variables of interest were excluded.

2.3. Data collection

Variables collected included demographic data, familial history, clinical characteristics, bone densitometry, anthropometric values, serological markers, and details of medical and surgical treatment. Patients were categorized according to Van Dijk's severity classification and according to Aglan's severity scale (*10,11*). We assessed severity of each patient with the help of a medical geneticist since the severity wasn't described in the medical record. Data were obtained from the patients' medical records, compiled in a collection form.

Two possible biases were identified: measurement bias due to errors in obtaining data from medical records, and selection bias by including only patients from the INSN-Breña, although this is a national pediatric reference center. The sample size was all patients with OI in the INSN-Breña during 2010-2021, given the low frequency of OI.

2.4. Statistical analysis

Categorical variables were presented with frequencies, and numerical variables with measures of central tendency and dispersion according to normality. It is necessary to mention that all clinical variables are described in their entirety due to the descriptive nature of the study.

An exploratory analysis was carried out, using chi-square tests with goodness of fit for the variables "sex" and "origin", taking into account that presentation of OI is the same in men and women (*2*), the proportion of inhabitants of Lima and other departments is 32.2% and 67.8%, respectively according to the last national census carried out by the National Institute of Statistics in 2017. In addition, to determine if there are differences between the "severity" categories and the values of bone densitometry, serological markers, and fractures, the Kruskal-Wallis's test was used. To identify variables that could be used as predictors of a higher number of fractures, the relationship with bone densitometry and body mass index was analyzed using multiple linear regression. For the multivariate analysis, Poisson regression with robust variance was used to investigate the existence of new human phenotypes associated with severity and response to treatment; likewise, the OpenEpi program was used to calculate the power of said regression.

For statistical analysis, the STATA v.15 program was used with a 95% confidence interval and a p value of less than 0.05.

3. Results

3.1. Sociodemographic characteristics

There were potentially 194 medical records of patients with OI, however, when reviewing them, only 91 medical records met the inclusion criteria. The main reason for exclusion was not having defined diagnoses. Of all patients, 60.4% were male. 52.8% came from Lima and 14.3% of all patients had asthma/bronchial obstruction syndrome as comorbidity. 24.2% had a family history of OI (Table 1). The median age of diagnosis was 20.5 months (male = 21 and female = 19.5); with a mean age of the father and mother at the birth of the patient of 31.9 and 27.8, respectively. The mean birth weight was 2,822 ± 288.3 g and the mean birth height was 45 ± 3.3 cm.

Regarding the variability of birth weight and the Aglan severity scale, a difference was observed (p = 0.0068), with a *post hoc* analysis of difference between the mild and severe group (p = 0.010) (Supplemental Table S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=219*). On the contrary, we did not observe differences in birth weight on the Van Dijk severity groups (p = 0.0988); and in the same sense when evaluating both scales, height (p = 0.5523

Table 1. General characteristics of pediatric patients with OI

Variables	*n*	%	95% CI	*p*
Gender				0.046*
Male	55	60.4	49.9-70.1	
Female	36	39.6	29.9-50.1	
Origin				< 0.001*
Lima (capital)	48	52.8	42.3-62.9	
Other city's	43	47.3	37.0-57.6	
Use of complementary exams				
N-telopeptide	47	51.6	41.4-61.9	
C-telopeptide	24	26.4	17.3-35.4	
Calcium	40	44.0	33.8-54.2	
Phosphorus	51	56.0	45.8-66.2	
Magnesium	14	15.4	8.0-22.8	
Vitamin D (25-OH)	14	15.4	8.0-22.8	
Alkaline phosphatase	24	26.4	17.3-35.4	
Bone densitometry	48	52.7	42.5-63.0	
Comorbidities				
Asma/BOS	13	14.3	4.5-142	
Hypotonia	7	7.7	0.3-7.7	
Malnutrition	7	7.7	0.3-7.7	
Anemia	5	5.5	-0.8-5.5	
Heart disease	5	5.5	-0.8-5.5	
Neurodevelopmental disorder	5	5.5	-0.8-5.5	
Nephrolithiasis	2	2.2	-1.9-2.2	
Prostration	5	5.5	-0.8-5.5	
Family history				
No	69	75.8	65.8-83.6	
Yes	22	24.2	16.4-34.2	
Van Dijk severity grade (*n* = 82)				
Mild	6	7.3	3.2-15.6	
Moderate	47	57.3	46.2-67.7	
Severe	25	30.5	21.3-41.5	
Extremely severe	4	4.8	1.8-12.5	
Aglan severity scale (*n* = 41)				
Mild	17	41.5	27.0-57.5	
Moderate	4	9.8	3.6-24.1	
Severe	20	48.7	33.4-64.3	
Fractures				
Total number	426			
Lower limbs	264	61.9	55.3-66.6	
Upper limbs	110	25.8	21.7-30.0	
Clavicle	32	7.5	5.0-10.0	
Head	13	3.0	1.4-4.7	
Thorax	8	1.9	0.6-3.2	
Anthopometry at birth	Mean/Median	SD/IQR		
Weight (g)	2,903§	582.6	1,500-4,300	
Height (cm)	46.6§	4.3	35-52	
Cephalic circumference (cm)	35.0	2.5	30-37	
Diagnostic age (months)	20.5	58.0	0-180	
Father's age	31.9§	8.1	29.9-34	
Mother's age	27.8§	7.7	25.9-28.7	

BOS, bronchial obstruction syndrome; CI, confidence interval; IQR, interquartile range; OI, osteogenesis imperfecta; SD, standard deviation. *Chi2 with adjusted residuals. §Mean and SD.

and 0.7170) and head circumference at birth ($p = 0.3290$ and 0.3614). These results are not presented in any table or supplementary material. It is important to note that the small sample size in the mild group of the Van Dijk severity scale and the moderate group of the Aglan scale may limit the power of these comparisons. This limitation should be considered when interpreting the results.

The mean of the standard deviations for height and body mass index at the time of diagnosis was -2.78 (95% CI = -3.47 to -2.08) and 0.66 (95% CI = 0.33-0.99), respectively.

3.2. Clinical characteristics & severity degree

The mean number of fractures was 4.68, and one patient reported up to 18 fractures. The most frequently fractured location was the lower limbs (61.9%), followed by the upper limbs (25.8%). A total of 147 emergency admissions due to fractures were observed, with a

mean of 1.6 admissions per patient. The main cause was fractures (57.9%), and one patient reported 19 admissions. About the severity, 57.3% of patients had a moderate Van Dijk severity grade and 48.7% had a severe Aglan severity scale. On the other hand, N-telopeptide and bone densitometry were performed in around 50% of patients and alkaline phosphatase and C-telopeptide were only reported in 25% of patients (Table 1).

In respect of the physical characteristics, the most frequent associated phenotypes were blue sclera's (72.5%), bowed legs (39.6%), joint hypermobility (24.2%), and triangular faces (23.1%) (Table 2). In the same matter, a frequency analysis was carried out based on the Aglan severity scale from moderate to severe, where a greater occurrence of umbilical hernia, scoliosis and clinodactyly was observed. On the contrary, in this same group of patients, hearing loss, microcephaly and hypermobility was less dominant.

The analysis of the frequency according to the Aglan severity scale from moderate to severe in the adjusted model, observed a higher frequency of umbilical hernia, single palmar crease, scoliosis, clinodactyly, and asymmetric lower limbs. In the opposite direction, in this group of patients, keratoconus, hypermobility, hearing loss, triangular faces, jaw asymmetry, microcephaly and genu varum were less frequent in the adjusted model (Table 3). Based on the Van Dijk severity scale, the frequencies of phenotypes did not show any significant differences (Supplemental Table S2, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=219*).

Table 2. Associated phenotypes in pediatric patients with OI

Associated phenotypes	*n*	%	95% CI
Blue sclera	66	72.5	60.15-72.53
Bowed legs	36	39.6	26.01-39.56
Joint hipermobility	22	24.2	12.31-24.18
Triangular face	21	23.1	11.4-23.08
Dentinogenesis imperfecta	19	20.9	9.61-20.88
Short height	12	13.2	3.81-13.19
Scoliosis	9	9.9	1.62-9.89
Bowed arms	9	9.9	1.62-9.89
Asymmetric upper limbs	9	9.9	1.62-9.89
Shortening of lower limbs	5	5.5	-0.82-5.49
Pectus carinatum	5	5.5	-0.82-5.49
Microcephaly	4	4.4	-1.29-4.4
Inguinal hernia	4	4.4	-1.29-4.4
Umbilical hernia	3	3.3	-1.65-3.3
Asymmetric thorax	3	3.3	-1.65-3.3
Coxa vara	3	3.3	-1.65-3.3
Clinodactyly	3	3.3	-1.65-3.3
Macrocephaly	3	3.3	-1.65-3.3
Single palmar crease	2	2.2	-1.87-2.2
Hearing loss	2	2.2	-1.87-2.2
Wide anterior fontanelle	2	2.2	-1.87-2.2
Bot foot	2	2.2	-1.87-2.2
Short thorax	2	2.2	-1.87-2.2
Keratoconus	1	1.1	-1.79-1.1
Sunken nose bridge	1	1.1	-1.79-1.1
Jaw disproportion	1	1.1	-1.79-1.1
Hip Dysplasia	1	1.1	-1.79-1.1
Flat foot	1	1.1	-1.79-1.1
Varying leg	1	1.1	-1.79-1.1
Valgus foot	1	1.1	-1.79-1.1
Cleft lip & palate	1	1.1	-1.79-1.1

CI, confidence interval; OI, osteogenesis imperfecta.

The prevalence of phenotypes associated with the Van Dijk severity degree and the Aglan severity scale was analyzed; however, no significance was observed in the frequency when determining the means of the total phenotypes according to the scale and degree of severity ($p = 0.608$ and 0.940, respectively).

3.3. Treatment

Of all patients 75.8% started a treatment with bisphosphonates, with alendronate (1 mg/kg weekly, oral) being the most frequently used (27.5%) followed by zoledronate (0.5 mg/kg every six months, intravenous). Only 42.9% completed at least one year of treatment and 12.1% completed three or more years. Surgical treatment was required in 34.1% of patients due to fractures. Regarding the use of adjuvant medications, such as calcium (30-75 mg/kg/day) and vitamin D (doses diverse, *e.g.* 600,000 UI stat), 41.8% of patients used them (Table 4).

When analyzing the treatment response in relation to the increase in the standard deviation scores of bone densitometry, no differences were found when comparing it with the Aglan severity scale and the Van Dijk severity grade ($p = 0.7639$ and 0.2470, respectively). In that same sense, no changes were observed in densitometry in relation to any associated phenotype ($p > 0.05$).

3.4. Bone densitometry analysis

Regarding bone density, a trend towards improvement over time was observed, however, some patients only had a single measurement (Figure 1A). We observed that those who presented a favorable evolution according to bone densitometry had a mean number of fractures of 3.6 (SD = 1.73) versus those who presented an unfavorable response, the average was 11.1 (SD = 4.87) ($p < 0.001$). In the same sense, the number of fractures increases if bone densitometry remains the same or decreases over time ($R^2 = 0.138$; coefficient = 0.213; 95% IC = -0.422 to -0.003; constant = 2.5; $p = 0.047$) (Figure 1B). Additionally, it was observed that as the body mass index increases, the response to treatment calculated, according to bone density, is lower ($R^2 = -0.043$; coefficient = 0.0; 95% CI = -0.029 to -0.059; constant = 0.24; $p = 0.078$) (Figure 1C).

3.5. Comparative analysis

A comparison was made of the median of the difference between the last and first value of the serological

Table 3. Prevalence of associated phenotypes in patients with osteogenesis imperfecta according to the Aglan severity scale

Phenotype	PRc	95% CI	*p*	PRa	95% CI	*p*
Umbilical hernia	0.667	0.130-3.407	0.5782	10,059	1.961-51.608	0.006
Inguinal hernia	2,105	1.519-2.916	0.2995	1,984	0.492-8.007	0.335
Blue sclera	1,857	0.773-4.460	0.1159	2,074	0.821-5.241	0.123
Dentinogenesis imperfecta	1,375	0.715-2.645	0.3869	1,178	0.439-3.162	0.745
Poor sphincter control	-	-	-	-	-	-
Keratoconus	-	-	0.3231	9.35 x 10-7	5.7 x10-8-1.51 x10-5	< 0.001
Joint hipermobility	0.682	0.291-1.596	0.3355	0.053	0.008-0.343	0.002
Single palmar crease	2,105	1.519-2.916	0.2995	201,156	12.82-3155.69	< 0.001
Hearing loss	0	-	0.3231	1.20 x 10-8	1.38 x10-9-1.03 x10-7	< 0.001
Triangular face	1,524	0.831-2.794	0.2243	0.232	0.083-0.648	0.005
Short height	1,271	0.574-2.813	0.5922	1,007	0.293-3.470	0.99
Scoliosis	1,458	0.745-2.853	0.3428	5,434	1.702-17.348	0.004
Sunken nose bridge	-	-	-	-	-	-
Jaw disproportion	2,105	1.519-2.916	0.2995	0.027	0.002-0.448	0.012
Wide anterior fontanelle	-	-	-	-	-	-
Macrocephaly	-	-	.	-	-	-
Microcephaly	0	-	0.3231	2.35 x 10-8	2.17 x10-9-2.56 x10-7	< 0.001
Shortening of lower limbs	0	-	0.157	3.13 x 10-9	2.51 x10-10-3.90 x10-8	< 0.001
Flat foot	-	-	-	-	-	-
Coxa vara	1,026	0.247-4.258	0.9718	0.392	0.063-2.417	0.313
Genu varum	0	-	0.3231	9.35 x 10-7	5.7 x10-8-1.51 x10-5	< 0.001
Valgus foot	0	-	0.3231	0.169	0.002-10.199	0.395
Pectus carinatum	2,105	1.519-2.196	0.2995	3,389	0.279-41.049	0.337
Bot foot	-	-	-	-	-	-
Clinodactyly	1,407	0.591-3.351	0.5197	6,624	1.649-26.618	0.008
Bowed arms	1,407	0.591-3.351	0.5197	1,868	0.555-6.290	0.313
Asymmetric lower limbs	1,944	1.151-3.283	0.0669	41,757	4.918-354.508	0.001
Asymmetric thorax	2,167	1.544-3.040	0.1373	0.307	0.0197-4.789	0.4
Short thorax	0	-	0.3231	4.66 x 10-6	2.12 x10-7-1.02 x10-5	< 0.001
Cleft lip/palate	0	-	0.3231	1.07 x 10-8	9.58 x10-10-1.19 x10-7	< 0.001
Bowed legs	0.923	0.461-1.848	0.8187	1,788	0.769-4.158	0.177
Severe short height	1,436	0.783-2.633	0.2655	0.794	0.278-2.268	0.667

PRa, adjusted prevalence reason; PRc, crude prevalence reason; CI, confidence intervals.

markers according to the Van Dijk severity grade and the Aglan severity scale. However, no significant difference was found between these values (Table 5). Another comparison of the median number of fractures was made according to the Van Dijk severity grade and the Aglan severity scale. It was observed that the median number of fractures was more frequent in the group that was of moderate severity, according to the Van Dijk severity grade (p = 0.0003) and the Aglan severity scale (p = 0.0957) (Supplemental Table S3, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=219*).

