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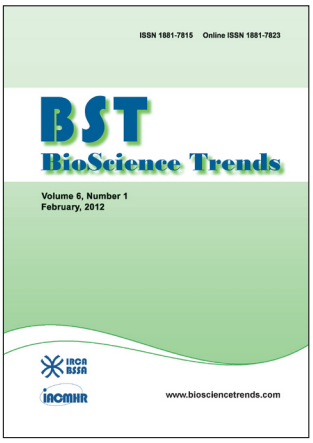

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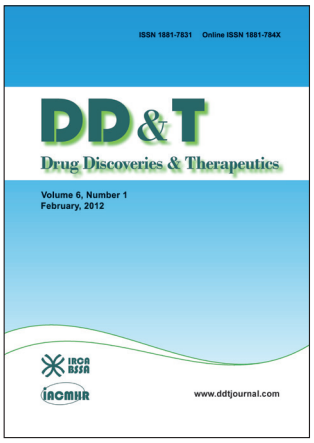

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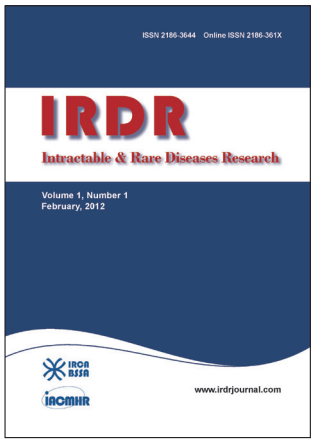

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Editorial Board

CONTENTS

Volume 6, Number 2, 2017

Reviews

Original Article

Brief Reports

Case Reports

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Review

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Idiopathic pulmonary fibrosis in East Asian

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Summary **Idiopathic pulmonary fibrosis (IPF) is a rare lung disease with a prognosis that can be worse than that of many cancers. Recent studies have improved our understanding of IPF and new treatment options have become available. However, most studies are conducted predominantly in Western countries while few are conducted in East Asian countries. The distribution, effectiveness of treatment, and prognosis for IPF differ among Westerners and East Asians, but whether the heterogeneity of IPF in East Asians is the result of ethnic differences and geographic variability is unclear. This study highlights the current prevalence of IPF and its characteristics in the East Asian population and it provides valuable information to understand the current clinical status of patients with IPF in light of recent advances in its diagnosis and treatment.**

Keywords: Idiopathic pulmonary fibrosis, East Asian population, heterogeneity, diagnosis, treatment

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a rare, chronic, progressive, and irreversible lung disease with no specific cause and pathophysiological mechanism. Most patients are males over the age of 60 and most patients have a history of smoking. IPF is the most common and severe form of usual interstitial pneumonia (UIP). Median survival is estimated to be 3 to 5 years from the time of diagnosis (*1*). Over the past few years, several medications have demonstrated the ability to slow the progression of this devastating disease, but there is no effective treatment for the disease itself (*2*). For now, lung transplantation seems to be the sole option for selected patients with advanced IPF (*3*).

Since the ATS/ERS/JRS/ALAT guidelines on the diagnosis and management of IPF were published in 2011, new data from major clinical studies, particularly with regard to epidemiology, and from clinical trials have been released from several Western countries. Only a few recent studies focused on East Asians. The distribution, effectiveness of treatment, and prognosis for IPF differ among Westerners and East Asians, but whether the heterogeneity of IPF in East Asians is the result of ethnic differences and geographic variability is unclear. To date, no systematic study has reviewed the prevalence and clinical course of IPF in the East Asian population. The current study has discussed the features and management of IPF in Chinese, Japanese, and Korean patients as a whole since those populations have a similar ethnicity. The aim of this study was to describe the current state of IPF in East Asia as well as recent advances in its diagnosis and treatment.

2. Epidemiology

IPF is a disease that affects the elderly and is more prevalent in males. The true incidence and prevalence of IPF have not been accurately determined because of the lack of a uniform definition, diagnostic criteria, and differences in methodologies and populations in previous studies (*4*). However, the 2001 ATS/ERS consensus statement offers an opportunity for more precise epidemiologic studies. Generally, the prevalence and incidence of IPF are higher in American studies than in European and Asian studies (*5,6*). Whether this is due to true differences in geography and ethnicity or simply due to methodological differences in the way the studies were conducted is unclear.

Data from the US indicate that IPF has a prevalence

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between 14 and 28 cases per 100,000 population. The annual estimated incidence in the US is between 6.8 and 8.8 cases per 100,000 population. IPF-related morbidity and mortality is higher in men and increases progressively with age (*5*). A study from South Korea in 2011-2012 indicated that the incidence of IPF was 1.7/100,000, based on the new ATS/ERS/JRS/ALAT statement published in 2011. There was an overall increase in morbidity and mortality in those years. Longevity, regular medical examinations, and increasing use of CT might contribute to changes in the epidemiology of IPF (*7*). An epidemiological survey in Japan from 2003 to 2007 indicated that the prevalence and cumulative incidence of IPF was 10.0 and 2.23 per 100,000 population, respectively, with 72.7% of cases involving males and an increase in frequency with age (*8*).

3. Risk factors

Although IPF is a condition of unknown origin. Exposure to risk factors such as cigarette smoking, environmental factors, and microbial agents as well as genetic factors and gastroesophageal reflux have been found to increase the risk of developing the disease according to different studies (*9*).

4. Diagnosis

4.1. *Clinical Presentation*

IPF primarily occurs between 60 and 70 years of age and the clinical symptoms of IPF are nonspecific. In the early stages, IPF clinically presents as progressive exertional dyspnea with a dry cough. In lung auscultation, bilateral inspiratory crackles ("Velcro crackles") at the lung base are characteristic and appear early in the disease. Finger clubbing is present in less than 50% of cases. Cyanosis and signs of right ventricular failure occur in the advanced stage with respiratory insufficiency, and precapillary pulmonary hypertension is often present, particularly if emphysema is associated with IPF. The disease progresses towards chronic restrictive respiratory failure and death (*1*).

4.2. *Pulmonary function tests*

Pulmonary function is usually evaluated with systematic tests when diagnosing IPF. The index of spirometry typically reveals restrictive ventilatory dysfunction with a reduced forced vital capacity (FVC) and total lung capacity (TLC) reflecting reduced lung function; the extent of the decrease is useful in quantifying the severity of disease and predicting outcome. Diffusing capacity for carbon monoxide (DLCO) almost invariably decreases due to both a contraction of the pulmonary capillary volume and ventilation and perfusion mismatching. The decline in DLCO may appear in the early or middle stages of IPF as the only functional abnormality. Resting arterial oxygen saturation is usually normal, but pulmonary function tests performed during exercise, such as a 6-min walk test (6MWT), reveal a reduced exercise capacity with increased alveolar-arterial oxygen tension difference and oxygen desaturation.

