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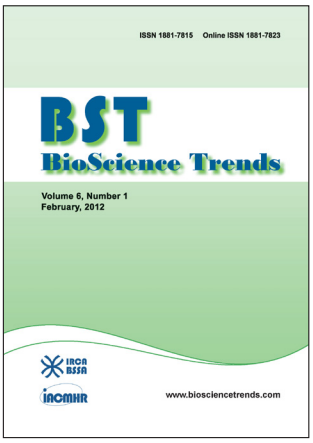

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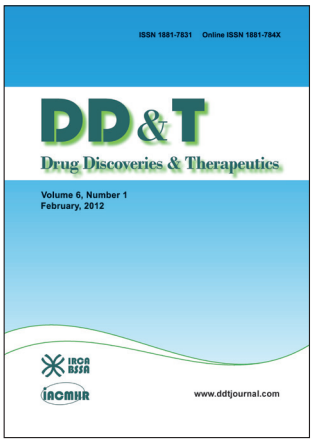

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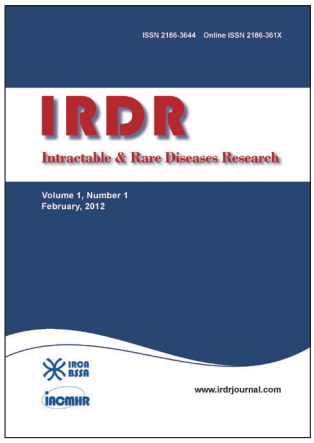

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Comparison of current guidelines and consensus on the management of patients with cholangiocarcinoma: 2022 update

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SUMMARY As a consequence of breakthroughs in the area of guidelines research, the therapy for cholangiocarcinoma has significantly improved the efficacy rate of diagnosis and survival outcomes. We compared the most recently updated clinical practice guidelines and consensus to provide recommendations based on the diagnostic and therapeutic equipment available in various countries. Following a systematic review, we discovered that these guidelines and consensus had both similarities and differences in terms of what organizations or groups drafted the guidelines and the approach, applicability, content and recent updates of the guidelines as well as in terms of diagnostic and treatment algorithms. The disparities could be attributable to a variety of etiological factors, high risk patients, health resources, medical technology, treatment options, and income levels. Additionally, while complete adoption of guidelines may benefit physicians, patients, and authorities, there remains a disconnect between expected goals and implementation.

Keywords cholangiocarcinoma, clinical practice guideline, diagnosis, treatment

1. Introduction

Cholangiocarcinoma (CCA) is a highly lethal, epithelial cell malignant tumor that can be derived from any point of the biliary tree. Due to the heterogeneity of malignancies, they are typically categorized according to primary anatomic subtype (intrahepatic, perihilar, and distal) (*1,2*). Intrahepatic cholangiocarcinoma (iCCA) is located proximally in the second-order bile ducts within the liver parenchyma. Perihilar cholangiocarcinoma (pCCA) arises between the second-order ducts and the insertion of the cystic duct. Distal cholangiocarcinoma (dCCA) is distal to the insertion of the cystic duct (*3,4*). Both pCCA and dCCA occur in the part of the bile duct outside the liver as an extrahepatic cholangiocarcinoma (ECC) (*4*). According to the 2019 WHO classification, mixed hepatocellular-cholangiocarcinoma (cHCC-CCA) was recently recognized as a distinct subtype of CCA (*5*). For each anatomic subtype, there is different epidemiology, biology, prognosis, and strategy for clinical management. Clinical practice recommendations for the management of CCA have been widely published globally. While the principle of the guidelines remains generally similar, the practice of different countries and the acceptance of recent research have presented a possible challenge to hepatobiliary surgeons. Several high-quality clinical practice guidelines have also been continually updated to reflect the most recent technological and drug advancement, as well as better understanding for the management of CCA. In addition to traditional treatments such as surgery and chemotherapy, targeted therapy and immunotherapy have made progress in the integrated management of CCA. The development of clinical trials and multicenter cross-regional collaboration provides high-level evidence-based medical evidence for new drug development and protocol optimization in CCA. Combined with the recent up-to-date guidelines, we have an updated review under the 2016 review version (*6*) to provide further treatment for the comprehensive management of CCA.

2. Literature search strategies

A literature search was conducted using PubMed, Embase, and Cochrane, as well as a bibliography search and manual search of association websites to identify guidelines and consensus for management of CCA. The search was limited to results with English or Chinese language since the year 2012 and is recent as of July 31, 2022. Key words included "cholangiocarcinoma", "biliary tract cancer", "hepatobiliary cancers",

"guideline", and "consensus". Selected guidelines were extensively examined in order to extract topics, relevant recommendations, and conclusions to the questions. Between each guideline, these recommendations were compared and contrasted, and main differences and gaps were identified. The main characteristics of clinical practice guidelines and consensus are summarized in Table 1 (*7-20*). After screening, there are 14 current guidelines and consensus for CCA around the world, including 3 guidelines from the USA, 4 from Asia, and 7 from Europe. The National Comprehensive Cancer Network (NCCN) guideline and Chinese Society of Clinical Oncology (CSCO) guideline is distributed as a manual and it will be revised every year.

The initial CCA guideline was originally published in 2002 (*21*) and revised in 2012. Despite that the British Society of Gastroenterology (BSG) guideline had not renewed since 2012, we still incorporate the guideline into our review. Due to the lack of diagnosis and treatment for cHCC-CCA, current guidelines do not establish standard recommendations. Only the American International Hepato-Pancreato-Biliary Association (AHPBA) consensus published the management of cHCC-CCA separately. The following sections will discuss CCA management in the context of the new guidelines, and key differences between the guidelines will be highlighted.

3. Epidemiology and risk factors

It is well-known that there is significant heterogeneity among patients with CCA, and this heterogeneity is present even within variable clinicopathologic phenotypes and natural history. Thus, the incidence and mortality of CCA varies by subgroup and geographic region. According to epidemiologic studies (*22-24*), the age-standardized incidence rate for iCCA is growing, although the incidence rate for ECCs may be increasing or plateauing in the majority of countries. Internationally, recent studies have shown an annual incidence of CCA ranging from 0.3 cases per 100,000 in Costa Rica and Israel to 85 per 100,000 in northeast Thailand (*25*). However, the mortality rate from iCCA increased as a result of changes in risk variables and improved clinical classification. Following the rise of laparoscopic cholecystectomy, mortality from ECC has stabilized or declined (*24*).

Most cholangiocarcinoma patients have no predisposing factors recognized, although there is evidence that some risk factors may be related to the disease in certain patients. The Italian guideline, the European Network for the Study of Cholangiocarcinoma (ENS-CCA) guideline and the BSG guideline summarized risk factors in table form, and the other guidelines did so in a description. All guidelines report that the development of CCA is associated with chronic inflammation. Leone *et al.* addressed how chronic inflammatory diseases favor hepatocyte malignant transformation, with a special focus on the immune cell compartment and oxidative stress, from the premalignant to full malignant stages (*26*). In western countries, primary sclerosing cholangitis (PSC) is the most well-known risk factor for CCA; nevertheless, some risk factors are recognized in all three subtypes. For instance, Caroli disease and choledochal cysts are strongly associated with all three CCA subtypes. In contrast, pancreaticobiliary maljunction (PBM), liver flukes, elderly people, non-alcoholic fatty liver disease (NAFLD), and hepatitis B and C are associated with iCCA, whereas choledocholithiasis is associated with ECCs. Globally increasing rates of obesity and NAFLD may be related to the rise in iCCA rates. A Japanese nationwide study of PBM revealed that the incidence of biliary tract cancer in adults was as high as 21.6% in

Table 1. Current guidelines and consesus on cholangiocarcinoma

Guidelines	Year	Country/Ge-ographical area	Language	Tumor	*Ref.*
NCCN Guideline	2022	USA	English	iCCA, pCCA, dCCA	(*7*)
CSCO Guideline	2020	China	Chinese	iCCA, pCCA, dCCA	(*8*)
ENS Guideline	2020	Europe	English	iCCA, pCCA, dCCA	(*9*)
SEOM Guideline	2020	Spain	English	PC, iCCA, pCCA, dCCA and GBC	(*10*)
Italian Guideline	2020	Italy	English	iCCA, pCCA, dCCA	(*11,12*)
JSHBPS Guideline	2019	Japan	English	iCCA, pCCA, dCCA	(*13*)
ESMO Guideline	2016	Europe	English	iCCA, pCCA, dCCA	(*14*)
CCHPBA Guideline	2015	China	Chinese	iCCA, pCCA, dCCA	(*15*)
AHPBA Guideline	2015	USA	English	iCCA, pCCA	(*16,17*)
ILCA Guideline	2014	Europe	English	iCCA	(*18*)
Asia-Pacific Guideline	2013	Asia-Pacific	English	pCCA	(*20*)
BSG Guideline	2012	UK	English	iCCA, pCCA, dCCA	(*19*)

CCA, cholangiocarcinoma; pCCA, perihilar cholangiocarcinoma; iCCA, intrahepatic cholangiocarcinoma; dCCA, distal cholangiocarcinoma; GBC, gallbladder carcinoma; PC, Pancreatic cancer; HCC, hepatocellular carcinoma; NCCN, National Comprehensive Cancer Network; CSCO, Chinese Society of Clinical Oncology; SEOM, the European Network for the Study (ENS), the Spanish Society of Medical Oncology; JSHBPS, the Japanese Society of Hepato-Biliary-Pancreatic Surgery; ESMO, the European Society of Medical Oncology; CCHPBA, the Chinese Chapter of International Hepato-Pancreato-Biliary Association; AHPBA, the American International Hepato-Pancreato-Biliary Association; ILCA, the International Liver Cancer Association; BSG, the British Society of Gastroenterology

patients with PBM with bile duct dilatation and 42.4% in patients with PBM without bile duct dilatation (*27*). Therefore, the Japanese Society of Hepato-Biliary-Pancreatic (JSHBP) guideline recommended that prophylactic surgical treatment should be performed at the earliest time after diagnosis to prevent cancer development. There is still controversy on the choice of treatment method for prophylactic surgery, which was not been mentioned in other guidelines.

Although there are multiple risk factors for CCA, the majority of CCAs lack an identifiable risk factor.

For patients with risk factors for CCA, both common and rare, targeted screening of high-risk individuals might be an alternative. Individuals with high-risk factors including PSC, liver cirrhosis, chronic inflammation of the biliary epithelium, cholestasis (*25*), and chronic hepatitis, by various tests should be considered for surveillance in the European Society for Medical Oncology (ESMO), the International Liver Cancer Association (ILCA) and BSG guidelines.

4. Screening and diagnosis

The lack of definitive diagnostic criteria and limited specificity of most diagnostic methods make cholangiocarcinoma challenging to diagnose. Early diagnosis of CCA is a critical point to improve the prognosis of patients. The guidelines and consensus contain distinctive diagnostic algorithms, which have been evaluated from a variety of viewpoints based on current guidelines. However, there is no accurate imaging examination that can be used for a comprehensive evaluation. All guidelines provide tests to diagnose CCA around the world include serological diagnosis, imaging diagnosis and histological diagnosis.

The clinical presentation of cholangiocarcinoma varies depending on the tumor stage, location, and growth pattern. ICCA patients usually have no specific clinical symptoms in the early stages, but as the disease progresses, abdominal discomfort, abdominal pain, fatigue, nausea, epigastric masses, malaise, night sweats, asthenia, weight loss and fever may occur (*28-30*). The most typical symptom of ECC is jaundice that is defined by the yellowing or greening of the skin and mucous membranes. From there, screening is theoretically the best way to detect asymptomatic CCA for early intervention.

The preliminary screening should include liver function tests, the carcinoembryonic antigen (CEA), the carbohydrate antigens 19-9 (CA19-9) (*31-33*), and abdominal ultrasound (US) in higher-risk groups. At present, CEA and CA19-9 are recommended as blood biomarkers for CCA, however their limited sensitivity and specificity make them ineffective for early identification. Serum CA 19-9 is neither highly sensitive nor specific for diagnosis, as CA 19-9 in patients with benign bile duct obstructions or acute cholangitis also could be slightly elevated (*31*). In the setting of bile duct obstruction, CA 19-9 levels should be reassessed after biliary intervention/drainage since the half-life of CA 19-9 is one to three days. Patients with iCCA who had either a high preoperative CA 19-9 or CEA had a very poor outcome with a 1-year survival of only 64.9% (*34*). Both pre- and postoperative serum CA 19-9 levels predict the survival of patients with resectable CCA, and may contribute to the establishment of a new therapeutic strategy (*35*). The descriptions of diagnostic tests with CA 19-9 reach a consensus in the current guidelines. The NCCN, CSCO, SEOM, CCHPBA and EASL also advise CEA for baseline blood tests. Only BSG guideline recommended CA125 to diagnose CCA. Other serum markers, such as cytokeratin-19 fragment (CYFRA 21-1), CA242, MK-1, Caudal homeobox 2(CDX2) and C-reactive protein, have been reported in a limited number of studies, but are not in routine clinical use (*36-39*). Thus, the first step to evaluate the usefulness of tumor biomarker (CA 19-9 and CEA) in the early diagnosis of CCA is to establish which high-risk population should be screened.

Several imaging examinations are also thought to be helpful in diagnosis, including Ultrasound (US), contrast-enhanced MRCP, contrast-enhanced CT, PET-CT scan, and endoscopic ultrasound (EUS). Their diagnostic accuracy is influenced by anatomic location and growth patterns of CCA.

US is the method of choice for the diagnosis of cholangiocarcinoma, which may appear as a limited intrahepatic mass, or as a portal tumor with dilated intrahepatic bile ducts and no dilated extrahepatic bile ducts. The advantage of US is that it can reliably differentiate between masses and stones and can initially identify the site of obstruction based on whether the bile ducts within or outside the liver are dilated. US can show lesions in and around the bile ducts and evaluate the degree of portal vein invasion. Testing of contrast enhanced ultrasound (CEUS) has been introduced into guidelines and recommendations for the diagnostic work-up of iCCA: the ENS guidelines, the Italy Society, and the EASL guidelines. CEUS increases the diagnostic performance in differentiation between iCCA and HCC significantly, in comparison with conventional ultrasound (*40,41*). US-screening is an effective technique for detecting CCA in its early stages, a comprehensive population-based program utilizing such screening in high incidence areas is recommended (*42*). A magnetic resonance cholangiopancreatography (MRCP) is considered the routine image study for staging CCA. Endoscopic ultrasound/fine needle aspiration EUS-FNA is effective to identify malignant regional lymph nodes (MRLNs) in patients with CCA, and should be routinely incorporated into staging of all CCA subtypes given the impact of MRLN on prognosis and management decisions (*43*). Due to difficulties in differential diagnosis between iCCA and

liver metastases, fluorodeoxyglucose positron emission tomography (FDG-PET) is also commonly used to rule out a primary tumor.

The NCCN, CSCO, and ESMO guidelines emphasize that a multidisciplinary team (MDT) of experts including experienced radiologists and surgeons needs to review examination results in order to stage the disease and determine potential treatment options, and shared decision-making consultation (*44,45*). MDT should be involved in the whole management.

5. Staging and classification

Accurate staging is critical for establish the appropriate treatment strategy for all cancer types. The American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC) staging system is the mostly commonly used staging system for CCA (*46,47*) (Table 2 and Table 3). Four of the eleven guidelines have been revised after publication. The JSHBPS guideline (3rd) did not revise the stage after it published the 3rd version of the Japanese classification of biliary tract cancers (*48*). The other 5 guidelines may not have been revised because the AJCC system was published in 2016 and made effective in 2018 (8th edition).

In 2018, the AJCC/UICC published modifications of the staging system. The most significant alteration has been made in the N stage. Traditionally, the AJCC/UICC classified regional lymph node stations depend on the anatomical site. In particular, in the 8th edition, the lymph node staging (N-category) of patients with CCA was altered, with the N1- and N2-stage categories based on the counts of positive lymph nodes (N1: one to three involved positive regional lymph nodes and N2: four or more involved positive regional lymph nodes). The 8th edition confirms that the anatomic extent of the tumor maintains to be the strongest predictor of outcome in CCA. The depth of tumor invasion is an independent predictor of prognosis in patients with dCCA and pCCA. Despite the fact that the present TNM classification provides a clinically useful categorization that is associated with prognosis, it has several drawbacks. A published study from two Western hepatobiliary centers evaluated the prognostic accuracy of the 8th TNM classification of the AJCC staging system in a cohort of 214 patients undergoing liver resection for CCA. In that study population, about 40% of patients changed their stages from the 7th to the 8th AJCC edition. The authors determined that the new 8th TNM edition was only slightly better than the previous 7th edition (*49*). The prognostic accuracy of the 8th edition of the AJCC staging system was similar to the 7th edition. Prognostic accuracy was particularly poor in unresectable patients (*50*). TNM classification has potential clinical implications during the preoperative stage, when it might still affect the choice to perform a resection or not. Thus, accuracy on imaging is therefore likely the most crucial variable. Future editions of the AJCC staging system should aim to improve the prognostic accuracy of the AJCC staging system on cross-sectional imaging.

The AHPBA guideline only suggests that staging laparoscopy should be routinely utilized in high-risk iCCA patients (*i.e*. patients with multicentric disease, high CA19-9, questionable vascular invasion or suspicion of peritoneal disease). The Italian guideline suggests against performing routine staging laparoscopy before surgery in CCA patients whose doctors will perform surgery (*12*). The others did not mention staging laparoscopy. Thus, staging laparoscopy is not recommended as a rule.

6. Treatment

Depending on CCA site of origin, each variety of CCA has different therapeutic strategies. A treatment algorithm is shown in Figure 1. This review is compared to recent research on guideline updated, treatment approaches, with an emphasis on treatment criteria and new therapy breakthroughs.

6.1. Biliary drainage and portal embolization

It is well-known that extended hepatectomy in patients with jaundice is related to a high risk of postoperative liver failure (PLF), morbidity and mortality (*51-54*). Therefore, preoperative biliary drainage and portal vein embolization (PVE) are frequently selected measures to prevent PLF.

Preoperative biliary drainage remains a matter for debate. Only 6 guidelines mention biliary drainage. In particular, the JSHPBS guideline emphasized that preoperative biliary drainage played an important role on the management of patients with CCA. And we summarized that the main selections of biliary drainage are *i*) cholangitis or sepsis originating from the biliary tract; *ii*) jaundice; *iii*) the need for preoperative anti-neoplastic therapy or PVE or ALPPS; *iv*) malnutrition, hepatic insufficiency; *v*) unresectable CCA. All 6 guidelines suggested biliary drainage, but only 3 mentioned total bilirubin concentration before drainage. The Italian guideline recommended patients biliary drainage with total bilirubin > 256.5μmol/L (mg/dL), and CCHPBA using a cut-off value of 200μmol/L. The CSCO guideline recommended that patients with hyperbilirubinemia more than 200μmol/L in pCCA and more than 380μmol/L in dCCA to perform biliary drainage. A randomized controlled trial was terminated because of higher all-cause mortality in the percutaneous transhepatic biliary drainage group in patients with pCCA (*55*). The results encourage further prospective trials and a reappraisal of the indications and approaches for biliary drainage. Despite the debate on whether to perform

Table 2. Definitions of American Joint Committee on Cancer (AJCC) staging system for iCCA, pCCA, and dCCA with 8th editions

	iCCA	pCCA	dCCA
T	Primary Tumor	Primary Tumor	Primary Tumor
T_X	Primary tumor cannot be assessed	Primary tumor cannot be assessed	Primary tumor cannot be assessed
T0	No evidence of primary tumor	No evidence of primary tumor	—
Tis	Carcinoma in situ (intraductal tumor)	Carcinoma in situ/high-grade dysplasia	
T1	Solitary tumor without vascular invasion, ≤ 5cm or >5 cm	Tumor confined to the bile duct, with extension up to the muscle layer or fibrous tissue	Tumor invades the bile duct wall with a depth less than 5 mm
T1a	Solitary tumor ≤ 5cm without vascular invasion		
T1b	Solitary tumor >5 cm without vascular invasion		
T2	Solitary tumor with intrahepatic		
T2a	vascular invasion or multiple tumors, with or without vascular invasion	Tumor invades beyond the wall of the bile duct to surrounding adipose tissue	Tumor invades the bile duct wall with a depth of 5-12 mm
T2b		Tumor invades beyond the wall of the bile duct to surrounding adipose tissue	
T3	Tumor perforation of the visceral peritoneum	Tumor invades unilateral branches of the portal vein or hepatic artery	Tumor invades the bile duct wall with a depth greater than 12 mm
T4	Tumor involving local extrahepatic structures by direct invasion	Tumor invades the main portal vein or its branches bilaterally, or the common hepatic artery, or unilateral second order biliary radicals with contralateral portal vein or hepatic artery involvement	Tumor involves the celiac axis, the superior mesenteric artery, and/or common hepatic artery
N	Regional lymph nodes	Regional lymph nodes	Regional lymph nodes
N_X	Regional lymph nodes cannot be assessed	Regional lymph nodes cannot be assessed	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis	No regional lymph node metastasis	No regional lymph node metastasis
N1	Regional lymph node metastasis present	One to three positive lymph nodes typically involving the hilar, cystic duct, common bile duct, hepatic artery, posterior pancreatoduodenal, and portal vein lymph nodes	Metastasis to one to three regional lymph nodes
N2	—	Four or more positive lymph nodes from the sites described for N1	Metastasis to four or more regional nodes
M	Distant Metastasis	Distant Metastasis	Distant Metastasis
M0	No distant metastasis	No distant metastasis	No distant metastasis
M1	Distant metastasis present	Distant metastasis present	Distant metastasis present

Table 3. Prognositc Groups of American Joint Committee on Cancer (AJCC) staging system for iCCA, pCCA, and dCCA

	iCCA			pCCA			dCCA		
Stage 0	Tis	N0	M0	Tis	N0	M0	Tis	N0	M0
Stage I				T1	N0	M0	T1	N0	M0
IA	T1a	N0	M0		—			—	
IB	T1b	N0	M0		—			—	
Stage II	T2	N0	M0	T2a-b	N0	M0			
IIA		—			—			T1N1M0 or T2N0M0	
IIB		—			—			T2-3N1M0 or T3N0M0	
Stage III									
IIIA	T3	N0	M0	T3	N0	M0		T1-3, N2M0	
IIIB	T4 or Any T	N0 or N1	M0	T4	N0	M0		T4, any N, M0	
IIIC		—		Any T	N1	M0	—	—	—
Stage IV	Any T	Any N	M1				Any T	Any N	M1
IVA		—		Any T	N2	M0			
IVB				Any T	Any N	M1			
Histologic Grade(G)									
GX				Grade cannot be assessed					
G1				Well differentiated					
G2				Moderately differentiated					
G3				Poorly differentiated					

biliary drainage, PVE remains consensus according to all current guidelines for sufficient future liver remnant (FLR) with patients who will perform hepatectomy in pCCA. PVE could cause FLR hypertrophy, which could improve the safety of the extended hepatectomy.

6.2. Resection & transplantation

According to recent research, the only potentially curative treatment method that is recommended by all guidelines is surgical resection. Surgical management is

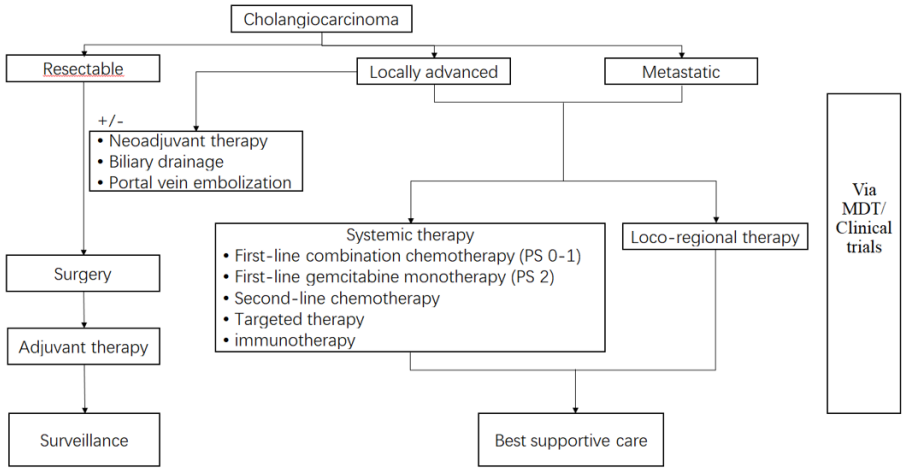

Figure 1. Algorithm for the management of patients with cholangiocarcinoma.

based on the location and extent of the tumor. However, the surgical treatment for CCA recently have had little progress. The initial surgical examination should include evaluation for multifocal liver disease, lymph node metastases, distant metastases, and biopsy not required before surgery. In summary, the main selection of surgical procedures is: *i*) iCCA, segment or lobe resection. Extensive hepatic resections are usually needed to confirm R0 resection; *ii*) pCCA, extended right or left hepatectomy combined with caudate lobectomy, the extent of the involved biliary tract determines the range of hepatectomy; *iii*) pCCA, pancreatoduodenectomy is generally performed. Few patients with CCA in the middle part of the extrahepatic bile duct are cured with isolated resection of the bile duct. En-bloc resection of the caudate lobe is recommended because the tumor typically extends into the caudate lobe *via* small branches draining into the right or left hepatic ducts or the biliary confluence (*56*).