4. Discussion

This is the second and largest study in the country about the pediatric population with OI and although it is not a national study, it allows us to have a great overview of this disease in Peru (*6*). In our country there is no information about the incidence of OI. However, Guio *et al.* determined that in one of the main Peruvian reference hospitals, 101 cases of skeletal dysplasia were diagnosed during the years 2014-2018, and it is worth mentioning that within this group of diseases is OI (*7*). Another study in a different national reference hospital observed 11 patients with OI over a period of 3 years (2007-2009) (*13*).

A greater frequency was observed in the male sex (60.4%), which is contrary to what is indicated in the international literature, which mentions that there is no sex predominance in OI, this could be because this disease is still underdiagnosed in our country (*14,15*), or it might be related to social or familial factors. However, in a Portuguese study (n = 21) results like ours were shown with a significant male population (61.9%) (*16*).

The average age of diagnosis of our study was 20.5 months of age, which is early compared to a national study of the Vietnamese population with OI (n = 146), where the most frequent age of diagnosis ranged from 6-10 years (27.4%) (*17*). In Spain (Valencia) it was observed (n = 40) that the average age of diagnosis was 8.4 ± 14.6 years, and 0.04 ± 0.3 years in severe cases where presentation at birth have only been seen in extremely severe cases (*18*). In a Turkish study (n = 29), average age of diagnosis was 3.6 years, which reflects a later age compared to our results (*19*). However, in a Portuguese hospital the mean age of diagnosis was 20.6 months, like this study (*16*). This may be explained since more than 60% of patients in this hospital debuted with a fracture before the first three years of life (*16*); or by the differences in population size and the types of OI across

Table 4. Therapy in pediatric patients with OI

	n	%	95% CI
Bisphosphonates			
No treatment	22	24.2	16.3-34.2
Only alendronate	25	27.5	19.1-37.7
Only zoledronate	19	20.9	13.6-30.6
Alendronate→Zoledronate	15	16.5	10.0-25.7
Only pamidronate	4	4.4	1.6-11.3
Alendronate→Pamidronate →Zoledronate	3	3.3	1.04-9.92
Pamidronate→Zoledronate	2	2.2	0.53-8.57
Alendronate→Pamidronate	1	1.1	0.14-7.6
Years of Bisphosphonates (*n* = 65)			
0	22	24.2	16.35-34.2
1	39	42.9	32.9-53.3
2	19	20.9	13.6-30.6
3	8	8.8	4.39-16.8
4	2	2.2	0.53-8.57
7	1	1.1	0.14-7.64
Treatment continuity for 3 or more years			
Yes	11	12.1	0.6-2.0
No	80	87.9	79.2-93.2
Surgeries for fractures			
No	60	65.9	55.4-75.1
Yes	31	34.1	24.9-44.6
Number of surgeries			
0	49	53.9	43.4-63.9
1	24	26.4	18.2-36.6
2	14	15.4	9.2-24.5
3	3	3.3	1.0-9.9
4	1	1.1	0.1-7.7
Adjuvants (vitamin D/ calcium)			
Yes	38	41.8	31.9-52.3
No	53	58.2	47.7-68.07

CI, confidence intervals. →, "followed by".

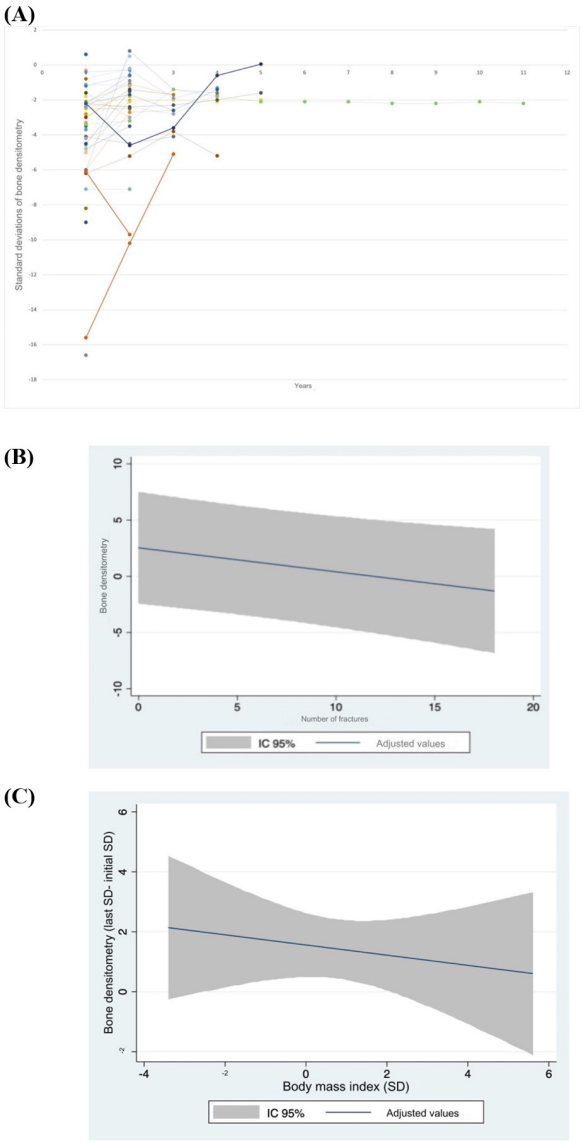

Figure 1. (A) Evolution of lumbar bone densitometry, each color represents a patient. **(B)** Relationship between the number of total fractures and the response of bone densitometry according to its standard deviation in patients with osteogenesis imperfecta. (R^2 = 0.138; coefficient = 0.213; 95% CI = -0.422 to -0.003; constant = 2.5; p = 0.047). **(C)** Relationship between body mass index and response to treatment according to bone densitometry (R^2 = -0,043; coefficient = 0.0; 95% CI = -0,029 to -0,059; constant = 0.24; p = 0.078).

the cohorts being compared.

It was also determined that most patients came from the capital of Peru (52.8%). We propose that this could be due to the shortage of specialists (mainly geneticists), in regional hospitals to diagnose and refer patients to specialized hospitals, and most of them are in our capital (*7*).

Most of our patients (75.8%) did not have any family history. Which would imply that many of the genetic variants in these patients are likely de novo and the frequency of mild type OI is low. A study carried out in Portugal also showed that 66.6% of its patients had no family history (*16*). On the contrary, a study carried out in Spain (Valencia) observed that more than 50% of its patients had a family history (*20*). This difference could be attributed to the fact that, depending on the population groups, there may be greater awareness of the importance of timely diagnosis, making family history more relevant. Alternatively, patients with rare diseases in high-income countries might generally have greater survival rates.

Regarding the extra-skeletal characteristics, we observed that the most prevalent were the presence of blue sclerae, bowed legs, joint hypermobility, triangular faces and dentinogenesis imperfecta (DI) with 72.5%, 39.6%, 24.2%, 23.1%, and 20.9%, respectively. Multiple studies agree with our findings since they observe that the most prevalent extra-skeletal characteristic of OI is blue sclerae (79%-86%), and DI (20.9% *vs.* 22.5%-61%), but this may vary depending on the population and type of OI (*16,18,20*) or the experience of clinical experience for found and registered phenotypes.

To evaluate the severity of OI in our study we used two grading scales. The first, developed by Van Dijk *et al.,* which allows us to establish a pre or post-natal severity based on clinical or ultrasound characteristics

Table 5. Comparison of median difference between the first and last values of serological markers according to grade and severity scale

Markers	*n*	median	*p**
Van Dijk severity grade			
N-telopeptide			
Mild	3	128	
Moderate	8	104.45	
Severe	5	-136	0.0274
Extremely severe	3	-41	
Total	19	67.4	
C-telopeptide			
Mild	0	0	
Moderate	7	-8.7	
Severe	3	24	0.7324
Extremely severe	0	0	
Total	10	7.65	
Alkaline phosphatase			
Mild	1	-417	
Moderate	5	-52	
Severe	3	-109	0.133
Extremely severe	1	-524	
Total	10	-108	
Bone densitometry			
Mild	2	2.2	
Moderate	13	1.4	
Severe	6	1.2	0.8066
Extremely severe	3	4.7	
Total	24	1.35	
Aglan severity scale			
N-telopeptide			
Mild	17	134.75	
Moderate	20	456.02	0.8104
Severe	4	294.7	
Total	41	226.8	
C-telopeptide			
Mild	3	195.7	
Moderate	3	-8.7	0.2752
Severe	0	0	
Total	6	17.65	
Alkaline phosphatase			
Mild	5	-82	
Moderate	2	-219.5	0.7408
Severe	1	-121	
Total	8	-108	
Bone densitometry			
Mild	5	1.6	
Moderate	4	0.8	0.0971
Severe	2	3.85	
Total	11	1.1	

PRa, adjusted prevalence reason; PRc, crude prevalence reason; CI, confidence intervals. *Median differences by Kruskall wallis.

(*10*). In this study, we observed that most cases were moderate (57.3%). In contrast, Caudevilla *et al.* observed that mild cases were the most frequent (68.3%) (*18*). This discordance may be due to the fact that the study mentioned before wasn't done in a completely pediatric population.

The second scale uses a scoring system based on five parameters and was developed by Aglan *et al.* with the severe form being the most frequent (48.7%) (*11*). The same author observed (n = 43) that, as in our study, the severe form was the most frequent (37.2%) (*11*). Otaify *et al.* observed in his study (n = 33) that all his patients presented a moderate or severe degree of severity (*21*).

In our study, 93.4% of patients had at least one fracture in their lives and one case even had 18. Furthermore, we observed that, like other studies, the most affected location were the lower limbs, followed by the upper limbs. In Spain (Valencia) the results were similar, with lower limbs slightly more frequent than upper limbs with 36.5% and 33.6%, respectively (*20*). In Vietnam, it was observed that 142/146 patients presented fractures with a total of 1,932 fractures. Likewise, fractures in this population were most common in the lower limb, especially the femur (*17*). Similarly, in Turkey, it was observed that the most frequent fractures were the lower limbs, especially the tibia, followed by the femur (*19*). In Portugal, results were similar with the lower limb (55.6%) being the most common, followed by the upper limb (37.8%) (*16*).

The treatment is based in the use of bisphosphonates, commonly being alendronate, pamidronate and zoledronate (*4*). In addition, many specialists opt for adjuvant therapy using calcium and vitamin D supplements (*9*). We observed that 75.8% of patients started a treatment with bisphosphonates. However, only 41.8% used adjuvant medications. Regarding bisphosphonates, in this study alendronate (27.5%) was the main one indicated, followed by zoledronate (20.9%). The therapeutic regimen of alendronate followed by zoledronate was used in 15 patients (16.5%). We emphasize that nearly 50% of patients did not complete the year of treatment with bisphosphonates. We propose that this could be due to multiple factors such as the shortage of medications, lack of follow-up, difficulty in scheduling appointments, distance to the hospital, limited knowledge about rare diseases or administrative barriers.

In Turkey, it was observed that 75% of patients started a treatment with bisphosphonates, where pamidronate (77%) and alendronate (23%) were the most indicated (*21*). In Vietnam, a low-resource country, only 25% of patients started treatment with bisphosphonates where zoledronate predominated, since it is the most accessible in the country (*17*). In said country access to bisphosphonates and specialists is difficult, which is why most patients do not complete treatment. It should be noted that these difficulties are like our national reality (*17*). It is important to emphasized that in Vietnam surgical treatments are the most frequently indicated with 163 surgeries performed in all 58 patients (*17*).