4.3. *Biomarkers*

There are no specific laboratory abnormalities in patients with IPF. Lung diseases with a specific etiology need to be systematically ruled out and extra-pulmonary signs and biomarkers need to be examined to eliminate connective tissue diseases (CTD) before IPF is diagnosed. Inflammatory and potential disease–associated biomarkers such as blood cell count, C-reactive protein, antinuclear antibodies, rheumatoid factor, and precipitin should be measured. Bronchoalveolar lavage is non-specific in patients with IPF and is characterized by the presence of macrophages and neutrophils with or without eosinophils. A bronchoalveolar lavage may be warranted and crucial to ruling out other diseases. Ohshimo *et al*. found that a high proportion of lymphocytes in the lavage fluid (more than 30%) tends not to indicate IPF (*10*).

4.4. *Radiology*

HRCT is an essential component to the diagnosis of IPF. The ATS/ERS/JRS/ALAT 2011 guidelines have assigned a primary diagnostic role to high-resolution computed tomography (HRCT) (*1*). Radiological criteria for IPF are classified into three categories: a UIP pattern, a possible UIP pattern, and a pattern inconsistent with UIP. On HRCT, UIP is characterized by the presence of reticular opacities, which are often associated with traction bronchiectasis. Honeycombing is a sufficient and persuasive sign of a definite UIP pattern on HRCT. Honeycombing on HRCT appears as clustered cystic airspaces 3-10 mm in diameter and is located below the pleura with well-defined walls (Figure 1). The distribution of UIP on HRCT is characteristically basal and peripheral, although it is often patchy (*11*).

4.5. *Surgical lung biopsy*

An HRCT and histopathologic correlate for IPF is UIP. If UIP is definitively diagnosed based on typical manifestations on HRCT, a surgical lung biopsy is not necessary. A video-assisted surgical lung biopsy after careful evaluation of the operative risk is required to confirm the diagnosis if the imaging features are not characteristic. In some cases, multidisciplinary team must consult to reach a definite diagnosis when HRCT findings are inconsistent with findings from a surgical

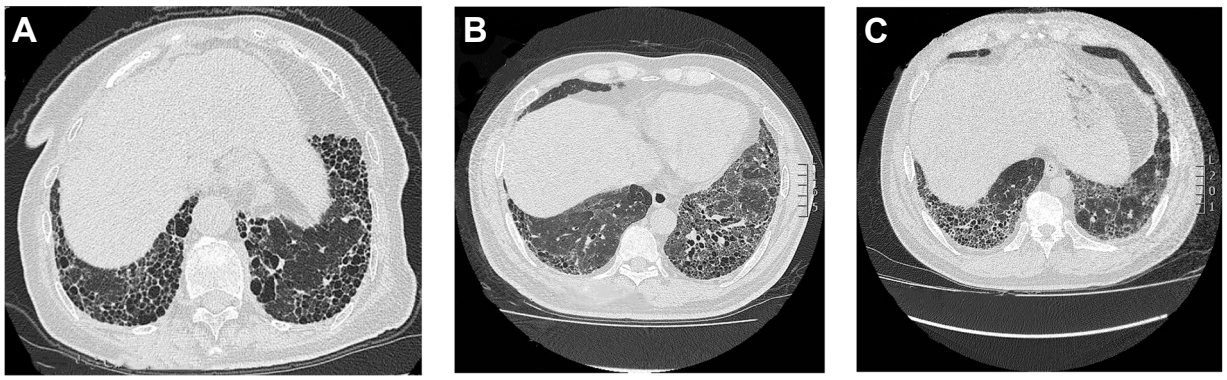

Figure 1. Chest computed tomography (CT) scans from patients with idiopathic pulmonary fibrosis (IPF). (A) A typical radiological pattern of usual interstitial pneumonia with subpleural honeycombing in the costophrenic angle in Patient A. Based on CT images from Patient B **(B)** and Patient C **(C)**, there is a familial cluster of IPF in two brothers age 50 and age 52, respectively.

lung biopsy (*1*).

5. Treatment

5.1. *Pharmacological treatment*

For many years, there were no effective treatments of IPF until two agents, pirfenidone and nintedanib, demonstrated the ability to slow the progression of the disease according to recent clinical trials.

Pirfenidone is an oral antifibrotic agent that inhibits the TGF-β pathway. The first multicenter, double-blind, placebo-controlled phase III randomized controlled trial of pirfenidone in IPF, which was conducted in 275 Japanese patients with well-defined IPF over 52 weeks, indicated that pirfenidone 1,800 mg/day slowed the decline in vital capacity (70 mL) and it may increase the PFS time. Photosensitivity was a major adverse event in that trial, though it was mild in most of the patients (*12*).

Nintedanib is an intracellular inhibitor that targets multiple tyrosine kinases, including PDGF, VEGF, and FGF. A randomized, double-blind, phase 3 trial to evaluate the efficacy and safety of 150 mg of nintedanib twice daily in comparison to a placebo was conducted over 52 weeks in a total of 1,066 patients with IPF. Nintedanib reduced the decline in FVC (125 ml FVC decline), which is consistent with slowing of the disease progression. There were no differences in DLCO measurements or in the distance walked in 6 minutes. Nintedanib use was frequently associated with diarrhea, which led to discontinuation of the study medication in less than 5% of patients (*13*). Patients who completed the trial were able to receive nintedanib in an open-label extension trial. In patients with a baseline FVC ≤ 50% and > 50% predicted at the start of the extension trial, the absolute mean change in FVC from the baseline to week 48 was –62.3 and –87.9 mL, respectively (n = 24 and n = 558, respectively). The decline in FVC in both subgroups by baseline FVC % predicted was similar to that in an earlier trial, suggesting that nintedanib may have a similar effect on disease progression in patients with advanced disease as in less advanced disease (*14*).

The decline in FVC was significantly slower in patients treated with pirfenidone or nintedanib compared to a placebo. Both pirfenidone and nintedanib were recommended for treatment of IPF in an update of an Official ATS/ERS/JRS/ALAT Clinical Practice Guideline in 2015 after a comprehensive analysis of a series of clinical trials involving these two medications (*2*). Furthermore, the FDA also has approved both pirfenidone and nintedanib for the treatment of IPF.

However, which drug is superior as the first-line therapy for IPF is still unclear. A recent systematic review that indirectly compared both treatments based on network meta-analysis reported that the two treatments had a beneficial effect; nintedanib appears to have superior benefit on FVC, but mortality did not differ significantly with nintedanib and pirfenidone (*15*). A superior first-line therapy for IPF has yet to be determined based on clinical efficacy. Furthermore, the two drugs cause slightly different adverse reactions. Gastrointestinal and skin-related events were more common in the pirfenidone group, whereas diarrhea and liver dysfunction were more common in the nintedanib group. The choice of pirfenidone or nintedanib for IPF should be individualized based on these results.

5.2. *Lung transplantation*

Lung transplantation is now a widely accepted treatment option for the management of a wide range of chronic end-stage lung disorders. Given the progressive and incurable nature of IPF, lung transplantation is commonly suggested as the most effective treatment for patients with moderate to severe IPF (*16*).

IPF accounts for the largest group of patients on the transplant list and median survival post-transplantation among patients with IPF is estimated to be 4.5 years.