The AHPBA guideline recommended that regional lymphadenectomy be performed in patients undergoing resection. De Jong *et al.* demonstrated that among patients who underwent routine lymphadenectomy, patients with lymphadenectomy had a worse median survival (*57*). However, some studies reporting the number of lymph nodes (LNs) retrieved affects patient survival (*58*). However, owing to the lack of the randomized controlled trials, there is still no consensus about the prognostic significance in iCCA with or without lymphadenectomy. In addition, it is unclear what is a standard lymph node dissection (LND) given the multiple potential lymphatic pathways for intrahepatic malignancies. Nevertheless, the effects of lymphadenectomy remain controversial, and the majority of guidelines still recommend routine LND in CCA. Staging laparoscopy is recommended by ENS-CCA and Asia-Pacific guidelines, especially in patients with a high CA19-9 level or major vascular invasion (*59*). Wu *et al.* demonstrated laparoscopic liver resection (LLR) associated lymphadenectomy for iCCA is safe and feasible compared with open liver resection (OLR) (*60*). The study reported LLR was used to reduce intraoperative blood loss and postoperative hospital stay. In addition, laparoscopic surgery is useful to detect occult metastasis with the peritoneum. Their application in preoperative staging is controversial. Thus, laparoscopic surgery is not routinely recommend in most guidelines.

Liver transplantation (LT) for cholangiocarcinoma has been an absolute contraindication worldwide due to poor results. However, in recent years thanks to improvements of patient management and treatments of CCA patients, this indication has been revisited. The CSCO, ESMO and Italian guidelines (*61*) recommend LT for iCCA patients. LT may be considered in patients with unresectable pCCA who fulfill the Mayo Clinic protocol (tumor diameter ≤ 3 cm without lymph node or distant metastases in the staging laparotomy, after external beam radiation, chemotherapy based in 5-fluorouracil, intra biliary radiation, and oral capecitabine until LT). The diameter of the tumor is tightly associated with post-LT recurrence. Only single-nodule tumors ≤ 2 cm without vascular invasion would be acceptable (*62*). In this interim analysis of an initial case series, patients with stable intrahepatic cholangiocarcinoma before liver transplantation had an overall survival of 83% and a recurrence-free survival of 50% at 5 years. These findings suggest that tumor stability over time and response to therapy might serve as surrogate markers of favorable tumor biology for liver transplantation, and that the Methodist–MD Anderson selection criteria might identify subpopulations of patients with intrahepatic cholangiocarcinoma who would benefit most from liver transplantation (*63*).

6.3. Loco-regional therapies

Recent literature suggests an emerging role for loco-regional therapies in iCCA, including radiation therapy

(RT), transcatheter arterial (TACE), radio embolization and radiofrequency ablation (RFA). While distant metastasis is a less frequent cause of mortality, many of these patients die of liver failure caused by tumor-related vascular involvement or biliary blockage. It is so necessary to try to obtain local control of the tumor to improve quality of life. Loco-regional treatment decisions must take into account both the conditions of patients (comorbidities, liver function, prior therapies) and the size of tumor, vascularity, and involvement of bile ducts, blood arteries, colon, and chest wall (*64*). All guidelines recommend loco-regional therapies for iCCA while guidelines encourage further research in these areas.

The palliative treatment of cholangiocarcinoma, with photodynamic therapy, is associated with an increased survival benefit, an improved biliary drainage, and a better quality of life (*65*). However, the quality of this evidence is low (*66*). Photodynamic therapy (PDT) is a new local-ablative, tumor-specific treatment that has shown promising results and is now the standard of care for unresectable cholangiocarcinoma. Moole *et al.* reported that PDT combined with biliary stenting improves the success of biliary drainage and improves the survival and quality of life in patients with nonresectable cholangiocarcinoma (*67*). Unfortunately, the combination of PDT with biliary stenting was reported to be associated with prolonged OS in patients with unresectable cholangiocarcinoma in relatively small sample studies. Photodynamic therapy (PDT) was recommended for routine use based on the most recent data by only BSG and NCCN guidelines.

6.4. Systemic therapies

CCA is a kind of digestive system tumor with high malignancy and poor prognosis. Despite significant advances in diagnostic modalities, the vast majority of patients present with metastases or with advanced locoregional disease that prevents surgical therapy. However, the recurrence rate is high, even for patients who have received treatment in the early stage, and the survival rate of patients with advanced cancer, including those who receive treatment, is poor. The main goals for the palliation of patients with advanced CCA are decompression of the biliary system and control of tumor growth. Currently, systemic therapies for advanced or metastatic CCA are ineffective due to molecular variants that define the biological characteristics of each CCA subtype.

At the time of assessment of patients with CCA for systemic therapies, the following three aspects need to be considered: patient fitness as assessed in terms of Eastern Cooperative Oncology Group Performance Status (ECOG PS), disease distribution and accessibility of tumor profiling. If patients with an ECOG PS ≥ 3 are unlikely to benefit from systemic treatment, guidelines recommend only supportive care.

6.4.1. Chemotherapy

Chemotherapy strategies for patients with CCA include: *i*) neoadjuvant chemotherapy; *ii*) post-operative adjuvant chemotherapy; *iii*) palliative chemotherapy for patients with unresectable or metastatic disease. A chemotherapy-based systemic treatment model has a proven clinical benefit in CCA, however, criteria of treatment remains controversial. Zhang *et al.* summarized that chemotherapy has a most significant effect on the systemic treatment of advanced or recurrent CCA (*68*). Appropriate patients are recommended to participate in clinical trials.

There is no evidence supporting the use of neoadjuvant systemic chemotherapy over upfront resection in patients with resectable iCCA (*69*). There is a lack of randomized controlled phase III clinical trials demonstrating the benefit of neoadjuvant therapy for CCA. This assessment of treatment response might be important in future trial designs (*70*). Interval from completion of neoadjuvant treatment to surgery varied from 3 days to 6 months. Resection was by hepatectomy with three studies reporting an R0 rate of 100%, 24% and 63%, respectively. Three studies reported histopathological evidence of prior treatment response. There were two treatment related deaths at 90 days. Median survival was 19 (95% CI: 9.9–28) months and 5-year survival 20% (*70*). In accordance with CSCO and ESMO guidelines, neoadjuvant treatment is also recommended for CCA. Nevertheless, neoadjuvant treatment for resectable CCA is not included in the guidelines: NCCN, SEOM, JSHBPS, ENS, Italian, AHPBA.

Patients with CCA are mostly unable to be cured due to recurrence after surgery, which is a significant basis for the options of adjuvant chemotherapy. NCCN guideline, CSCO guideline, ENS guideline, SEOM guideline, and Italian guideline recommend capecitabine as the first approach for patients with resectable CCA. Although the evidence of an optimal regimen has not yet been established in Japan, JSHBPS guideline noted adjuvant chemotherapy may be considered (*13*). It is with regret that most guidelines except for SEOM guideline and ENS guideline do not illustrate a duration of chemotherapy. Based on evidence from a phase III (BILCAP) randomized controlled trial, patients with resected BTC should be offered adjuvant capecitabine chemotherapy for a duration of 6 months (*71,72*). Besides, CSCO guideline, SEOM guideline, CCHPBA, and AHPBA guidelines recommend that radiotherapy for patients with lymph node-positive disease or with microscopically involved margins (R1 resection) could improve the poor prognosis. Findings from a nationwide retrospective study showed that adjuvant radiotherapy was associated with a survival benefit in

patients with resected dCCA, regardless of pathological nodal involvement, resection margin status, and receipt of adjuvant chemotherapy (*73*).

Unresectable CCA is classified as locally advanced or metastatic disease. The combination of gemcitabine and cisplatin chemotherapy is still recommended as standard first-line treatment for advanced and metastatic CCA patients with an ECOG PS of 0-1. Durvalumab was approved as an orphan drug to treat BTC. Before that, a phase I study published results of combination therapy with durvalumab and tremelimumab to treat BTC (*74*). Based on the convincing data of the AC-02 trial which revealed a significantly increased median overall survival compared to gemcitabine monotherapy (11.7 *vs.* 8.1 months, respectively; hazard ratio 0.64; 95% CI: 0.52–0.8; $p < 0.001$). Additionally, the combination therapy had an 81.4% disease control rate compared to 71.8% for monotherapy (*75*). Some interesting trials such as a BTC trial, a phase II trial focused on triplet therapy cisplatin, gemcitabine and nab-paclitaxel (*76*), as well as the phase III trial of gemcitabine plus S1 (*77*). These provide a new option for patients with BTC as a convenient standard therapy. The most important independent prognostic factor for advanced BTC is ECOG PS, which can guide therapeutic choices. Indeed, patients with ECOG PS 2 should be preferred to gemcitabine monotherapy in CSCO, ENS, SEOM guidelines.

Patients with tumor progression under first-line chemotherapy might be suitable for a second-line treatment, especially young patients and those with a good performance status (*78*). However, there are no consensus guidelines that help in choosing an appropriate second-line therapy. In addition, a systematic review of 25 studies, which included 761 patients, evaluated the role of secondline therapy in advanced biliary tract cancer. The study showed an overall response of 8%, indicating that there could be a cohort of patients who might benefit from empirically selected secondline therapy (*79*). The ABC-06 trial demonstrated the effectiveness of second-line chemotherapy (adjusted HR 0.69). Although variations in median OS between study arms were modest (5.3 *vs.* 6.2 months), differences in survival at 6 months (35.5% versus 50.6%) and 12 months (11.4% *vs.* 25.9%) were clinically significant. Based on these findings, FOLFOX can be considered a new standard of care in the second- line setting. FOLFOX was recommended for the treatment of advanced and metastatic patients by NCCN, CSCO, ENS, SEOM guidelines, but the JSHBPS guideline and Italian guideline respectively recommended fluoropyrimidine-based chemotherapy as second-line treatment.

6.4.2. Targeted therapy and immunotherapy for CCA

In terms of treatment algorithms, targeted therapy and immunotherapy have received considerable interest, and targeted therapy or immunotherapy as recommended by NCCN guideline, CSCO guideline, ENS guideline and SEOM guideline.

Because of the significant inter-tumoral and intra-tumoral heterogeneity of CCA, no effective targeted medicines are currently available for treating this disease. Alterations of isocitrate dehydrogenase (IDH)1, IDH2, fibroblast growth factor receptor FGFR1, FGFR2, FGFR3, epoxide hydrolase (EPH)A2, and biofilm-associated surface protein (BAP)1 genes have been reported in the intra-hepatic subtype, while in perihilar and dCCA genetic alterations of AT-rich interactive domain (ARID)1B, E74-like factor (ELF)3, protein polybromo-1 (PBRM1), protein kinase cAMP-activated catalytic subunit alpha (PRKACA), and PRKACB were described (*80*).

AG-120 (Ivosidenib) (*81*) was tested in 73 patients with IDH1-mutant advanced CCA in a phase I study. Four (5%) patients had a partial response, 56% experienced stable disease, and the median overall survival was 13.8 months. Results of the cross-over phase III study (ClarIDHy) of Ivosidenib compared to placebo were reported at ESMO 2019. Ivosidenib significantly improved PFS compared with placebo. The median OS was 10.8 months for Ivosidenib and 9.7 months for placebo, with 57% of placebo patients crossing over to Ivosidenib. In the intention to treat population, there was a trend in favor of Ivosidenib, but it was not yet significant. At present the drug has been recommended by CSCO and ENS guidelines. The distinguished genetic profile, histological characteristics, and clinical results observed in these different anatomical areas may lead one day to individualized treatment strategies.

Recently, immunotherapy has developed rapidly, especially the introduction of immune checkpoint inhibitors, which have achieved good efficacy in many solid tumors. Some phase I and II clinical studies on biliary system malignancies have demonstrated good safety and effectiveness of immunotherapy. There has been a surge of interest in targeted therapies and immune therapies for CCA. The anti-programmed cell death 1 (PD-1) antibody pembrolizumab has been approved by the United States Food and Drug Administration for previously treated patients with DNA mismatch repair (MMR) deficiency and/or microsatellite instability (MSI)-high advanced solid tumors, independent of histology, which would include those with CCA. Of note, MMR deficiency has been reported to occur in 5% to 10% of CCA (*82,83*). Pembrolizumab is a highly selective, humanized monoclonal antibody against PD-1 that is designed to block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. On the basis of these results, this is similar to the recommendation in the updated NCCN, CSCO, JSHBPS, and ENS guidelines. The panelists of

the consensus agree that all advanced, metastatic CCA in patients who are medically fit should be screened for MSI-H/dMMR, and those with MSI-H solid tumors should receive pembrolizumab monotherapy (*84*). The discovery of targeted therapies for this diverse and relatively uncommon cancer remains a challenging task. Precision medicine efforts have discovered the disease's underlying mutational landscape and prepared the way for targeted therapy and immunotherapy trials.

7. Future perspectives

According to our review, there are significant and notable variances. The goals of this review were to identify and emphasize the distinctions that lead to the development of these guidelines and to support the practicing surgeons in understanding these guidelines in order to provide superior treatment for patients with CCA. Unfortunately, comprehensive, randomized controlled trials comparing these guidelines in well-defined clinical studies do not exist. Therefore, it is challenging to recommend one guiding principle above others. The availability of various imaging modalities in addition to new biomarkers, enables the early detection of CCA, while developments in the treatment modalities have bolstered a multidisciplinary strategy for hepatobiliary surgeons. Identifying molecular biomarkers that indicate primary or secondary resistance to CCA remains an active research topic. Despite these developments, recurrence following curative treatment remains a significant drawback, and more effective adjuvant therapies are required. The potential efficacy of systematic therapy may revolutionize the systemic treatment protocol. As the number of effective systemic drugs continues to increase, the challenge is to determine which order of sequential systemic therapy can offer optimal efficacy with minimal toxicity. There are a few systemic chemotherapy studies dedicated to all anatomic subtypes of CCA, and the majority comprise GBC. These differences in guidelines also help to identify issues for future researches that will hopefully reconcile these controversial issues. Updated guidelines have had randomized controlled trials to improve prognosis of patients with iCCA, pCCA, dCCA separately.

8. Conclusion

Management of CCA remains a significant challenge, and as well as source of uncertainty and anxiety for both surgeons and patients. It's reassuring to notice such remarkable uniformity between various kinds of recommendations. However, no major advances in surgical treatment of CCA have occurred over the past 10 years. We analyzed the similarities and differences between the clinical practice guidelines for CCA from different countries to clarify the status of management. The systemic therapy of CCA remains a key clinical problem, and a promising breakthrough has not yet occurred. The management of locally advanced and metastatic CCA does need further research. Targeted therapy may become established in this field. Immunotherapy has obtained good results for treating CCA, but more clinical evidences are needed before these can be recommended for CCA. Distinctive treatment guidelines dependent on the regional scale, and clinical trials provide more evidence in the era of individualized treatment. The purpose of guidelines is not to replace doctor' expertise but to present physicians with the most up-to-date options for their patients. Therefore, they must address crucial issues and advise physicians on optimal treatment options for each specific individual. To advance standard management for CCA, governments should develop and implement domestic recommendations that are evidence-based, resource-constrained, appropriate to specific patients, and subject to systematic evaluation.

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Review

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Do patients with Hirayama disease require surgical treatment? A review of the literature

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SUMMARY The main clinic characteristic of Hirayama disease (HD) is atrophy of the distal muscles in the upper limbs. Recently, an increasing number of HD cases have been reported. Many HD patients have persistently progressive symptoms and conservative treatments failed. This article aims to review the current status of the field and summarizes the main surgical treatment options for patients with HD. A comprehensive search of the PubMed and the Web of Science databases was conducted from their inception to September 15th, 2022. Search terms included "juvenile muscular atrophy of upper extremity", "Hirayama disease" and "surgery". A total of 169 relevant publications were identified and 29 articles were finally reviewed. Current surgical treatments for HD are either anterior cervical surgery or posterior cervical surgery. The two approaches can effectively stop the disease. However, no studies have compared the advantages and limitations of the two surgical methods. The previous view that HD can be improved with conservative treatment has been challenged. In many studies, surgical treatment has been shown to improve the hand function in patients with HD. However, there is still controversy about the methods of anterior and posterior cervical surgery. Future research could focus on exploring the advantages and limitations of different surgeries.

Keywords Hirayama disease, posterior cervical surgery, anterior cervical surgery

1. Introduction

Hirayama disease (HD), also referred to as juvenile muscular atrophy of the distal upper extremity, was first reported by Keizo Hirayama (*1*) in 1959. Previously, most reports of HD were in Asian countries such as Japan, China and India (*2-4*). Recently, an increasing number of HD cases have been reported in regions outside Asia. Table 1 summarizes cases of HD reported in PubMed in regions outside Asia in the past five years (*5-32*). Developments in imaging and electromyography (EMG) examination have improved the understanding of HD. Although the pathogenesis of HD is not fully understood, two main hypotheses have been proposed. The first is the hypothesis of dynamic cervical flexion compression. Repeated or continuous flexion of the cervical spine causes the posterior wall of the dural sac to move forward causing compression of the spinal cord that results in microcirculation disorders and chronic injury in the anterior horn of the spinal cord (*33,34*). The study of Sun *et al.* used diffusion tensor imaging (DTI) to scan patients with HD also supported the hypothesis (*35*). The other hypothesis is based on growth and development. The spinal cord and the dura mater are unbalanced during rapid growth and development in adolescence. During this time, the posterior roots become shortened. In the neutral position, the posterior root is in a relaxed state. When the neck is flexed, the shortened posterior root pulls the cervical spinal cord forwards resulting in compression of the cervical spinal cord and HD (*2,36*). Both hypotheses suggest that repeated and persistent cervical flexion is an important causative factor in HD.

Based on the above hypotheses, many physicians began to use cervical collar therapy to treat HD patients. In 1992, Tokumaru *et al.* compared 14 HD patients who had worn a cervical collar with 18 untreated patients (*37*). The study showed that cervical collar therapy can stop the progression of HD in a short period. Another study by their team also supported the reliability of this conclusion (*38*). However, conservative treatment has limitations. First, some reports showed that many patients still have continuous progress of symptoms 10 years after the onset of the disease. A small number of patients experience rapid progression months to years after symptoms have developed (*39,40*). Second, the efficacy of cervical collar

Table 1. Summary of the cases of HD published in PubMed in regions outside Asia in the past five years (2016-2022)

References	Country	Cases	Treatment
Kieser DC, *et al.* (*6*)	New Zealand	1	Collar therapy
Kumar M, *et al.* (*8*)	United States	1	Collar therapy
Baumann M, *et al.* (*9*)	Austria	1	Collar therapy
Lewis D, *et al.* (*5*)	United Kingdom	1	Collar therapy
Vachon C, *et al.* (*11*)	United States	1	Conservative treatment
Brems M, *et al.* (*12*)	Belgium	1	No treatment
Filiz MB, *et al.* (*13*)	Turkey	1	Physical therapy
McGregor S, *et al.* (*14*)	Canada	2	ACDF
Lolli VE, *et al.* (*15*)	Belgium	1	Not reported
Antonioni A, *et al.* (*16*)	Italy	1	Conservative treatment
Galletta K, *et al.* (*17*)	Italy	1	ACDF
Salome M, *et al.* (*18*)	The Netherlands	1	ACDF
Ayas ZO, *et al.* (*19*)	Turkey	1	Conservative treatment
Kapetanakis S, *et al.* (*20*)	Greece	1	Conservative treatment
Alpaydin Baslo S, *et al.* (*21*)	Turkey	1	Conservative treatment
Chanson JB, *et al.* (*22*)	France	1	Conservative treatment
Ay H, *et al.* (*23*)	Turkey	1	Conservative treatment
Tolu S, *et al.* (*24*)	Turkey	1	Collar therapy
Kandukuri GR, *et al.* (*25*)	United States	1	Conservative treatment
Witiw CD, *et al.* (*26*)	United States	1	ACDF
Macey MB, *et al.* (*27*)	United States	1	Not reported
Cabona C, *et al.* (*7*)	Italy	3	Conservative treatment
Wang H, *et al.* (*10*)	United States	1	Rehabilitation treatment
Koutsis G, *et al.* (*28*)	Greece	1	Not reported
Ashour M, *et al.* (*29*)	Canada	1	Posterior surgery
Abreu Tanure A, *et al.* (*30*)	Brazil	1	Nerve transfer
Hayden ME, *et al.* (*32*)	United States	1	Tendon transfer

HD, Hirayama disease; ACDF, anterior cervical discectomy and fusion.

therapy is greatly affected by patient compliance. A study showed that most patients wear cervical collars for less than half a year due to factors including appearance, inconvenience and progression of the disease (*41*). Finally, a study published in 2021 suggested that surgery may be better at helping patients with HD achieving symptomatic improvement than conservative treatment (*42*).Therefore, in patients with progressive disease, surgery should be considered for treatment. At the same time, a large number of studies including guidelines established by clinical multidisciplinary teams have highlighted that surgical treatment can effectively limit abnormal flexion of the cervical spine and expand the volume of the dural sac (*43-46*). Based on these studies, many HD patients who have failed conservative treatment have benefited from surgery. HD has become an area of intense research for spinal surgeons. In this article, we review the current knowledge of HD and provide a review of surgical treatments.

We conducted a structured search of PubMed and Web of Science. Publication dates were included from the inception of each database to September 15th, 2022. Search terms included "juvenile muscular atrophy of upper extremity", "Hirayama disease" and "surgery". A total of 169 relevant publications were identified. After the exclusion of duplicate publications and a further detailed review of the articles, 29 articles were finally included in the review. The inclusion criteria were case reports focusing on patient surgical treatment and prognosis, observational studies and randomized controlled trials (RCTs). Reviews, systematic reviews and meta-analyses were excluded.

2. Indications for surgical treatment

At present, the surgical treatment of HD aims to relieve the compression of the cervical spine and reconstruct normal cervical spine alignments. The guidelines established by the clinical multidisciplinary team showed that surgical treatment can be performed when the disease continues to progress after long-term wearing of a cervical collar, in patients who cannot tolerate the long-term wearing of a cervical collar and when symptoms progress (*43*). In addition, the Huashan clinical classification system for patients with HD was established in 2021 and its internal consistency was primarily verified (*47*). According to this system, HD patients can be divided into types I, II and III. Patients with type I HD have typical symptoms such as unilateral muscular atrophy without pyramidal tract signs or sensory disturbances. If the symptoms do not progress within 6 months, the patient is classified as subtype Ia; however, if the patient progresses, then the classification is subtype Ib. Patients with type II HD have typical symptoms accompanied by sensory disturbances or pyramidal tract signs. Patients with type III HD have an atypical form of HD involving the proximal muscles of the upper limbs or bilateral symptoms. Some physicians believe patients with HD of type Ib or above need surgical treatment (*47-49*).

3. Posterior cervical surgery

The literature and key conclusion on posterior cervical surgery are summarized in Table 2. Masaki *et al.* began to treat HD patients with surgery as early as 1990 (*50*). They believed that the main cause of the disease was increased imbalance of the cervical spine alignment. A posterior C2-C6 segmental fusion was performed on a HD patient. After surgery, the patient showed signs of improved muscular strength. In 1999, Kohno *et al.* performed posterior C4-C5 decompression and fusion on 3 HD patients (*51*). The surgery was successful and stopped the progression of the disease. Xu *et al.* performed posterior cervical fixation from the C3-C7 segment on a patient who had not benefited from 2-years of cervical collar therapy (*52*). Follow-up at three months and four years after surgery showed improvements in muscle atrophy. Goel *et al.* postulated that HD patients have multiple levels of cervical spine alignment imbalance that are not limited to the diseased segment. Therefore, they performed C1-C6/C7 posterior fixation on 4 patients. The patients were followed after surgery and showed no progression of hand symptoms (*53*).

Based on the clinical and imaging characteristics of HD patients, Konno *et al.* proposed that the dura mater lacks elasticity causing the spinal cord to become compressed and flattened and the spinal cord moves forward when patients flex their necks. They performed a duraplasty and fusion in patients and showed improvement of symptoms after surgery (*54*). In 2014, Ito *et al*. performed cervical duraplasty with tenting sutures *via* laminoplasty on HD patients. Their surgery achieved positive results (*44*). In 2009, Patel *et al.* performed C4-T1 laminectomy and duraplasty to treat HD patients. The muscle strength of the patients improved significantly after the surgery (*55*). Brandicourt *et al.* performed a posterior cervical laminectomy and posterior venous plexus micro-resection on HD patients. All of the patients' symptoms stopped progressing after the surgery (*56*).

In general, most of the studies on posterior cervical surgery are case reports or case series. Compared to anterior cervical surgery, there is still a lack of advanced evidence-based medical studies to support its effectiveness and reliability.

4. Anterior cervical surgery

The literature and key conclusion on anterior cervical surgery are summarized in Table 3.