In Portugal, 85% of patients started a treatment with bisphosphonates, specifically with pamidronate, and the mean age at which treatment began was 50 months (*16*). In Valencia evidence showed that 52.1% of patients received treatment, where bisphosphonates were the most common (44.8%). In addition, 24.7% of patients received exclusively adjuvant treatment with vitamin D,

calcium or parathyroid hormone (*20*). Finally, in Spain it was observed that 19 patients (47.5%) started treatment with bisphosphonates, six of which added recombinant growth hormone (pamidronate + rhGH). All patients treated with bisphosphonates showed a decreased fracture rate and increased activity (*3*).

As shown in the studies mentioned before, the rate of patients starting treatment with bisphosphonates in our hospital is in line with the international literature. However, we can observe that the main problem is related to its continuity, since around 50% of our patients do not complete the year of treatment for the reasons mentioned above. Furthermore, the follow-up of patients must be improved, given that about 50% of our patients do not have control tests such as bone densitometry or bone resorption markers. In our hospital, compared to others abroad, we used alendronate more frequently due to its availability, although in recent years, zoledronate has been used in most patients.

In the bivariate analyzes it was observed that there is no significant difference with respect to the difference of the first and last value of the serological markers, with respect to the degree and severity scale mentioned above. On the other hand, we have managed to establish in our population that if bone densitometry remains the same or decreases over time, it means a greater risk of presenting new fractures. Likewise, we determine which patients have a favorable and unfavorable evolution based on bone densitometry and the average number of fractures. We also observed a higher median number of fractures in patients with moderate severity according to the Van Dijk severity grade (p = 0.0003). It should be emphasized that such analyzes have not been previously reported in similar OI studies, although these differences are likely due to sample size.

Although the Aglan scale showed differences in birth anthropometry based on the severity of OI, these findings could be influenced by variations in the frequency of occurrence by sex.

About the clinical manifestations (human phenotype), a frequency analysis was carried out based on the Aglan severity scale from moderate to severe, where a greater predominance of umbilical hernia, scoliosis and clinodactyly was observed. Conversely, in this same group of patients, hearing loss, microcephaly and hypermobility are less common. It should be emphasized that these types of analyzes have not been done before in similar OI studies.

Among the limitations we had a small sample, although it is the study with the largest number of participants with OI in Peru. Furthermore, data collection was based exclusively on clinical records (loss of information), and since this is a national reference center, the concentration of cases in a single center could induce a selection bias, therefore the patients treated in this institute may not be representative of all patients with osteogenesis imperfecta in Peru. Although, being one of the few reference centers, the results let us estimate the characteristics of patients with OI in Peru.

Among the limitations, it is important to mention that not all patients had adequate follow-up, some even had only one consult, and this may cause a lack of data on the variables and the analysis of the treatment response may not be precise. On the other hand, this is a small sample, despite being the study with the largest number of participants with OI in Peru. Furthermore, data collection was based exclusively on clinical records (loss of information), and since the institution is a national reference center, the concentration of cases in a single center could introduce a selection bias, since the patients treated in this institute may not be representative of all patients with osteogenesis imperfecta in Peru. Although, being one of the few reference centers, it could show what happens in patients with OI, especially those with severe OI.

In conclusion, the present study represents a significant advance in the understanding of OI in the pediatric population of Peru. When compared with international studies, some similarities were observed in the degrees of severity, clinical and therapeutic characteristics, but also differences in prevalence, proportion in men, and family history. The use of tools such as the Van Dijk severity grade and the Aglan severity scale is recommended since they are easy to apply and will help improve the classification of patients with a higher risk of fractures and response to treatment. It is necessary to ensure the care of these patients, the monitoring and evaluation of the use of bisphosphonates as the first line of treatment in all patients, given that encouraging results in the reduction of fractures and improvement of quality of life have been reported in other countries.

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References

1. Caudevilla Lafuente P, Labarta Aizpún JI, Garagorri Otero JM. Osteogénesis imperfecta. Patrones clínicos y genéticos. Revisión de nuestra experiencia. Zaragoza: Universidad de Zaragoza; 2013. *https://zaguan.unizar.es/record/12044/files/TAZ-TFM-2013-712.pdf* (accessed October 6, 2024). (in Spanish)
2. Marini JC, Forlino A, Bächinger HP, Bishop NJ, Byers PH, De Paepe A, Fassier F, Fratzl-Zelman N, Kozloff K, Krakow D, Montpetit K, Semler O. Osteogenesis imperfecta. Nat Rev Dis Primers. 2017; 3:17052.
3. Marom R, Rabenhorst BM, Morello R. Osteogenesis imperfecta: an update on clinical features and therapies. Eur J Endocrinol. 2020; 183:R95-R106.
4. Hoyer-Kuhn H, Netzer C, Semler O. Osteogenesis imperfecta: pathophysiology and treatment. Wien Med Wochenschr. 2015; 165:278-284.

5. Sarmiento J, Rojas J, Wandurraga E, Parra G, Sarmiento J. Osteogénesis imperfecta (OI) en una mujer adulta y su hija: reporte de caso. Revista Colombiana de Endocrinología, Diabetes & Metabolismo. 2016; 3:37-40.
6. Lizaraso F, Fujita R. Enfermedades Raras o Huérfanas, en Perú más huérfanas que raras. Horizonte Médico (Lima). 2018; 18:4-5.
7. Guio H, Poterico JA, Levano KS, *et al*. Genetics and genomics in Peru: Clinical and research perspective. Mol Genet Genomic Med. 2018; 6:873-886.
8. Trejo P, Rauch F. Osteogenesis imperfecta in children and adolescents-new developments in diagnosis and treatment. Osteoporos Int. 2016; 27:3427-3437.
9. Etich J, Leßmeier L, Rehberg M, Sill H, Zaucke F, Netzer C, Semler O. Osteogenesis imperfecta – pathophysiology and therapeutic options. Mol Cell Pediatr. 2020; 7:9.
10. Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A. 2014; 164A:1470-1481.
11. Aglan MS, Hosny L, El-Houssini R, Abdelhadi S, Salem F, ElBanna RAS, Awas SA, Zaki ME, Temtany SA. A scoring system for the assessment of clinical severity in osteogenesis imperfecta. J Child Orthop. 2012; 6:29-35.
12. Hackley L, Merritt L, Osteogenesis imperfecta in the neonate. Adv Neonatal Care. 2008; 8:21-30; quiz 31-2.
13. Condori ER. Osteogénesis imperfecta en niños tratados en el Hospital Nacional Daniel Alcides Carrión entre los años 2007 y 2009 : Callao, Lima-Perú. [Tesis]. 2011. (in Spanish)
14. Martin E, Shapiro JR. Osteogenesis imperfecta: Epidemiology and pathophysiology. Curr Osteoporos Rep. 2007; 5:91-97.
15. Lindahl K, Åström E, Rubin CJ, Grigelioniene G, Malmgren B, Ljunggren Ö, Kindmark A. Genetic epidemiology, prevalence, and genotype-phenotype correlations in the Swedish population with osteogenesis imperfecta. Eur J Hum Genet. 2015; 23:1042-1050.
16. Escobar C, Malveiro D, Salgado A, Santos MI, Lameirão J, Cassiano M. Osteogénese Imperfeita – Experiência do Serviço de Ortopedia do Hospital Dona Estefânia. Acta Médica Portuguesa. 2013; 26:5-11. (in Portuguese)
17. Binh HD, Maasalu K, Dung VC, Ngoc CTB, Hung TT, Nam TV, Nhan LNT, Prans E, Reimann E, Zhytnik L, Kõks S, Märtson A. The clinical features of osteogenesis imperfecta in Vietnam. Int Orthop. 2017; 41:21-29.
18. Caudevilla P, De Arriba A, Izquierdo S, Ferrer M, Medrano M, Labarta JI. Osteogenesis imperfecta: Review of 40 patients. Med Clin (Barc). 2020; 154:512-518.
19. Erbaş İM, İlgün Gürel D, Manav Kabayeğit Z, Koç A, Ünüvar T, Abacı A, Böber E, Anik A. Clinical, genetic characteristics and treatment outcomes of children and adolescents with osteogenesis imperfecta: A two-center experience. Connect Tissue Res. 2022; 63:349-358.
20. Gimeno-Martos S, Pérez-Riera C, Guardiola-Vilarroig S, Cavero-Carbonell C. Epidemiología de la Osteogénesis Imperfecta: una enfermedad rara en la Comunitat Valenciana. Revista Española de Salud Pública. https://enfispo.es/servlet/articulo?codigo=7020816 (accessed October 6, 2024). (in Spanish)
21. Otaify GA, Aglan MS, Ibrahim MM, Elnashar M, El Banna RA, Temtamy SA. Zoledronic acid in children with osteogenesis imperfecta and Bruck syndrome: A 2-year prospective observational study. Osteoporos Int. 2016; 27:81-92.

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Brief Report

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The value of contrast-enhance ultrasound in the diagnosis of hepatic post-transplant lymphoproliferative disease: Four case reports

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SUMMARY Post-transplant lymphoproliferative disease (PTLD) is a rare but life-threatening disease that occurs after organ transplantation. Histopathology is the gold standard for the diagnosis of PTLD. Because of its rarity and atypical symptoms, many patients are misdiagnosed with liver abscess, liver cancer, or missed diagnosis long before pathological diagnosis is obtained, thus delaying treatment. Early and accurate diagnosis, in addition to histopathological examination, is difficult. Contrast-enhanced ultrasound (CEUS) imaging techniques have overwhelming advantages of being safe (noninvasive, radiation-free) and sensitive for evaluating the microcirculation of lesions, thus making them widely used in the diagnosis of hepatic lesions. Unfortunately, there are few reports of CEUS data on hepatic PTLD (HPTLD). This study reported and analyzed four cases of HPTLD in detail, all of which underwent pre-biopsy CEUS examinations and had a complete diagnosis and treatment process. By offering readers comprehensive knowledge of CEUS in the diagnosis of HPTLD, our study aims to help reduce misdiagnoses and missed diagnoses, thereby improving patient treatment and prognosis.

Keywords post-transplant lymphoproliferative disease, transplant liver, contrast-enhanced ultrasound, diagnosis, image

1. Introduction

Post-transplant lymphoproliferative disease (PTLD) develops in only 1–3 % of liver transplant recipients (*1*). However, as PTLD shows characteristics of high malignancy and mortality rates, early diagnosis is expected to be life-saving (*2*). Contrast-enhanced ultrasound (CEUS) has the advantages of non-radiation, non-invasiveness, cost-effectiveness, portability, good repeatability at frequent intervals, and few side effects (*3*). As CEUS has high accuracy in the detection and diagnosis of hepatic lesions, it is recommended as a first-line examination for high-risk populations of HCC according to the Chinese guidelines (*4*) and World Federation for Ultrasound in Medicine & Biology (WFUMB) guidelines (*5*). In particular, microbubbles of CEUS do not metabolize through the liver; therefore, CEUS (even if used continuously and cumulatively) has no hepatotoxicity. From the perspective of liver preservation, CEUS is safer than contrast-enhanced computed tomography (CT) and magnetic resonance (MR) imaging for follow-up of patients undergoing liver transplantation. CEUS should be used as the first-line imaging modality to screen patients for post-orthotopic liver transplantation complications (*6*).

Currently, there are no English articles describing the imaging features of CEUS for hepatic PTLD (HPTLD) after transplant liver. Research, even with a small-sized case report, is important as it provides a reference for the CEUS diagnosis of HPTLD. To date, 1,300 cases of adult liver transplantations have been performed at the First Affiliated Hospital of Xi'an Jiaotong University, ranking among the leading positions in China (*7*). With abundant sources of liver transplantation cases at hand, we searched for HPTLD cases that had undergone CEUS examinations over the past seven years (when CEUS technology was introduced as a routine clinical examination for patients). We hope that this research can provide valuable information and inspiration for doctors/scholars studying PTLD from a pre-operative imaging perspective.

2. Patients and Methods

2.1. Clinical data

Patient data were retrospectively collected from the First Affiliated Hospital of Xi'an Jiaotong University, China. The research period was from January 2017 (Since CEUS began to be performed in our hospital) to June 2024, based on the pathologic diagnoses of HPTLD by searching the hospital's electronic pathology reporting system. From this period, only four patients had a definite histopathological diagnosis of PTLD and underwent pre-biopsy CEUS examinations. We retrospectively collected their general clinical data (sex, age, etiology, medical history, and treatment process, especially the regimen of using immunosuppressants after transplantation), as well as data on laboratory examinations (tumor markers, liver function, *etc*.) from the electronic medical record system.

Written consent was obtained from the selected patients or their immediate families for the publication of this case report and any accompanying images. All data collection and diagnostic and therapeutic procedures were performed in accordance with the principles of the Declaration of Helsinki. This study was approved by the Research Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University (XJTU1AF2023LSK-363) on June 6, 2023.

2.2. Grayscale ultrasound (US) and CEUS examination

A LOGIQ E9 US system (GE Healthcare, Milwaukee, WI, USA), equipped with native tissue harmonic grayscale imaging and CEUS functions, was used. Convex and microconvex transducers with frequencies of 1–6 and 2–5 MHz, respectively, were used. A 2.0–2.5 mL dose of sulfur hexafluoride microbubbles (SonoVue, Bracco, Milan, Italy) was injected into the antecubital vein at 0.2 mL/s *via* a 20-gauge cannula, followed by 5 mL of 0.9% sterile sodium chloride solution. CEUS images were acquired during three contrast phases: arterial phase (AP) (10–20 s to 30–50 s after contrast injection), portal venous phase (PP) (30–50 s to 120 s), and delayed phase (DP) (>120 s, until bubble disappearance).