Post-transplant survival is lower for patients with IPF than for patients with other pre-transplant conditions, with a 5-year survival rate of about 50% internationally (*17*). According to data from the International Society for Heart and Lung Transplantation (ISHLT) on lung transplants received by patients with IPF, bilateral lung transplantation has been increasingly performed at most facilities because it provided a better long-term survival than single lung transplantation (*18*). A pooled survival analysis of three observational studies revealed no differences between patients who received single versus bilateral lung transplantation. The improved survival of bilateral lung transplantation over single lung transplantation may be the result of selection bias (*1*).

In East Asia, a shortage of lung donors is a key problem that has yet to be resolved because of the difficulty in accepting the concept of brain death and strict laws.

A systematic review in Japan reported that lung transplantation was performed in 464 patients at 9 lung transplant centers in Japan between 1998 and 2015. Cadaveric lung transplantation was performed in 283 patients (61%) and living-donor lobar lung transplantation was performed in 181 patients (39%). The upper age limit in Japan is stricter due to the severe shortage of cadaveric donors. Candidates should be under the age of 55 for bilateral lung transplantation and under the age of 60 for single lung transplantation when registered with the Japan Organ Transplant Network waiting list. Single lung transplantation has been chosen more often than bilateral lung transplantation to maximize the number of transplants by sharing scarce donors, with a donor/recipient ratio approaching 80%. Over the past several years, living-donor lobar lung transplantation has been performed extensively as a life-saving procedure for critically ill patients in Japan because of the long average waiting time, which is more than 800 days for a cadaveric lung. There was no significant difference in survival between patients who underwent single lung transplantation and those who underwent bilateral lung transplantation (*19*).

A retrospective review of a total lung transplant database from a medical facility in China indicated that IPF accounts for 47% of all lung transplants. Single lung transplantation accounted for 72% of transplants. The proportion of bilateral lung transplants is consistently low (less than 30% of all procedures), and this proportion is much lower than that noted in the ISHLT Registry (*20*).

Based on evidence of favorable long-term survival in patients with IPF who have received a lung transplant, current statements recommend that patients with progressive IPF and limited diffusion capacity should be evaluated early for lung transplantation (*1*). International recommendations specify that transplantation should be considered in patients younger than age 65 if their DLCO is less than 39% predicted and FVC has decreased by more than 10% over 6 months of follow-up (*21*).

6. Complications and comorbidities

6.1. *Acute exacerbation*

Acute exacerbation of IPF (AE-IPF) is a life-threatening event with no identified cause. In a large retrospective study, the 1-year and 3-year incidence of AE-IPF was as high as 14.2% and 20.7%, respectively. The presence of AE-IPF results in a poor prognosis, with mortality exceeding 60% during hospitalization and 90% within 6 months of discharge (*22*). A study in Japan reported that acute exacerbation is the most frequent cause of death in up to 40% of patients with IPF (*8*).

AE-IPF is characterized by acute (less than 30 days) worsening of dyspnea with new opacities, and ground-glass opacities in particular, on HRCT after ruling out specific causes such as infection, pulmonary embolism, and left heart failure. High-dose steroid treatment is recommended for AE-IPF, but evidence of its efficacy is lacking. Bronchoalveolar lavage may be warranted to rule out pulmonary infection before starting steroid therapy if the patient's condition allows it. Antibiotic treatment depends on the clinical status of the patient or the results of a bronchoalveolar lavage. Mechanical ventilation may be needed in spite of the difficulty of weaning from patients (*1,3*).

6.2. *Pulmonary hypertension*

Pulmonary hypertension is present in less than 10% of patients with IPF at the time of diagnosis and in 30-45% during evaluation prior to lung transplantation. Pulmonary hypertension is associated with increased mortality, dyspnea and hypoxemia, decreased exercise capacity and DLCO, and a risk of acute exacerbation. Doppler echocardiography is the first-line noninvasive examination to diagnose pulmonary hypertension, but it has low positive and negative predictive values. Whether right heart catheterization is indicated for a patient with IPF patient should be decided at specialized facilities (*3*). Currently, there is no specific treatment recommended for pulmonary hypertension in patients with IPF. Further evidence is needed to make a clinical decision (*2*).

6.3. *Gastro-esophageal reflux*

Abnormal gastroesophageal reflux (GER), either symptomatic or asymptomatic, has been noted in more than 90% of patients with IPF (*23*). GER is a risk factor for aspiration and microaspiration, which may play a significant role in the pathogenesis of IPF (*24*). Antiacid treatments such as proton pump inhibitors (PPIs) or histamine-2 blocker receptor antagonists (H2RAs) may decrease the risk of microaspiration-associated lung injury or damage. Two retrospective studies have indicated that pulmonary function and oxygen requirements stabilized with medical and surgical

management of GER (*25,26*). The updated Official ATS/ERS/JRS/ALAT Clinical Practice Guideline recommends regular antiacid treatment for patients with IPF (*2*).

6.4. *Lung cancer*

The risk of lung cancer increases in patients with IPF. Compared to the risk of lung cancer in the general population, patients with IPF have a 7-fold higher incidence of lung cancer (*27*). The prevalence of lung cancer in patients with IPF is between 4.4% and 9.8% (*28*), and a retrospective study reported that the 10-year risk of developing lung cancer was 55% (*29*). A prospective study of complications among patients with IPF in Japan reported that the prevalence of lung cancer was 3.1% (*30*). The physician in charge of follow-up should be made aware of the frequent occurrence of lung cancer in patients definitively diagnosed with IPF (*3*). Management of lung cancer including surgical resection, radiation therapy, and chemotherapy is hampered by IPF and by the risk of acute respiratory failure and/or acute exacerbation associated with treatment of cancer.

7. Pulmonary rehabilitation and palliative treatment

Pulmonary rehabilitation consists of aerobic conditioning, strength and flexibility training, educational lectures, nutritional advice, and psychosocial support. In a study in Japan, a rehabilitation group had marked improvement in their 6-minute walking distance and their total score for health-related quality of life (*31*). Although only a few studies have investigated the effects of pulmonary rehabilitation on patients with IPF, pulmonary rehabilitation seems to be beneficial with respect to exercise capacity and quality of life in patients with IPF (*32*).

Dyspnea and coughing are the main symptoms of progressive disease, reducing the quality of life for patients and hampering treatment. Supplemental oxygen alleviates symptoms, and particularly dyspnea on exertion. Despite the lack of properly designed studies, oxygen supplementation is currently recommended by international guidelines (*1*). Treatment of coughing in IPF is problematic, and particularly so in the later stages of the disease. For the patients with a dry cough that is not alleviated by codeine, transient, low-dose oral corticosteroid therapy is an option for patients with IPF, but the efficacy and tolerance of this therapy should be monitored (*3*).