4.1. Is anterior cervical surgery effective?

Paredes *et al.* (*57*) admitted a Caucasian male with HD, and cervical collar therapy was ineffective. They chose C5-C6 anterior cervical discectomy and fusion (ACDF). One year after the surgery, the lower limb muscle strength in the patient had completely recovered and the symptoms of upper limbs stopped progressing. Imamura *et al.* (*58*) reported a case in a 16-year-old HD patient who underwent anterior cervical corpectomy decompression and fusion (ACCF). The patient was in good condition after surgery without complications and follow-up at six months showed the symptoms had stopped progressing. Wu *et al.* (*59*) reported a 34-year-old patient with advanced HD who underwent ACCF surgery and recovered well after surgery. Kohno *et al.* (*51*) performed ACCF on 4 patients with HD which stopped the progression of the disease. Their case illustrates the effectiveness of anterior cervical surgery.

Zhang *et al.* (*60*) performed anterior cervical internal fixation with or without fusion on 19 HD patients. For the internal fixation group, titanium plates and screws were used for fixtures during the operation, while the fusion group used intervertebral fusion cages or autologous iliac bone graft. After an average of 70 months of follow-up, the patient had a reduced range of flexion in the cervical spine, the lower cervical spine mobility angle was reduced, the cervical lordosis was restored and the cross-sectional area of the spinal cord increased. Liu *et al.* (*61*) investigated short-term to midterm clinical outcomes after ACDF surgery among 115 patients with HD. Their study found that most patients showed improvement in postoperative cervical sagittal alignments, hand function rating scales, and EMG data. Therefore, they concluded HD patients can benefit from ACDF surgery. These data indicated that anterior cervical surgery has significant clinical efficacy and is associated with improvements detected on imaging.

In addition to case control and cohort studies, a RCT showed that anterior cervical surgery is effective in treating HD. In 2013, Lu *et al.* carried a prospective RCT (*45*) involving anterior cervical decompression and fusion in 48 male HD patients. 24 patients underwent ACDF and 24 underwent ACCF. Symptoms were assessed and EMG was performed before and after surgery. Both groups showed improvements of > 60% demonstrating that anterior cervical surgery is effective in treating HD.

4.2. What factors affect the effectiveness of anterior cervical surgery?

As mentioned above, many physicians tend to perform posterior cervical surgery on HD patients based on the fact that posterior surgery can reduce cervical spine alignments imbalance. Anterior surgery is also an active area of research. A retrospective study by Song *et al.* (*62*) included 23 HD patients. ACDF were performed and the alignment of the cervical spine in sagittal position was compared to 21 healthy participants before and after the surgery. Specifically, the alignments included the C2-C7 Cobb angle, the thoracic inlet angle, the C2-C7 sagittal vertical axis and the neck tilt angle. The study showed an

Table 2. Summary of the studies about posterior cervical surgery

References	Type of study	Key conclusion
Ashour M, *et al.* (*29*)	Case report	Patient got symptom improvement with Posterior cervical surgery.
Masak T, *et al*. (*50*)	Case report	Patient got symptom improvement with posterior C2-C6 segmental fusion.
Kohno M, *et al.* (*51*)	Case report	Patient got symptom stabilization with posterior C4-C5 decompression and fusion.
Xu Q, *et al.* (*52*)	Case report	Patient got symptom improvement with posterior cervical fixation from the C3-C7 segment.
Goel A, *et al.* (*53*)	Case report	Patient got symptom improvement with posterior C1-C6/C7 posterior fixation.
Konno S, *et al.* (*54*)	Case report	Patient got symptom improvement with posterior duraplasty and fusion surgery.
Ito H, *et al.* (*44*)	Case report	Patient got symptom improvement with posterior duraplasty surgery.
Patel TR, *et al.* (*55*)	Case report	Patient got symptom improvement with C4-T1 laminectomy and duraplasty.
Brandicourt P, *et al.* (*56*)	Case report	Patient got symptom improvement with posterior cervical laminectomy and posterior venous plexus micro-resection.
Thakar S, *et al.* (*42*)	Case control study	Compared with conservative treatment, cervical duraplasty can help HD patients get better clinical and electrophysiological improvement.

HD, Hirayama disease.

Table 3. Summary of the studies about anterior cervical surgery

References	Type of study	Key conclusion
McGregor S, *et al.* (*14*)	Case report	Patient got symptom stabilization with ACDF.
Galletta K, *et al.* (*17*)	Case report	Patient got symptom stabilization with ACDF.
Salome M, *et al.* (*18*)	Case report	Patient got symptom stabilization with ACDF.
Witiw CD, *et al.* (*26*)	Case report	Patient got symptom improvement with ACDF.
Paredes I, *et al.* (*57*)	Case report	Patient got symptom improvement with C5-C6 ACDF.
Imamura H, *et al.* (*58*)	Case report	Patient got symptom improvement with ACCF.
Wu W, *et al.* (*59*)	Case report	Patient got symptom improvement with ACCF.
Kohno M, *et al.* (*51*)	Case report	Patient got symptom improvement with ACCF.
Zhang H, *et al.* (*60*)	Cohort study	Patients can benefit from anterior cervical surgery; fusion and fixation have similar efficacy.
Liu S, *et al.* (*61*)	Case control study	Patients showed improvement in cervical sagittal alignments, hand function rating scales, and electromyographic data after ACDF surgery.
Lu F, *et al.* (*45*)	RCT	Patients can benefit from anterior cervical surgery; ACDF and ACCF have similar efficacy.
Song J, *et al.* (*62*)	Cohort study	HD patient have an imbalance of the cervical spine alignments compared to healthy individuals and the imbalance can be corrected by ACDF.
Lu X, *et al.* (*49*)	Case control study	Preoperative sagittal alignments of the cervical spine in HD patients may be a predictor of postoperative outcomes.
Zheng C, *et al.* (*46*)	Cohort study	ACDF for HD patients can restore nerve conduction in the cervical spinal cord and restore muscle strength.
Wang HL, *et al.* (*64*)	Cohort study	ACDF for HD patients can promote the functional reconstruction of upper motor neurons.
Zou F, *et al.* (*65*)	Case control study	The degree of spinal cord flatness, spinal atrophy, and postoperative spinal cord recovery is related to the outcome of ACDF for HD patients.
Song J, *et al.* (*62*)	Case control study	Age of onset, course of the disease, physiological reflexes and pathological reflexes were risk factors that affect the outcome of ACDF for HD patients.
Yu Q, *et al.* (*66*)	Case control study	Loss of attachment is an important factor affecting surgical outcomes in patients with HD.
Wang H, *et al.* (*67*)	Case control study	In addition to neck flexion MRI, dynamic X-ray is also an important reference index for selecting surgical segments in patients with HD.
Lu X, *et al.* (*63*)	Cohort study	HD patient have increased stress in the entire cervical spine and can be relieved by ACDF.

HD, Hirayama disease; ACDF, anterior cervical discectomy and fusion; ACCF, anterior cervical corpectomy decompression and fusion.

imbalance of the cervical spine alignments in HD patients compared to healthy individuals, and the imbalance can be corrected by ACDF. Another study carried out by the team concluded that preoperative sagittal alignment of the cervical spine in HD patients may be a predictor of postoperative outcomes (*49*). In addition, the study of Lu *et al.* pointed out that HD patients have increased stress in the entire cervical spine and it can be relieved by ACDF (*63*).

A study by Zheng *et al.* (*46*) showed that ACDF for HD patients can immediately eliminate the abnormality of the F wave in the flexion position of the neck. The results showed ACDF can help to restore not only the nerve conduction in the cervical spinal cord but also the muscle strength of the upper limbs. Also, Wang *et al*. (*64*) showed that ACDF can promote the functional reconstruction of upper motor neurons. By measuring brain functional magnetic resonance imaging (fMRI) and handgrip strength before and after surgery, they found that the contralateral and ipsilateral cortex were simultaneously activated before surgery. After surgery, the observed pathological activation decreased and the handgrip strength increased.

Studies have analyzed the factors that affect surgery outcomes. Zou *et al*. (*65*) retrospectively analyzed 40 HD patients and performed ACDF on these patients. The patients were followed for an average of 18 months and Odom scores were recorded after surgery. Based on the scores, the patients were divided into effective and ineffective groups and the imaging findings were

compared across the two groups. The data indicated that the degree of spinal cord flatness, spinal atrophy, and postoperative spinal cord recovery is related to the outcome of the surgery. Another retrospective study by Song *et al.* (*62*) included 210 patients who underwent ACDF. The Odom score was recorded after surgery, and the patients were divided into effective and ineffective groups. The age, gender, age of onset, course of the disease, cervical alignment, physiological and pathological reflexes, and involvement of the bilateral extremities were compared across the groups. The authors concluded that four factors, age of onset, course of the disease, physiological reflexes and pathological reflexes were risk factors that affected the outcome of the surgery.

The above studies have confirmed reliability and effectiveness of anterior surgery for HD. In addition, a preliminary exploration of the factors affecting the surgical effect was also carried out in these studies. However, the choice of surgical segments remains controversial.

4.3. How many segments and which segments should be chosen?

The first question is how many segments should be chosen. Paredes (*57*) chose one segment. In the study of Zhang *et al.* (*60*), there are examples of choosing three segments. Lu *et al.* (*45*) and Zheng *et al.* (*46*) chose two segments and believed that fusion of the two segments could limit the abnormal movement of the cervical spine while retaining sufficient cervical spine mobility. The study of Yu *et al.* (*66*) showed that when the longitudinal separation range is ≥ 5 cervical segments, longer segment fusion may be required to further improve the surgical efficacy in such patients. Wang *et al.* (*67*) proposed that as the cervical spine pressure of HD patients changes when standing and lying down, previous studies only using magnetic resonance imaging (MRI) to determine stability of the cervical spine are not comprehensive. They then conducted a retrospective analysis of 50 HD patients and measured the Cobb angle of the cervical spine and the range of motion of the cervical spine with dynamic X-ray imaging and MRI. The study concluded that two imaging techniques should be used to select the most appropriate surgical segments. The unstable segment of the dynamic position X-ray is also a factor that should be considered. Clinical guidelines (*43*) and update reviews (*48*) indicate that patients with HD always have an imbalance in the cervical spine sagittal alignments. In addition, the patient also has imaging manifestations characterized by loss of attachment between the posterior dural sac and the subjacent lamina, and the clinical symptoms are mainly hand dysfunction. Therefore, the selection of surgical segments needs to combine dynamic X-ray, neck flexion MRI and EMG.

In summary, many studies have confirmed the effectiveness and reliability of anterior surgery. However, there is still a lack of reports comparing anterior and posterior cervical surgery.

5. Conclusions

With an improved understanding of the clinical manifestations and pathogenesis of HD, an increasing number of cases are being diagnosed. The treatment of HD can be divided into conservative and surgical treatments. When conservative treatment fails to stop progression, surgery can be used. However, the indications for surgery remain controversial and more evidence is required to support its use. Surgical treatments can be divided into anterior and posterior cervical surgery. These two surgical methods can effectively stop the disease. Anterior cervical surgery has more detailed studies concerning the choice of surgical methods, surgical segments, and prognosis. However, no study has demonstrated superiority of either approach. Future research should focus on exploring surgical indications and comparing the advantages and limitations of anterior and posterior cervical surgery.

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Original Article

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Knowledge level of medical students and physicians about rare diseases in Lima, Peru

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SUMMARY Rare diseases (RDs) affect up to 8% of the world's population, and unfortunately, health professionals have a low level of knowledge regarding the impacts of RDs on the social, psychological, and economic spheres of the patients and their families; hence, RD management is inadequate, consistently empirical, and precarious. The objective of this study was to determine the knowledge level of the medical students from a non-state university and physicians from Lima, Peru of RDs through a virtual survey for an analytical cross-sectional study. A total of 338 medical students and 382 physicians were surveyed. Results showed that several of the respondents (68.1% of students and 48.7% of physicians) had heard of the term "rare disease", but only a few stated that they had received any kind of training specific to it. Of the physicians, 46.6% considered that there should be a course about RDs in medical curricula, and more than 60% considered RDs a public health problem. Most respondents prioritized the planning of a higher budget for common diseases and believe it is convenient to allocate a specific fund for RDs. More than half of the participants had a very poor knowledge level. Due to students and physicians' low level of general knowledge of RDs, it is important to raise awareness and improve their education about these pathologies because this will have beneficial effects for RD patient care.

Keywords rare diseases, orphan drugs, medical students, physicians, knowledge

1. Introduction

A rare disease (RD), also known as an infrequent disease or minority disease, is one in which there is a risk of death or chronic disability and low incidence (*1*), with heterogeneous clinical manifestations such as congenital hypotonia, intellectual disability, autism spectrum disorder, congenital anomalies, altered anthropometry, signs of neuroregression, or the appearance of common diseases that do not correspond to the patient's age group (*2*).

The threshold for defining an RD varies among regions. For example, the European Union defines a disease as rare when it affects no more than 1 person per 2,000 people (≈250,000 people) (*3*), whereas, in the United States, a disease is considered rare when it affects less than 200,000 people (≈1 per 1,600) (*1*). Regarding Asia, in Japan, an RD is a disease that affects less than 50,000 people (≈1 per 2,500) (*4*). Between 3.5-8% of the world population has an RD and approximately 7,000 RDs exist, 80% of which are estimated to be of a genetic origin (*5,6*).

In Spain, future healthcare and non-healthcare professionals have a low level of general knowledge of RDs, and none prioritize the allocation of funds to these diseases (*7*); in contrast, another study conducted on Spanish primary care physicians found a high level of interest in prevention, improvement of the family environment, genetic counseling, and medical education (*8*).

In Norway, 24% of the general population showed little interest in prioritizing RDs (*9*). Conversely, Norwegian doctors showed a preference for prioritizing the treatment of common diseases (77.4%) instead of RDs (*10*).

Counselors of the National Institute for Clinical Excellence (NICE) rarely have a favorable predisposition toward the reimbursement of drugs for RDs, although more than half would agree to reimbursement if the use of the drug is well documented or based on disease severity that is severe (*10*).

The lack of interest in recognizing RDs is a result of inadequate instruction and administrative management of people who are unaware of the prevalence, etiology, and manifestations of RDs as well as the forms of adequate and timely management, which directly harm the patients and their families, affecting them socially, psychologically, and economically and portraying

them as rarely able to recover or are beyond recovery. Therefore, it is important to demystify what is related to RDs in order to generate universality and equity in this group of pathologies.

The aim of this study was to determine the knowledge level of medical students and physicians of RDs and its relationship with the need to include training, prioritization of resource allocation, and the degree of interest in these pathologies.

2. Materials and Methods

The study design was cross-sectional and analytical. The research was carried out between the months of December 2020 and March 2022, among medical students above the age of 18 from the Universidad Científica del Sur (UCSUR) and physicians from Lima, Peru through a virtual survey using Google Forms®.

A survey was designed based on previous reports (*11-15*) and was validated by 11 experts in the field of RDs with a Cronbach's alpha of 0.92. The survey was anonymous and contained 38 questions comprising of open, dichotomous, and multiple-choice questions. The survey was organized into four groups:

i) The first group comprised eight questions about personal information, including sex, occupation, career year of study, medical specialties, career length (years as a physician and years as specialist), level and sector of healthcare in which they practice (the last four were addressed only to the physicians).

ii) The second group comprised 17 questions that investigated the participants' general knowledge of RDs. Among them, polychotomous questions were included on the correct definition of RDs and orphan drugs (ODs), the most frequent etiology, and the estimated number of RDs. Other questions were about the typical manifestations, the percentage of the population affected by these, and treatments available in Peru and worldwide. In addition, participants were asked to mention three rare diseases. A score (between 4-9 points) was assigned to the 17 questions, which gave a total sum of 100 points.

iii) The third group included seven questions related to academic education and participants' self-perceptions of their competence in the RD field. The participants were asked whether they had ever heard the term "rare disease" or "orphan drug", whether they consider it necessary to include a course on RDs in the MD curriculum, and whether they had ever attended a conference on RDs. They were also asked whether they had ever encountered a patient with an RD and whether they felt prepared to care for/treat a patient with any of these pathologies. These last two questions had the same purpose but were formulated differently for the students and the physicians.

iv) The fourth group of questions referred to organizational issues and the participants' attitude towards RDs. It included 2 dichotomous questions asking whether participants consider RDs to be a public health problem and whether they believe that resources should be allocated for RDs and ODs. In addition, three Likert scale-type questions were added about the importance of RD coverage by insurance systems and the importance of the etiological diagnosis (1 = *very unimportant*, 5 = *very important*); and the support of pharmaceutical laboratories in the diagnosis of RDs (1 = *strongly disagree*, 5 = *strongly agree*). Finally, a question was included only for the physicians, asking whether they consider it important to allocate the same budget for both RDs and common diseases.

Ethics approval and research approval were obtained from the Ethics Committees of the Universidad Científica del Sur and the Instituto Nacional de Salud del Niño de Breña. Informed consent was obtained from all the participants included in this study.

The dependent variable was the knowledge level, with a scale of 0-100 points and was categorized as: A - *excellent* (90-100 points); B - *good* (80-89 points); C - *sufficien*t (70-79 points); D - *poor* (60-69 points); and E - *very poor* (0-59 points) (*16*).

The independent variables were sex, occupation, medical specialty, years of experience as a physician, years of experience as a specialist, career year of study, level and sector of healthcare. The specialties were categorized into six groups: *i*) resident doctors and MD without specialty; *ii*) general surgery; *iii*) pediatrics and neonatology; *iv*) internal medicine and related (cardiology, dermatology, endocrinology, gastroenterology, geriatrics, hematology, infectious diseases, family medicine, intensive and emergency medicine, legal medicine, pneumology, nephrology, oncology, psychiatry, rheumatology, genetics); *v*) other surgical specialties (anesthesiology, gynecology and obstetrics, ophthalmology, orthopedics and traumatology, otorhinolaryngology, urology); and *vi*) others (health administration and management, allergy and immunopathology, pathology and laboratory anatomy, epidemiology, radiology, public health).

The sample size was calculated using the OpenEpi version 3 software (*www.openepi.com*). The medical student population at UCSUR in 2020 was 2,759 (*17*); and the medical professional population in Lima in 2019 was 47,465 physicians (*18*). Since there were no references that analyzed global knowledge, the hypothetical frequency was 50%, and a sample of 338 students and 382 physicians was obtained, with a confidence interval of 95%.

The answers obtained on Google Forms® were automatically transferred to Google Sheets, and the database was then relocated to Microsoft Excel for encoding. The statistical analysis was performed with Stata, with a statistical significance of $p < 0.05$ and a 95% confidence interval. First, the frequencies of each of the questions in both populations were calculated, and the means and standard deviations of the years of experience

and scores were obtained. The bivariate analysis was performed using the student's *t*-test and the variance analysis test and the corresponding post-hoc analysis. In addition, robust Poisson regression was used to calculate the crude and adjusted prevalence ratio (PR). To carry out this analysis, the dependent variable (knowledge level) was divided into two groups: deficient (0-69 points) and sufficient (70 points or more), and the PR was calculated for this new variable with the following questions: "Do you consider that RDs are a public health problem?"; "Have you ever attended a course, workshop, or educational congress on RDs?"; and "Do you think the government should designate a fund specifically for RDs and ODs?".

3. Results

3.1. Sociodemographic characteristics of the participants

The survey was answered by a total of 338 students and 382 physicians, the majority of whom were women (in both groups; 61.5% and 53.4%, respectively). As shown in Table 1, 59.4% of the physicians worked in the public sector and 78.3% belonged to the third level of healthcare.

Regarding knowledge level, 75.2% of the medical students and 61.8% of the physicians obtained a score reflecting a very poor level (Table 1). The mean score achieved was 51.3 (SD: 12.6) for the students and 55.6 (SD: 12.6) for the physicians.

3.2. General knowledge of RDs

About 87% of both groups provided the correct definition of RD. However, less than half of the participants knew that the most common cause of RDs is genetic (48.5% of the students, 41.9% of the physicians). Only 9.5% and 6.3% of the students and physicians, respectively, knew the frequency of the prevalence of RDs, while 25.2% of the students and 20.7% of the physicians correctly estimated the number of RDs that exist. Similarly, a low number of participants in both groups knew that RDs mostly affect children (11.8% of the students and 19.4% of the physicians). More than 50% of the students and physicians believed that RDs did not discriminate against countries with low or high resources (Table 2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

More than 90% of the participants in both groups knew that the treatment of RD is expensive. The majority was not aware of the existence of a law on RDs in Peru (75.1% and 50.5% of the students and physicians, respectively).

Among the typical manifestations of RDs, of the 11 options given, the most recognized by the students were manifestations from birth (congenital anomalies), and in the case of the physicians, it was altered anthropometry (Table 2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

Table 1. General characteristics of the medical students and physicians

Items	*n* (%)
Students (*n* = 338)	
Gender	
Female	208 (61.5)
Male	130 (38.5)
Year of studies	
First year	35 (10.4)
Second year	35 (10.4)
Third year	35 (10.4)
Fourth year	40 (11.8)
Fifth year	60 (17.8)
Sixth year	88 (26.0)
Seventh year	45 (13.3)
Knowledge level	
Very deficient (0-59 points)	254 (75.2)
Deficient (60-69 points)	57 (16.9)
Sufficient (70-79 points)	23 (6.8)
Good (80-89 points)	3 (0.9)
Excellent (90-100 points)	1 (0.3)
Physicians (*n* = 382)	
Gender	
Female	204 (53.4)
Male	178 (46.6)
Medical specialty	
Residents doctors and MD without specialty	185 (48.4)
Internal medicine and others medical specialties	89 (23.3)
Other surgical specialties	42 (11.0)
General surgeons	25 (6.5)
Pediatric and neonatology	25 (6.5)
Other	16 (4.2)
Healthcare sector	
Public sector	227 (59.4)
Both	90 (23.6)
Private sector	65 (17.0)
Healthcare level	
First level	49 (12.8)
Second level	34 (8.9)
Third level	299 (78.3)
Career length (years of medical doctor)	
0-5	159 (41.6)
6-10	80 (20.9)
11-15	38 (9.9)
16-20	23 (6.0)
More than 20	82 (21.5)
Career length (years of specialist)	
0-5	65 (17.0)
6-10	34 (8.9)
11-15	25 (6.5)
16-20	26 (6.8)
More than 20	46 (12.0)
No specialty	186 (48.7)
Knowledge level	
Very deficient (0-59 points)	236 (61.8)
Deficient (60-69 points)	89 (23.3)
Sufficient (70-79 points)	42 (11.0)
Good (80-89 points)	15 (3.4)
Excellent (90-100 points)	0 (0.0)

Regarding the studies available in Peru on the diagnosis of RDs, neonatal screening and karyotyping were the most remembered methods by both groups. When questioning the participants about the informatic programs that recognize the clinical diagnosis, the best-known platform among the students and physicians was PubMed. On the other hand, Phenomizer, OMIM,

FDNA (Face2Gene), and Possum were the least known (Table 2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

The answers to the question about the most recognized treatments available, in Peru as well as worldwide, were special formulas and gene therapy. An open response was placed on this question where another treatment mentioned was immunoglobulin therapy (Table 2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

Finally, regarding the open-ended question that asked the participants to mention three RDs, 10% of the participants did not answer or did not know the answer. Among the most reported RDs were Marfan syndrome, Prader Willi syndrome, fragile X syndrome, amyotrophic lateral sclerosis, and phenylketonuria (Table 2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

3.3. General opinion on RDs and ODs

Although several of the respondents (68.1% of students and 48.7% of physicians) had heard of the term "rare disease", only a small percentage of the doctors and students claimed to have received any kind of education about it. Most of the participants were of the opinion that a course about RDs should be included in medical curricula and more than 60% of them considered RDs to be a public health problem. On the other hand, a considerable number of participants were in favor of allocating a specific fund for RDs and ODs (Table 3).

More than 75% of the students and physicians believed that RD coverage by insurance systems is important. A minority (2.1% of the students and 10% of the physicians) considered that the etiological diagnosis of RD is not important (Table 3). More than 60% of the students agreed that laboratories support the diagnosis of RDs, while a few of them (17.5%) thought the opposite and 18.3% had a neutral stance (Table 3).