2.3. Histological diagnosis

Shortly after the CEUS examination, all four patients underwent US-guided percutaneous transhepatic needle biopsy. Hematoxylin-eosin (HE) staining and immunohistochemical examination with cluster of differentiation (CD)19, CD20, and Epstein–Barr virus-encoding region in situ hybridization (EBER-ISH) were performed. Pathologists with more than 10 years of experience in liver pathology reviewed all specimen slices.

3. Results and Discussion

3.1. Clinical data

Baseline patient characteristics, treatment, and prognosis are summarized in Figure 1. All four patients with HPTLD were middle-aged and had no gender differences. All the patients underwent classic orthotopic liver transplantation for cirrhosis. They all have preexisting conditions, such as viral hepatitis or alcohol use, which precipitate end-stage liver disease and are reported to be non-negligible incentives for PTLD (*8*). After liver transplantation, all four patients were treated with continuous immunosuppressive therapy.

The medication regimens are displayed in Supplemental Table S1 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=213*). The lymph system is widely known as a part of the immune system, which explains why immunosuppressive therapy is widely recognized as a critical cause of PTLD. Whole blood tacrolimus (FK506) concentrations were measured regularly to maintain the level between 50 and 80 ng/mL. All patients had persistent fever of unknown origin (body temperature > 39°C) after liver transplantation. The quantitative DNA tests of Epstein-Barr virus (EBV) and cytomegalovirus (CMV) in these patients before liver transplantation were within normal limits. However, EBV infection was confirmed by increased levels of IgG antibodies of NA and VCA against EBV, which are serologic tests most often used for EBV serostatus assignment (*9*). However, except that patients No. 1 and 4 had a high level of EBV DNA quantification (1.01×10^4 copies and 3.17×10^4 copies, respectively), DNA quantification of EBV and Cytomegalovirus (CMV) for two other patients was within the normal range. CMV-IgG was positive in all four patients. A thorough laboratory workup on admission showed that the routine blood, urine, feces, serum glucose levels, electrolytes, liver function, and kidney function were all within normal ranges. Tumor markers, including alpha-fetoprotein, carcinoembryonic antigen, protein induced by vitamin K absence or antagonist-II, and carbohydrate antigens 19-9 were also unremarkable.

Patient 1 had better prognosis. This finding suggests that if HPTLD occurs late, therapy begins when the lesion is small, and chemotherapy is repeated regularly, the patient would enjoy favorable survival. In this setting, an accurate diagnosis at the early onset of HPTLD is of crucial importance.

3.2. Grayscale US and CEUS examination

Three patients had solitary lesions in the hilar part of the liver, whereas one patient had multiple lesions in both lobes of the liver. As US is the first-line routine follow-up examination for patients after liver transplantation, all HPTLD lesions were first detected using US. Early

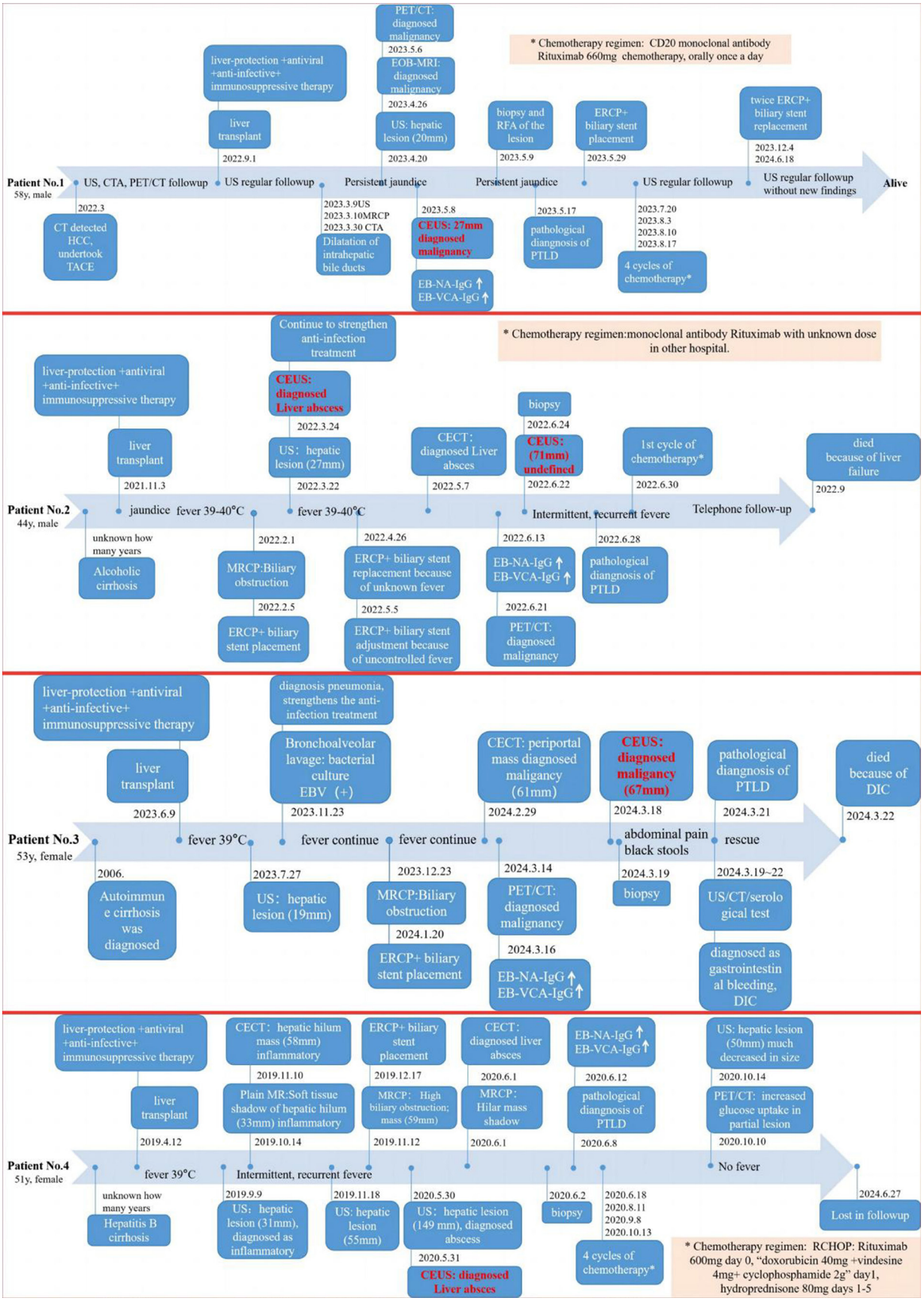

Figure 1. Time-line of diagnosis and treatment of the four patients. Abbreviations PTLD, hepatic post-transplant lymphoproliferative disease; EB(V), Epstein-Barr virus; CMV, Cytomegalovirus; US, ultrasound; CEUS, contrast-enhanced US; CECT, contrast-enhanced computed tomography; MR, magnetic resonance; MRCP, magnetic resonance cholangiopancreatography; ERCP, endoscopic retrograde cholangiopancreatography; PET, positron emission tomography; DIC, disseminated intravascular coagulation; NA, nucleoantigen; VCA, capsid antigen.

lesions usually occur within one year of transplantation, and the first year post-transplant is a risk factor for PTLD (*10*). Consistently, all HPTLD lesions in our study were first detected within one year. From the difference between the size when first discovered and the size when CEUS was performed, it can be seen that the lesions grew rapidly in a short period of time.

The diagnosis of PTLD based on radiological studies, especially at the early stage of the disease course, is thought to be challenging. Positron emission tomography (PET)/CT is recommended for PTLD staging according to guidelines and expert consensus (*9,11*). However, perhaps because PTLD is rare and poorly understood, the best imaging modality for diagnosing it has not yet been determined. Four patients in our study underwent imaging examinations including endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance cholangiopancreatography (MRCP), CECT, PET/CT, and CEUS. However, PET/CT is radioactive, while CECT and MRI have the allergic and nephrotoxic potential of the employed contrast materials (*12*). Even worse, none of the patients in this study made a diagnosis, or even a suspected diagnosis, of PTLD.

Encouragingly, there were some common US and CEUS features in these four cases. As seen in Table 1 and Supplemental Figure S1–S4 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=213*), all these lesions displayed similar appearances as inhomogeneous hypoechic/extremely hypoechoic with clear boundaries and irregular shapes in the US images. The appearance of hypoechic/extremely hypoechoic lesions can be explained by the histopathological features of PTLD. The lesion is characterized by high cellularity and single-cell composition (mature medium-large B cells). Therefore, the difference in US acoustic impedance was small (*13*). All lesions grew in hilar areas. Owing to the rapid growth and volume of the lesions, they gradually develop to closely surround and even compress the hilar biliary tract and blood vessels. This is the reason why patients 1 and 2 presented with jaundice, and patient 4 had severe life-threatening post-puncture bleeding complications.

As shown in Table 2 and Supplemental Figure S1–S4 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=213*), on CEUS, the mass was enhanced earlier than that in the surrounding tissues. In contrast to hepatocellular carcinoma or hepatic hemangioma, there are intratumoral perfusion defects in AP that are independent of lesion size; in other words, even small lesions (No.1 patient and the first CEUS of No. 2 patient) have perfusion defects. The clearance of contrast microbubbles was rapid and occurred during the late AP phase. These characteristics may play a role in the diagnosis of common hepatocellular carcinoma and other benign lesions. There was no significant change in the lesion range after CEUS compared with grayscale US, which was different from that of inflammatory/abscess lesions. In DP, the contrast with the surrounding

Table 1. US features of four enrolled HPTLD lesions

Patient No.	Size (mm)*	Time interval (US first detection~transplantation)	Location	Intrahepatic biliary tract dilatation	Numbers of lesions	Echogenicity	Tumor border	Homogeneity
No. 1	20	7.6 months	S4	Yes	Solitary	Hypoechoic with peritumoral "halo" sign	Ill-defined	Heterogeneous
No. 2	27	4.6 months	S4	Yes	Solitary	Hypoechioc with internal scattered hyperechoic	same**	same**
No. 3	19	1.6 months	S4	Yes	Solitary	Extremely-hypoechoic	same**	same**
No. 4	31	4.9 months	/	Yes	Multiple	Hypoechioc with internal scattered hyperechoic	same**	same**

*The size measured when first detected by US. **This index is exactly the same as the above row of the same column. HPTLD, hepatic post-transplant lymphoproliferative disease; US, ultrasound; S4, segmental 4.

Table 2. CEUS features of four enrolled HPTLD lesions[1]

Patient No.	Size (mm)*	Time interval (CEUS~transplantation)	Perfusion level in AP	Wash-in perfusion pattern in AP	Perfusion level in PP	Perfusion level in DP	Homogeneity	Tumor border	Vascular floating sign
No. 1	37	8.2 months	Local hyperenhancement	Peritumoral ring-like	Hypoenhanced	Hypoenhanced	Heterogeneous	Ill-defined	Absence
No. 2	71	4.6 and 7.6 months**	same***	Peritumoral ring-like +internal sparse scattered dot-like	same***	same***	same***	same***	Presence in the 2nd CEUS
No. 3	67	9.3 months	same***	same***	same***	same***	same***	same***	Presence
No. 4	149	13.7 months	same***	same***	same***	same***	same***	same***	Presence

*The size measured when undertaken CEUS examination. **This patient has taken CEUS examination twice at different time. ***This index is exactly the same as the above row of the same column. HPTLD, hepatic post-transplant lymphoproliferative disease; CEUS, contrast-enhanced ultrasound; AP, arterial phase; PP, portal venous phase; DP, delayed phase.

tissue is very strong and seems to show the sign of "black hole" as metastatic carcinoma.

Deep explored the CEUS images of four patients and reviewed the related literature, and we concluded two typical characteristics of HPTLD. The first is the wash-in perfusion pattern of the microbubbles in the AP. All lesions in the four patients showed peritumoral ring-like perfusion, as described above. Three of these exhibited internal sparse-scattered dot-like enhancement. Peritumoral ring-like enhancement is not well demarcated and has been detected in some cases of liver lymphoma. This marginal enhancement in AP was attributed to vasculitis due to involvement of the liver parenchyma adjacent to the lesion (*14*). This may explain why the lesions were misdiagnosed as inflammatory/abscess. The few areas of intratumoral enhancement in the AP are consistent with the fact that, based on cellular morphology and tissue of origin, lymphoid neoplasms are classified as "round cell neoplasia" rather than "mesenchymal neoplasia" and "epithelial neoplasia" (*15*); thus, they rarely invade the blood vessels. In agreement with our study, under the microscope, HPTLD lesions showed dense and tightly arranged large B cells and lacked vascular endothelial cells. It is worth noting that the second feature is "vascular floating sign" or "vessel penetration sign", which was reported as a specific characteristic for the diagnosis of hepatic lymphoma by CECT (*16*) or color doppler US imaging (*17*). The "vascular floating sign" is a blood vessel passing through the tumor without signs of stenosis or invasion in the blood vessel itself (*18*). The cause of the "vascular floating sign" in the images may be that extranodal lymphoma originates from the interstitium of the organs, which infiltrates and grows along the stroma (rather than the blood vessels). Therefore, the original vascular anatomical structure of the organ remains intact (*19*). In patients No. 2 (2nd CEUS), No.3, and No.4, the vessels walked through the lesion completely, naturally, and without invasion. The reason why the lesions of No.1 patient and the 1st CEUS for No.2 patient did not manifest this phenomenon might be that the lesions were at an early stage and had a relatively small size (patient 1:37 mm, patient 2:27 mm). At that time, the lesion had not grown to the extent surrounding the blood vessels.