8. Prognosis

Currently, IPF remains an incurable disease with varying rates of progression and a poor prognosis. Several clinical phenotypes of IPF have been described, including slow progression in patients with a history of worsening dyspnea and/or a dry cough lasting for months to years and rapid progression (referred to accelerated IPF) characterized by shortened survival (Figure 2) (*33,34*). Indices such as symptoms, respiratory function, and imaging are used to evaluate the clinical progression and outcome of IPF. Studies are underway to determine the heterogeneity of the disease (*35*). A prognostic staging system named after the GAP score

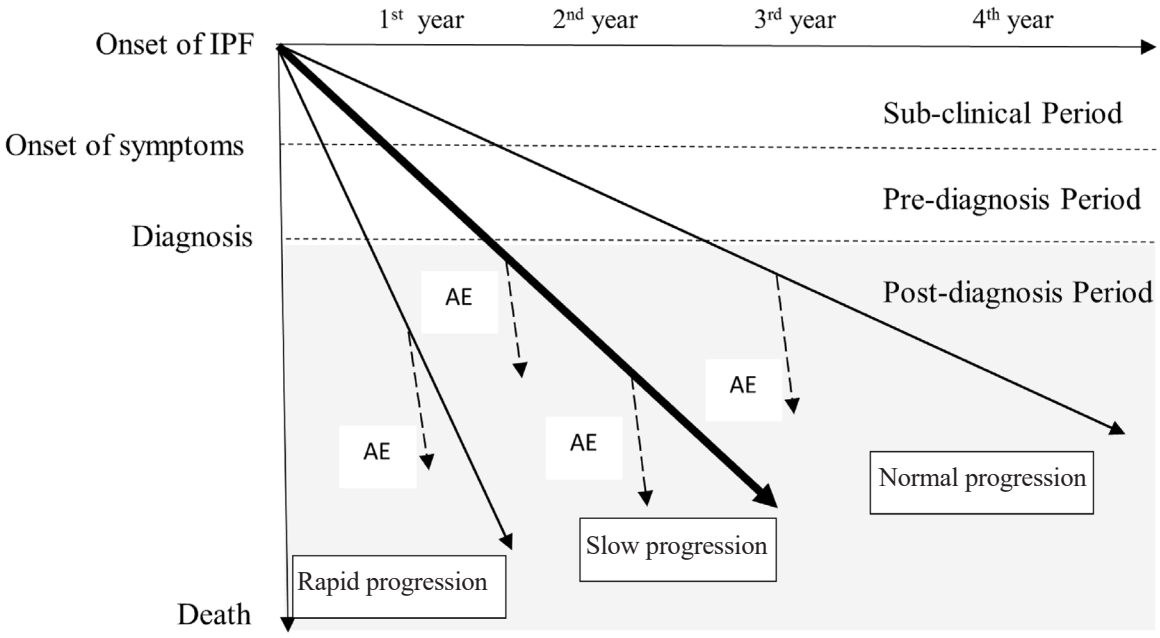

Figure 2. The disease course of idiopathic pulmonary fibrosis (IPF) varies. Predicting the exact phenotype in individuals is difficult. The disease progresses rapidly in some patients, slowly in some patients, and normally in other patients experience. Some patients may experience acute exacerbation (AE) of IPF in an unpredictable stage.

risk prediction model has been developed for IPF and it has been validated in three large, geographically distinct cohorts of patients (*36*). The GAP score risk prediction model is a feasible and useful marker that includes four factors: gender (G), age (A), and two lung physiologic (P) variables (FVC and DLCO). Improvement in pulmonary function as a result of pharmacologic and non-pharmacologic therapies is crucial to improving the prognosis for patients with IPF because gender and age are non-modifiable variables.

The clinical course of IPF is highly variable and unpredictable in individuals. Different patterns of IPF progression may determine survival time and the cause of death. In a Japanese study, a total of 328 patients (59.3% of those patients had IPF) died from various causes. Among patients with IPF, the most common cause of death was an acute exacerbation of the disease (40% of events), followed by lung cancer (11%), pneumonia (7%), and cardiovascular diseases (3%). The percentage of patients with acute exacerbation was higher than that in American studies, which may suggest an ethnic difference between East Asian and Western populations (*8*).

9. Conclusion

IPF is a rare and intractable respiratory disease with a poor prognosis. The incidence of that disease, the rate of lung transplants, and mortality rates and causes of death differ between East Asians and Westerners. These differences may be the result of ethnic and geographical factors. Regional studies and trials should be conducted to accurately diagnose and treat IPF. Since the East Asian population is rapidly aging, the prevalence and incidence of IPF will presumably increase in the near future. Thus, more effective therapies need to be developed to improve survival from this devastating disease. This development must come from advanced and collaborative clinical and basic scientific research. Although East Asians accounts for 1/4 of the world's population, patients with IPF are less likely to receive a lung transplant due to national shortages in lung donors. As in European countries, coordination and collaboration among networks should be promoted in East Asia to facilitate more lung transplants.

Acknowledgements

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References

1. Raghu G, Collard HR, Egan JJ, *et al*. An official ATS/ERS/JRS/ALAT statement: Idiopathic pulmonary fibrosis: Evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011; 183:788-824.
2. Raghu G, Rochwerg B, Zhang Y, *et al*. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: Treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. Am J Respir Crit Care Med. 2015; 192:e3-e19.
3. Cottin V, Crestani B, Valeyre D, *et al*. Diagnosis and management of idiopathic pulmonary fibrosis: French practical guidelines. Eur Respir Rev. 2014; 23:193-214.
4. Caminati A, Madotto F, Cesana G, Conti S, Harari S. Epidemiological studies in idiopathic pulmonary fibrosis: Pitfalls in methodologies and data interpretation. Eur Respir Rev. 2015; 24:436-444.
5. Nalysnyk L, Cid-Ruzafa J, Rotella P, Esser D. Incidence and prevalence of idiopathic pulmonary fibrosis: review of the literature. Eur Respir Rev. 2012; 21:355-361.
6. Hutchinson JP, McKeever TM, Fogarty AW, Navaratnam V, Hubbard RB. Increasing global mortality from idiopathic pulmonary fibrosis in the twenty-first century. Ann Am Thorac Soc. 2014; 11:1176-1185.
7. Gjonbrataj J, Choi WI, Bahn YE, Rho BH, Lee JJ, Lee CW. Incidence of idiopathic pulmonary fibrosis in Korea based on the 2011 ATS/ERS/JRS/ALAT statement. Int J Tuberc Lung Dis. 2015; 19:742-746.
8. Natsuizaka M, Chiba H, Kuronuma K, Otsuka M, Kudo K, Mori M, Bando M, Sugiyama Y, Takahashi H. Epidemiologic survey of Japanese patients with idiopathic pulmonary fibrosis and investigation of ethnic differences. Am J Respir Crit Care Med. 2014; 190:773-779.
9. Spagnolo P, Sverzellati N, Rossi G, Cavazza A, Tzouvelekis A, Crestani B, Vancheri C. Idiopathic pulmonary fibrosis: An update. Ann Med. 2015; 47:15-27.
10. Ohshimo S, Bonella F, Cui A, Beume M, Kohno N, Guzman J, Costabel U. Significance of bronchoalveolar lavage for the diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2009; 179:1043-1047.
11. Funke M, Geiser T. Idiopathic pulmonary fibrosis: The turning point is now! Swiss Med Wkly. 2015; 145: w14139.
12. Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, Suga M, Taguchi Y, Takahashi H, Nakata K, Sato A, Takeuchi M, Raghu G, Kudoh S, Nukiwa T; Pirfenidone Clinical Study Group in Japan. Pirfenidone in idiopathic pulmonary fibrosis. Eur Respir J. 2010; 35:821-829.
13. Richeldi L, du Bois RM, Raghu G, *et al*. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. N Engl J Med. 2014; 370:2071-2082.
14. Wuyts WA, Kolb M, Stowasser S, Stansen W, Huggins JT, Raghu G. First data on efficacy and safety of nintedanib in patients with idiopathic pulmonary fibrosis and forced vital capacity of ≤ 50 % of predicted value. Lung. 2016; 194:739-743.
15. Loveman E, Copley VR, Scott DA, Colquitt JL, Clegg AJ, O'Reilly KM. Comparing new treatments for idiopathic pulmonary fibrosis--A network meta-analysis. BMC Pulm Med. 2015; 15:37.
16. Raghu G. Idiopathic pulmonary fibrosis: Guidelines for diagnosis and clinical management have advanced from consensus-based in 2000 to evidence-based in 2011. Eur Respir J. 2011; 37:743-746.
17. Kistler KD, Nalysnyk L, Rotella P, Esser D. Lung transplantation in idiopathic pulmonary fibrosis: A systematic review of the literature. BMC Pulm Med.