The participants were also asked whether they had

Table 3. Frequency of the general opinion on RDs among the medical students and physicians

Characteristics	*n* (%)	
	Students *n* = 338	Physicians *n* = 382
Have you ever heard the term rare diseases or orphan drugs?		
Rare diseases	230 (68.1)	186 (48.7)
Orphan drugs	15 (4.4)	30 (7.9)
Both	45 (13.3)	131 (34.3)
None of the above	48 (14.2)	35 (9.2)
Do you consider that there should be a subject about rare diseases in medical curricula?		
Yes	247 (73.1)	178 (46.6)
No	14 (4.1)	64 (16.8)
Maybe	77 (22.8)	140 (36.7)
Have you ever been on a conference, course, or congress about rare diseases?		
Yes	31 (9.2)	86 (22.5)
No	307 (90.8)	209 (77.5)
Do you consider rare diseases as a public health problem?		
Yes	220 (65.1)	233 (61.0)
No	118 (34.9)	149 (39.0)
Do you consider that the government allocate a specific fund for rare diseases and orphan drugs?		
Yes	312 (92.3)	308 (80.6)
No	26 (7.7)	74 (19.4)
Likert scale questions		
Do you think is important for coverage of rare diseases by insurance systems?		
Very Unimportant	1 (0.3)	7 (1.8)
Unimportant	3 (0.9)	12 (3.1)
Neutral	30 (8.9)	63 (16.5)
Important	76 (22.5)	110 (28.8)
Very Important	228 (67.5)	190 (49.7)
Do you consider important the etiological diagnosis?		
Very Unimportant	3 (0.9)	14 (3.7)
Unimportant	4 (1.2)	24 (6.3)
Neutral	30 (8.9)	80 (20.9)
Important	85 (25.2)	104 (27.2)
Very Important	216 (63.9)	160 (41.9)
Do you agree that pharmaceutical laboratories support the diagnosis of rare diseases?		
Strongly Disagree	12 (3.6)	14 (3.7)
Disagree	46 (13.9)	37 (9.7)
Neutral	62 (18.3)	102 (26.7)
Agree	62 (18.3)	109 (28.5)
Strongly Agree	155 (45.9)	120 (31.4)

Table 3. Frequency of the general opinion on RDs among the medical students and physicians (continued)

Characteristics	*n* (%)	
	Students *n* = 338	Physicians *n* = 382
Only for students		
During your studies have you ever suspected a rare disease in your medical practice?		
Yes	177 (52.4)	
No	79 (23.4)	
Maybe	82 (24.3)	
Do you think that your training gives you the ability to care for a patient with a rare disease in the future?		
Yes	143 (42.3)	
No	195 (57.7)	
Only for physicians		
Have you ever treated a patient with a rare disease?		
Yes		215 (56.3)
No		51 (13.4)
Maybe		116 (30.4)
Do you feel prepared to care for a patient with a rare disease?		
Yes		121 (31.7)
No		88 (23.0)
Maybe		173 (45.3)
Do you think a budget should be assigned for rare diseases?		
It does not matter if you assign a higher budget for one or another		22 (5.8)
Higher Budget for common diseases		236 (61.8)
Higher Budget for rare diseases		30 (7.9)
Same Budget for both		94 (24.6)

ever come across a patient with an RD and whether they felt prepared to care for patients with any of these pathologies. Regarding the former, more than 50% of both the students (52.4%) and physicians (56.3%) reported having known a patient with an RD during their training or professional practice. And regarding the latter, 23% of the physicians considered themselves not trained to care for a patient with an RD, while more than half of the students (57.7%) considered that their training did not give them the skills to care for a patient with an RD in the future (Table 3).

Finally, a question was included on whether the participants considered it important to designate the same budget for both RDs and common diseases, taking into account that the treatment of some RDs is more expensive than that of common diseases, but this was addressed only to the physicians. Most of the physicians (61.8%) believed the largest budget should be allocated to common diseases, and only 7.9% thought that it should be allocated to RDs (Table 3).

3.4. Analysis of the knowledge level compared to previous education, degree of empathy, and general characteristics of the participants

Among the students, it was observed that men had a greater knowledge about RDs in comparison to women ($p = 0.02$) (Table 4). On the other hand, no significant differences between the sexes regarding the knowledge level were found among the physicians ($p = 0.18$). The students and physicians who indicated having received some type of training with respect to RDs had better scores on the survey (Table 4).

Regarding the scores obtained by the students in relation to whether they considered RDs to be a public health problem, no significant differences were found ($p = 0.83$). Conversely, there was a significant difference ($p < 0.001$) among the physicians, as those who did consider it a public health issue obtained a higher score in the survey (Table 4).

When we asked if they agreed that the government should designate a fund specifically for RDs and ODs, the mean knowledge level was higher in the group that agreed with the question in the students and physicians (Table 4).

3.5. Analysis of the knowledge level regarding the allocation of resources, demographic characteristics, and the participants' self-perceptions about their competencies in RDs

Both the students and physicians who positively answered the question "Have you ever heard the term RD or ODs?" obtained a significantly higher score on the survey ($p < 0.001$) (Table 5 *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

For the question of whether they thought a course on RDs should be included in the medical curricula, no significant differences ($p = 0.56$) were observed in the general scores of the physicians. However, among the students, those who disagreed with the need for a course on RDs were the ones who obtained the lowest scores (42.9) (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

Regarding the relationship between the knowledge level and importance of the etiological diagnosis of RDs, no significant differences were found ($p = 0.35$) between the physicians who did not consider it important and those who believed it to be very important (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

Regarding the relationship between the knowledge level and whether the participants considered that pharmaceutical laboratories should support the diagnosis

Table 4. Analysis between the knowledge level and sociodemographic characteristics, previous training, and level of empathy of the medical students and physicians

Variables	Sample survey					
	Students			Physicians		
	Mean	SD	*p*	Mean	SD	*p*
Gender						0.18
Female	50.2	12.4	0.02	55.0	13.1	
Male	53.2	12.8		56.2	12.6	
Have you ever been on a conference, course, or congress about rare diseases?						< 0.001
Yes	57.0	12.1	0.009	61.1	12.7	
No	50.8	12.6		53.9	12.5	
Do you consider rare diseases as a public health problem?						< 0.001
Yes	51.4	13.2	0.83	58.2	13.2	
No	51.1	11.5		51.3	11.1	
Do you consider that the government allocates a specific fund for rare diseases and orphan drugs?						< 0.001
Yes	51.5	12.7	0.50	56.1	12.9	
No	49.7	11.5		53.3	12.5	
Do you have a specialty?						0.05
Yes				56.6	13.4	
No				54.4	12.3	

of RDs, physicians with the highest score in the survey were in favor of pharmaceutical laboratories supporting RDs ($p = 0.003$). Despite this, no significant differences were found among the students ($p = 0.92$) since almost all of them obtained the same score in the knowledge survey regardless of how much they agreed or disagreed (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

The physicians who worked in both public and private sectors had greater knowledge about RDs (59.2 points) in contrast to those who worked only in the public or private sector (54 points) ($p = 0.01$), (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

For the relationship between the healthcare level (I, II, or III) and the score achieved in the RDs survey, no significant differences were found ($p = 0.84$), with a similar score (approximately 55 points) (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

No differences were found regarding the knowledge level of general practitioners and specialists ($p = 0.05$). Regarding the years of experience as a physician and the knowledge level, there was a significant difference ($p = 0.003$) between those who had more than 20 years of experience and those who had between zero and five years of experience (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*). The years of experience as a medical specialist did not affect the knowledge level (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

In reference to the question addressed to the physicians about whether they had ever treated a patient with an RD, a statistically significant difference was found ($p < 0.001$), as those who stated that they had treated a patient with one of these pathologies obtained a higher score on the survey than those who had not treated a patient with an RD. The students were asked whether, at any time during their training, they had suspected any RD in their clinical rotations, and significant differences were found ($p < 0.001$) since those who had commented on it in their pre-professional practices were those with the highest scores (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

We also assessed the relationship between the score achieved and the ability physicians feel to care for a patient with an RD; no significant differences were found ($p < 0.001$) since those who felt qualified had greater knowledge (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

The additional question addressed to only the physicians revealed that those who were indifferent to providing a supporting budget to RDs obtained the lowest scores (47.7 points) (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

When comparing the variable *year of studies* with the score achieved in the survey, the knowledge level increased slightly from the first to sixth year; however, in the seventh year the knowledge level dropped to 51.7 ($p < 0.001$) (Table 5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=121*).

11% of the students and 25% of the physicians who previously attended training on RDs demonstrated a sufficient knowledge level. Only 6% of the students and 17% of the physicians who considered RDs to be a public health problem had a sufficient knowledge level. In the case of whether the government should allocate a fund specifically for RDs and ODs, only 8% of the students and 3% of the physicians who were in favor of it showed scores indicative of sufficient knowledge (Table 6).

4. Discussion

This research confirms the previous findings of other countries that have shown that both medical students and health professionals lack knowledge of RDs. For example, research carried out in La Rioja, Spain concluded that future healthcare and non-healthcare professionals have a low level of general knowledge of this subject and none of them prioritize the allocation of funds to RDs (*7*). Also, another study conducted in Kazakhstan showed deficient knowledge if the epidemiology of RDs, since only 5% of the physicians had mastered the frequency of prevalence and correctly estimated the number of RDs (*19*). In this research, 92% of the students and 85.6% of the physicians obtained scores indicative of an insufficient or low level of knowledge, as demonstrated in a study carried out in Spain, where the primary health care professionals lack knowledge about RDs and this lack is consciously perceived by physicians (*20*).

The results of our study indicate that, although almost all the participants knew the correct definition of RDs, they tend to underestimate the epidemiological burden of RDs. Additionally, less than 25% of the participants did not know the current number of existing RDs, and very few students knew that RDs develop mainly in the childhood population. Similar findings were reported in China, where doctors did not know how many RDs exist (58.1%), and that the most frequent age of onset is in childhood (30.9%) (*21*).

The results regarding the participants' knowledge level show that the more knowledge the participants have, the more sensitive or empathetic they are to support RDs in terms of diagnosis by pharmaceutical laboratories and coverage of these diseases by insurance systems. This idea was based on a previous study carried out in China among nine RD experts, where all of them expressed concern about the high cost of ODs and the majority of them (seven out of nine participants, 77.8%) supported the creation of a special insurance program for RDs (*22*).

A study carried out in Norway indicated that almost

Table 6. Analysis between insufficient knowledge level and academic education, perception of RDs as a public health problem and the allocation of resources of the medical students and physicians

Items	PR	*p*	95% CI
Have you ever been on a conference, course, or congress about rare diseases?			
Students			
Crude	1.11	0.08	0.95-1.29
Adjusted	1.11	0.21	0.95-1.29
Physicians			
Crude	1.28	< 0.001	1.11-1.48
Adjusted	1.25	0.002	1.08-1.45
Do you consider rare diseases a public health problem?			
Students			
Crude	1.06	0.06	1-1.13
Adjusted	1.06	0.06	1-1.12
Physicians			
Crude	1.19	< 0.001	1.11-1.29
Adjusted	1.17	< 0.001	1.08-1.26
Do you consider that the government allocates a specific fund for rare diseases and orphan drugs?			
Students			
Crude	1.09	0.12	1.06-1.13
Adjusted	1.08	< 0.001	1.04-1.11
Physicians			
Crude	1.08	0.14	0.99-1.18
Adjusted	1.03	0.43	0.95-1.13

The statistical significances are bold. PR: prevalence ratio.

half of the participants (48.3%) believed that funds should be allocated to common diseases, since this group of pathologies is the most prevalent; however, a lower percentage (44.4%) agreed with assigning a small portion of the funds to RDs (*9*). The same occurred in our study: although more than 90% of the participants believed that RDs incur high costs, they prioritized the allocation of a larger budget for common diseases. However, they also believed it is convenient to allocate a specific fund for RDs and ODs.

In Peru, although the subject of genetics is included in the respective curricula of medical careers, it only encompasses general topics and occasionally touches on some genetically-related RDs, but no workshop or course specifically covers the topic. Therefore, the participants in our study believe that the inclusion of RDs in medical study programs is necessary. Similarly, in a previous study conducted among medical students in Poland, almost half of the participants (46.5%) agreed with adding an extra course on RDs in medical curricula (*13*). In this study, more than 50% of the participants considered that their training did not give them the skills to care for a patient with an RD, which coincides with research carried out in Spain, Iran, and Poland that showed that most future healthcare professionals (81%, 73%, and 92%, respectively) did not feel prepared to care for patients with an RD (*20,23,24*).

Another finding was that, although most of the participants perceived RDs as a public health problem, less than half were aware of the existence of a law in Peru related to RDs, which reflects the paltry effect that this rule has on the care for patients with RDs (*25*).

Regarding the studies available in our country, neonatal screening was one of the diagnostic methods for detecting RDs that was most recognized by both groups; however, this technology is limited to a group of only approximately 50 conditions, out of more than 7,000 RDs (*5*), although these pathologies are potentially treatable (*26*).

One point that both the students and physicians agreed on is that there is the same number of RDs in both developed and developing countries. Although the definition of RDs tells us that they are infrequent diseases and that the proportion of people affected could vary according to geographical area, there are no epidemiological studies in that respect. Rather, this assumption is the result of the lack of information, the absence of certain diagnostic methods throughout the world, and an inefficient registration system, as reported in China, where there is still very little documented information on the epidemiology of RDs (*27*).

Since RDs have diverse clinical manifestations and usually take time to be diagnosed, the lack of knowledge resulted in 38% of patients with RDs in Australia having to consult more than six different doctors before receiving the correct diagnosis; 37% believed that their diagnosis was delayed and 27% initially received an incorrect diagnosis (*28*). In Brazil, administrative obstacles for patients and their families caused delays in diagnosis (*29*), which can take between four to six years (*30*), and only 5% of RDs have treatment, as some of these pathologies can incur high costs (*9*).

Therefore, having a greater number of RD specialists would greatly impact the management of RDs. Although in our study, most of the participants considered geneticists to be the specialists to whom a patient should

be referred to for a definitive diagnosis of an RD, in our environment the physician-geneticists per million inhabitants is 1,1, which is below the reported figure for Latin American countries (1,9) and worldwide (12,2) (*31-33*).

Some important limitations to note are that this study cannot be generalized to the entire population of medical students or health professionals, since the sample included students from a single private medical university of Lima and only physicians who worked in Lima city, in addition to the fact that the study only represents the opinion of those who agreed to participate, for which further investigations would be necessary. Another limitation of the study is that being a virtual survey, the participants completed the survey without supervision and could have used additional information resources to provide the answers. Nevertheless, this study provides new insights into the knowledge level of RDs in a low- and middle-income country.

Although in 2011, a law was created in Peru that proposed a national plan for comprehensive care and a national registry of patients with RDs as well as budgetary guarantees for treatment, it remains scarcely known. Even the knowledge level of RDs in both future health professionals and physicians is very poor. The United Nations Educational, Scientific and Cultural Organization (UNESCO) tells us that health is one of the fundamental rights of every human being, which includes timely and affordable access to quality health care services (*34*); however, this is not reflected in patients with RDs because they must confront a utilitarian system with many difficulties, which have an impact on various areas of their lives. At a social level, due to the diversity of their condition, an individual patient with an RD experiences different disabilities, which often leads to exclusion with psychological implications. Another important problem is that there is a lack of information both in relation to the disease itself and scientific research, which is reflected in the delays in detection from the moment of the onset of symptoms until receiving the correct diagnosis. There is also a lack of knowledge in the reference centers from which patients can obtain support, which has a serious impact on the economic sphere of the families affected due to the high cost of the few existing medicines (due to the lack of profitability for the pharmaceutical companies) added to the deficit of social benefits and reimbursement due to the deficiency of support from the usual insurance systems. These show the inequity that exists between treating a patient with a common disease and a patient with an RD. Therefore, it is imperative to make medical students aware of RDs and educate them about this regard, since this will have a beneficial effect on the quality of patient care, quality of life, and family environment of those affected by RDs. In addition, there is an urgent need to create a cooperative network with the main hospitals in Lima and other regions, as well as with international institutions to improve the care of these patients.

In conclusion, this study shows the preference of physicians to prioritize the treatment of diseases with the largest/biggest number of patients (*i.e.*, common diseases), even though some of them reserve a small part of resources for RD. On the other hand, the lack of knowledge in both students and physicians causes concern, since most considered RDs as a public health problem, but they did not feel prepared to care for this type of patient, so we consider that the existence of a course that covers the topic of RDs in the curriculum of the medical career is essential. Finally, this study provides new insights into the knowledge level of rare diseases in a low-and middle-income country.

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Original Article

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Assessment of health-related quality of life in patients with spina muscular atrophy in China

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SUMMARY Spinal muscular atrophy (SMA) is a rare disease that has attracted considerable interest in China due to its severity and hefty treatment costs. Few studies have been conducted on Chinese patients. The objective of this study was to assess the quality of life of SMA patients in China and to investigate the real impact of new treatments. We used the Pediatric Quality of Life Inventory (PedsQL) to analyze the Health-related quality of life (HRQoL) of patients with SMA in China. Information on demographics, disease-specific characteristics, and treatment were collected using a child-reported or proxy-reported questionnaire. The mean scores of HRQoL for the Nusinersen treatment group and conventional treatment groups are 55.6 and 48.4, respectively. Patients with SMA type I have the lowest scores, while those with type III have the highest scores. A higher proportion of the medication group showed improvement in the condition in the past six months (56.9% *vs.* 17.1%). Our results show that the clinical type, motor function and treatment strategy have a significant influence on HRQoL. The findings imply that Nusinersen benefits patients by slowing the progression of the disease and increasing their quality of life in the real world.

Keywords spina muscular atrophy, quality of life, patient-reported outcome, rare diseases, Nusinersen

1. Introduction

Spinal muscular atrophy (SMA) is a rare genetic disease that causes muscle weakness. For children with severe SMA, respiratory failure is a common cause of death. Patients with SMA type I are unable to sit by themselves, and significant muscle weakness impairs breathing and swallowing, they will not survive 2 years without respiratory support. Patients with SMA type II can achieve unassisted sitting, but they gradually lose mobility and live shorter than the general population. SMA III can gain independence in walking without assistance (*1*).

SMA causes a considerable deterioration in HRQoL, affecting both patients and their caregivers (*2,3*). According to López (*2*), the average EQ-5D social tariff score for SMA patients was 0.16, significantly lower than that of young Spanish people (0.987). Using the youth version of the EQ-5D and Australiana utility weights Chambers (*1*) measured the HRQoL for SMA patients and found that the average score was 0.115. A systematic review conducted by Erik (2019) (*4*) confirmed that poor physical health contributes to HRQoL impairment in SMA.

Before the approval of Nusinersen, the management of SMA was limited to symptomatic treatment, and the conventional treatment is supportive care. Nusinersen is the first disease-modifying medication for SMA, significantly improving motor function (*5,6*). A huge potential for improving HRQoL can be reached if the new therapeutic intervention results in a less severe course of the disease (*7*). The National Medical Products Administration approved Nusinersen in February 2019 to treat 5qSMA.

There is little knowledge of HRQoL in Chinese SMA patients. Only one study for Chinese patients was published: Yao (*8*) recruited 101 children aged 0-17 years with SMA in China and measured their HRQoL using the Pediatric Quality of Life Inventory 3.0 Neuromuscular Module. Only six patients received Nusinersen treatment in this study, and further comparison analysis was constrained by the limited sample size. We aimed to assess the HRQoL of patients with SMA in China, and explore the effectiveness of Nusinersen in the real world.

2. Methods

2.1. Study design and participants

Two groups of subjects were surveyed, namely

medication and non-medication groups. The medication group included patients, who received Nusinersen during the study horizon, the non-medication group included patients, who received conventional treatment without Nusinersen. The allocation was non-randomized, and the patients' decision to receive Nusinersen was dependent on their own choices and willingness. The dosage and administration followed the instructions and medical prescription.

This study was approved by the Ethics Review Committee of The Chinese Academy of Medical Sciences and Peking Union Medical College Hospital (JS-1233), and informed consent was obtained prior to the investigation. Participants were enrolled from December 2019 to September 2020, and all participants were the members of Beijing Meier Advocacy & Support Center for SMA (Meier). We conducted three surveys in December 2019, April 2020, and September 2020 to evaluate the disease-specific characteristics and HRQoL. With the promotion of the new treatment, more patients were willing to choose the new drug. As an observational study, we did not set strict inclusion and exclusion criteria for participants. As a result, the total number of patients measured might change over time. The first measurement was considered as the baseline. We concentrated our further study on the third measurement in order to reflect the effectiveness of treatment more properly.

2.2. Measures

The basic information was extracted from Meier's Chinese SMA patient registry database. The main fields included the patient's age, gender, SMA clinical subtypes, onset age, motor function, the use of nasal or gastric tube feeding, and whether a non-invasive or invasive ventilator is used regularly.

The PedsQL 4.0 Generic Core Scales (PedsQL GCS) and the PedsQL 3.0 Neuromuscular Module (PedsQL NMM) are primarily designed to measure the HRQoL for SMA patients, according to a systematic review conducted by Erik (2019) (*4*). The PedsQL (Pediatric Quality of Life Inventory) was designed to integrate the relative merits of generic and disease-specific approaches. It demonstrated feasibility, reliability, and validity in the SMA population. The PedsQL NMM was designed to measure HRQoL dimensions specific to children ages 2 to 25 years with neuromuscular disorders, including SMA. The feasibility, reliability, and validity of the PedsQL NMM in children with SMA were supported by Susan (*9*). Hu (*10*) evaluated the reliability and validity of the Chinese version of PedsQL NMM. The study came to the conclusion that the instrument would be useful for measuring the HRQoL of Chinese children with neuromuscular diseases. PedsQL GCS was designed for both healthy and patient populations, and utilized for children with numerous disorders (*11*). The PedsQL Infant Scales (PedsQL IS) were designed as a generic HRQoL instrument specifically for healthy and ill infants ages 1-24 months. It was the first and only instrument developed specifically for infants. James (*12*) demonstrated the initial measurement properties of the PedsQL IS in healthy and ill infants and concluded that these instruments might be utilized to evaluate HRQoL in infants ages 1-24 months.

In this study, PedsQL was used to evaluate the HRQoL. PedsQL NMM was applicable for ages 2-25 years and encompassed three dimensions: *i*) about my neuromuscular disease (17 items), *ii*) communication (3 items), and *iii*) about our family resources (5 items). The PedsQL IS was applicable for infants (ages 1-24 months), composed of 36 items for infants ages 1-12 months and 45 items for infants ages 13-24 months, comprising five dimensions: *i*) physical functioning, *ii*) physical symptoms, *iii*) emotional functioning, *iv*) social functioning, and *v*) cognitive functioning. For adults ages over 26, PedsQL GCS was used, which encompasses four sizes: *i*) physical functioning (8 items), *ii*) emotional functioning (5 items), *iii*) social functioning (5 items), and *iv*) school functioning (5 items). Each multiple-answer was scored using a 5-point Likert scale from 0 (never a problem) to 4 (almost always a problem). Item scores were reversed and transformed on a scale from 0 to 100 (0 = 100, 1 = 75, 2 = 50, 3 = 0). Therefore, higher scores indicate better HRQoL.

2.3. Statistical analysis

Descriptive statistics were used to describe the characteristics of patients at baseline and compare the difference across subgroups. Continuous variables were summarized as mean ± standard deviations (SDs), and categorical variables were summarized using frequencies with percentages. Differences between groups were compared using a two-sample *t*-test or one-way ANOVA for continuous variables. Normality test and homogeneity of variance test were performed before comparing. For categorical variables, differences between groups were compared using Pearson's chi-squared test. As for hierarchical variables, Cochran-Mantel-Haenszel chi-square (CMH χ^2) test was used, and odds ratio (OR) was reported. Multiple linear regression was conducted to identify factors influencing HRQoL scores. The significance for all statistical tests was indicated by $p < 0.05$ (two-tailed); all the analyses were performed using Stata 16 MP.

3. Results

3.1. General characteristics

Three hundred and twenty-two patients were enrolled in our study at the first measurement time, including

59 SMA type I, 197 SMA type II, and 76 SMA type III. There were 281 patients in the non-medication group and 51 patients in the medication group. The baseline number of patients who used each scale is shown in Figure 1. A total of 64 patients completed the PedsQL IS, 249 patients completed the PedsQL NMM, and 19 completed the PedsQL GCS.

Two hundred seventy patients participated in the last survey, including 61 patients who received Nusinersen treatment. Compared to the baseline, the number of patients in the medication group increased, implying that acceptability of the new treatment increased.

Demographics and baseline results are provided in Table 1. More than 78.43% patients in the medication group received Nusinersen treatment at the first survey. It can be seen from the data that the medication group reported significantly higher HRQoL scores (55.6 *vs.* 48.4), and more patients achieved improvements in their condition (56.9% *vs.* 17.1%, $p < 0.01$). Also, the medication group shows that more patients used nasal or gastric tube feeding for nutrition support, and more patients used ventilators, implying that patients with more serious health conditions were more likely to use high-value treatments.

3.2. HRQoL stratification by SMA clinical subtypes

Two hundred and seventy patients finished the third measurement. As shown in Table 2, the severity of the disease condition has a negative impact on the HRQoL. The type I patients have the lowest scores. For SMA type I, II, and III, the average scores are 46.17, 48.48, and 54.26, respectively.

3.3. HRQoL stratification by motor function

The average scores of HRQoL stratified by motor function are summarized in Table 2. There was a significant difference between the three conditions, patients who could walk scored the highest. The average scores are 46.66, 48.60, and 55.40 for non-sitter, sitter, and walker, respectively.

3.4. Impact of Nusinersen

Figure 2 shows an overview of changes in health conditions in the past six months for two treatment groups. The proportion of patients who achieved any condition improvement is higher in the medication group. Additionally, the disease condition deteriorates over time in the non-medication group, while the medication group has the opposite situation, more patients benefit from the treatment. In terms of HRQoL, Figure 3 shows that the medication group has higher HRQoL scores, and scores improve over time, and these results can be seen in all clinical subtypes.