We regrettably found that there is still some appearance of CEUS for our HPTLD that cannot be well explained. The first is the anechoic "halo" sign surrounding the lesion in No. 1 case, which has not been reported in any published studies. Secondly, compared with the surrounding liver tissue, all these four cases exhibited the rapid "wash in" and "wash-out" phenomenon, even though perfusion was sparse. This "rapid wash-in and wash-out" phenomenon is usually explained by the low resistance of blood flow and tumor neovascularization (*20*). However, the specific reason for this phenomenon from the perspective of HPTLD formation and progression cannot be clearly explained.

3.3. Histological diagnosis

As shown in Supplemental Figure S1–S4 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=213*), hematoxylin-eosin (HE) staining showed diffuse infiltration of dysplastic lymphocytes (most medium-large B cells), with a large nucleus, scant cytoplasm, and abundant mitoses. The routine immunohistochemical markers for diagnosing a lymphocyte tumor are cytoplasmic CD19 and CD20, which appear to be focal or diffusely positive for staining of the cytoplasm of larger lymphoid cells. EBER-ISH further classifies lymphocyte tumors as a source of EBV-positive large B-cell lymphoma. Both HE staining and CD series immunohistochemistry staining only detected large and medium-sized B cells with dysplasia and relatively simple shapes, and no plasma cells, monocytes, or T cells were found. Therefore, these findings consistently support the diagnosis of PTLD with clear categories of monomorphic B cells. According to the literature, the most common biopsy proved that the histopathological type and subtype are monomorphic and diffuse large B-cell lymphomas, respectively (*21*). After solid organ transplantation, EBV-specific cytotoxic T cells may be completely lost within 6 months of transplantation, which induces the proliferation of latently infected B cells and results in PTLD.

In conclusion, we found that the four cases in this study had some common characteristics in the diagnosis of HPTLD. This is the first English study to summarize the CEUS features of HPTLD after liver transplant. The typical features of CEUS that suggest the possibility of HPTLD are "vascular floating signs" and wash-in perfusion patterns in the AP. In particular, most CEUS findings of HPTLD found in our study can be reasonably explained from the perspective of the histopathology of lymphoma development. Nevertheless, further case studies are needed to confirm whether these imaging findings are unique to the diagnosis of HPTLD. In summary, we recommend CEUS as the preferred modality for the preoperative diagnosis of HPTLD.

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References

1. Camacho JC, Moreno CC, Harri PA, Aguirre DA, Torres WE, Mittal PK. Posttransplantation lymphoproliferative disease: Proposed imaging classification. Radiographics. 2014; 34:2025-2038.
2. Scarsbrook AF, Warakaulle DR, Dattani M, Traill Z. Post-transplantation lymphoproliferative disorder: The spectrum of imaging appearances. Clin Radiol. 2005; 60:47-55.
3. Wang F, Numata K, Nihonmatsu H, Okada M, Maeda S. Application of new ultrasound techniques for focal liver lesions. J Med Ultrason (2001). 2020; 47:215-237.
4. Zhou J, Sun H, Wang Z, *et al*. Guidelines for the diagnosis and treatment of primary liver cancer (2022 Edition). Liver Cancer. 2023; 12:405-444.
5. Dietrich CF, Nolsoe CP, Barr RG, *et al*. Guidelines and good clinical practice recommendations for contrast-enhanced ultrasound (CEUS) in the liver-update 2020 WFUMB in cooperation with EFSUMB, AFSUMB, AIUM, and FLAUS. Ultrasound Med Biol. 2020; 46:2579-2604.
6. Girometti R, Como G, Bazzocchi M, Zuiani C. Post-operative imaging in liver transplantation: State-of-the-art and future perspectives. World J Gastroenterol. 2014; 20:6180-6200.
7. The introduction of the First Affiliated Hospital of Xi'an Jiaotong Universtiy. *http://www.en.jdyfy.com/About_Us/Introduction.htm* (accessed on July 5, 2024).
8. Brookmeyer CE, Bhatt S, Fishman EK, Sheth S. Multimodality imaging after liver transplant: Top 10 important complications. Radiographics. 2022; 42:702-721.
9. Allen UD, Preiksaitis JK. Post-transplant lymphoproliferative disorders, Epstein-Barr virus infection, and disease in solid organ transplantation: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019; 33:e13652.
10. Kamdar KY, Rooney CM, Heslop HE. Posttransplant lymphoproliferative disease following liver transplantation. Curr Opin Organ Transplant. 2011; 16:274-280.
11. Shah N, Eyre TA, Tucker D, *et al*. Front-line management of post-transplantation lymphoproliferative disorder in adult solid organ recipient patients - A British Society for Haematology Guideline. Br J Haematol. 2021; 193: 727-740.
12. Chen W, Li J, Fan X, Zhang Y, Wang L, Liu Y, Cui A, Wang L. Application of contrast-enhanced ultrasound in the diagnosis of post-transplant lymphoproliferative disease after hematopoietic stem cell transplantation: A case report. Medicine (Baltimore). 2021; 100:e24047.
13. Qiao X, Chen K, Chen G, Xue L, Cheng G, Ding H. Hepatic reactive lymphoid hyperplasia and primary hepatic lymphoma: Ultrasound features and differentiation diagnosis. Advanced Ultrasound in Diagnosis and Therapy. 2021; 5:63-72.
14. Maher MM, McDermott SR, Fenlon HM, Conroy D, O'Keane JC, Carney DN, Stack JP. Imaging of primary non-Hodgkin's lymphoma of the liver. Clin Radiol. 2001; 56:295-301.
15. Barger A. Cytology of neoplasia: an essential component of diagnosis. Today's Veterinary Practice. 2012; 12-18.
16. Rajesh S, Bansal K, Sureka B, Patidar Y, Bihari C, Arora A. The imaging conundrum of hepatic lymphoma revisited. Insights Imaging. 2015; 6:679-692.
17. Taiji R, Marugami N, Marugami A, Itoh T, Shimizu S, Nakano R, Hoda Y, Kunichika H, Tachiiri T, Minamiguchi K, Yamauchi S, Tanaka T. Multimodality imaging of primary hepatic lymphoma: A case report and a literature review. Diagnostics (Basel). 2024; 14:306.
18. Hamada K, Yamamoto S, Seno H. Primary hepatic mucosa-associated lymphoid tissue lymphoma in a patient with primary biliary cholangitis. Clin Gastroenterol Hepatol. 2022; 20:e352-e353.
19. Rong X, Cai J, Dong J, Liu Y, Ren H. CT and MR imaging characteristics of 11 cases with primary liver lymphoma. Chinese Hepatolgy. 2021; 26:296-298. (in Chinese)
20. Kang MK, Kim MY, Kang SH, Baik SK. Contrast-enhanced ultrasonography: The third modality for differentiation of liver mass. J Liver Cancer. 2019; 19:91-96.
21. Haider MZ, Zamani Z, Shahid H, Taqi M, Ahmed Z, Mirza HM, Irfan A, Sami KN, Kumar D, Kiran F, Abdullah SM, Anwer F. Post-transplant lymphoproliferative disorder after liver transplant: A systematic review. Blood. 2020; 136 (Supplement I): 34-35.

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Extrachromosomal DNA: Molecular perspectives in aging and neurodegenerative diseases

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SUMMARY Extrachromosomal DNA (ecDNA) refers to a class of circular, non-chromosomal DNA that has recently gained widespread attention due to its potential role in aging and neurodegenerative diseases. The generation of ecDNA is closely associated with processes such as double-strand breaks, micronuclei formation, and the breakage-fusion-bridge (BFB) cycle, all of which are integral to regulation of gene expression, genetic stability, and clonal evolution. In neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease, the aberrant formation of ecDNA is closely linked to defects in DNA repair, alterations in synaptic plasticity, and neuronal dysfunction. The distinct distribution and functional roles of ecDNA in these conditions make it a potential diagnostic biomarker and therapeutic target. This review provides an overview of the mechanisms underlying ecDNA formation and its functions in the nervous system. Additionally, it explores the clinical potential of ecDNA in disease diagnosis, targeted therapy, and personalized medicine, offering new insights for future research and treatment strategies.

Keywords extrachromosomal DNA (ecDNA), aging, neurodegenerative diseases, DNA repair, micronuclei, breakage-fusion-bridge cycle

1. Introduction

With the advancement of life sciences research, extrachromosomal DNA (ecDNA) has emerged as an important biological phenomenon that is attracting increasing attention. From normal physiological activities to pathological conditions, ecDNA plays a critical role in regulating gene expression, maintaining cellular genetic stability, and influencing disease progression. Recent studies on the role of ecDNA in aging and neurodegenerative diseases have provided new insights into the understanding of these complex diseases.

2. Biological characteristics and mechanisms of ecDNA formation

ecDNA refers to circular DNA molecules that originate from chromosomes and lack centromeres and telomeres. These DNA fragments are generated through various mechanisms, including double-strand DNA breaks, asymmetric chromosome segregation, micronuclei formation, and the breakage-fusion-bridge (BFB) cycle (*1-3*). Studies have shown that ecDNA generation significantly increases under stress or during repair of DNA damage (*4*). For example, drug-induced stress, such as methotrexate treatment, can trigger the amplification of the *DHFR* gene in the form of ecDNA, thereby enhancing cellular resistance to the drug (*5*).

ecDNA is typically distributed unevenly within subcellular compartments, a feature that exacerbates genetic diversity between cell clones and provides a selective advantage for cellular evolution (*6*). In tumors, ecDNA containing oncogenes such as *MYC*, *EGFR*, and *HER2* has been closely linked to tumor progression (*7,8*). However, ecDNA is not limited to cancer cells. Small polydispersed circular DNAs (spcDNA) have also been found in normal tissues, such as muscle and blood, although their functions remain largely unexplored (*9,10*).

The formation of ecDNA within cells is closely associated with DNA repair mechanisms. Studies have shown that double-strand breaks (DSBs) are one of the primary triggers for ecDNA formation (*11*). Under stresses such as chemotherapy or radiation exposure, non-homologous end joining and microhomology-mediated end joining (MMEJ) DNA repair mechanisms may incorrectly stitch together DNA fragments, resulting in circular structures (*12-14*). Additionally, micronuclei formation and the BFB cycle are also key mechanisms in

ecDNA generation. In micronuclei, residual chromosome fragments may transform into ecDNA due to replication delays or chromatin breaks.

In normal cells, ecDNA, such as spcDNA and t-circles, primarily originates from repetitive sequences and may play a role in regulating genomic stability and maintaining telomere integrity (*9,15*). In cancer cells, however, ecDNA is typically larger and contains oncogenes or enhancer elements. Studies have shown that the amplification of genes such as *MYC* and *HER2* is closely associated with the malignancy of tumors (*16,17*). Due to their circular structure, ecDNAs exhibit higher gene expression activity and genetic instability, which provide cells with an adaptive advantage in response to the microenvironment (*18*).

3. EcDNA and genetic stability in aging

Aging is a biological process closely associated with the accumulation of DNA damage (*19*). Studies have shown that, as individuals age, the capacity for DNA repair gradually declines, while DNA damage, and DSBs in particular, increases significantly (*20*). This damage may lead to the formation of ecDNA through inaccurate repair pathways and can also impact chromosomal stability. Extrachromosomal ribosomal DNA circles (ERCs), which were first discovered in model organisms like yeast, have been directly linked to the aging process (*21,22*). In aging yeast cells, ERCs accumulate in large quantities and accelerate the aging process by disrupting cellular metabolism and gene expression (*23*).

In human neurons, the generation of DSBs is considered a normal physiological process that is involved in the expression of early response genes (ERGs) (*24*). With advancing age, however, the DNA repair capacity of neurons declines, and the generation of ecDNA may have profound effects on neuronal function. Research has shown that small ecDNAs containing regulatory gene fragments play a role in modulating gene expression and epigenetic modifications. This may help explain the significance of ecDNA in neural plasticity.

4. Role of ecDNA in neurodegenerative diseases

In neurodegenerative diseases, the accumulation of DNA damage and the impairment of DNA repair are considered to be key factors in disease pathogenesis. Aberrant generation of ecDNA has been linked to the onset and progression of diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD).

4.1. AD

In patients with AD, elevated levels of markers of DNA damage such as γH2AX have been observed in neurons and glial cells, suggesting that dysfunctional DNA repair plays a critical role in disease progression (*25*). Studies have shown that β-amyloid can inhibit DNA-PK-dependent non-homologous end joining (NHEJ) repair, leading to the accumulation of DNA damage (*26*). The generation of ecDNA in this context may exacerbate neuronal dysfunction by affecting gene expression or epigenetic modifications. Additionally, AD mouse models have revealed increased ecDNA formation after exposure to a new environment, but these DNA fragments are poorly repaired, suggesting that ecDNA could serve as a novel marker of defects in DNA repair (*27*).

4.2. PD

In PD, key proteins, including α-synuclein, are closely linked to DNA damage and ecDNA formation (*28*). Overexpression of α-synuclein has been found to induce both single-strand and double-strand DNA breaks and to interfere with the repair of DSBs (*29*). EcDNA detected in patients with PD may contain gene sequences that regulate synaptic plasticity, thereby impacting the functional stability of dopaminergic neurons.

4.3. HD

In HD, the mutant huntingtin protein (mHTT) impairs NHEJ repair, resulting in the accumulation of DSBs in primary neurons and increased formation of ecDNA (*30-32*). Studies have shown that mHTT interacts with the Ku70 protein, hindering the repair of DSBs and subsequently increasing the amount of ecDNA (*32*). These ecDNA fragments may further disrupt gene expression networks, contributing to neuronal degeneration.