2014; 14:139.
18. Yusen RD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Goldfarb SB, Levvey BJ, Lund LH, Meiser B, Rossano JW, Stehlik J. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Lung and Heart-Lung Transplantation Report--2015; Focus Theme: Early Graft Failure. J Heart Lung Transplant. 2015; 34:1264-1277.
19. Date H. Current status and problems of lung transplantation in Japan. J Thorac Dis. 2016; 8:S631-S636.
20. Mao W, Chen J, Zheng M, Wu B, Zhu Y. Initial experience of lung transplantation at a single center in China. Transplant Proc. 2013; 45:349-355.
21. Orens JB, Estenne M, Arcasoy S, *et al*. International guidelines for the selection of lung transplant candidates: 2006 update--A consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2006; 25:745-755.
22. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: Incidence, risk factors and outcome. Eur Respir J. 2011; 37:356-363.
23. Tobin RW, Pope CE, 2nd, Pellegrini CA, Emond MJ, Sillery J, Raghu G. Increased prevalence of gastroesophageal reflux in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 1998; 158:1804-1808.
24. Raghu G, Meyer KC. Silent gastro-oesophageal reflux and microaspiration in IPF: Mounting evidence for anti-reflux therapy? Eur Respir J. 2012; 39:242-245.
25. Lee JS, Ryu JH, Elicker BM, Lydell CP, Jones KD, Wolters PJ, King TE Jr, Collard HR. Gastroesophageal reflux therapy is associated with longer survival in patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011; 184:1390-1394.
26. Lee JS, Collard HR, Anstrom KJ, Martinez FJ, Noth I, Roberts RS, Yow E, Raghu G; IPFnet Investigators. Anti-acid treatment and disease progression in idiopathic pulmonary fibrosis: An analysis of data from three randomised controlled trials. Lancet Respir Med. 2013; 1:369-376.
27. Hubbard R, Venn A, Lewis S, Britton J. Lung cancer and cryptogenic fibrosing alveolitis. A population-based cohort study. Am J Respir Crit Care Med. 2000; 161:5-8.
28. Harris JM, Johnston ID, Rudd R, Taylor AJ, Cullinan P. Cryptogenic fibrosing alveolitis and lung cancer: The BTS study. Thorax. 2010; 65:70-76.
29. Ozawa Y, Suda T, Naito T, Enomoto N, Hashimoto D, Fujisawa T, Nakamura Y, Inui N, Nakamura H, Chida K. Cumulative incidence of and predictive factors for lung cancer in IPF. Respirology. 2009; 14:723-728.
30. Bando M, Sugiyama Y, Azuma A, Ebina M, Taniguchi H, Taguchi Y, Takahashi H, Homma S, Nukiwa T, Kudoh S. A prospective survey of idiopathic interstitial pneumonias in a web registry in Japan. Respir Investig. 2015; 53:51-59.
31. Nishiyama O, Kondoh Y, Kimura T, Kato K, Kataoka K, Ogawa T, Watanabe F, Arizono S, Nishimura K, Taniguchi H. Effects of pulmonary rehabilitation in patients with idiopathic pulmonary fibrosis. Respirology. 2008; 13:394-399.
32. Vainshelboim B, Oliveira J, Yehoshua L, Weiss I, Fox BD, Fruchter O, Kramer MR. Exercise training-based pulmonary rehabilitation program is clinically beneficial for idiopathic pulmonary fibrosis. Respiration. 2014; 88:378-388.
33. Selman M, Carrillo G, Estrada A, Mejia M, Becerril C, Cisneros J, Gaxiola M, Pérez-Padilla R, Navarro C, Richards T, Dauber J, King TE Jr, Pardo A, Kaminski N. Accelerated variant of idiopathic pulmonary fibrosis: Clinical behavior and gene expression pattern. PloS One. 2007; 2:e482.
34. Ley B, Collard HR, King TE Jr. Clinical course and prediction of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2011; 183:431-440.
35. Fujimoto H, Kobayashi T, Azuma A. Idiopathic pulmonary fibrosis: Treatment and prognosis. Clin Med Insights Circ Respir Pulm Med. 2016; 9(Suppl 1):179-185.
36. Ley B, Ryerson CJ, Vittinghoff E, Ryu JH, Tomassetti S, Lee JS, Poletti V, Buccioli M, Elicker BM, Jones KD, King TE Jr, Collard HR. A multidimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med. 2012; 156:684-691.

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Review

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Aortic intramural hemorrhage: A distinct disease entity with mystery

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Summary **Aortic intramural hemorrhage (IMH) is one of the disease processes that comprise the spectrum of acute aortic syndrome (AAS) with clinical manifestations and a mortality rate similar to those of classic aortic dissection (AD). However, IMH should be considered as a distinct disease entity rather than a precursor to classic dissection because of differences in their pathology, etiology, natural history, and imaging findings. Multidetector computed tomography (CT) is recommended as the first-line diagnostic imaging modality for IMH, but transesophageal echocardiography (TEE) and magnetic resonance imaging (MRI) are also helpful. There is still debate over the appropriate treatment of IMH. Medical treatment of type B IMH appears effective and safe, while surgical treatment is recommended for type A IMH. Thoracic endovascular aortic repair (TEVAR) is a promising treatment for selected patients, and more clinical evidence needs to be assembled.**

Keywords: Aortic intramural hemorrhage, pathology, imaging, treatment

1. Introduction

Aortic intramural hemorrhage (IMH), characterized by the absence of an intimal tear and thus of flow communication between the true and false lumen, is one of the disease processes that comprise the spectrum of acute aortic syndrome (AAS). IMH was first described by Krukenberg in 1920 as "aortic dissection (AD) without an intimal tear" (*1*). IMH is a hemorrhage into the medial layer and can propagate longitudinally or circumferentially, but it does not rupture into the lumen. IMH presents with symptoms similar to those of classic (typical) AD and may have similar morbidity and mortality rates (*2,3*), but the pathologic differences between IMH and AD are well established (Figure 1). IMH has a lower morbidity, but some aspects of IMH are still a mystery.