3.5. Regression analysis

We performed a multiple linear regression analysis to identify factors influencing the HRQoL score. Factors for regression included demographics, disease-specific, and treatment variables. We also created dummy variables for multi categorical variables. The best model

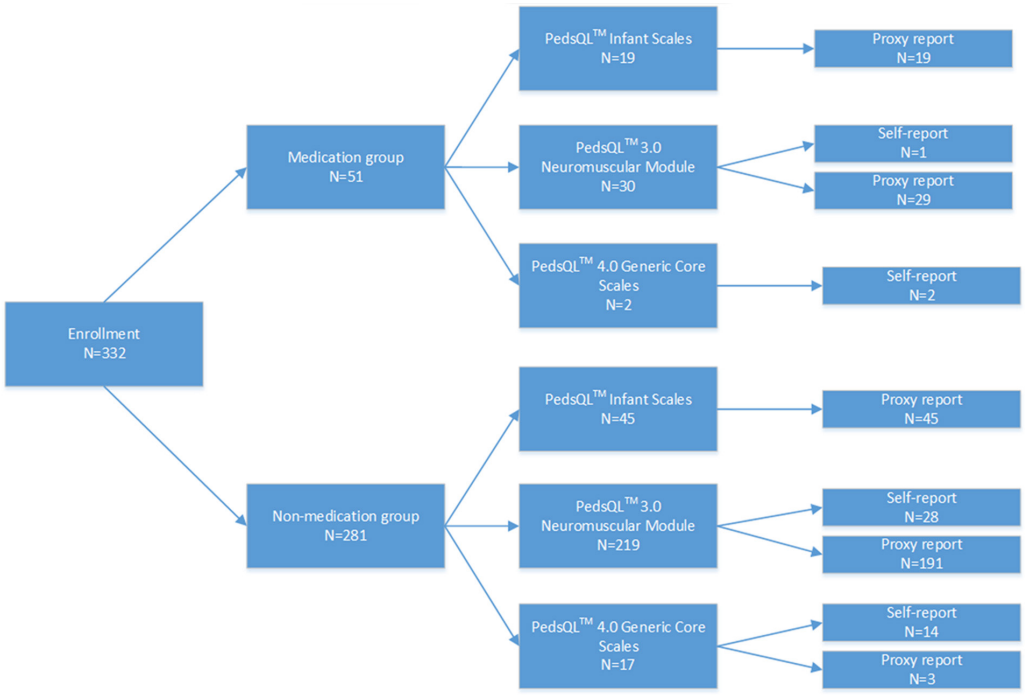

Figure 1. number of patients on each scale.332 patients were enrolled and divided into 2 groups: medication and non-medication groups. A total of 64 patients completed the PedsQL IS, 249 patients completed the PedsQL NMM, and 19 completed the PedsQL GC.

Table 1. Demographics and baseline results

Variable	Non-medication (n = 281)	Medication (n = 51)	p-value	test
Sex (male)	121 (43.06%)	19 (37.25%)	0.44	Pearson's χ^2
Clinical Subtypes			< 0.01	CMH χ^2
SMA type I	45 (16.01%)	14 (27.45%)	-	
SMA type II	167 (59.43%)	30 (58.82%)	0.58 (OR)	
SMA type III	69 (24.56%)	7 (13.73%)	0.32 (OR)	
Onset age			< 0.01	CMH χ^2
< 6 months old	64 (22.78%)	16 (31.37%)	-	
7~18 months old	170 (60.50%)	33 (64.71%)	0.78 (OR)	
19 months~ 10 years old	33 (11.74%)	2 (3.92%)	0.24 (OR)	
10~30 years old	13 (4.63%)	0 (0.00%)		
> 30 years old	1 (0.36%)	0 (0.00%)		
Current age			< 0.01	CMH χ^2
1~12 months old	11 (3.91%)	4 (7.84%)	-	
13~24 months old	34 (12.10%)	15 (29.14%)	1.32 (OR)	
2~4 years old	77 (27.40%)	16 (31.37%)	0.62 (OR)	
5~7 years old	52 (18.51%)	5 (9.80%)	0.29 (OR)	
8~12 years old	38 (13.52%)	5 (9.80%)	0.39 (OR)	
13~17 years od	30 (10.68%)	4 (7.84%)	0.40 (OR)	
18~25 years old	22 (7.83%)	0 (0.00%)		
> 26 years old	17 (6.05%)	2 (3.92%)	0.35 (OR)	
Motor function			< 0.05	CMH χ^2
Non-sitter	75 (26.69%)	17 (33.33%)		
Sitter	148 (52.67%)	28 (54.90%)		
walker	58 (20.64%)	6 (11.78%)		
Use of nasal tube or gastric tube feeding	0 (0.00%)	4 (7.84%)	< 0.01	Pearson's χ^2
Use of non-invasive ventilator			< 0.01	CMH χ^2
Never	270 (96.09%)	46 (90.20%)	-	
Sometimes	11 (3.91%)	4 (7.84%)	5.96 (OR)	
All-day	0 (0.0%)	1 (1.96%)		
Use of invasive ventilator			< 0.01	CMH χ^2
Never	280 (99.64%)	47 (92.16%)	-	
Sometimes	1 (0.36%)	1 (1.96%)	2.13(OR)	
All-day	0 (0.0%)	3 (5.88%)		
Change of disease condition in the past six months			< 0.01	CMH χ^2
Significant regression	51 (18.15%)	7 (13.73%)	-	
Regression	34 (12.10%)	6 (11.76%)	1.20 (OR)	
Slight regression	89 (31.67%)	4 (7.84%)	0.32 (OR)	
No changes	59 (21.00%)	5 (9.80%)	0.61 (OR)	
Slight improvements	41 (14.59%)	20 (39.22%)	3.55 (OR)	
Improvements	3 (1.07%)	4 (7.84%)	9.71 (OR)	
Significant improvements	4 (1.42%)	5 (9.80%)	9.10 (OR)	
HRQoL score	48.4 (16.1)	55.6 (18.1)	< 0.01	Two sample t-test

Table 2. HRQoL stratified by (A) SMA clinical subtypes and (B) motor function

(A)

Variable	SMA type I, Mean (SD) (n = 43)	SMA type II, Mean (SD) (n = 173)	SMA type III, Mean (SD) (n = 54)	p-value (ANOVA)
HRQOL score	46.17 (18.09)	48.48 (15.78)	54.26 (18.72)	0.04

(B)

Variable	Non-sitter, Mean (SD) (n = 75)	Sitter, Mean (SD) (n = 147)	Walker, Mean (SD) (n = 48)	p-value (ANOVA)
HRQoL score	46.66 (18.08)	48.60 (15.31)	55.40 (18.57)	0.02

indicated the use of Nusinersen and SMA clinical subtype as significant variables, which explained 13.46% of the variance in HRQoL (Table 3). The use of Nusinersen increased HRQoL, and patients with SMA type 1 and type 2 reported lower HRQoL as compared to SMA type 3.

4. Discussion

This study assessed HRQoL for patients with SMA

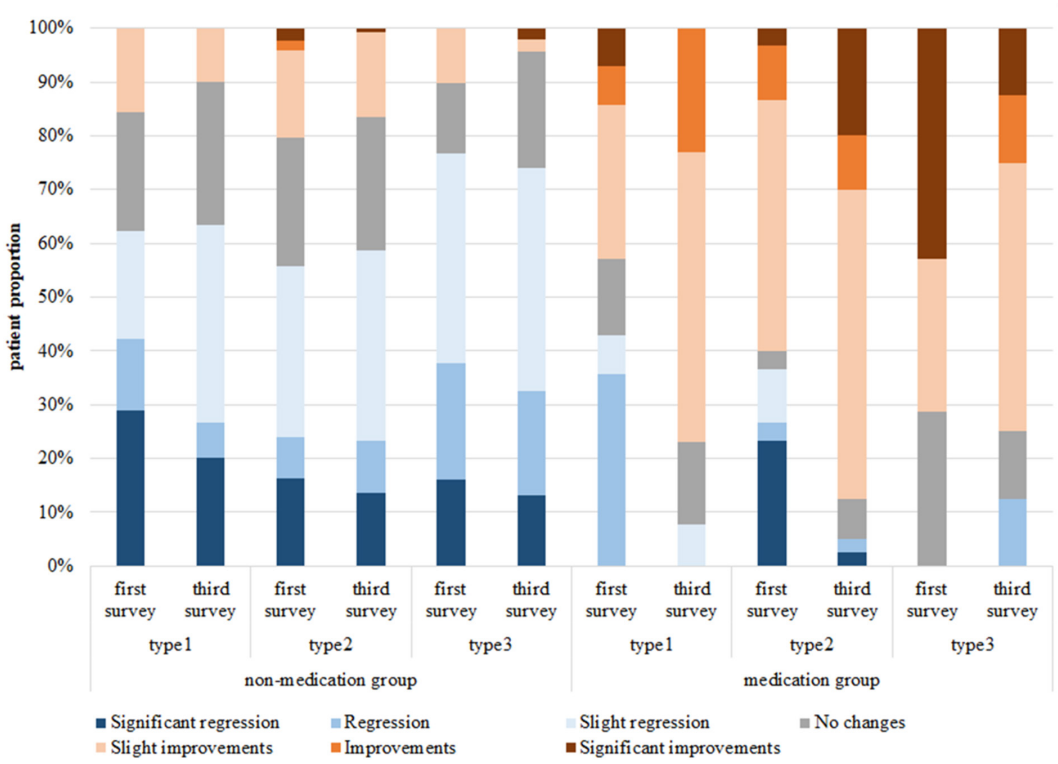

Figure 2. Change of disease condition in the past six months sorted by treatment group. The proportion of patients who achieved any condition improvement is higher in the medication group. In addition, the disease condition gets worse in the non-medication group over time.

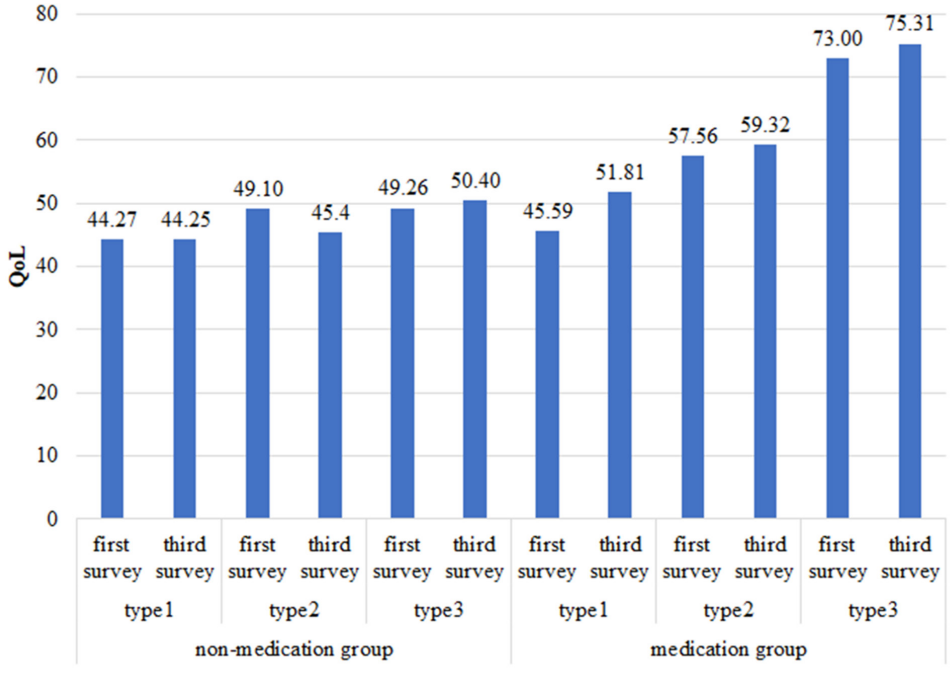

Figure 3. HRQoL score sorted by treatment group. Medication group has higher HRQoL scores, and the score increased with time. At the same time, the score decreased with time in the non-medication group.

in China. Consistent with the previous Chinese study conducted by Yao M, *et al.* (*8*), we confirmed that the HRQoL differed across SMA clinical subtype, motor function, and treatment regimen. Yao M, *et al.* (*8*) measured the HRQoL for children aged 0-17 years with SMA by PedsQL NMM. In our study, we chose a specific scale for each age group of patients according to the official description of PedsQL.

Table 3. Multivariate linear regression: predictors of HRQoL score

variables	Coef.	*t*	$p > \|t\|$	Beta
Use of Nusinersen	12.53	5.36	0.000	0.31
SMA type 1	-9.59	-2.90	0.004	-0.21
SMA type 2	-6.82	-2.74	0.007	-0.19
-cons	52.41	23.86	0.000	-

This study shows a meaningful difference in HRQoL across SMA clinical subtypes. Patients with SMA type I have the lowest scores, while those with type III have the highest scores. HRQoL decreases with disease severity, consistent with earlier publications (*8,13,14*). The HRQoL stratified by motor function lends support to the hypothesis that motor function affects the HRQoL. Patients with less motor disability showed better scores, and the walkers had the highest HRQoL scores.

Another important finding was that the patients could benefit from Nusinersen treatment. This result supports evidence from previous observations (*5,8,15*). Compared to the conventional treatment, patients receiving Nusinersen showed higher HRQoL scores, more patients achieved improvement in their health condition in the past six months, and the benefits grew over time.

Our regression analysis confirmed the association between SMA clinical subtype, Nusinersen treatment, and HRQoL. SMA type1 or type 2 would lower the HRQoL scores. While the Nusinersen treatment would increase the scores.

The main strength of our study is that we enrolled 332 SMA patients, a relatively large sample size, especially for rare diseases. Our sample covered the main age groups of SMA, and we adapted the survey instruments to the patients' ages, which can reflect their survival condition more accurately. This was the first study to explore the real effectiveness of Nusinersen for SMA patients in China, especially the patient-reported outcomes like improvement of motor functions and HRQoL.

The study is limited by the lack of HRQoL information on family or caregivers. SMA is a severe disease with high caregiver demands, with implications across all aspects of the family (*2*). Farrar (*16*) gained insights into the effects of caring for a child with SMA from the carer's perspective. The impact of taking care of an SMA patient includes changes in career choices and ongoing physical, social, and psychological consequences (*17*). These costs are high, and the impact on families can be devastating. Yao (*8*) measured the caregiver's HRQoL by Pediatric Quality of Life Inventory Family Impact Module (PedsQL FIM), they concluded that the more severe the SMA disorder, the lower the average scores on PedsQL FIM, which was consistent with the HRQoL of patients with SMA. The second limitation of this study is the time horizon. We conducted 3 surveys in ten months, which means the longest observation time is less than 1 year. SMA is so far a non-curable disease in China needing continuous treatment. We were unable to trend the quality longitudinally due to the short time horizon. Thus, the long-term effects of Nusinersen are still unknown. Lastly, the three Scales we used to measure HRQoL have not been validated in children with SMA in China.

In conclusion, the HRQoL differed across SMA clinical subtype, motor function, and treatment regimen. The HRQoL scores decreased with increasing disease severity. SMA patients can benefit from Nusinersen treatment, by slowing disease progression and improving HRQoL.

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Insights from a patient with chronic lymphocytic leukemia complicating ALK$^+$ anaplastic large cell lymphoma

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SUMMARY Chronic lymphocytic leukemia (CLL) that transforms into a more aggressive lymphoma has been termed Richter syndrome (RS). CLL with T-cell neoplasia is rarely reported; those with ALK$^+$ anaplastic large cell lymphoma (ALCL) are also exceedingly rarely reported. A 63-year-old woman from the south of China presented with generalized lymphadenectasis and fever; she already had a prior diagnosis of CLL 9 years ago. As per her current diagnosis, it was CLL with ALK$^+$ ALCL. The two-lymph node and bone marrow biopsies presented two types of cellular groups: *i*) left cervical lymph node biopsy suggested CLL (Ki67: 10%), along with bone marrow biopsy exhibited enhancement of the small lymphocytes (30%) with scant cytoplasm, round or irregular cell nuclei, and massive amounts of chromatin. Large cells (< 1%) that expressed CD30 and ALK were visible; The results of immunohistochemistry were as follows: CD20 (weak positive); PAX5 (positive); CD23 and CD5 (weak positive); and CD3, CD10, and CyclinD1 (negative); *ii*) left supraclavicular lymph node biopsy suggested ALK$^+$ ALCL (Ki67: 70%). The final diagnosis was CLL with ALCL. The mechanisms of this condition are not fully understood, which might be associated with chronic stimulation of T cells by CLL cells along with immune dysfunction.

Keywords chronic lymphocytic leukemia, Richter syndrome, ALK, anaplastic large cell lymphoma, fludarabine

1. Introduction

Chronic lymphocytic leukemia (CLL) that transforms into a more aggressive lymphoma has been termed Richter syndrome (RS) or Richter transformation (*1*). This occurs approximately in 5-10% of patients with CLL (*2*) and is commonly associated with a worse clinical outcome (*3-5*). Approximately 80-90% of RS cases are clonally related to CLL, whereas only 10-20% are clonally unrelated (*6*). Diffuse large B-cell lymphoma is the most common RS subtype, accounting for 95% of total RS cases, of which less than 5% may develop into classical Hodgkin's lymphoma (*7*). CLL with T-cell neoplasia is rarely reported; those with ALK$^+$ anaplastic large cell lymphoma (ALCL) are also exceedingly rarely reported; however, whether CLL with ALK$^+$ ALCL can be attributed to RS remains controversial (*8*).

2. Clinical manifestation of a rare case

A 63-year-old woman from the south of China was diagnosed with ALK$^+$ ALCL 9 years after she was diagnosed as having CLL (Binet B) in 2011. She complained of lymphadenectasis throughout her body, including in the neck, axilla, and inguinal region. She was untreated (Binet B) as per the National Comprehensive Cancer Network (NCCN) guidelines (*9*) until 2018, when CLL progressed. She underwent six courses of fludarabine, cyclophosphamide, and rituximab chemotherapy and achieved alleviation of the symptoms. In 2020, she felt extreme fatigue with generalized lymphadenectasis. Pathological slices of the left cervical lymph node exhibited the morphological manifestation of CLL/SLL (Figure 1 A1). The results of immunohistochemistry were positive for CD20, CD5, CD23, Bcl-2, Bcl-6, CD21, and Ki67$^+$ cells (10%) and negative for CD3, CD10, Mum1, CyclinD1, and TdT. The results of hybridization in situ were EBER negative. The immunophenotyping data of the bone

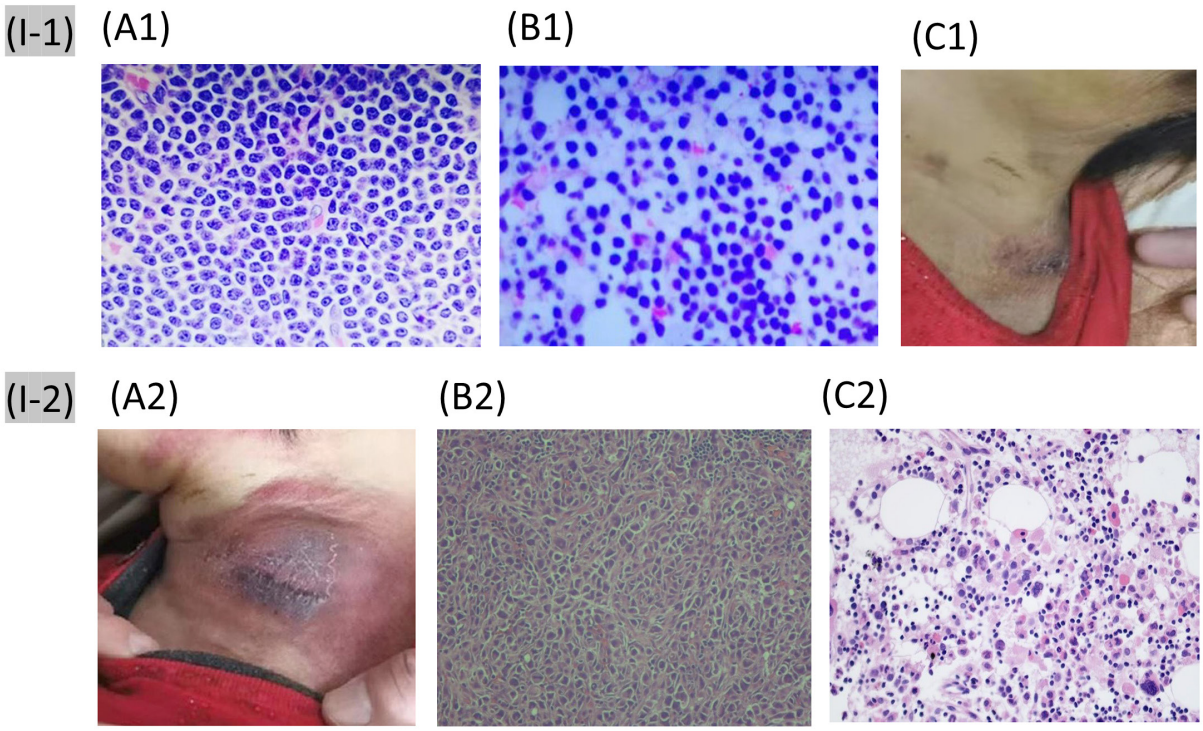

Figure 1. I-1, Images in the first hospitalization (2020); I-2, Images in the second hospitalization (2021). **(A1)** The pathological slices of the left cervical lymph node; **(B1)** The pathological slices of the bone marrow; **(C1)** The skin of the biopsy location of the left cervical lymph node during the first febrile episode; **(A2)** The skin of the biopsy location of the left cervical lymph node at the second febrile episode; **(B2)** The pathological slices of the left supraclavicular lymph node; **(C2)** The pathological slices of the bone marrow. (hematoxylin & eosin staining, 400×).

marrow exhibited increased expression of CD5, CD22, CD19, CD79a, CD23, CD200, IgM, and Kappa and non-expression of CD10, FMC7, CD79b, and Lambda, which indicated that these cells were monoclonal mature B cells (CLL scoring 4-5). Fluorescence in situ hybridization of the marrow revealed the following: CEP12, 68%; D13S319/LAMP1, 85%; ATM/CEP11, P53/CEP17, and IGH/CCND1, negative; TP53 gene mutation, negative; IGH rearrangement, IGHV3-30; and IGH somatic mutation rate, 9.1%. The pathological slice of the bone marrow suggested recurrence of CLL (Figure 1 B1). The patient then underwent treatment with zanubrutinib. After 1 month of zanubrutinib treatment, the biopsy location of the left cervical lymph node began to swell accompanied by tenderness and fever of 39°C (Figure 1 C1).

However, 1 week after antibiotics administration, fever recurred, which was now accompanied by cough, and the lymph node began to swell again. Antibiotics were again administered, but the symptoms did not improve. The skin in the left neck is shown in Figure 1 A2. Lymphadenectasis was noted at the bilateral neck, axilla, supraclavicular area, and inguinal area. She was then diagnosed with pulmonary infection and underwent several anti-infective treatments, including anti-virals and anti-fungals. The cough improved, but fever persisted. Hence, zanubrutinib treatment was maintained. A second biopsy of the left supraclavicular lymph node was conducted, which revealed heteromorphic cell nests. The cells were large with irregular nuclei and abundant karyokinesis (Figure 1 B2). The results of immunohistochemistry were positive for ALK, CD30, EMA, and $Ki67^+$ cells (70%) and negative for AE1/AE3, Bcl-2, Bcl-6, CD10, CD20, CD21, and CD3. The immunophenotyping data of the peripheral blood revealed that 88.41% of cells expressed CD19, CD200, and Kappa; weakly expressed CD5, CD20, CD23, and CD43; and did not express CD10, FMC7, CD79b, Lambda, CD22, CD103, CD38, and CD138. The results indicate that these cells were monoclonal mature B cells. The values of forward scatter and side scatter were small. The immunophenotyping assay of the bone marrow revealed that 86.15% of the cells expressed CD19 and Kappa; weakly expressed CD5, CD20, CD23, CD43, and CD200; and did not express CD10, FMC7, CD79b, Lambda, CD22, CD103, CD38, and CD138, thus also indicating that these cells were monoclonal mature B cells. The lymphoma gene rearrangement of the marrow fluids showed that TCRD, TCRβ, and TCRγ were negative. Human T-cell leukemia virus type I in the peripheral blood was negative. The pathological slices of the bone marrow revealed 60% bone morrow hyperplasia, enhancement of small lymphocytes (30%) with less cytoplasm, round or irregular nuclei, and massive amounts of chromatin. Large cells (< 1%) were visible and expressed CD30 and ALK (Figure 1 C2). The results of immunohistochemistry were as follows: CD20 (weak positive); $PAX5^+$ (positive); and CD23 and CD5 (weak positive). Meanwhile, CD3,

Table 1. The included literature and patients

Literatures	Age, gender	Time from CLL to ALCL (years)	ALCL location	ALK (+ or −)	ALCL Immunophenotype	Chemotherapy before ALCL	Chemotherapy after ALCL	Outcome
Nai *et al*. 1998 (*3*)	61, F	3	Spleen	UK	CD30 (+), CD3 (+), CD45(+), CD45RO (+), EMA (+); CD15 (+/−); CD20 (−), κ (−), λ (−)	Fludarabine	No	LTF
van den Berg *et al*. 2002 (*4*)	76, M	4	Lymph node	−	CD30 (+), CD45 (+), TIA-1 (+), UCHL-1 (+); EMA (+/−); CD20 (−), CD3 (−), CD15 (−), ALK (−), TARC (−)	UK	UK	UK
Marschalkó *et al*. 2007 (*5*)	75, M	7	Cutis	UK	CD30 (+), TIA-1(+); CD4 (+/−); CD3 (−), CD5 (), CD7 (-), CD8 (-), CD79a (-)	No	UK	MF appeared (1.5 years later)
Liu *et al*. 2008 (*18*)	59, M	8	Lymph node	+	CD30 (+), CD45 (+), CD45RO (+), CD4(+), ALK (+); CD5 (-), CD8 (-), CD20 (-), CD23 (-), CD79a (-), EBER (-)	Chlorambucil/prednisone; fludarabine; fludarabine/ rituximab; pentostatin/ cyclophosphamide/ rituximab; weekly rituximab	ICE	Dead (2 months)
Persad & Pang 2014 (*19*)	47, M	0	Lymph node	−	CD30 (+), granzyme (+), CD43 (+), CD23 (+); CD2 (+/-), CD4 (+/-), CD45 (+/-), Bcl-2 (+/-); CD3 (-), CD5 (-), CD7 (-), CD8 (-), ALK (-), EBER (−), B-F1 (−), CD56 (−), CD57 (−), CD10 (−), CD15 (−), CD20 (−), PAX5 (−)	No	R-EPOCH; Autologous stem cell transplant	CR
Boyel *et al*. 2014 (*8*)								
Case 1	56, F	0	Lymph node	+	CD30 (+), Perforin (+), CD5 (+), EMA (+), ALK (+); granzyme (+/−), CD56 (+/−); CD3 (−), CD4 (−), CD8 (−), CD20 (−), EBER (−), Ki67: 80%	No	R-CHOP	ANED (15 months later)
Case 2	66, F	0.7	Lymph node/bone	+	CD30 (+), Perforin (+), CD5 (+), ALK (+); granzyme (+/−), CD4 (+/−), CD7 (+/−), CD45 (+/−); CD3 (−), CD8 (−), CD20 (−), EBER (−)	Cyclophosphamide, vincristine, prednisone, fludarabine, rituximab	Cisplatin, etoposide, cytarabine	Dead (7 months later)
Mant *et al*. 2015 (*17*)								
Case1	60, F	5	Lymph node/ marrow/blood	−	CD30 (+), Perforin (+), CD2 (+), CD3 (+), CD43 (+); granzyme (+/−); ALK (−), CD4 (−), CD5 (−), CD8 (−), CD56 (−), EBER (−)	FCR	Palliative therapy	Dead (2 months later)
Case 2	44, M	2	Lymph node	−	CD30 (+), CD2 (+); granzyme (−), Perforin (−), ALK (−), CD4 (−), CD5 (−), CD8 (−), CD3 (−), EBER (−)	Fludarabine; CHOP	CHOP	Dead (1 months later)

AD: Alive with disease; ANED: alive with no evidence of disease; CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone; CR: complete remission; FCR: fludarabine, cyclophosphamide, rituximab; ICE: ifosfamide, carboplatin, and etoposide; LTF: loss to follow-up; MF: mycosis fungoides; R-EPOCH: rituximab, etoposide, prednisone, vincristine, doxorubicin, and cyclophosphamide; UK: unknown.