5. Clinical and research implications

The role of ecDNA in aging and neurodegenerative diseases opens new avenues for disease diagnosis and treatment. First, the specific sequences and structures of ecDNA can serve as molecular biomarkers for early disease diagnosis (*33,34*). Monitoring the abnormal presence of ecDNA in the brain tissue of patients with AD may provide insights into disease progression, helping to inform personalized treatment strategies. Moreover, interventions aimed at modulating ecDNA formation or clearance or influencing the regulation of gene expression associated with ecDNA, could represent novel therapeutic approaches. Studies have shown that targeted deletion of ecDNA carrying oncogenes can reduce cancer invasiveness, and this strategy may potentially be adopted to regulate abnormal gene expression in neurodegenerative diseases.

Furthermore, drugs that enhance DNA repair capacity

may protect neurons from aging and degenerative damage by reducing ecDNA formation (*35,36*). NAD+ supplementation strategies have been found to improve DNA repair and delay disease progression in mouse models of AD and PD (*27*). Use of next-generation sequencing (NGS) technology to analyze patient-specific ecDNA profiles can assist in formulating personalized treatment plans (*37*). Additionally, ecDNA sequencing can provide valuable data with which to understand the molecular characteristics of various diseases (*38*).

Finally, researchers can, through use of CRISPR technology to precisely simulate the mechanisms of ecDNA formation, better construct cell or animal models of human diseases, thereby advancing drug development (*11*). The progress of these studies and technologies not only provides new tools for functional research on ecDNA but also opens new avenues for the diagnosis and treatment of neurodegenerative diseases.

6. Future directions

Despite significant progress in ecDNA research over the past years, many unanswered questions remain. How does ecDNA precisely regulate neural plasticity? Which types of ecDNA are critical for neuronal function? What are the mechanisms of interaction between ecDNA and gene expression regulation in disease states? Future research could further integrate single-cell sequencing, CRISPR/Cas9 technology, and high-resolution microscopy to explore the mechanisms of ecDNA formation and its functions in the nervous system. In neurodegenerative diseases in particular, understanding the biological role of ecDNA will provide new insights into the molecular pathogenesis and potential therapeutic strategies.

7. Conclusion

As a unique molecular phenomenon, ecDNA bridges the complex relationships between DNA damage, repair, gene amplification, and cellular function. In aging and neurodegenerative diseases, the generation and accumulation of ecDNA may be key drivers of pathological processes. Gaining a deeper understanding of the mechanisms and functions of ecDNA may offer valuable insights to improve human health.

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References

1. Holland AJ, Cleveland DW. Chromoanagenesis and cancer: Mechanisms and consequences of localized, complex chromosomal rearrangements. Nat Med. 2012; 18:1630-1638.
2. Tanaka H, Watanabe T. Mechanisms underlying recurrent genomic amplification in human cancers. Trends Cancer. 2020; 6:462-477.
3. Vukovic B, Beheshti B, Park P, Lim G, Bayani J, Zielenska M, Squire JA. Correlating breakage-fusion-bridge events with the overall chromosomal instability and *in vitro* karyotype evolution in prostate cancer. Cytogenet Genome Res. 2007; 116:1-11.
4. Zuo S, Yi Y, Wang C, Li X, Zhou M, Peng Q, Zhou J, Yang Y, He Q. Extrachromosomal circular DNA (eccDNA): From chaos to function. Front Cell Dev Biol. 2021; 9:792555.
5. Goker E, Waltham M, Kheradpour A, Trippett T, Mazumdar M, Elisseyeff Y, Schnieders B, Steinherz P, Tan C, Berman E, *et al*. Amplification of the dihydrofolate reductase gene is a mechanism of acquired resistance to methotrexate in patients with acute lymphoblastic leukemia and is correlated with p53 gene mutations. Blood. 1995; 86:677-684.
6. Crasta K, Ganem NJ, Dagher R, Lantermann AB, Ivanova EV, Pan Y, Nezi L, Protopopov A, Chowdhury D, Pellman D. DNA breaks and chromosome pulverization from errors in mitosis. Nature. 2012; 482:53-58.
7. Albertson DG. Gene amplification in cancer. Trends Genet. 2006; 22:447-455.
8. Von Hoff DD, McGill JR, Forseth BJ, Davidson KK, Bradley TP, Van Devanter DR, Wahl GM. Elimination of extrachromosomally amplified MYC genes from human tumor cells reduces their tumorigenicity. Proc Natl Acad Sci U S A. 1992; 89:8165-8169.
9. Wang T, Zhang H, Zhou Y, Shi J. Extrachromosomal circular DNA: A new potential role in cancer progression. J Transl Med. 2021; 19:257.
10. Cohen S, Regev A, Lavi S. Small polydispersed circular DNA (spcDNA) in human cells: Association with genomic instability. Oncogene. 1997; 14:977-985.
11. Oobatake Y, Shimizu N. Double-strand breakage in the extrachromosomal double minutes triggers their aggregation in the nucleus, micronucleation, and morphological transformation. Genes Chromosomes Cancer. 2020; 59:133-143.
12. Horowitz MP, Milanese C, Di Maio R, Hu X, Montero LM, Sanders LH, Tapias V, Sepe S, van Cappellen WA, Burton EA, Greenamyre JT, Mastroberardino PG. Single-cell redox imaging demonstrates a distinctive response of dopaminergic neurons to oxidative insults. Antioxid Redox Signal. 2011; 15:855-871.
13. Fishel ML, Vasko MR, Kelley MR. DNA repair in neurons: So if they don't divide what's to repair? Mutat Res. 2007; 614:24-36.
14. Paulsen T, Kumar P, Koseoglu MM, Dutta A. Discoveries of extrachromosomal circles of DNA in normal and tumor cells. Trends Genet. 2018; 34:270-278.
15. Basenko EY, Cesare AJ, Iyer S, Griffith JD, McEachern MJ. Telomeric circles are abundant in the stn1-M1 mutant that maintains its telomeres through recombination. Nucleic Acids Res. 2010; 38:182-189.
16. Storlazzi CT, Fioretos T, Surace C, *et al*. MYC-containing

double minutes in hematologic malignancies: Evidence in favor of the episome model and exclusion of MYC as the target gene. Hum Mol Genet. 2006; 15:933-942.
17. Savelyeva L, Schwab M. Amplification of oncogenes revisited: From expression profiling to clinical application. Cancer Lett. 2001; 167:115-123.
18. Turner KM, Deshpande V, Beyter D, *et al*. Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. Nature. 2017; 543:122-125.
19. Chen Y, Geng A, Zhang W, Qian Z, Wan X, Jiang Y, Mao Z. Fight to the bitter end: DNA repair and aging. Ageing Res Rev. 2020; 64:101154.
20. Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, Yankner BA. Gene regulation and DNA damage in the ageing human brain. Nature. 2004; 429:883-891.
21. Hull RM, Houseley J. The adaptive potential of circular DNA accumulation in ageing cells. Curr Genet. 2020; 66:889-894.
22. Sinclair DA, Guarente L. Extrachromosomal rDNA circles--A cause of aging in yeast. Cell. 1997; 91:1033-1042.
23. Hull RM, King M, Pizza G, Krueger F, Vergara X, Houseley J. Transcription-induced formation of extrachromosomal DNA during yeast ageing. PLoS Biol. 2019; 17:e3000471.
24. Madabhushi R, Gao F, Pfenning AR, *et al*. Activity-induced DNA breaks govern the expression of neuronal early-response genes. Cell. 2015; 161:1592-1605.
25. Thompson LH LC. Origin, recognition, signaling and repair of DNA double-strand breaks in mammalian cells. In: Madame Curie Bioscience Database. Austin (TX): Landes Bioscience; 2000-2013. *https://www.ncbi.nlm.nih.gov/books/NBK6555/*
26. Cardinale A, Racaniello M, Saladini S, De Chiara G, Mollinari C, de Stefano MC, Pocchiari M, Garaci E, Merlo D. Sublethal doses of beta-amyloid peptide abrogate DNA-dependent protein kinase activity. J Biol Chem. 2012; 287:2618-2631.
27. Hou Y, Lautrup S, Cordonnier S, Wang Y, Croteau DL, Zavala E, Zhang Y, Moritoh K, O'Connell JF, Baptiste BA, Stevnsner TV, Mattson MP, Bohr VA. NAD^+ supplementation normalizes key Alzheimer's features and DNA damage responses in a new AD mouse model with introduced DNA repair deficiency. Proc Natl Acad Sci U S A. 2018; 115:E1876-E1885.
28. Vasquez V, Mitra J, Hegde PM, Pandey A, Sengupta S, Mitra S, Rao KS, Hegde ML. Chromatin-bound oxidized alpha-synuclein causes strand breaks in neuronal genomes in *in vitro* models of Parkinson's disease. J Alzheimers Dis. 2017; 60:S133-S150.
29. Schaser AJ, Osterberg VR, Dent SE, *et al*. Alpha-synuclein is a DNA binding protein that modulates DNA repair with implications for Lewy body disorders. Sci Rep. 2019; 9:10919.
30. Polidori MC, Mecocci P, Browne SE, Senin U, Beal MF. Oxidative damage to mitochondrial DNA in Huntington's disease parietal cortex. Neurosci Lett. 1999; 272:53-56.
31. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, Bird ED, Beal MF. Oxidative damage and metabolic dysfunction in Huntington's disease: Selective vulnerability of the basal ganglia. Ann Neurol. 1997; 41:646-653.
32. Enokido Y, Tamura T, Ito H, *et al*. Mutant huntingtin impairs Ku70-mediated DNA repair. J Cell Biol. 2010; 189:425-443.
33. Frater JL, Hoover RG, Bernreuter K, Batanian JR. Deletion of MYC and presence of double minutes with MYC amplification in a morphologic acute promyelocytic leukemia-like case lacking RARA rearrangement: Could early exclusion of double-minute chromosomes be a prognostic factor? Cancer Genet Cytogenet. 2006; 166:139-145.
34. Pang J, Nguyen N, Luebeck J, *et al*. Extrachromosomal DNA in HPV-mediated oropharyngeal cancer drives diverse oncogene transcription. Clin Cancer Res. 2021; 27:6772-6786.
35. Wang J, Jacob NK, Ladner KJ, Beg A, Perko JD, Tanner SM, Liyanarachchi S, Fishel R, Guttridge DC. RelA/p65 functions to maintain cellular senescence by regulating genomic stability and DNA repair. EMBO Rep. 2009; 10:1272-1278.
36. Jiang X, Zhu D, Okagaki P, Lipsky R, Wu X, Banaudha K, Mearow K, Strauss KI, Marini AM. N-methyl-D-aspartate and TrkB receptor activation in cerebellar granule cells: An *in vitro* model of preconditioning to stimulate intrinsic survival pathways in neurons. Ann N Y Acad Sci. 2003; 993:134-145; discussion 159-160.
37. Noer JB, Horsdal OK, Xiang X, Luo Y, Regenberg B. Extrachromosomal circular DNA in cancer: History, current knowledge, and methods. Trends Genet. 2022; 38:766-781.
38. Wang Y, Wang M, Zhang Y. Purification, full-length sequencing and genomic origin mapping of eccDNA. Nat Protoc. 2023; 18:683-699.

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PytheasDB: An open-access graphical database of clinical data on rare pediatric digestive diseases

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SUMMARY Advances in genetic testing over the past decades are driving a continuing increase in the diagnosis and reporting of rare genetic diseases, but no tool has yet been developed to aggregate published molecular and phenotypic data, a task that is nevertheless essential to optimize patient care. In this article, we present PytheasDB, an online database of published clinical data from patients with rare digestive diseases. At the time of writing (August 2024), the database contains data from 833 patients with progressive familial intrahepatic cholestasis or trichohepatoenteric syndrome, collected from 172 articles. Users can compare the phenotypic profiles, sex ratios, survival curves, ages at first symptoms, and consanguinity rates of the included diseases. PytheasDB is the first ever online resource providing access to aggregated clinical data from case reports of rare digestive diseases in the literature. The database is currently being expanded to cover ultra-rare pediatric digestive diseases with regular updates to optimize the study and treatment of these diseases.

Keywords PytheasDB, database, rare diseases, PFIC, THE

1. Introduction

The diagnosis of many rare digestive diseases in pediatrics has been revolutionized in recent years by advances in genetic analysis techniques, with next-generation sequencing (NGS) allowing the parallel testing of a panel of genes in a matter of hours. In progressive familial hepatocellular cholestasis (PFIC) for instance, the diagnostic yield of NGS is about 30% (*1*), compared with just a few percent for traditional Sanger sequencing. PFIC is a group of rare disorders, caused by defects of bile secretion or of primary bile acid synthesis. A wide range of genes have been implicated in PFIC since the first genetic cause was identified in *ATP8B1*, in 1998 (*2*), and the NGS panel currently used in clinical practice contains about 50 genes.

As well as being required to establish genotype-phenotype correlations, molecular diagnosis can also inform prognosis and allow personalized treatment. In some cases however, the pathogenicity of identified variants is ambiguous, notably for missense variants, complicating clinical decision making (*3*). The challenge then for clinicians is to analyze molecular results alongside available phenotypic data (clinical, laboratory, radiographic and histological) to make a precise diagnosis and adapt treatments.