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2. IMH: A precursor to classic dissection or a distinct disease entity?

It's reported that IMH progressed to AD in 12-47% patients (*4*). IMH is associated with a clinical profile and prognosis similar to those of classic dissection, and the same treatment strategy was recommended for both IMH and AD (*5,6*). IMH was assumed to be a hyperacute stage of AD and a precursor to classic dissection, but in fact the pathology, etiology, and natural history of IMH and AD differ considerably (Table 1).

The location of an IMH within the media is quite different from AD. The distinguishing feature of IMH is its exterior location within the media near the adventitia, whereas AD extends into the media in closer proximity to the intima (*7*). Data from the International Registry of Acute Aortic Dissection (IRAD) have revealed that IMH tends to involve the descending aorta (42% type A IMH and 58% type B), in contrast to classic AD, which principally involves the ascending aorta (73% type A AD and 27% type B) (*8*). A similar conclusion was reached by Song *et al*. (*9*). The average age of patients presenting with intramural hematoma ranged from 58 to 71 years (median age: 68 years) (*2,8,10-15*), which is much older than the average of patients with classic AD (median age: 61 years) (*16*). The prevalence

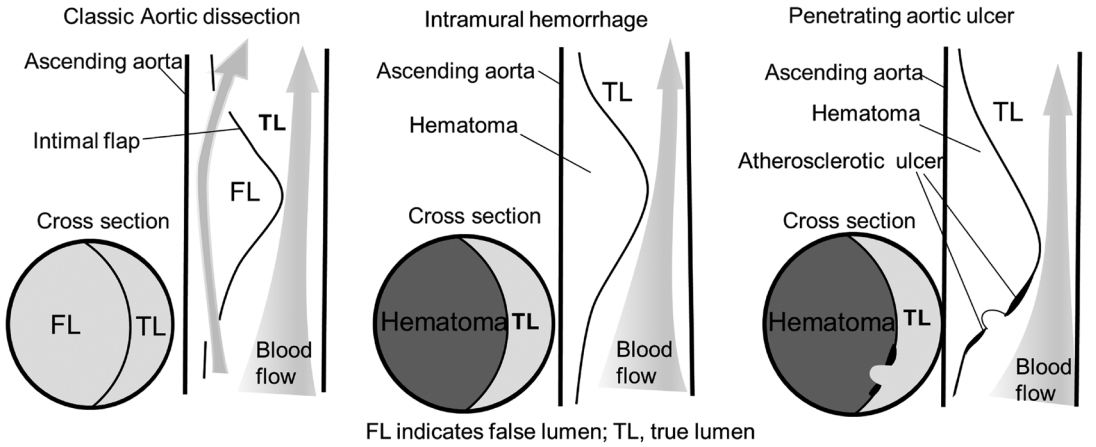

Figure 1. Pathogenesis of acute aortic syndromes.

Table 1. Differences between aortic IMH and AD

Items	IMH	AD
Pathology		
Intimal flap	absent	present
Communication between the true and false lumen	absent	present
Location within the media	exterior	interior
Descending aorta involvement	more	less
Epidemiology		
Mean age (y)	68	61
Prevalence of Marfan syndrome	lower	higher
Clinical manifestation		
Aortic regurgitation	less	more
Pulse deficits	less	more
Pericardial effusions	more	less
Cardiac tamponade	more	less
Natural history		
Complete resorption	high rate	very low rate

AD, aortic dissection; IMH, intramural hemorrhage.

of Marfan syndrome is significantly lower in patients with intramural hematoma (*9*). Symptoms of chest pain, frequently severe and abrupt, are noted in most patients with IMH or AD. Unlike patients with classic AD, patients with type A IMH are less likely to present with aortic regurgitation, and patients with IMH are less likely to present with pulse deficits (*8*). Pericardial effusions or cardiac tamponade are more likely to occur in patients with type A IMH than in those with type A AD (21% *vs*. 9%) (*3,17*). There are marked differences in the natural remodeling process in AD and IMH when medically treated (*18,19*). Without surgical intervention, the most common and expected remodeling process in AD is a persistent double-channel aorta with the frequent development of aortic aneurysm as a result of uninterrupted flow communication between the true and false lumen through an intimal tear. In IMH, a high rate of complete resorption of the initial aortic pathology is observed in imaging follow-up, which is unimaginable in AD.

All of this evidence supports the conjecture that IMH is a distinct disease entity rather than a precursor to classic dissection.

3. Epidemiology: Does IMH vary geographically and among ethnic groups?

AAS is rare, occurring in 2 to 3.5 per 100,000 person-years, and IMH varies widely as a form of AAS (*20-22*). In early studies, the incidence of IMH as a form of AAS appeared to differ geographically. South Korean and Japanese studies reported that its incidence generally ranged from 22.9% to 53% (*3,18,23,24*), whereas Western studies reported a lower incidence of 5.7-10% (*8,10,21*). This discrepancy may be due to ethnic or geographical differences. In addition, the lack of a uniform definition of IMH, as well as different methods of evaluation and treatment algorithms, may also account for this discrepancy. Moreover, most studies on IMH from Japan and South Korea are based on data from a few centers rather than an overall nationwide database, so the potential for publication bias should

also be seriously considered. However, recent studies from China and South Korea have cast some doubt on this conclusion. Data from 1,812 patients at 19 large hospitals in China indicated that the incidence of IMH was 4.7% (*25*), while Cho *et al*. in South Korea reported an incidence of 10.7% (*26*). These rates seem to roughly coincide with the reported incidence in the West. Thus, there may be no geographic or ethnic variability in IMH.

4. Pathology: Due to rupture of the vasa vasorum or a micro intimal tear?

Early studies did not find a typical intimal tear or flow communication between the true and false lumen through the tear in patients with IMH, so the hemorrhage may be the result of a rupture of the vasa vasorum, a contention which is substantiated by computed tomography (CT) and transesophageal echocardiography (TEE) findings (*27,28*). However, there is little direct clinical or experimental evidence to substantiate this contention. Crescentic aortic wall thickening mimicking an intramural hemorrhage has been reported due to iatrogenic injury such as coronary angioplasty (*29*) or insertion of a balloon pump (*30*). Recent studies using more sensitive imaging modalities have identified intimal tears in many cases of IMH (*31-34*). Moreover, Park *et al*. (*33*) and Uchida *et al*. (*31*) found a small intimal communication at the time of surgery.

All of these findings indicate that IMH may originate from small intimal tears rather than a rupture of the vasa vasorum. Thus, the ACC/AHA guideline states that "When the term IMH is used strictly, no intimal defect such as a tear or an ulcer is present. But in practice, the term is used loosely to mean a thrombosed false lumen regardless of a small defect" (*35*). In Japan, IMH is referred to as "thrombosed-type aortic dissection" (*31*). As imaging sensitivity improves, the rate at which very small intimal tears are detected will continue to increase. If this occurs, the incidence of "true IMH" as has been reported may decline. In actual clinical practice, IMH should mainly be diagnosed based on imaging findings if surgical inspection or autopsy cannot be performed.