Table 1. The included literature and patients (continued)

Literatures	Age, gender	Time from CLL to ALCL (years)	ALCL location	ALK (+ or −)	ALCL Immunophenotype	Chemotherapy before ALCL	Chemotherapy after ALCL	Outcome
Case 3	86, M	1	SNose	−	CD30 (+), CD43 (+); CD4 (+/−), granzyme (+/−); ALK (−), CD5 (−), CD8 (−), CD3 (−), CD45 (−), CD15 (−), CD56 (−), EBER (−)	UK	No	AD (3 months later)
Case 4	64, M	8	Lymph node/ Ascitic fluid/CNS	+	CD30 (+), CD4 (+), granzyme (+), Perforin (+), ALK (+), EMA (+); CD43 (+/−), CD45 (+/−); CD2 (−), CD5 (−), CD8 (-), CD3 (-), EBER (-)	FCR	CHOP; High dose methotrexate; intrathecal chemotherapy	ANED (16 months later)
Case 5	63, F	8	Lymph node	+	CD30 (+), CD43 (+), Perforin (+), ALK (+); CD4 (+/-); CD45 (-), CD56 (-), CD5 (-), CD8 (-), CD3 (-), granzyme (-), EBER (−)	Chlorambucil and prednisone	CHOP	ANED (10 months later)
Thakra & Konoplev. 2017 (*6*)	56, M	UK	Marrow	+	CD30 (+), ALK (+), CD4(+), CD5 (+), CD45 (+), CD43 (+); CD2 (+/−); CD3 (−), CD7 (−), CD8 (−), CD15 (−), Pax-5 (−)	UK	UK	UK
Van Der Nest *et al*. 2019 (*11*)								
Case 1	77, F	4	Lymph node	+	CD30 (+), MUM1 (+), granzyme (+), perforin (+), ALK1 (+); CD4 (+/−), CD45 (+/−); CD20 (−), PAX5 (−), CD79a (−), BOB1 (−), OCT2 (−), CD3(−), CD5 (−), CD138 (−), BCL2 (−), EBER (−), CD15 (−), C-MYC (−), CD34(−), CD117(−), TIA1(−)	UK	Mini-CHOP	A high-grade neuroendocrine tumor occurred
Case 2	74, M	8	Cutis	−	CD30 (+), CD3 (+), CD4 (+), granzyme (+); CD2 (+/−), CD5 (+/−), CD7 (+/−); CD20 (−), ALK (−), EBER (−), CD8 (−), BF1 (−)	No	CHOP, R-CHOP, dexamethasone, cytarabine, carboplatin, gemcitabine/vinorelbine, high dose methotrexate	Dead (3 years later)
Case 3	66, M	16	Cutis/Lymph node/Bone	−	CD30 (+), CD 4(+), CD43 (+), CD2 (+), CD7 (+), TIA (+), granzyme B (+), (focal) and EMA (+), (patchy); CD20 (−), CD45RO (−), CD3 (−), CD5 (−), CD8 (−), CD56 (−), ALK1 (−), CD163 (−), CD123 (−), BCL11A (−), CD2AP (−), CD303 (−), EBER (−), HHV8 (−)	No	Cyclophosphamide; vincristine/gemcitabine and brentuximab vedotin.	Dead (4 years)

AD: Alive with disease; ANED: alive with no evidence of disease; CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone; CR: complete remission; FCR: fludarabine, cyclophosphamide, rituximab; ICE: ifosfamide, carboplatin, and etoposide; LTF: loss to follow-up; MF: mycosis fungoides; R-EPOCH: rituximab, etoposide, prednisone, vincristine, doxorubicin, and cyclophosphamide; UK: unknown.

CD10, CyclinD1 were negative. Hence, the patient was finally diagnosed as CLL with ALK^+ ALCL. She then underwent cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) plus zanubrutinib chemotherapy. After 4 days of treatment, fever abated. After two courses of treatment, generalized lymphadenectasis was resolved, and efficacy was evaluated as "partial remission". The patient is well and alive after the last follow-up (2022 August).

This study was approved by the ethics committee of the First Hospital of Putian City, and informed consent was signed to present her agreement reported in this study.

3. Experience and insights

CLL with ALCL is rarely reported. By far, only 16 cases have been reported in the literature (Table 1); of those, 10 are males and 6 are females. The median age of patients was 63.5 years (range, 47-86 years). The average time from CLL to diagnosis of ALCL was 4 years (range, 0-16 years). With respect to ALK expression, seven cases were ALK^+, seven cases were ALK^-, and two cases were unknown. In this case, the patient was 63 years old, and the time from CLL to diagnosis of ALK^+ ALCL was 9 years.

To date, mechanisms of the CLL complicating aggressive T lymphoma remain unclear. Some authors believe that it is associated with the chronic stimulation of T cells by CLL cells along with immune dysfunction (*10-12*). In addition, abnormal proliferation of T cells might cause new mutations that may develop into aggressive T-cell lymphoma (*12*). This theory is also supported by a previous report that the cytotoxic T-cell expansions in the peripheral blood are closely associated with the occurrence of T-cell lymphoma (*13*). In total, 12 of 16 CLL patients that developed ALCL expressed cytotoxic T lymphocyte-related genes such as TIA-1, granzyme, and perforin (Table 1). Unfortunately, the expression of these genes was not investigated in the patient in this study. The immune dysregulation inherent to CLL also contributes to T-cell oncogenesis due to its capacity to induce mixed neoplastic clones (*10*). A high occurrence of lymphoma is also observed in other diseases with immune dysregulation, such as sicca syndrome, rheumatoid arthritis, and chronic lymphocytic thyroiditis. All of this evidence indicates that immune dysregulation plays a role in the occurrence of lymphoma. Moreover, T cells from patients with CLL (*vs*. healthy subjects) seem to be more resistant against cellular apoptosis (*14*). All these factors might have contributed to the development of aggressive T lymphomas. Another factor that must be seriously considered is that whether the treatment for CLL has secondarily induced the occurrence of ALCL. Indeed, this is a controversial issue. The present case underwent six courses of fludarabine treatment before the occurrence of ALK^+ ALCL. Of all the 16 patients included in the literature review, 5 underwent treatment with fludarabine. Gassner *et al*. reported that the administration of fludarabine continuously reduced the number of $CD4^+$ and $CD8^+$ T cells, which then correlates with the cytotoxic effects of fludarabine on T cells *in vitro*. Moreover, fludarabine plays a role in immune modulation, which might be a double-edged sword to T-cell oncogenesis (*15*). The role of CLL treatment in T-cell oncogenesis requires further investigation.

Before this patient could be diagnosed with ALCL, fever was a predominant symptom, and lymph node swelling recurred after zanubrutinib treatment, which we considered as both being due to the skin and soft tissue infections. Unfortunately, the subsequent anti-infective therapy was ineffective. We had to determine the final diagnosis by performing lymph node and bone marrow biopsies. When the patient had fever and recurrence was considered, the lymph node biopsy exhibited ALK^+ cells. Moreover, the bone marrow biopsy found that there were < 1% large cells (Figure 1 C2). These results suggested that the lymph nodes and bone marrow were involved.

Generalized ALCL has a poor clinical outcome. Patients with ALK^+ may have better outcomes than those of ALK^- with the development of treatments such as brentuximab vedotin (BV) and crizotinib. The 5-year survival rate of ALK^+ ALCL is 70-80%; in contrast, those with ALK^- ALCL is only 40-60% (*16*). Our case is well and alive. However, due to the small sample size and the heterogeneity of the included studies, the effects of ALK expression on the prognosis of CLL complicating ALCL as well as the efficacy of the related treatments require further investigation.

Taken together, CLL with ALCL is rare, and the mechanisms involved are not fully understood. Chronic stimulation of T cells by CLL cells, along with immune dysfunction in CLL, might play a role. Several clinical problems, such as whether fludarabine treatment will increase the risk of CLL developing ALCL and the difference in the prognosis between idiopathic ALCL and ALCL complicated from CLL, have not been elucidated. Further investigation is required in the future.

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Insights into clinical diagnosis and treatment of malignant hepatic perivascular epithelioid cell tumor

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SUMMARY Perivascular epithelioid cell tumors (PEComas) are infrequent mesenchymal tumors. They are usually benign, and only a few are malignant. These tumors are more commonly found in middle-aged women. PEComas are mainly composed of differentiated perivascular epithelioid cells arranged radially around the vascular cavity, and they are usually positive for melanocyte markers and smooth muscle cell differentiation markers. Among the PEComas, hepatic PEComas generally have no obvious symptoms and no typical imaging manifestations. Malignant hepatic PEComas are even rarer. So, we explained our insights into clinical diagnosis and treatment of malignant hepatic PEComas, in order to help clinicians and pathologists to further understand PEComas.

Keywords PEComa, liver tumor, hepatectomy, mTOR inhibitors, TFE3

Perivascular epithelioid cell tumors (PEComas) are extremely infrequent (roughly equivalent to 1 case per 4 million population) mesenchymal tumors and typically diagnosed in females of an age range of 39-56 years old. PEComas are difficult to diagnose, as their morphology can be analogous to that of smooth muscle tumors (*1,2*). The 2016 World Health Organization (WHO) Classification of Soft Tissue Tumors defined PEComa as a mesenchymal tumor composed of unique cells that focal associate with blood vessel walls and typically express melanocytic and smooth-muscle markers (*3*).

According to existing statistics, most PEComas are benign, and commonly occur in the uterus and gastrointestinal tract. Moreover, PEComas can also develop in organs such as the kidney, lung and liver. These tumors should be considered malignant only if any two or more of the following situations are found: a. tumor diameter > 5 cm; b. infiltrative growth; c. pronounced heteromorphic nuclei; d. number of mitotic figures ≥ 1/50 HPF; e. necrosis; and f. vascular invasion.

1. Clinical manifestation of a rare case

A 49-year-old female patient started to experience upper right abdominal pain and distention four months ago. Her symptoms worsened in the two weeks prior to admission. However, she did not present with fever, nausea, weight loss, general numbness or weakness. In terms of her past medical history, she had a laparoscopic fenestration and drainage of a liver cyst ten months prior and denied a history of hepatitis. Secondary infection of the liver cyst was reported in her last postoperative pathology. The laboratory data on admission were all within the normal range except that albumin was 28.7 g/L (normal range, 30-50 g/L), and CA-125 was 374.3 U/mL (normal range, 0-35 U/mL). The clinical abdominal examination showed normoactive bowel sound, no tenderness, no rebound pain, no muscle guarding and tympanic sound to percussion. Because her former cyst was 12 cm × 9 cm and the surgical wound was large, we first hypothesized that the abdominal pain and distention were caused by exudation accumulation. Therefore, we immediately completed the ultrasonography for the patient. The results indicated that there was indeed peritoneal effusion, so we gave the patient ultrasound-guided abdominal puncture catheter drainage. Then, approximately 1,000 mL of ascites was drawn out in a short time period. Her symptoms were relieved as well. However, in the next several days, 400 mL ascites was drawn out continuously every day. To determine the reason, we completed abdominal computed tomography (CT) and reperformed ultrasonography for the patient. CT revealed a mixed cyst and solid mass in the right lobe of the liver, and the lesion was obviously bulging out of the liver (Figure 1). Ultrasonography revealed a 16.1 cm × 13.2 cm mixed echo in the right lobe of the liver. After

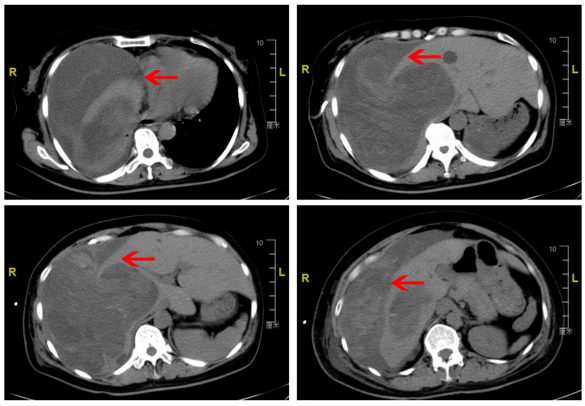

Figure 1. CT revealing a mixed mass of cysts and solids in the right liver, the largest diameter was approximately 15 cm.

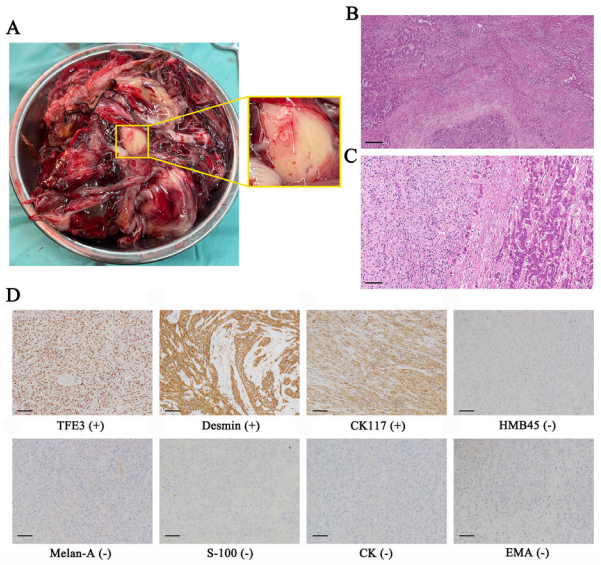

Figure 2. Specimens and Pathological Results. (A) The tissue specimens showed numerous cord-shaped lesions, accompanied by abundant blood filling. **(B)** Hematoxylin-eosin staining specimen under the 4× microscope. Scale Bar = 250 μm. **(C)** Hematoxylin-eosin staining specimen under the 10× microscope. Scale Bar = 100 μm. **(D)** Immunohistochemical results. Scale bar = 25 μm.

communicating with the patient and her daughter, we decided to perform an exploratory laparotomy.

At the moment of entering the abdominal cavity, a large amount of drainage gushed out. Then, we could see that not only the right hemiliver but also the patient's abdominal wall and omentum were covered with messy disordered cord-shaped lesions. We completely resected her right hemiliver, and all visible lesions. Furthermore, the excised specimens were subjected to pathological analysis. The pathological results revealed that the lesions were mesenchymal malignant tumors and considered malignant tumors with perivascular epithelioid differentiation (malignant PEComa). We found a large amount of tumor tissue and abundant blood vessels in some areas through observing the hematoxylin-eosin staining specimen under a 4× microscope. In addition, the tumor grew around the blood vessels and invaded the liver tissue. We also found some large solid areas in which blood vessels were not abundant, which is not typical of PEComas. Under a 10× microscope, we found residual normal liver tissue and abnormal blood vessels, and there was tumor tissue surrounding the abnormal blood vessels. In addition, large areas of necrosis were seen in the tumor tissue, indicating a high degree of tumor malignancy. In addition, the immunohistochemical expression profile was atypical. The tissue was positive for TFE3, Desmin and CD117, but it was negative for other typical markers, such as HMB45, Melan-A, S-100, CK and EMA (Figure 2). After the patient was discharged from the hospital, the medical oncologist advised her to take sirolimus and follow up closely. Currently, the patient's quality of life is good, and there is no recurrence. The informed consent was obtained from the patients for all descriptions.

2. Insights into hepatic PEComas

Hepatic PEComas are very rare, and usually these patients have no history of hepatis or factors that can damage the parenchyma (*4-6*). Most cases of hepatic PEComas are benign and have no specific clinical manifestations. Additionally, the onset is insidious and difficult to detect. However, when the lesion is large, abdominal distension, abdominal pain, ileus and other symptoms of abdominal compression may occur. In addition, many abdominal organs, such as the omentum, can be simultaneously affected by malignant PEComas originating from the liver. This can ultimately lead to massive bleeding into the abdominal cavity (*7*). However, there are generally no specific abnormalities observed in the laboratory tests (*8*).

Primary PEComas are usually well demarcated. Ultrasonography of the abdomen usually shows hyperechogenic cystic solid lesions with abundant blood supply. These tumors are hypodense to isodense on CT. MRI shows hypointense to isointense in comparison to skeletal muscle on T1-weighted imaging, and these tumors are heterogeneously hyperintense on T2-weighted imaging (*1,4*). Due to abundant vascularization from hepatic artery branches, we can observe that the lesion is strongly and heterogeneously enhanced in ultrasonography involving intravenous contrast media. Similarly, in CT and MRI, these tumors are characterized by intense enhancement in the arterial phase and an absence of contrast media in the veno-portal and delayed phases. (*5,9*). These imaging manifestations add to the difficulty of differentiating hepatic PEComas from hepatocellular carcinomas and hepatic adenomas. Furthermore, there are multiple enhancement characteristics of hepatic PEComas, and they may show persistent enhancement in the veno-portal and delayed phases in some cases. Thus, PEComas could be misdiagnosed as benign masses, such as focal nodular hyperplasia and hemangioma (*1,10*). Therefore,

the heterogeneous enhancement characteristic of hepatic PEComas is the main feature that distinguishes PEComas from other liver tumors.

In terms of its lack of clinical, laboratory and imaging manifestations, only histological and immunohistochemical analysis can provide a precise final diagnosis. PEComas are characterized by differentiated perivascular epithelioid cells arranged radially around the vascular cavity. These cells are typically epithelioid and spindle-shaped, which is analogous to smooth muscle cells. They also present with abundant transparent to eosinophilic granular cytoplasm and the specific expression of melanin cell markers and smooth muscle cell markers (*11*). On histopathology, adipose tissue and thick-walled vessels can be identified in most PEComas. Additionally, we found epithelioid cells with abundant transparent to eosinophilic cytoplasm forming nests and trabeculae. In smaller neoplastic lesions, there are fewer atypical epithelioid cells and more adipose tissue (*4,7,12,13*). Immunohistochemically, PEComas are usually positive for melanocyte markers (HMB45, Melan-A, Mitf) and smooth muscle cell differentiation markers (SMA, Desmin), while epithelial cell markers (CK, EMA) and endocrine markers (S-100, Syn and CgA) are negative. A novel marker, TFE3, which indicates the presence of TFE gene rearrangement, is highly expressed in 15% of PEComas. PEComas with TFE3 gene rearrangement can demonstrate robust staining for HMB45 and TFE3, but the staining of Melan-A and smooth muscle markers are focal or negative (*14*). Similar to other TFE3 translocation-associated tumors, acinar structure and epithelioid cell morphology can be found in TFE3 (+) hepatic PEComas. Moreover, TFE3 (+) hepatic PEComas usually exhibit aggressive biological behaviors and has a poor prognosis. However, existing research reveals that compared with TFE3 (+) PEComas in other organs, TFE3 (+) hepatic PEComas may be less malignant (*13*).

Surgical resection of the lesion is currently the most recommended treatment for PEComas. Simple resection of the tumor can be operated for small hepatic PEComas that are considered to be benign. However, extensive segmental hepatectomy or hepatic lobectomy is used for large and malignant hepatic PEComas. For benign hepatic PEComas, complete resection of the lesion can achieve a good therapeutic effect, and there are relatively few postoperative complications. However, even if the final diagnosis is benign, these patients need to be followed up as closely as patients with malignant liver tumors (*5,13,15*). It is recommended that patients with malignant hepatic PEComas should be treated with mTOR inhibitors, such as sirolimus and everolimus, regardless of whether the primary lesion can be surgically removed. The complex formed by tuberin and hamartin can regulate the mTOR signaling pathway. However, in most cases of PEComas, the function of tuberin and/or hamartin is lost. This induces the dysregulation of the mTOR signaling pathway and increases its activity. Activation of the mTOR pathway leads to increased ribosomal biogenesis, translation, proliferation and angiogenesis (*15,16*). Based on current research, mTOR inhibitors are expected to be the first-line therapy for advanced and metastatic PEComas (*13,15,16*). Beyond the above treatments, in some situations, hormonotherapy, transarterial chemoembolization and radiofrequency ablation are used (*17,18*).

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Monoclonal gammopathy of undetermined significance (MGUS) characterized by refractory lower gastrointestinal postoperative bleeding with coagulopathy

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SUMMARY Bleeding is a common complication after lower gastrointestinal surgery, and cases due to coagulation dysfunction are rare. The current authors encountered a 54-year-old Chinese man with refractory bleeding after endoscopic rectal polypectomy, and multiple endoscopic and surgical interventions failed to control that bleeding. An APTT mixing test could not be corrected and there was no evidence of autoimmune-related disease, so the presence of nonspecific antibodies was considered. After empiric therapy with a cyclophosphamide and glucocorticoid, APTT was corrected and gastrointestinal bleeding stopped. Based on laboratory results and therapeutic results, the patient was ultimately diagnosed with prolonged APTT induced by monoclonal gammopathy of undetermined significance (MGUS). MGUS and coagulopathy characterized by a prolonged APTT has rarely been reported. Here, studies noting elevated monoclonal immunoglobulins and coagulopathy have been reviewed. If a prolonged APTT of undetermined significance cannot be corrected with an APTT mixing test and if autoimmune-related factors are excluded, then plasma cell-related diseases such as MGUS need to be considered.

Keywords lower gastrointestinal bleeding, postoperative bleeding, abnormal coagulation mechanism, MGUS

Lower gastrointestinal bleeding (LGIB) occurs in the small intestine, colon, or anorectum and accounts for 30 to 40% of gastrointestinal bleeding. Its incidence is approximately 33-87 per 100,000 (*1,2*). Endoscopic colorectal polypectomy is a routine clinical procedure, and bleeding is the most common postoperative complication. The incidence of bleeding after endoscopic polypectomy has been reported to be approximately 1-6% (*3*). Reported here is a case of post-polypectomy bleeding, and that bleeding recurred after multiple endoscopic and surgical interventions. Based on a multidisciplinary discussion, an APTT correction test could not be corrected, and there was no evidence of autoimmune-related disease. Relevant tests revealed the presence of nonspecific antibodies. After empiric therapy with a cyclophosphamide and glucocorticoid, APTT returned to normal and bleeding stopped. Relevant tests were conducted and the patient was ultimately diagnosed with monoclonal gammopathy of undetermined significance (MGUS).