This often involves a painstaking literature review because no aggregate resource has yet been developed for these diseases. While the Online Mendelian Inheritance in Man (OMIM) website catalogs published articles by disease, it does not provide classified patient-level clinical data and is not regularly updated. For trichohepatoenteric syndrome (THES) type 1 for instance, the latest of the 13 listed references (as of September 2024) dates from 2018, whereas more than 20 articles have since been published on this topic.

Our recent study of microvillus inclusion disease (*4*) (MVID; *MYO5B* and *STX3* mutations) highlights the value of regrouping published data on rare diseases. Our literature review of published cases (323 patients in 86 articles), clarified the natural history of the disease and revealed a previously overlooked association between *MYO5B* variants and preterm birth, leading to the recommendation that preterm birth and the associated risks should be considered in patient management and may help to better estimate prognosis.

Our aim in developing PytheasDB was therefore to gather, classify and provide access to relevant and regularly updated clinical data from published cases of rare digestive diseases. This resource should save time in clinical practice, facilitate comparisons between diseases and the study of phenotypic patterns and associations,

improving research and patient care.

2. Studied diseases

2.1. Included diseases

This initial version of PytheasDB was built by gathering published data on patients with PFIC or THES, associated with variants in *ATB8B1* (PFIC1), *ABCB11* (PFIC2), *ABCB4* (PFIC3), *TJP2* (PFIC4), *NR1H4* (PFIC5), *SLC51A* (PFIC6), *USP53* (PFIC7), *KIF12* (PFIC8), *ZFYVE19* (PFIC9), *MYO5B* (PFIC10), *SEMA7A* (PFIC11), *SKIC3* (THES1) and/or *SKIC2* (THES2). Searches were conducted in the PubMed database for articles published in English before 1 January 2023, using the gene and associated disease as separate search terms, *i.e.* for PFIC1, either "PFIC 1" or "ATP8B1". Articles that did not report clinical data, literature reviews, and animal studies were excluded. Care was taken to identify patients described in several articles and gather all relevant data while avoiding duplication.

2.2. Studied variables

The studied variables are listed in Supplemental Table S1 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=215*). These were chosen based on our previous work on rare digestive diseases (*4*), and describe patients' genetic (associated variants) and clinical (symptoms, weight and height growth) characteristics.

3. Website

PytheasDB is accessible at *www.pytheasdb.com*. The website is a client-side application, written in Vue JS, with tables and graphics created on the fly from patient data stored in JSON files. Users can analyze patient data for the included diseases/genes, in the form of tables and figures, or compare data between any number of diseases. The graphical representations include bar and pie charts of reported symptoms at different HPO (Human Phenotype Ontology) (*5*) branch levels and Kaplan–Meier survival curves.

Among the 491 articles identified using search terms in PubMed, 172 matched the inclusion criteria, from which the data for 833 patients were extracted, classified and added to PytheasDB (Supplemental Figure S1, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=215*).

The first version of the website only provides information on a subset of accessible variables, namely the phenotypic profile (reported symptoms), sex ratio, age at first symptoms, survival, and consanguinity. Supplemental Table S2 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=215*) lists the number of patients and data points included for each disease, along with a completeness score (the proportion of considered characteristics reported in the articles for each group of patients), devised as a semi-quantitative measure of the completeness of patient descriptions in the literature for each gene/disease.

The number of included patients varies from just 1 for PFIC11 (*SEMA7A*) to 159 for PFIC2 (*ABCB11*). Roughly twice as many patients were included for THES1 (*SKIC3*) as for THES2 (*SKIC2*). The number of included data ranges from 5 for PFIC11 to 622 for PFIC11. The completeness of the datasets range from 47.2% for PFIC4 to 100% for PFIC5–9 and PFIC11, indicating that some reports do not include all the basic characteristics (sex, consanguinity, symptoms, age at first symptoms, survival status) considered here.

Examples of PytheasDB outputs are presented in Figures 1-2 and Supplemental Figures S2-S3 (*https://www.irdrjournal.com/action/getSupplementalData.php?ID=215*). Figure 1 presents the list of symptoms reported for PFIC10 patients (*MYO5B*) analyzed at HPO (*5*) branch level 3 (user adjustable) as bar and pie charts, color-coded by HPO branch level 2 category. Figure S2 compares the sex ratio of patients with PFIC1–5 and Figure S3, the corresponding consanguinity rates. Note that the bars are color-coded by disease (and gene), and the outputs are ordered by the chosen category to facilitate comparisons.

Figure 2 compares the Kaplan–Meier survival curves of patients with THES1 and THES2. The survival rates of the 106 THES1 patients are 71% and 68%, at 5 and 10 years respectively, and for the 54 THES2 patients 84%, both at 5 and 10 years, with a most deaths occurring in the first three years of life.

4. Discussion

PytheasDB is the first ever open-access resource providing aggregate information from the literature on patients with rare pediatric digestive diseases. The database continues to grow and will be updated with data from relevant publications. For these rare but increasingly diagnosed diseases indeed, the number of articles in the literature has been growing regularly (Supplemental Figure S4, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=215*). Fabre *et al.*'s 2018 review of THES (*6*), based on roughly 50 patients, is now obsolete, since PytheasDB already contains data on 179 patients. The aim is to provide easy access to exhaustive up-to-date patient information to clinicians, researchers and the general public, simplifying the utilization of medical literature data. Our results for the survival outcomes for THES1 and THES2 patients are similar to those reported by Caralli *et al.* in their 2021 review of THES (*4*).

The main limitation of this approach is that it relies completely on the quality and completeness of patient

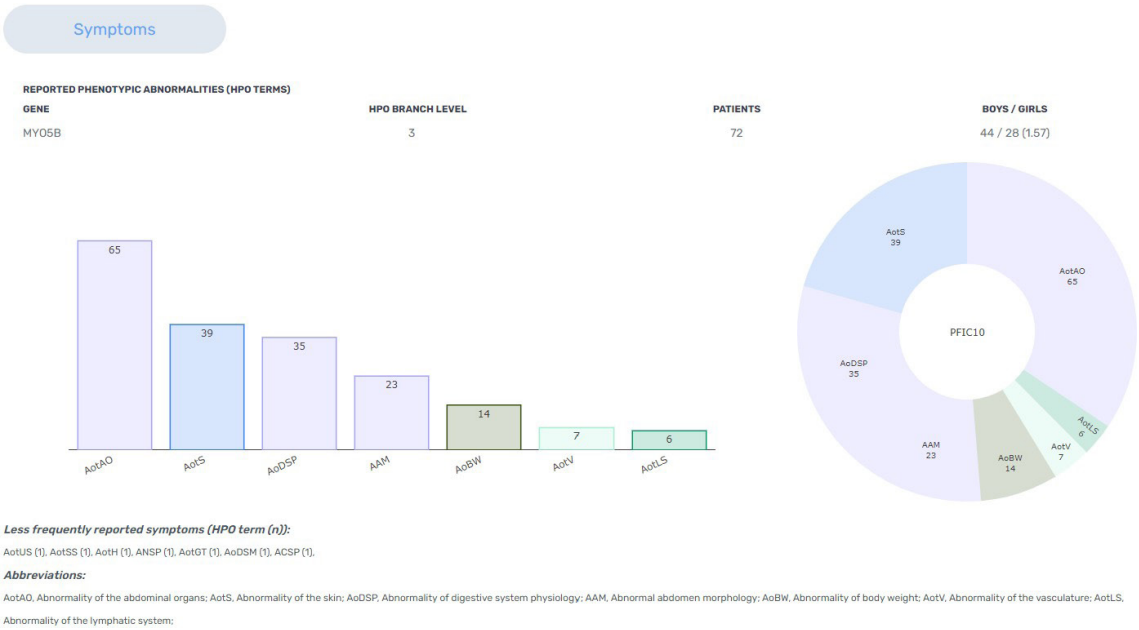

Figure 1. Screenshot of PytheasDB output for PFIC10 symptoms (phenotypic abnormalities reported for PFIC10 patients), classified at HPO (*5*) branch level 3 (user adjustable from 2 to 6). The bars and pie segments are color coded according to the HPO branch level 2 category of the symptom: abnormalities of the digestive system in mauve, abnormalities of the integument in blue, growth abnormalities in olive green; abnormalities of the cardiovascular system in light green, and abnormalities of the immune system in forest green.

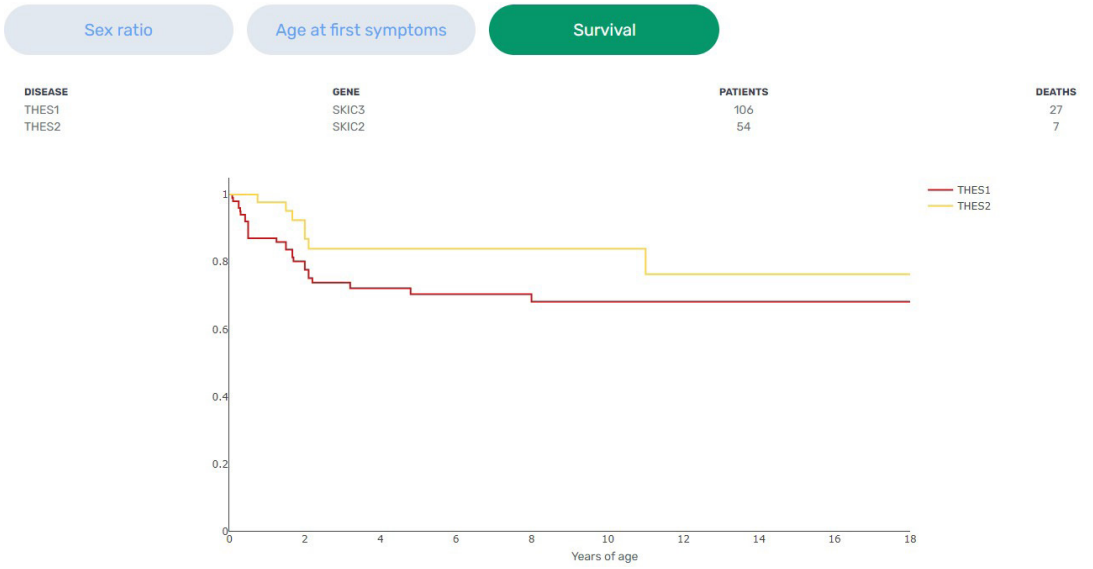

Figure 2. Screenshot of PytheasDB output for the comparison of survival rates between patients with THES1 (red line) and THES2 (yellow).

descriptions in the literature. For the "oldest" diseases for instance, PFIC1–3, first described in the late 1990s, far fewer patients were included than initially expected, as only 79 of the 350 eligible articles were found to report individualized patient characteristics. The absence of individual patient data, as in the results of the ongoing NAPPED study (*7*), reduces the phenotypic resolution of the data. Furthermore, while it is sometimes possible to interpret the non-reporting of a characteristic as absence of this characteristic, this does not generally hold true. In this regard, it is encouraging that according to our estimates of the completeness of data reporting for the different diseases (Supplemental Table S2, *https://www.irdrjournal.com/action/getSupplementalData.php?ID=215*), those that have been described more recently tend to be described in greater detail in the literature. It remains to be seen however whether this reflects an improvement in reporting standards or simply greater diligence in initial case reports.

Another limitation lies in the uniform weighting of patient entries regardless of the level of detail of the descriptions in the original article (*e.g.* subject of an entire paragraph compared with a line in a supplemental table). A more nuanced approach would perhaps be to weight data points according to the number of words devoted to the corresponding patient, thereby assigning greater importance to data from more detailed articles.

Our approach is also limited by the difficulty of distinguishing phenotypes associated with variants in the same gene. For example, mutations in *ATP8B1* and *ABCB11* (PFIC1 and PFIC2) can also cause benign recurrent intrahepatic cholestasis types 1 and 2 (BRIC1 and BRIC2), autosomal recessive disorders at the opposite end of a clinical spectrum ranging from intermittent cholestatic episodes to chronic, progressive cholestasis. It may therefore be interesting to include information on clinical progression to offer a more subtle classification of patients' phenotypes.

In conclusion, this first version of PytheasDB shows that it is possible to aggregate and present clinical data on rare digestive diseases in a way that enriches our understanding of the phenotypic profile and natural history of these disorders and will hopefully contribute to improving patient care. The public availability and regular updating of the data should also stimulate further research. Work is ongoing to expand the database to include additional clinical characteristics, including laboratory findings and treatment types and efficacy, and the long-term aim is to include a range of genes associated with ultra-rare pediatric digestive diseases.

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References

1. Almes M, Spraul A, Ruiz M, *et al.* Targeted-capture next-generation sequencing in diagnosis approach of pediatric cholestasis. Diagnostics (Basel). 2022; 12:1169.
2. Bull LN, van Eijk MJ, Pawlikowska L, DeYoung JA, Juijn JA, Liao M, Klomp LW, Lomri N, Berger R, Scharschmidt BF, Knisely AS, Houwen RH, Freimer NB. A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis. Nat Genet. 1998; 18:219-224.
3. Chen HL, Li HY, Wu JF, Wu SH, Chen HL, Yang YH, Hsu YH, Liou BY, Chang MH, Ni YH. Panel-based next-generation sequencing for the diagnosis of cholestatic genetic liver diseases: Clinical utility and challenges. J Pediatr. 2019; 205:153-159.e6.
4. Caralli M, Roman C, Coste ME, Roquelaure B, Buffat C, Bourgeois P, Badens C, Fabre A. Genetic enteropathies linked to epithelial structural abnormalities and enteroendocrine deficiency: A systematic review. J Pediatr Gastroenterol Nutr. 2021; 72:826-832.
5. Gargano MA, Matentzoglu N, Coleman B, *et al.* The human phenotype ontology in 2024: Phenotypes around the world. Nucleic Acids Res. 2024; 52:D1333-D1346.
6. Fabre A, Bourgeois P, Chaix C, Bertaux K, Goulet O, Badens C. Trichohepatoenteric Syndrome. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews®. Seattle (WA): University of Washington, Seattle, 1993-2024.
7. van Wessel DBE, Gonzales E, Hansen BE, Verkade HJ. Defining the natural history of rare genetic liver diseases: Lessons learned from the NAPPED initiative. Eur J Med Genet. 2021; 64:104245.