5. Imaging: Which modality to choose?

IMH is diagnosed based on imaging. Contemporary imaging modalities, such as magnetic resonance imaging (MRI), CT, and TEE are used to diagnose IMH. Each has its advantages and limitations in clinical practice.

5.1. *CT*

Because of its widespread availability, high spatial resolution, rapid examination time, and complete anatomic evaluation of the thoracoabdominal aorta and major branch vessels, CT scan is the most common diagnostic modality with which to diagnose IMH (*22,36*), with a negative predictive value of approximately 100% (*37*). The hallmark non-contrast multidetector CT (MDCT) finding of IMH is eccentric crescent-shaped thickening of the aortic wall with high attenuation (60–80 HU) (indicative of blood products), which extends in a longitudinal fashion (*5,38-40*). The thickened wall is typically > 7 mm and doesn't enhance following contrast medium injection. IMH is classified using the Stanford classification. IMH is Stanford type A if eccentric (crescent-shaped) to circumferential thickening and brightness of the aortic wall are observed in the ascending aorta on unenhanced CT images, and all other forms are classified as Stanford type B (*8*). MDCT with a systematically delayed phase and millimeter-thin slices can increase the rate at which intimal anomalies are detected (*41*). Some CT imaging finding may be related to the poor prognosis of IMH, such as intimal erosion measuring > 10 mm (*41*), ulcer-like projections (ULPs; also referred to as intimal erosions) (*34,41-45*), an aortic diameter (with different cutoff, ≥ 50 mm (*46*), ≥ 48 mm (*47*), ≥ 41 mm (*48*)), and an intramural hematoma thickness > 10 to 11 mm (*4,41,44,45,49*).

5.2. *TEE*

2D Transthoracic echocardiography (TTE) is not recommended for diagnosis of IMH (*22*); IMH can be detected and monitored with TEE but not with aortography (*27,50*). TEE has a sensitivity of 96.5-100% and a specificity of 91-98.5% in the diagnosis of IMH(*12,16*). TEE is superior to other imaging modalities because it allows direct observation of the aortic intima, and flow communication can be depicted with the Doppler technique. TEE is widely available, portable, convenient, fast, and it is exceptional at depicting pericardial effusion, the presence, degree, and mechanism(s) of aortic regurgitation, and LV function, which is important for unstable patients. Moreover, TEE can also be used in the operating room, facilitating early detection of IMH as well as possible complications (*51*). One characteristic TEE finding of IMH is the presence of an "echo-free space or echo-lucent area" within the thickened aortic wall (*22*). There is no Doppler evidence of communication between the hematoma and the true lumen, but there may be some color Doppler flow within the hematoma (*22,52*). Although TEE requires esophageal intubation, images are acquired at the bedside and can immediately be interpreted. In a limited number of patients, contrast injection helps to reveal direct flow communication through the microtear into the echo-free space (*9*). The main disadvantage of TEE is the "blind spot" of

Table 2. Reported outcomes for treatment of intramural hemorrhage (IMH)

Items	Medical				Open Surgery				TEVAR			
	Patient NO.	Mortality 1 (%)†	Mortality 2 (%)††	*Ref.*	Patient NO.	Mortality 1 (%)†	Mortality 2 (%)††	*Ref.*	Patient NO.	Mortality 1 (%)†	Mortality 2 (%)††	*Ref.*
Country												
China	13-25	0-32	0-8	(*13,14,69*)	9	22.2	/	(*69*)	6-23	0	0	(*13,14*)
Japan	17-50	4-23.5	14	(*23,46*)	10-165	0.9-16.7	0.9-25.1	(*7,11,23,31, 46,47,70*)	/	/	/	/
South Korea	18-150	0-7.1	0-12.7	(*3,18,19,26, 48,71,72*)	4-16	0-33.3	0-6.7	(*3,18,26,72*)	/	/	/	/
IRAD	40-90	3.8-7.8	10	(*8,21*)	14-59	23.7-42.9	27.1	(*8,21*)	2-5	0	0	(*8,21*)
Italy	94	6.4		(*73*)	5	0	/	(*73*)	7	0	/	(*73*)
France	/	/	/	/	/	/	/	/	15-15	0	0-25	(*74,75*)
Sweden	/	/	/	/	/	/	/	/	7	28.5	/	(*76*)
Germany MR	/	/	/	/	/	/	/	/	18	5.6	/	(*75*)
Austria	/	/	/	/	/	/	/	/	8	0	0	(*32*)
Canada	/	/	/	/	/	/	/	/	4-10	0	25	(*77*)
USA	/	/	/	/	/	/	/	/	6-44	4.5-16.7	/	(*42,60*)
Argentina	21	19	0	(*2*)	6	17	/	(*2*)				
Classification												
Type A/B	13-150	0-23.5	/	(*8,14,21,23, 26,46,71,72*)	4-34	0-6.3	6.3	(*23,26,46*)	6-44	0-5.6	0	(*14,60, 74,75*)
Type A	9-85	4.3-40	11.8-40	(*3,8,18,21,23, 69,71,72*)	2-165	0-42.9	0-27.1	(*3,7,8,11,18, 21,23,31,47, 69,70,72*)	4-8	0	0-25	(*32,74, 77*)
Type B	6-107	0-19	0-11.7	(*2,13,19,21,23, 48,71-73*)	1-32	0-20	0-40	(*2,8,23,72, 73*)	2-23	0-28.5	0	(*8,13,21, 42,73-76, 78*)

† Mortality in hospital; †† Mortality during follow-up. Germany MR: Germany Multicenter Registry.

the distal ascending aorta and proximal arch because of interposition of the air-filled trachea and the main bronchus (*22,53*). A further disadvantage is that TEE may also cause upper gastrointestinal injuries (*54*).

5.3. *MRI*

MRI has a sensitivity of approximately 100% in the diagnosis of IMH, and MRI has the advantage of a very high signal to-noise ratio and a high contrast resolution with the ability to characterize the vascular wall far better than CT or TEE. However, MRI is less frequently used because of its relative lack of availability and longer scan time in comparison to CT (*55*). MRI is able to diagnose intramural bleeding in the acute phase (< 7days) because the hematoma shows an isointense signal (black) on T1-weighted images (blood appears black) and a hyperintense signal (bright white) on T2-weighted images (blood appears white) (*22*). In contrast, a chronic IMH (> 7 days) on T1-weighted images appears hyperintense (bright) as the hematoma evolves and degenerates, whereas a chronic IMH on T2-weighted images is less intense than an acute IMH (*55,56*). MRI can also be useful in follow-up. Ma *et al*. (57) reported a 6-year follow-up of a spontaneous IMH with a cardiovascular magnetic resonance examination.

5.4. *Recommendation*

In conclusion, CT is recommended as the first-line diagnostic imaging modality for IMH, particularly in the emergency room. TEE can be available at bedside or in the operating room. When findings on CT or TEE are equivocal, MRI may prove helpful.