MGUS refers to the clonal proliferation of plasma cells or B cells to secrete monoclonal immunoglobulins (*4*), and it is characterized by the presence of serum M-protein less than 3 g/dL, bone marrow (BM) clonal plasma cells less than 10%, an absence of myeloma-defining events or amyloidosis, and end-organ damage (*5*). A study has found that almost all patients with multiple myeloma (MM) suffered from MGUS before the onset of the disease (*6,7*). Other patients with MGUS may develop Waldenström macroglobulinemia, light chain amyloidosis, or related disorders (*8*). Although prolonged PT can occur in patients with MGUS, prolonged APTT occurs in only a few cases (*9*). Moreover, cases involving postoperative bleeding are rarer and harder to diagnose. The current case may offer clinicians insight into this condition.

Clinical manifestations: A 55-year-old male visited a local hospital for gastroscopy and rectal polypectomy. A routine examination before gastrointestinal endoscopy indicated that APTT was 42.4 seconds (24-36 seconds) and TT was 26.8 seconds (14-21 seconds). A slight abnormality of coagulation was considered as a

possibility. The patient had a history of health and no history of abnormal bleeding, no family history of hemophilia, and no history of von Willebrand disease. The patient had not received antiplatelet or anticoagulant therapy. Before the examination was complete, gastrointestinal endoscopy was performed. Gastroscopy revealed atrophic gastritis and a gastric angle ulcer. Colonoscopy revealed a sessile polyp, located about 8 cm from the margin in the rectum, and 12 flat polyps in the rectum, with a size of about 0.2 cm × 0.3 cm. Endoscopic rectal polypectomy and argon ion coagulation were performed, and the procedures were successful.

On the fourth day after polypectomy, the patient had multiple dark red loose stools. Endoscopy revealed multiple blood clots in the rectal lumen and obvious bleeding. After treatment with endoscopic clips, bleeding persisted. The patient was treated with medications. Endoscopic clips were used and endoscopic electrocoagulation was performed three times for hemostasis, and the bleeding temporarily subsided. After 5 days, the patient suddenly had a large amount of bloody stools again. The patient was urgently referred to this hospital and treated with somatostatin and terlipressin to stop the bleeding. A hemostatic clip was applied with immediate obvious hemostatic effect. Bleeding recurred, and colonoscopy revealed bleeding at the polyp wounds and other margins of the polyp wounds. The hemostatic effect was still significant after re-hemostasis. When bleeding persisted after repeated hemostatic treatment (Figure 1A), the possibility of a vascular malformation was considered. Mesenteric arteriography and embolization were performed, and an arteriovenous malformation was considered.

After embolization with gelatin sponge particles, blood in the stool was still present. Considering the possibility that a vascular malformation had caused the bleeding, after several multidisciplinary conferences the decision was made to perform surgery. The patient was subsequently transferred to Colorectal Surgery for laparoscopically assisted low anterior resection with prophylactic loop ileostomy. Pathology results suggested that there was an ulcer 3.5 cm × 2.2 cm in size on the surface of the intestinal mucosa. Microscopy revealed erosion necrosis of the surface mucosa, inflammatory exudation and hyperplasia of interstitial small blood vessels and fibrous tissue, and infiltration of a large number of acute and chronic inflammatory cells (Figure 1B). Pathology results revealed no vascular malformation. However, on the 7th and 10th days after surgery, the patient suffered from sudden gastrointestinal bleeding again. Additional colonoscopy revealed anastomotic oozing, and hemostasis was performed. Other treatments were provided, including repeated plasma transfusions, vitamin K supplementation, and aminocaproic acid hemostasis.

During those treatments, laboratory results indicated that APTT was prolonged and that von Willebrand

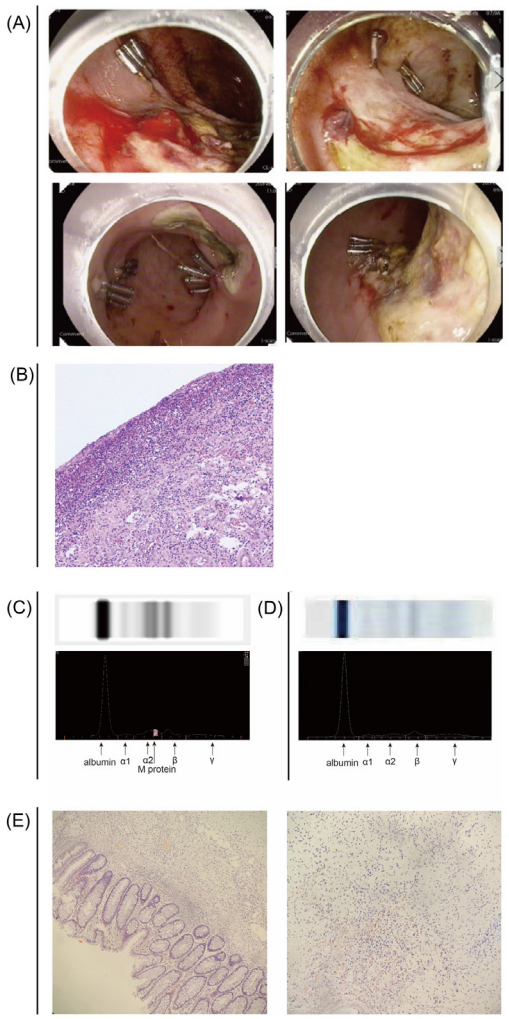

Figure 1. (A) Colonoscopy images. **(B)** Removed intestinal tissues (hematoxylin & eosin staining, medium magnification). (C) Serum M protein assay. Images of electrophoresis bands of serum protein and optical density scanning images of electrophoresis bands. **(D)** Normal protein electrophoresis. Images of electrophoresis bands of serum protein and optical density scanning images of electrophoresis bands. **(E)** Removed intestinal tissues (Congo red staining, medium magnification).

factor was normal. An APTT mixing test revealed that APTT could not be corrected to the normal range, and coagulation factor XI and XII activity decreased (Table 1). After a multidisciplinary discussion, postoperative bleeding was deemed to have been caused by abnormal coagulation. After consultation with an experienced hematologist, the presence of immediately acting clotting inhibitors was considered (including heparin, lupus anticoagulants, or several clotting factor antibodies). Combined with negative autoimmune results, the presence of non-specific antibodies resulting in abnormal coagulation was considered. The patient's TT was prolonged, and the clotting time was normal after treatment with protamine. Thus, the presence of heparin antibodies was excluded. Due to repeated bleeding, the patient's condition was critical, and hemoglobin decreased from normal to 61g/L within 1 week.

Table 1. Basic coagulation profile and coagulation factor activity assay

Items	Before disease	After endoscopic hemostasis	After surgery	After CTX	Normal range
Basic coagulation profile					
PT	13	14.1	14.5	12.2	11-15s
APTT	42.4 (24-36)	57.1	52.3	40.1	28-42s
Normal reference APTT	NA	35.2	31.2	31.5	
APTT 50/50 mixed immediately	NA	46.8	44.9	36.1	
APTT 50/50 mixed within 2 h	NA	50.8	45.6	38.4	
Fibrinogen	3.02	3.42	4.82	2.63	2-4g/L
Coagulation factor activity assay					
Plasma coagulation factor VIII activity assay	-	201	113	NA	60-150%
Plasma coagulation factor IX activity assay	-	165	173	NA	60-150%
Plasma coagulation factor X activity assay	-	92	91	NA	70-120%
Plasma coagulation factor XI activity assay	-	43	45	114	60-150%
Plasma coagulation factor XII activity assay	-	51	43	110	60-150%
Von Willebrand factor	-	231	NA	NA	50-160%

PT, prothrombin time. APTT, activated partial thromboplastin time. CTX, cyclophosphamide.

After empiric therapy with a cyclophosphamide and glucocorticoid, APTT and TT returned to their normal range, and gastrointestinal bleeding did not recur.

Since the cause of bleeding due to a prolonged APTT was not clear, the relevant tests were completed. Immunofixation electrophoresis revealed monoclonal IgG and light chain λ in blood and urine. M protein in blood was 2.8 g/L. Serum free light chain λ was 165.27 mg/L. Serum free light chain κ was 10.06 mg/L. The free light chain ratio λ/κ was 16.4:1 (Figure 1C and D). The proportion of plasma cells in bone marrow was 0.5%, and there were no abnormalities in bone marrow pathology, MM FISH, or flow cytometry. Plain body bone radiographs revealed no significant abnormalities. Bone marrow pathology, an abdominal wall skin biopsy, and the pathology of the removed intestine were negative for Congo red staining (Figure 1E). Based on comprehensive clinical data, the patient was diagnosed with MGUS and a prolonged APTT. At present, the patient has a normal APTT without bleeding, and factor XI and XII activity has returned to its normal range. Elective enterostomy was subsequently performed, and the patient was followed up. This study was approved by the ethics committee of the Fujian Medical University Union Hospital, and informed consent was obtained prior to this study.

Experience and insights: The patient reported in this case suffered from refractory bleeding at the site of rectal polyp resection after colonoscopy due to slight prolongation of APTT and an absence of medical attention. After surgery, bleeding continued at the surgical site. Repeated endoscopic hemostasis was ineffective, and a vascular malformation was considered, but postoperative bleeding persisted after surgery. Pathology revealed no vascular malformation. One week after surgery, bleeding recurred. A multidisciplinary conference deemed that postoperative bleeding was not related to the procedure. Thus, repeated lower gastrointestinal bleeding was considered to have been mainly related to coagulopathy. The APTT mixing test suggested the presence of non-specific antibodies, causing an acquired coagulation dysfunction. Although the antibodies were unclear, the patient's condition was critical, and the only option was to eliminate autoantibodies to anticoagulation factors through empirical use of immunosuppressive therapy. Fortunately, the patient's APTT has returned to the normal range and postoperative bleeding stopped.

Moreover, an examination revealed elevated monoclonal IgG and lambda light chains, an abnormal serum free light chain ratio (κ/λ), M protein less than 30g/L, bone marrow (BM) clonal plasma cells less than 10%, and an absence of end-organ damage. Thus, the patient was diagnosed with low-risk MGUS. Based on Mayo risk stratification, the International Myeloma Working Group (IMWG) developed clinical follow-up guidelines for MGUS patients in 2010, recommending re-examination of M protein 6 months after initial diagnosis. According to those guidelines (*10*), the current patient is in the low-risk group and should only be followed up. Due to repeated bleeding after lower gastrointestinal surgery and failure to correct the prolonged APTT using normal plasma, the presence of non-specific antibodies and reduced factor XI activity was considered. Thus, empirical therapy with a cyclophosphamide and glucocorticoid was provided. The APTT returned to normal, bleeding stopped, and treatment was effective. Factor XI and XII activity decreased slightly, and those factors are only slightly correlated with bleeding (*9*). Whether M protein was involved in the prolonged APTT and whether M protein was the direct cause of bleeding is still uncertain. A handful of studies have suggested that MGUS is associated with prolonged APTT. McCaughan *et al*. reported that a patient with MGUS and detectable IgG and kappa also had uncorrectable APTT and reduced factor XI activity (*9*). However, that patient did not suffer from obvious bleeding.

Studies have found that patients with MGUS may develop an acquired bleeding disorder similar to congenital von Willebrand disease. These patients have low plasma levels of factor VIII or von Willebrand factor, and measures to improve hemostasis should be taken to prevent or treat bleeding. Two patients with MGUS and

severe recurrence of chronic gastrointestinal bleeding were treated with long-term IVIg, and their laboratory results improved and chronic gastrointestinal bleeding stopped (*11*). Monoclonal binding proteins may bind to von Willebrand factor in the body.

MGUS is a precancerous lesion that may become a plasma cell disease. For patients with MM, prolonged PT or prolonged APTT is an independent factor for a poor prognosis in patients with newly diagnosed MM (*12,13*). Five to 86% of patients with MM have abnormal PT, and 8.9-69% of patients have abnormal APTT (*14-16)*. A study on abnormal coagulation in patients with plasma cell tumors such as MM and MGUS found that prolonged PT was more common and that prolonged APTT alone accounted for less than 1% (*17*). Laboratory results can detect coagulation abnormalities in more than half of patients with AL amyloidosis, with prolonged APTT and bleeding. Abnormal deletion of factor X is the most common cause, which is due to the selective adsorption of factor X by amyloid fibrils. However, a deficiency in factor X is not the only cause of bleeding in patients with AL amyloidosis because vascular wall amyloidosis, fibrinogen abnormalities, abnormal platelet aggregation, and deficiencies in other factors including factors II, VII, IX, and V can lead to bleeding. An acquired coagulation factor deficiency can be explained by coagulation factor adsorption by amyloid fibrils, but the specific pathophysiological mechanism remains unclear (*18*). The current patient was negative for Congo red staining, and AL amyloidosis was ruled out by pathology.

The pathogenic mechanisms involved in MGUS are diverse, manifesting as autoantibody activity against tissue antigens, immune complex formation, and complement activation (*11*). The current patient required long-term follow-up, and more studies need to be conducted to examine the correlation between APTT prolongation and reduced factor XI activity and MGUS in order to determine the underlying cause of bleeding.

Based on the principles of perioperative management of hemophilia and the current authors' experience, alternative therapy is required to treat and prevent perioperative bleeding in a patient with a coagulation factor deficiency alone and the absence of specific or non-specific antibodies. Depending on the type of surgery, the plasma level of factors needs to be increased to at least 50-100% of the normal value, thereby reducing the risk of bleeding. For patients with specific and non-specific antibodies and an uncorrectable APTT undergoing elective surgery, the cause needs to be ascertained, the condition needs to be controlled, coagulation abnormalities need to be corrected, and surgery needs to be performed. If emergency surgery is performed, immunosuppressive drugs can be added as appropriate via fresh frozen plasma transfusions to reduce the risk of postoperative bleeding. If routine examinations fail to identify the cause, further testing for disorders associated with the clonal proliferation of plasma cells or B cells should be performed.

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Updated information regarding management of hepatic epithelioid hemangioendothelioma

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SUMMARY Hepatic epithelioid hemangioendothelioma (HEHE) is a rare hepatic vascular tumor with a borderline biological behavior between hemangioma and hemangiosarcoma. It tends to be multiple or diffuse subcapsular lesions across the liver but has no characteristic clinical manifestations or imaging findings. On computed tomography and magnetic resonance imaging, these lesions usually have a hypodense appearance with heterogeneous enhancement and a "halo sign" or "lollipop sign" may be evident in some cases. HEHE is diagnosed mainly based on a pathological examination along with differential immunohistochemical markers such as CAMTA1, CD31, CD34, CD10, vimentin, and factor VIII antigen. Currently, there are no standardized treatment guidelines for HEHE, and surgery (curative resection and liver transplantation) remains the mainstay of treatment. Studies have indicated that extra-hepatic metastasis might not be a contraindication for resection or transplantation. Systemic chemotherapeutic agents including doxorubicin, vincristine, interferon-a, 5-fluorouracil, and thalidomide, as well as VEGF-related agents are being investigated, but no agents have been approved for the treatment of HEHE.

Keywords hepatic epithelioid hemangioendothelioma, differential diagnosis, pathology, curative resection, liver transplantation

Epithelioid hemangioendothelioma (EHE) is a rare vascular tumor with an estimated incidence of less than 0.01 per million (*1*). The World Health Organization has classified it as a tumor condition with a full potential of malignancy (*2*). It can occur anywhere in the body but mostly affects the liver (*3,4*). Hepatic epithelioid hemangioendothelioma (HEHE) is diagnosed predominantly in females (female-to-male ratio: 3:2) (*1*). The hallmark of HEHE is its unpredictable clinical course, which can be indolent, stable, or aggressive due to its borderline biological behavior between hemangioma and hepatic hemangiosarcoma (*5*). Clinical diagnosis and management of HEHE is a challenge. Due to its rarity and inconsistent behavior, there are no standardized treatment guidelines for HEHE at present. This article has reviewed the recent literature and analyzed updated data regarding the diagnosis and management of HEHE.

1. Clinical presentation and diagnosis

HEHE has no characteristic clinical manifestations and may present as right upper quadrant or epigastric pain (60-70%), weight loss (20%), an impaired general condition (20%), or jaundice (10%) (*1*). A study has noted that approximately one-quarter of patients are asymptomatic at the time of diagnosis (*6*). HEHE has a more aggressive course than EHE arising in bone or soft tissue. Clinically, HEHE is misdiagnosed in approximately 60-80% of cases (*7*). HEHE is most often confused with disease entities such as angiosarcoma, cholangiocarcinoma, metastatic carcinoma, and hepatocellular carcinoma. HEHE tends to be multiple or diffuse throughout the liver with a peripheral or subcapsular growth pattern. One study found that HEHE was multifocal in 81% of patients and solitary in the remaining 19% (*7*). Another study found that the multinodular type of HEHE is an intrahepatic metastatic disease arising from a single clone (*8*). The tumor size (> 3cm) and mitotic index (> 3mitoses/50HPF) have been found to be poor prognostic factors for HEHE (*9*). Extrahepatic involvement was found in 36.6% of HEHE cases (*7*). The most common sites of metastasis are the lungs, regional lymph nodes, peritoneum, bone,

and the spleen.

1.1. Imaging features of HEHE

On a CT scan, HEHE lesions usually display a hypodense appearance and heterogeneous enhancement. Tumors larger than 3 cm in size frequently exhibit delayed heterogeneous enhancement, tumors 2 to 3 cm in size exhibit ring-like enhancement, and tumors smaller than 2 cm exhibit homogeneous enhancement (*10*). A "lollipop" sign with an enhancing portal vein terminating at the edge of a hypodense lesion may be evident in some cases, and a "halo sign" with ring enhancement during the arterial phase and central filling on the delayed phases may be evident in others (*11*). HEHE lesions typically have a hypoechoic appearance on sonography. HEHE displays intratumoral vascularity and is better depicted on color Doppler (*12*). Contrast-enhanced ultrasound (CEUS) can be used to detect multiple foci of HEHE, with high enhancement in the arterial phase and low enhancement in the portal or delayed phase (*13*). 18F-FDG PET-CT is useful in detecting metastatic lesions and determining the spread of HEHE (*14*).

1.2. Histology and pathological differential diagnosis

Due to its nonspecific clinical manifestations and radiological features, HEHE is mainly diagnosed based on a pathological examination (*2*). EHE was first described by Dail and Liebow in 1975 as an epithelial lesion with an epithelioid morphology (*15*). The term "epithelioid hemangioendothelioma" was coined by Weiss and Enzinger in 1982 (*16*). The histology of HEHE is relatively distinctive from normal liver parenchyma, and it displays an infiltrative growth pattern consisting of epithelioid, dendritic, and intermediate cells interspersed in a hyaluronic acid–rich myxoid matrix (*7*). The epithelioid cells in HEHE contain rounded vesicular nuclei, an eosinophilic cytoplasm, and occasional intracytoplasmic vacuoles (*17*). These cells tend to grow along vascular structures and infiltrate hepatic sinusoids, causing atrophy and replacement of hepatocytes (*7*). A subset of HEHE displays a histologic overlap with hepatic angiosarcoma (HA) containing necrosis or cytonuclear atypia, without the typical myxoid stromal component. Pathologists often have difficulty differentiating HEHE from HA (*3*). Immunohistochemical and special staining can help with differentiation. Around 90% of HEHEs harbor the CAMTA1- WWTR1 fusion gene, which has been consistently identified in hemangioendothelioma, irrespective of the primary site (*15,18*). Other vascular endothelial markers include CD31, CD34, CD10, vimentin, and factor VIII antigen (*7*). Immunohistochemical staining of a fine-needle aspiration or biopsy specimen is the best method for diagnosing nonoperative patients. However, a false negative rate of 10% has been observed after a biopsy (*19*). The disease entities that tend to be confused with HEHE and their differential diagnosis are summarized in Table 1.

2. Current management of and the prognosis for HEHE

A standardized treatment algorithm for HEHE has yet to be devised, partly due to its rarity and inconsistent biological behavior. Therapeutic options in clinical practice include radiotherapy, chemotherapy, transcatheter arterial chemoembolization (TACE), anti-angiogenic drugs, locoregional ablation, hepatic resection, and liver transplantation (LT). The treatment modality is determined based on the tumour burden, extrahepatic involvement, resectability, and the condition of the patient's major organs (*20*). Currently, surgery including curative resection and transplantation remains the mainstay of treatment. Curative resection and LT have the best survival rates, with 5-year survival rates of 54.5% and 75%, respectively. In contrast, chemo/radiotherapy and observational follow-up have 5-year survival rates of 30% and 4.5%, respectively (*21*). According to a multivariate analysis, surgery was the only independent prognostic factor for overall survival of HEHE patients (*22*). Chemotherapeutic agents such as doxorubicin, vincristine, interferon-a, 5-fluorouracil, and thalidomide and therapy targeting vascular endothelial grow factor (VEGF) are being investigated (*23*). Thalidomide has been reported to offer potential for the treatment of HEHE (*24*). However, there are no approved systemic treatments for HEHE at present.

2.1. Curative resection for HEHE

Lesions that are multiple foci or extensive involvement of the liver pose a challenge to surgical resection. A detailed preoperative plan needs to be devised or surgery needs to be simulated preoperatively, like colorectal liver metastasis (CRLM). Optimal surgery is a radical resection of all HEHE lesions with maximum preservation of liver parenchyma (*2*). The long-term prognosis for patients with extra-hepatic metastasis who underwent surgery was not inferior to the long-term prognosis for patients without extra-hepatic involvement, indicating that extra-hepatic metastasis might not be a contraindication for surgery (*25*).

2.2. LT for HEHE

LT is seldom performed for a benign liver tumor worldwide, and it accounts for only 1% of all LTs in Europe and the US (*26*). However, HEHE is not necessarily considered to be a contraindication for LT when it is bilobar foci or diffuse disease throughout the

Table 1. Differential diagnosis of HEHE and other hepatic disease entities (*4,12,21*)

Entities	Imaging features	IHC markers	Histology
HEHE	Lollipop sign, halo sign, subcapsular growth, capsular retraction	(+)CAMTA1, WT-1, CD31, CD34, vimentin, F VIII,ERG, D2-40; (-)GLUT-1, cytokeratin	Cords and nests of epithelioid cells in a variable fibromyxoid stroma; Occasional intracytoplasmic vacuoles/lumina; minimal cytologic atypia with low mitotic rates; portal tracts intact.
Hepatic angiosarcoma	Heterogeneous centripetal enhancement	(+)WT-1, CD31, CD34, F VIII,FLI-1, ERG; (-)GLUT-1, D2-40, alpha-1-antitrypsin, pan-cytokeratin	More nuclear pleomorphism; more atypia and mitotic activity; well-formed, anastomosing vessels; more solid areas composed of pleomorphic spindle cell; hypercellular whorls of spindled cells; intracytoplasmic eosinophilic globules.
Hepatic hemangioma	Posterior shadowing and centripetal filling	(+)WT-1, CD34, CD31, FVIII,ERG; (-)GLUT-1,D2-40	Circumscribed proliferation of variably sized, dilated and thin-walled vessels lined by a single layer of flat endothelial cells; no cytologic atypia or mitosis.
ICC	Cholangiectasis, capsular retraction	Pan-cytokeratin	Abundant desmoplastic stroma and gland formation or nested neoplastic cells.
HCC	Wash-out	HepPar-1; CD10, CK8/18, glypican3,pCEA	Well-vascularized tumors with wide trabeculae (> 3 cells), a prominent acinar pattern, small cell changes, cytologic atypia, mitotic activity, vascular invasion, absence of Kupffer cells, and the loss of the reticulin network.
CRLM	Bull's-eye sign	Resemble original tumors	Similar to original tumors.

Abbreviation: IHC immunohistochemical staining; ICC intrahepatic cholangiocarcinoma; HCC hepatocellular carcinoma; CRLM colorectal cancer liver metastasis; WT-1: Wilms' tumor 1; FVIII: factor VIII; ERG: Erythroblast transformation, specific-related gene; GLUT-1: glucose transporter-1; D2-40: podoplanin; CAMTA1: calmodulin-binding transcription activator1; TFE3: transcription factor E3.

liver (*27*). Good outcomes of LT for HEHE have been reported from the West, with a 5-year survival of 67% in the US (1987–2005) and 79.5% in Europe (1989–2017) (*28,29*). Both studies found that extrahepatic metastasis did not represent an absolute contraindication for LT (*2*). After transplantation, the 5-year survival rate of patients with HEHE and extra-hepatic metastasis was as high as 72% (*17*). In a study of 110 patients with HEHE, the recurrence rate after LT was 11% (*29*). The independent risk factors for post-LT recurrence of HEHE include macrovascular invasion at pathology, a pre-LT waiting time longer than 120 days, and hilar lymph node metastasis (*29*). Therefore, that study emphasized the importance of routine extensive lymphadenectomy during LT.

In conclusion, HEHE is a rare borderline liver vascular tumor with an unpredictable clinical course (indolent to progressive), and its pathogenesis is not completely known. Its diagnosis depends mainly on a pathological examination. Immunohistochemistry is helpful in making a diagnosis, along with vascular endothelial markers such as CD10, CD31, CD34, and factor VIII antigen. Currently, there are no standardized guidelines for treating HEHE, and surgery including curative resection and transplantation remains the mainstay of its treatment.