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Letter

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Inferior vena cava leiomyosarcoma mimicking an exophytic intrahepatic cholangiocarcinoma

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SUMMARY Inferior vena cava (IVC) leiomyosarcomas are rare smooth muscle neoplasms that account for 0.5% of adult soft tissue sarcomas. They present with nonspecific symptoms and have poor prognosis. We present a case of leiomyosarcoma arising from the retrohepatic IVC that was difficult to diagnose on imaging, showing similar characteristics to intrahepatic cholangiocarcinoma including heterogenous arterial phase enhancement and delayed enhancement on contrast-enhanced magnetic resonance imaging. Important differentiating features of IVC leiomyosarcomas on imaging include dilated IVC, imperceptible IVC lumen, and development of prominent venous collaterals. Despite this, imaging features may be similar to other retrohepatic mass etiologies so IVC leiomyosarcoma should be included in the differential diagnosis of any retrohepatic mass and biopsy should be pursued.

Keywords inferior vena cava leiomyosarcoma, magnetic resonance imaging

Vascular leiomyosarcomas are rare smooth muscle neoplasms of the vessel wall, accounting for 5% of all leiomyosarcomas (*1*). Specifically, primary leiomyosarcoma of the inferior vena cava (IVC) make up 0.5% of adult soft tissue sarcomas (*2,3*). Characteristics of IVC leiomyosarcomas include a female-to-male ratio of 3 to 1, a peak incidence in the sixth decade of life, predilection for arising from the middle segment of the IVC (between the hepatic and renal veins) and extraluminal growth (*3-5*).

Common presenting symptoms including abdominal pain, weight loss, an abdominal mass, nausea, and vomiting are non-specific and can lead to delayed diagnosis (*2,6*). Symptomatology may depend on tumor location. Tumors arising from the upper segment of the IVC (from the right atrium to the hepatic veins) may cause valvular obstruction, lower limb edema, and constrictive pericarditis. Tumors arising from the middle segment may cause Budd-Chiari syndrome. Tumors arising from the lower segment of the IVC (below the renal veins to the iliac confluence) may cause renovascular hypertension due to renal vein obstruction (*2,3,6*).

Ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI) are typically used to diagnose, stage, and surveil IVC leiomyosarcomas. US generally shows a hypoechoic mass with tumoral vascularity. The IVC may demonstrate abnormal flow due to the intraluminal tumor. Lower extremity doppler may show abnormal waveform due to upstream tumoral obstruction. CT typically demonstrates an irregularly distended IVC with an enhancing lobulated soft tissue mass. While it is often difficult to differentiate IVC leiomyosarcomas from other retroperitoneal etiologies, important imaging findings include a dilated IVC and an imperceptible IVC lumen (*7,8*). In contrast, a negative embedded organ sign, where the IVC is narrowed due to extrinsic compression, suggests that the mass does not arise from the IVC (*7*). On MRI, the tumor is typically T1 hypointense, with heterogenous enhancement on post-contrast T1 images. The tumor also demonstrates T2 hyperintensity and diffusion restriction. Additional features include a prominent collateral circulation (*2*). There is typically no internal fat or calcification (*8*).

Prognosis is generally poor, with five-year disease-free survival and overall survival ranging from 6-28% and 34-55%, respectively (*3,5*). Surgical resection remains the mainstay of treatment. Larger tumor size, resection with positive margins, and older patient age are associated with decreased disease-free survival. Isolated involvement of the middle segment of the IVC is associated with increased disease-free survival (*5*). The use of adjuvant and neoadjuvant chemoradiotherapy is not well studied, likely related to tumor rarity.

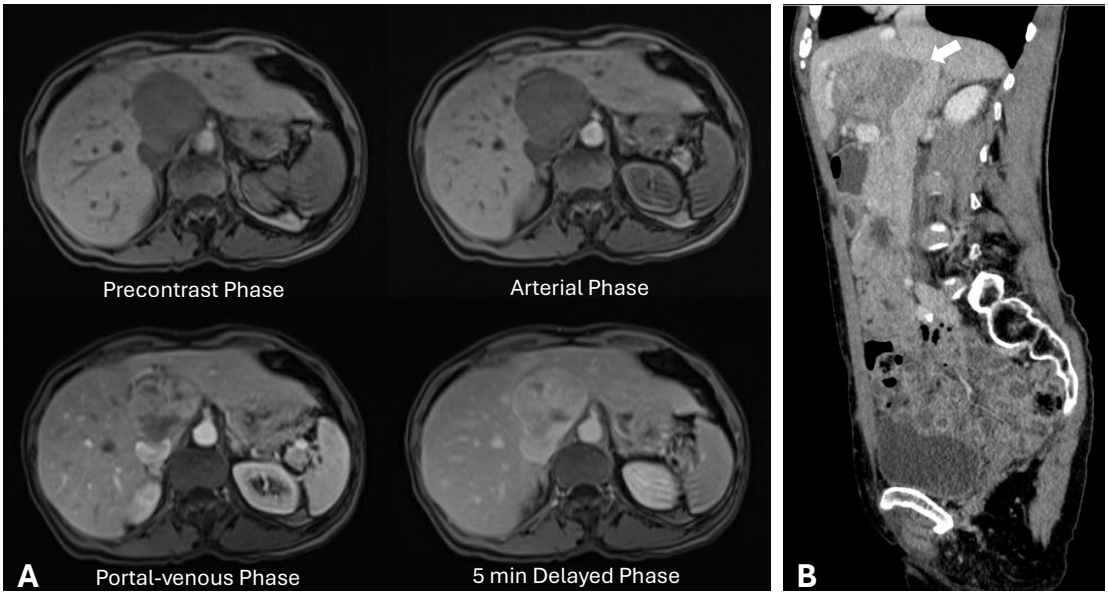

Figure 1. Imaging features of an IVC leiomyosarcoma. (A) Axial T1-weighted pre- and post-gadolinium contrast MRI images demonstrate delayed hyperenhancement of the large retrohepatic mass abutting the IVC; **(B)** Portal-venous phase sagittal CT image shows suspected intraluminal invasion (arrow) along the anterior IVC.

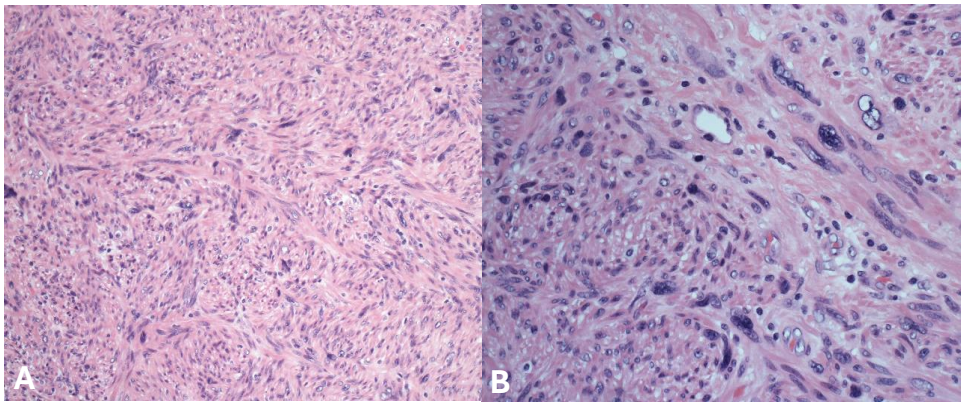

Figure 2. Pathologic characteristics of the IVC leiomyosarcoma. (A) 200×; **(B)** 400×. The sections show infiltration by a malignant neoplasm showing interlacing bundles of atypical smooth muscles with associated markedly atypical spindle cells with moderate amount of eosinophilic cytoplasm and occasional large, bizarre nuclei.

A 68-year-old woman presented with chronic right upper quadrant pain. She reported a 10-pound weight loss in the past year. She had no jaundice, nausea, vomiting or change in abdominal girth. Her laboratory results showed a normal blood count, electrolytes and liver function tests. On US, a heterogeneously echogenic mass with mild internal vascularity was seen and was initially thought to be arising from the left hepatic lobe. She was referred to gastroenterology and repeat blood work demonstrated normal alpha-fetoprotein, cancer antigen 19-9 and hepatitis serology. MRI demonstrated a large mass along the posterior aspect of segment 4A of the liver. There was low-grade heterogeneous arterial enhancement with more progressive hyperenhancement on the portal and delayed phases (Figure 1A). The mass abutted the anterior wall of the intrahepatic IVC with no definite evidence of invasion. The MR findings were non-specific with the main differentials being an exophytic intrahepatic cholangiocarcinoma (ICC) or IVC leiomyosarcoma.

At the time of the proposed US-guided biopsy 1.5 months after initial presentation, the mass was found to move independently from the liver and was intimately associated with the intrahepatic IVC. The biopsy showed an atypical smooth muscle tumor consistent with either a primary hepatic leiomyosarcoma or an atypical smooth muscle tumor from elsewhere. A staging CT could not definitively determine whether the mass was an exophytic hepatic lesion or a mass of extrahepatic origin (Supplemental Figure S1, *https://www.irdrjournal.com/supplementaldata/214*). There was suspicion of intraluminal invasion along the anterior IVC (Figure 1B).

The patient underwent in vivo primary resection of the tumor 2.5 months after initial presentation including excision of a portion of IVC with concurrent primary repair and cholecystectomy. No hepatectomy was

required. Final pathology revealed a FNCLCC grade 2 leiomyosarcoma (Figure 2) with negative margins; however, the distance from the tumor to the closest margin was less than 0.1 cm. The patient received a dose of adjuvant external beam radiation to the surgical bed post-operatively. The patient remained disease-free at the 8-month follow-up CT (Supplemental Figure S2, *https://www.irdrjournal.com/supplementaldata/214*).

In our case, the delayed centipedal enhancement on MRI confounded diagnosis as both sarcomatous tumors and ICC can show delayed hyperenhancement due to abundant fibrous stroma. Although definite intraluminal tumor was not identified on initial imaging, IVC leiomyosarcomas are often predominantly extraluminal. As a result, the diagnosis of IVC leiomyosarcoma must be considered in the differential when evaluating a retrohepatic mass, despite its rarity. Potential differentiating features on imaging between IVC leiomyosarcoma and other retrohepatic masses include dilated IVC, imperceptible IVC lumen, and development of a prominent collateral venous system from IVC obstruction. Any liver lesion with suspicious imaging features should be recommended for biopsy.

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References

1. Matsuyama A, Hisaoka M, Hashimoto H. Vascular leiomyosarcoma: Clinicopathology and immunohistochemistry with special reference to a unique smooth muscle phenotype. Pathol Int. 2010; 60:212-216.
2. Wang MX, Menias CO, Elsherif SB, Segaran N, Ganeshan D. Current update on IVC leiomyosarcoma. Abdom Radiol N Y. 2021; 46:5284-5296.
3. Laskin WB, Fanburg-Smith JC, Burke AP, Kraszewska E, Fetsch JF, Miettinen M. Leiomyosarcoma of the inferior vena cava: Clinicopathologic study of 40 cases. Am J Surg Pathol. 2010; 34:873-881.
4. Mingoli A, Cavallaro A, Sapienza P, Di Marzo L, Feldhaus RJ, Cavallari N. International registry of inferior vena cava leiomyosarcoma: Analysis of a world series on 218 patients. Anticancer Res. 1996; 16:3201-3205.
5. Wachtel H, Gupta M, Bartlett EK, Jackson BM, Kelz RR, Karakousis GC, Fraker DL, Roses RE. Outcomes after resection of leiomyosarcomas of the inferior vena cava: A pooled data analysis of 377 cases. Surg Oncol. 2015; 24:21-27.
6. Das SAP, Paleela PS, Kuppuswamy MK, Venugopal B, Krishnan E. Retrohepatic caval leiomyosarcoma antesitum resection: A case report and a review of literature. J Clin Exp Hepatol. 2021; 11:742-746.
7. Webb EM, Wang ZJ, Westphalen AC, Nakakura EK, Coakley FV, Yeh BM. Can CT features differentiate between inferior vena cava leiomyosarcomas and primary retroperitoneal masses? AJR Am J Roentgenol. 2013; 200:205-209.
8. Bednarova I, Frellesen C, Roman A, Vogl TJ. Case 257: Leiomyosarcoma of the inferior vena cava. Radiology. 2018; 288:901-908.

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Inagaki Y, Tang W, Zhang L, Du GH, Xu WF, Kokudo N. Novel aminopeptidase N (APN/CD13) inhibitor 24F can suppress invasion of hepatocellular carcinoma cells as well as angiogenesis. Biosci Trends. 2010; 4:56-60.

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Darby S, Hill D, Auvinen A, *et al*. Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005; 330:223.

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Shalev AY. Post-traumatic stress disorder: Diagnosis, history and life course. In: Post-traumatic Stress Disorder, Diagnosis, Management and Treatment (Nutt DJ, Davidson JR, Zohar J, eds.). Martin Dunitz, London, UK, 2000; pp. 1-15.

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