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Letter

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The aberrant behavior profile in Indonesian individuals with fragile X syndrome with limited genetic services

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SUMMARY Fragile X syndrome (FXS) is caused by the full mutation in the fragile x messenger ribonucleoprotein 1 (*FMR1*) gene leading to the absence of the fragile X protein (FXP). Previous studies show that individuals with FXS exhibit changing behavior over time; therefore, this study aimed to elucidate the aberrant behavior profile of FXS individuals. The Aberrant Behavior Checklist-Community (ABC-C) was used to measure the aberrant behavior profile of individuals with FXS, which was rated by the parent/caregiver combined with clinical impression. A total of 58 items were used to assess aberrant behaviors across five subscales. Forty-nine individuals with FXS were included (32 males, 17 females) with a mean age of 32.9 ± 14.62 years in males and 33.4 ± 13.98 years in females. The average score of irritability and hyperactivity was significantly higher in male FXS individuals (5.37 ± 6.231 and 10.28 ± 8.524) than in female individuals (3.24 ± 7.093 and 3.76 ± 3.327) with $p = 0.046$ and $p = 0.001$, respectively. Overall irritability in FXS individuals significantly decreased over time ($\beta = -0.141$; $p = 0.032$). A modest worsening in lethargy/social withdrawal in males across age and a gentle improvement in hyperactivity/noncompliance in male of FXS individuals were observed. FXS males had higher hyperactivity problems than FXS female individuals across age.

Keywords aberrant behaviors overtime, fragile X syndrome, hyperactivity, irritability

Fragile X syndrome (FXS) is the most frequent cause of inherited intellectual disability (ID), a well-known single-gene disorder associated with autism spectrum disorders (ASDs) (*1*). The frequency of FXS is approximately 1 in 4,000 males and 1 in 8,000 females worldwide (*2*). Males typically have a more severe phenotype in cognitive, physical, and behavioral features compared to females (*3*). Most males meet the criteria for severe ID and have more distinctive physical characteristics, macroorchidism, and more challenging behavior including ASD, ADHD, and self-injurious and aggressive behaviors (*4*). FXS is usually due to the expansion of CGG repeats of more than 200 units (full mutation) in the promotor region of the fragile x messenger ribonucleoprotein 1 (*FMR1*) gene that leads to transcriptional silencing and results in lack of fragile X protein (FXP) (*5*).

Developmental trajectories in speech, fine and gross motor, cognition, and behavior in FXS individuals have been studied to acknowledge the severity of the actual problem behavior and also to discover the need of services over time (*6*). Clinical manifestations change with age, some behaviors remain *stable* from childhood to adulthood, *i.e.*, unusual eye contact, difficulty in showing emotion and unusual/inappropriate moods, inattentiveness, impulsivity, and social withdrawal. Other behaviors increase with age, *i.e.*, overeating, social withdrawal, panic attack, and violent outburst/temper tantrums, and some appear to reduce post-puberty: sleep problems and hypersensitivity (*7,8*). Hustyi *et al*. carried out an interesting study in the United States, where clinical trials and standard interventions were available to address the longitudinal trajectories of aberrant behaviors (*9*).

In Indonesia, no specific therapy or standard intervention is available for children with FXS. Accordingly, documentation of persistent and nonpersistent behavioral problems in FXS individuals across age is needed to guide effective and strategic interventions. We conducted a study to obtain an aberrant

behavior profile of individuals with FXS who did not undergo interventions to document the severity of behavioral problems that occur over time.

Study Design

Forty-nine individuals with FXS were included in this a cross-sectional study. The age of participants ranged from 6.56 to 60 years, and then were categorized and ordered from youngest to oldest by ten year intervals and analysis was done to obtain occurrence of behavioral problems in age categories. This study was approved by the Health Research Ethics Committee (Approval No.1.032/EC/FK-RSDK/XII/2016). All parents/caregivers signed a consent form prior to the study.

Male and female individuals with FXS who had been confirmed genetically were recruited. The inclusion criteria for the respondents were as follows: having a child with FXS or caregivers who spent at least 6 h per day and were closely involved in daily living activities, health care, and social interactions for more than a year.

The Aberrant Behavior Checklist-Community (ABC-C) was used to assess the presence and severity of behavioral problems in children and adults with FXS. The ABC-C comprised five subscales that made up a 58-item questionnaire: irritability, lethargy/social withdrawal, stereotypic behavior, hyperactivity/noncompliance, and inappropriate speech (*10*). Each item was scored 0 (never a problem), 1 (slight problem), 2 (moderately serious problem), or 3 (severe problem). Each score was based on the parent or caregiver's perception of their child/client's behavior for the last 4 weeks.

The demographic characteristics of FXS individuals and respondents were obtained during the interview. The parent or caregiver completed the ABC-C through a semi-structured interview to rate their child/client's behavior conducted by experienced physicians (TIW and TAS). During and after the interview, TIW and TAS characterized the dominant aberrant behaviors to ensure that the information provided by the parent/caregiver was appropriate.

Each individual score was assumed as a baseline score, considering that all individuals received no standard therapy. The Mann-Whitney *U* test was applied to calculate the differences in subscale I, II, III, IV, and V scores between males and females because the data were not normally distributed. The Mann Whitney *U* test was also applied to analyze the difference of mean ABC-C score according to sex, rater, seizure co-morbidity, and education. Regression analysis was used to analyze the rate of change of subscales across age. *P*-values < 0.05 were considered statistically significant.

Core research findings

Forty-nine FXS individuals were included consisting of 32 males (65.3%) and 17 females (34.7%), the majority of participants were adults (87%) and only 12.2% were children. The mean age was 32.9 ± 14.62 years in males and 33.4 ± 13.98 years in females; the difference of age between males and females was not significant. Approximately 10% of FXS individuals had a history of seizure, more than 20% had no education/training, and none of them were treated with medications.

According to sex, the average score of irritability was significantly higher in male FXS individuals (5.37 ± 6.231) than in female individuals (3.24 ± 7.093) (-2.000; $p = 0.046$), and hyperactivity was significantly higher in male FXS individuals (10.28 ± 8.524) than in female individuals (3.76 ± 3.327) (-3.382; $p = 0.001$). The difference of irritability and inappropriate speech according to education category were found significant, with z value = 2.136, $p = 0.035$ and z values -2.283, $p = 0.030$, respectively. The difference of age according to education category was found statistically significant (Kruskal-Wallis H = 15.697; df = 3; $p = 0.001$). The remaining subscales demonstrated no significant difference according to the role of rater and seizure comorbidity (Table 1).

The analysis revealed that the aberrant behavior in FXS individuals changed over time: the irritability, stereotypic behavior, and hyperactivity subscales were mildly improved in males and females, the lethargy somewhat increased in males, while the inappropriate speech subscale remained steady in males. However, only the trajectory of decreasing irritability in all FXS individuals across age groups was found significant ($\beta = -0.141$; $p = 0.032$).

This study documented the behavioral changes based on ABC-C subscales according to sex and age. The five ABC-C subscales are presented in Figure 1. The graph shows the change (increase and decrease) in scores over time in male and female FXS individuals.

Challenging behaviors are very common in individuals with FXS, and prior studies have indicated differences between sexes (*9*). In contrast with previous studies, the present study indicates that sex did not have a robust effect on the behavioral profile. Only the hyperactivity subscale was significantly more severe in males than in females. Males with full mutation allele will demonstrate complete penetrance of FXS phenotype, while females will demonstrate reduced penetrance of 50% with phenotype ranging from mild to moderate. The lack of FXP has been associated with FXS clinical characteristics, *i.e.*, limited cognitive ability, behavioral problems, and social functioning (*11*).

This study revealed that age is an important factor in the behavioral phenotype. This study documented the variability in the aberrant behavior according to sex, especially with regards to irritability and hyperactivity subscales. Hyperactivity is a more significant problem in younger males than in females with FXS and it tended to decrease over time. The irritability in females and males

Table 1. The mean and median ABC-C subscale scores according to sex, the role of rater, seizure comorbidity, and education/training

Characteristics	Irritability	Lethargy	Stereotypic Behavior	Hyperactivity	Inappropriate Speech	Total
Sex						
Male	5.37 ± 6.231	14.81 ± 8.299	2.66 ± 2.659	10.28 ± 8.524	2.56 ± 2.873	59.63 ± 29.469
	4.50 (0-26)	13.50 (1-36)	2.00 (0-12)	7.00 (1-34)	2.00 (0-9)	52.50 (14-132)
Female	3.24 ± 7.093	11.94 ± 10.183	2.00 ± 2.828	3.76 ± 3.327	1.24 ± 2.107	58.88 ± 41.041
	1.00 (0-30)	10.00 (1-38)	1.00 (0-12)	3.00 (0-14)	0.00 (0-7)	52.00 (3-134)
(*z* value; *p*)*	(-2.000; $p = 0.046$)	(-1.010; $p = 0.312$)	(-1.652; $p = 0.098$)	(-3.382; $p = 0.001$)	(-1.622; $p = 0.105$)	(-0.399; $p = 0.690$)
Rater						
Parents	5.96 ± 7.815	13.25 ± 10.515	2.64 ± 3.082	8.39 ± 8.148	2.04 ± 2.755	59.54 ± 38.005
	3.50 (0-30)	12.00 (1-38)	2.00 (0-12)	5.50 (0-33)	1.00 (0-9)	51.50 (3-134)
Caregiver	2.86 ± 3.838	14.57 ± 6.630	2.14 ± 2.151	7.52 ± 7.414	2.19 ± 2.657	59.14 ± 27.273
	1.00 (0-13)	13.00 (4-29)	2.00 (1-11)	5.00 (1-34)	1.00 (0-9)	53.00 (19-115)
(*z* value; *p*)*	(-1.893; $p = 0.058$)	(-0.891; $p = 0.373$)	(-0.405; $p = 0.685$)	(-0.122; $p = 0.903$)	(-0.203; $p = 0.839$)	(-0.344; $p = 0.731$)
Seizure Comorbidity						
None	4.71 ± 6.818	14.18 ± 9.161	2.53 ± 2.785	8.42 ± 7.976	2.27 ± 2.742	59.42 ± 32.442
	2.00 (0-30)	13.00 (1-38)	2.00 (0-12)	6.00 (0-34)	1.00 (0-9)	53.00 (3-134)
Seizure	3.75 ± 1.893	9.75 ± 6.344	1.25 ± 1.258	3.50 ± 2.082	0.25 ± 0.500	58.75 ± 50.222
	4.50 (1-5)	10.00 (2-17)	1.00 (0-3)	3.50 (1-6)	0.00 (0-1)	40.00 (22-133)
(*z* value; *p*)*	(-0.573; $p = 0.585$)	(-0.823; $p = 0.426$)	(-1.145; $p = 0.277$)	(-1.337; $p = 0.190$)	(-1.487; $p = 0.166$)	(-0.511; $p = 0.634$)
Education/Training						
Not educated	2.00 ± 3.682	14.00 ± 8.894	2.40 ± 3.169	7.30 ± 9.967	0.60 ± 1.578	60.90 ± 38.336
	0.00 (0-11)	14.00 (1-29)	1.00 (0-11)	3.50 (1-34)	0.00 (0-5)	49.50 (18-115)
Educated	5.31 ± 6.978	13.77 ± 9.138	2.44 ± 2.624	8.21 ± 7.255	2.49 ± 2.790	58.97 ± 32.695
	3.00 (0-30)	12.00 (1-38)	2.00 (0-12)	6.00 (0-33)	2.00 (0-9)	52.00 (3-134)
(*z* value; *p*)*	(-2.136; $p = 0.035$)	-0.273; $p = 0.798$)	(-0.676; $p = 0.517$)	(-1.108; $p = 0.274$)	(-2.283; $p = 0.030$)	(-0.074; $p = 0.951$)

Value in the table are mean ± SD, median (min-max). *Mann Whitney *U* test

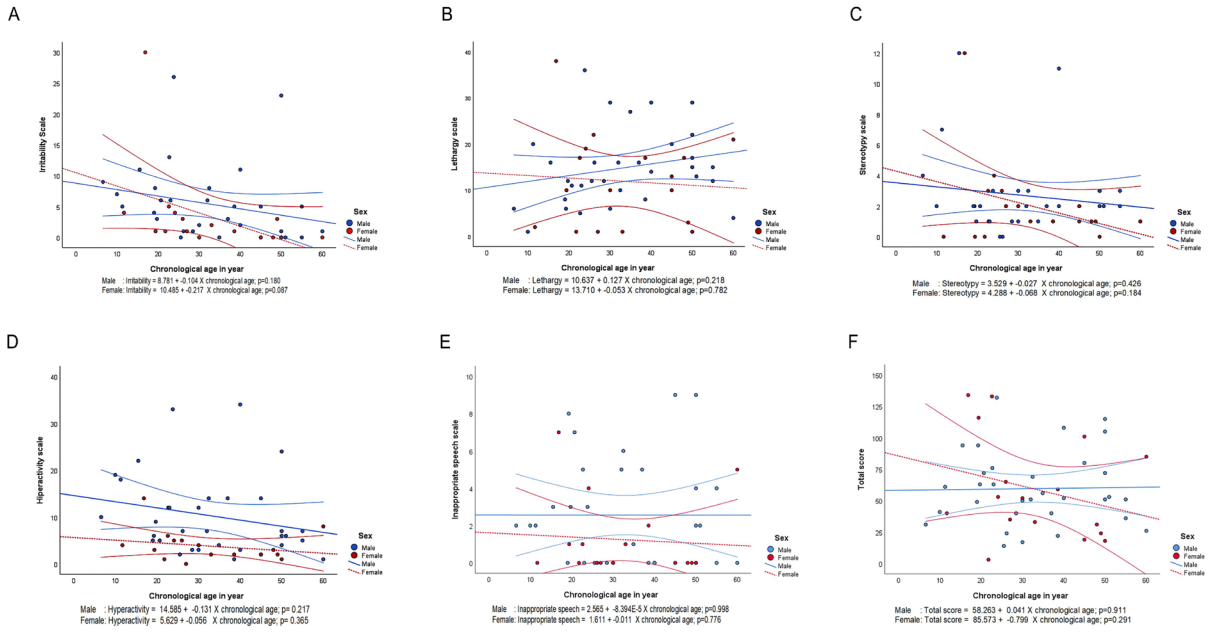

Figure 1. Trajectory score of five aberrant behavior subscales using the Aberrant Behavior Checklist-Community in FXS males and females across chronological age. (A) I, irritability; **(B)** II, lethargy/social withdrawal; **(C)** III, stereotypic behavior; **(D)** IV, hyperactivity/noncompliance; **(E)** V, inappropriate speech; and **(F)** Total score. Trajectory is presented in a blue dashed line for males with FXS and in a red dashed line for females with FXS, while a 95% confidence interval is presented in a blue solid line for males with FXS and in a red solid line for females with FXS.

(overall) across age groups significantly improved, while according to sex the change of irritability is modest. Previous studies reported improvement in hyperactivity and irritability subscales over time (*9*). From a parent and clinical perspective, irritability and hyperactivity behavior are their greatest concerns, including self-injurious behavior, aggression, disobedience, and noncompliance besides anxiety problems, because these behaviors negatively impact FXS individuals (*12*). There is an increased demand for medical care and support

from very young ages, and financial problems arising from such behavioral challenges have been reported, however, Hatton *et al*., in 2002, found that no differences exist between FXS children on or off medication in externalizing behavior and attention problems. Moreover, those who were on medication had a higher score for problems (*13*).

Interestingly, an increase of the lethargy/social withdrawal subscale in males over time was documented in this study. The score in the present study is higher (almost three to four times) compared to that in previous findings (*9*). Perhaps our findings are related to the lack of medical care in our country. The social withdrawal subscale consisted of 16 items representing lack of social interaction skills, which were found to be the strongest predictor of independence in adults with FXS and a marker of quality of life (QoL) (*14*). Our study shown some similar behavioral trajectories; however, sex differences have only been documented on the hyperactivity/noncompliance subscale, and problems on lethargy/social withdrawal behaviors are already prominent in younger males and females. In addition, the trend increased at older ages, especially in male FXS individuals.

Literature searches were conducted in the PubMed database and google search engine using two keywords *i.e.*, fragile X behavioral profile and limited medical care, and we found this is the first study to investigate the aberrant behavioral profile in FXS individuals who had received inadequate care. Factors that may impact the developmental trajectory, that is, environmental factors such as parent/caregiver education level and responsivity, IQ, and FXP, were not measured.

In conclusion, this study shows the variability in aberrant behavior according to sex, specifically irritability and hyperactivity subscales. The hyperactivity subscale is a more significant problem in younger male FXS individuals and it tended to decrease over time, while, the irritability subscale in females and males (overall) improves with age.

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KCNB1 frameshift variant caused inherited intellectual disability, developmental delay, and seizure

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SUMMARY Potassium voltage-gated channel subfamily B member 1 (*KCNB1*) encodes Kv2.1 potassium channel. KCNB1 mutations are known to cause global developmental delay, behavioral disorders, and various epilepsies. Most variants occur de novo and are rarely inherited. Here, we report a 14-year-old male patient who was admitted to our clinic with seizures, developmental delay history, and intellectual disability. Brain magnetic resonance image (MRI) was normal and electroencephalogram (EEG) showed spike and sharp-wave complexes emerging in the left hemisphere parietooccipital areas, which were paroxysmally generalized. We performed whole exome sequence analysis (WES) and identified a heterozygous frameshift mutation c.522delA in exon 1 of *KCNB1* (NM_004975.4) predicting a premature stop codon p.Lys174Asnfs*20 in the proband. Sanger sequencing confirmed the heterozygous c.522delA mutation in the proband and his mother who also had epilepsy and learning difficulties. His 45 year old mother had used antiepileptic drugs for 9 years after a seizure episode at 12 years old. Also, his mother's uncle's son is nonverbal and has developmental delay and epilepsy. Our study shows that frameshift mutation cytoplasmic domain of *KCNB1* gene can cause intrafamilial phenotypic variability and relatively mild clinical findings in these patients.

Keywords *KCNB1*, epilepsy, intellectual disability, intrafamilial phenotypic variability

Developmental and epileptic encephalopathies (DEE) are a broad and genetically heterogeneous group of neurodevelopmental disorders, which are characterized by social, cognitive, motor, language, and behavioral problems with or without epileptic seizures (*1*). Recent studies with next-generation sequencing reveal the involvement of gene-encoding ion channels in the pathogenesis of DEE (*1*). Potassium voltage-gated channel subfamily B member 1 (*KCNB1*) encodes Kv2.1 potassium channel and is expressed in various neurons and organs (*2*). *KCNB1* mutations are known to cause global developmental delay, behavioral problems, and various epilepsies (Developmental and epileptic encephalopathy-26) (*3*). To date, 51 distinct *KCNB1* pathogenic variants have been reported in 74 unrelated patients with DEE. Most common findings in these patients are epilepsy, early developmental delay, autism spectrum disorder (ASD), and other psychiatric and behavioral disorders (*4*).

Here, we report a child and his 45 year old mother with a novel *KCNB1* mutation identified by whole-exome sequencing (WES). The child presented with seizures, developmental delay history and borderline intellectual functioning whereas the mother just presented with seizure history, and learning difficulties.

A 14-year-old male patient was admitted to our medical genetic outpatient clinic with seizures, developmental delay history and mild intellectual disability. He was born to non-consanguineous parents as a first child after a full-term pregnancy due to fetal macrosomia through cesarean section. At presentation, his height was 55 cm (> 97 percentile), weight was 4.6 kg (> 97 percentile) and head circumference was 38 cm (> 97 percentile). He was able to walk independently at 17 months. He could speak his first meaningful word's at 3 years. He was always ahead of his peers in terms of height and weight. Apart from these, he did not have any problems until 14 years old. He started primary school at the normal time and learned to read and write on time. At the age of 14, he had afebrile generalized tonic-clonic seizures and repeated twice after 10 days from the first seizure. At this time, electroencephalogram (EEG) showed spike and sharp-wave complexes emerging in the left hemisphere parietooccipital areas, which were paroxysmally generalized. He has started to use levetirasetam therapy after a second seizure attack. He

still continues to use levetirasetam and has had no other seizure attack until now. He caught up with his peers in terms of speaking and other developmental parameters later. The patient has no dysmorphic features or other noteworthy findings revealed at physical examination. Neurological examination was normal except for mild intellectual disability. Laboratory investigations including hemogram, biochemical and metabolic investigations, cranial MRI, echocardiogram, and EEG was reported normal at 15 years old.

His 45 year old mother had used an antiepileptic drug (Maliasin) for 9 years after a seizure episode at 12 years old. The mother of the proband had the first seizure attack at 12 years old and had used antiepileptic drug (Maliasin) for 9 years without a further epilepsy attack. The mother stated that she had experienced learning difficulties throughout the education process. Other growth and developmental parameters of the mother were normal for her age. We decided to perform a whole exome sequence analyses for the proband to elucidate underlying genetic etiology. The parents of the patient provided written informed consent for participation in this study. The study was performed according to the Declaration of Helsinki protocols.

After karyotype analyses with normal results, we decided to perform a WES analyses on this patient. After variant prioritization in the WES data, we identified a heterozygous frameshift mutation c.522delA in exon 1 of *KCNB1* (NM_004975.4) predicting a premature stop codon p.Lys174Asnfs*20 in the proband. Sanger sequencing confirmed the heterozygous c.522delA mutation in the proband and his mother who also had epilepsy and learning difficulties (Figure 1). KCNB1 mutation in this study was not annotated in databases of human variation [Exome Variant Server (*http://evs.gs.washington.edu/EVS/*), 1000 genomes (*http://www.1000genomes.org/*), dbSNP (*http://www.ncbi.nlm.nih.gov/projects/SNP/*)]. Additionally, the mutation was not seen in the Acıbadem Labgen in-house exome database.

The variant in our cases p.Lys174Asnfs*20 is located in the cytoplasmic domain of the *KCNB1* gene. In the literature, there is only a case who carried a de novo missense *KCNB1* variant at cytoplasmic N-terminal region (p.Glu43Gly). In contrast to our current case, the case by Bar *et al*. was nonverbal and had early-onset epilepsy, and developmental delay. But this patient also carried a de novo variant in *GABRA5* gene (p.Thr301Met), which causes developmental and epileptic encephalopathy-79 (DEE79). So, both variants were considered to be contributing to the patient's phenotype and we do not know the exact effect of the *KCNB1* (p.Glu43Gly) variant in this case. It is postulated that truncating variants in the C-terminal domain correlate with less severe epilepsy outcomes (*5,6*). Similar to this finding, truncating variants in the N-terminal domain may correlate with less severe

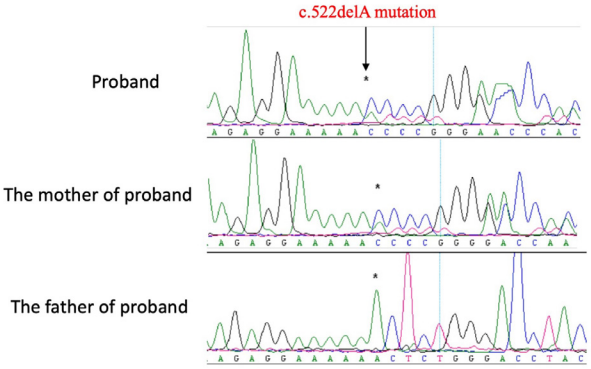

Figure 1. Electropherogram of the *KCNB1* mutation identified in the proband and his mother with his father's electropherogram.

epilepsy and milder developmental problems.

All the *KCNB1* variants identified occurred de novo, except for one maternally inherited variant (p.Arg583*) reported in 2019 (*6*).Our case is the second report that a *KCNB1* mutation in a patient was maternally inherited. In the case by Bare *et al*., the mother of the patient presented with a milder phenotype like intellectual disability with delayed language skills without epilepsy compared to his daughter who had a severe neurodevelopmental disorder with no language acquisition, autism spectrum disorder and behavioral disorders (*6*). This study emphasized that patients carrying a *KCNB1* variant with a relatively mild phenotype can transmit a severe disease to their offspring. Similar to this case, the mother in our study transmitted a relatively severe disease to his son supporting the view that inherited a *KCNB1* variant can be associated with intrafamilial variable expressivity.

Most of the reported cases with a *KCNB1* variant were relatively younger patients. Therefore, knowledge regarding its long-term outcome is limited. According to our current knowledge, this is the oldest patient with a *KCNB1* pathogenic variant. She had seizure history at 12 years old and learning difficulty history during her education period. No other difficulties have been experienced until now. Also no regression of verbal skills was noted during her life time in contrast to the case in the previous report by Lu *et al*. (*7*). We consider that type of variant and affected domain has a major impact on the patients' phenotype for patients with *KCNB1* variants. Truncating variants at the N terminal of the *KCNB1* gene may be more correlated with a milder disease phenotype. Further *KCNB1* cases with genotype-phenotype correlation are needed to prove this view. Also, the patient and his mother in our study had late age seizure onset supporting the view that late age seizure onset might correlate with a more benign disease course for patients with *KCNB1* variants (*7*).

To conclude, we report a proband and his 45 year old mother carrying a c.522delA variant in the *KCNB1*gene. Our study shows that frameshift mutation cytoplasmic domains of *KCNB1* gene can cause intrafamilial

phenotypic variability and relatively mild clinical findings in these patients. Further studies are needed to elucidate the effect of the *KCNB1* variants in different domains and to understand the course of the disease in elderly patients with *KCNB*1 variants.

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

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