6. Treatment: Medical treatment or surgical intervention, open surgery or thoracic endovascular aortic repair (TEVAR)?

IMH is a potentially lethal condition with a mortality of 24% (36% with type A IMH and 12% with type B) (*58*). Studies have reported that the mortality of IMH is similar to that of classic AD (*8,18,21*). Twenty years of experience in Japan indicated that the 30-day mortality rate was 6% with emergent open surgery and 4% with supportive medial therapy; the actuarial survival rate of all patients was 96.3% at 1 year, 94.3% at 5 years, and 89.5% at 10 years (*46*). Medical treatment may alleviate an IMH by decreasing the hematoma thickness (*59*). All patients with IMH should receive initial medical therapy to control pain and blood pressure (level I, grade C) (*36*). TEVAR is safe and effective in treating IMH and also contributes to ideal remodeling of the affected aorta (*60,61*).

Overall consensus regarding a treatment strategy has yet to be reached. Medical therapy is recommended more often in Asia (*62*) , while surgical treatment is recommended more often in Europe and the US (*35,36*). The clinical outcomes for treatment of IMH are shown in Table 2, and differences due to geography and

Stanford classification are evident.

The management of IMH depends on the clinical status of the patient and Stanford classification. Management will be difficult if one of the following aspects is present: persistence of chest pain despite medical treatment, hemodynamic instability, signs of an aortic rupture (periaortic hemorrhage), depth of ULP > 10 mm, maximum aortic diameter > 55 mm, or a rapid increase in the aortic diameter during hospitalization (*63*).

6.1. *Type B*

The mortality rate for initial medical treatment of type B IMH is 0-19% in hospital and 0-11.7% during follow-up (Table 2). The mean mortality rate related to aortic events was 5.4% within 3 years (*63*). Patients diagnosed with type B IMH were initially treated medically with beta blockers and other antihypertensive therapies. A study has reported a mortality rate of 8% for medical treatment and that type B IMH has a better prognosis without surgical intervention (*58*). Open surgical treatment of type B IMH results in a mortality rate of 0-20% in hospital and a mortality rate of 0-40% during follow-up (Table 2). The mean mortality rate is 23.2% within 3 years (*63*). TEVAR is an effective and promising way to manage IMH because it is relatively less invasive and causes fewer complication (*13*). A study on TEVAR has noted a mortality rate of 0-28.5% in hospital and a mean mortality rate of 7.1% within 3 years (*63*). Although TEVAR causes complication similar to those noted in other aortic diseases, endoleaks might occur more frequently when using TEVAR to treat an acute IMH (*64,65*).

Initial medical treatment is recommended for type B IMH with no complications (*35,36*). If comorbidities are present with type B IMH, the European Society of Cardiology (ESC) (*36*) and Mussa *et al*. (*16*) recommend TEVAR, but the 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM Guidelines recommend surgery without distinguishing between open surgery and TEVAR (*35*).

6.2. *Type A*

The optimal initial treatment strategy for type A IMH may still be individualized. A study has reported a high mortality rate for type A IMH in hospital (26.6 %) and a higher rate for medical treatment (40%) than for surgical treatment (24.1%) (*8*). A study in Japan indicated that 47.2% (17/36) of patients with type A IMH who received medical treatment also needed surgery. Urgent surgical repair is not necessary for all patients with type A IMH to achieve favorable surgical outcomes with careful follow-up using imaging (*11*). The mortality rate for initial medical treatment of type A IMH ranges from 4.3% to 40% in hospital and 11.8-40% during follow-up. Initial medical treatment and timed surgical therapy seem to be associated with a higher early mortality rate in patients with type A IMH, although the mortality rates did not differ significantly (14.4% *vs*. 10.1%, $p = 0.36$) (*66*). However, another study in Japan reported that medical treatment and timed surgical therapy resulted in no aortic dissection-related mortality and no aortic dissection-related events in patients who underwent surgical repair with a mean follow-up of 24.3 months (*67*). This suggests that type A IMH is more likely to progress, regardless of the diameter of the aorta, and, thus that prompt surgical repair should be performed (*6,68*). However, Uzuka *et al*. (*67*) recommended that emergency or urgent surgery not be considered for a hemodynamically stable patient unless the diameter of the ascending aortic was ≥ 50 mm or the thickness of the thrombosed false lumen was ≥ 10 mm. The mortality rate for open surgical treatment of type A IMH was 0-42.9% in hospital and 0-27.1% during follow-up (Table 2). The clinical use of TEVAR in selected patients with type A IMH resulted in good clinical outcomes (Table 2). This procedure offers low morbidity and mortality rates, representing a feasible therapeutic option especially for elderly patients with comorbidities.

Open surgery is recommended for type A IMH with comorbidities (*16,35,36*). Management of type A IMH with no comorbidities has yet to be determined, although Mussa *et al*. (*16*) recommended medical treatment.

7. Conclusion

IMH is one of the disease processes that comprise the spectrum of AAS with clinical manifestations and a mortality rate similar to those of classic AD. However, IMH should be considered as a distinct disease entity rather than a precursor to classic dissection. Imaging and surgical findings have revealed that a micro intimal tear may be the cause of IMH. IMH appeared to vary geographically and among ethnic groups, but recent studies have called that conclusion into question. MDCT is recommended as the first-line diagnostic imaging modality for IMH, while TEE and MRI are also helpful. There is still debate over the treatment for IMH. Medical treatment seems to be effective and safe for type B IMH, while surgical treatment is recommended for type A IMH. TEVAR is a promising treatment for selected patients, and more clinical evidence needs to be assembled.

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References

1. Krukenberg E. Beiträge zur Frage des Aneurysma dissecans. Beitr Patho Anat Allg Pathol. 1920; 67:329-351.
2. Falconi M, Oberti P, Krauss J, Domenech A, Cesáreo V,, Bracco D, Pizarro R. Different clinical features of aortic intramural hematoma versus dissection involving the descending thoracic aorta. Echocardiography. 2005; 22:629-635.
3. Song JK, Yim JH, Ahn JM, Kim DH, Kang JW, Lee TY, Song JM, Choo SJ, Kang DH, Chung CH, Lee JW, Lim TH. Outcomes of patients with acute type a aortic intramural hematoma. Circulation. 2009; 120:2046-2052.
4. Choi SH, Choi SJ, Kim JH, Bae SJ, Lee JS, Song KS, Lim TH. Useful CT findings for predicting the progression of aortic intramural hematoma to overt aortic dissection. J Comput Assist Tomogr. 2001; 25:295-299.
5. Nienaber CA, von Kodolitsch Y, Petersen B, Loose R, Helmchen U, Haverich A, Spielmann RP. Intramural hemorrhage of the thoracic aorta. Diagnostic and therapeutic implications. Circulation. 1995; 92:1465-1472.
6. von Kodolitsch Y, Csösz SK, Koschyk DH, Schalwat I, Loose R, Karck M, Dieckmann C, Fattori R, Haverich A, Berger J, Meinertz T, Nienaber CA. Intramural hematoma of the aorta: Predictors of progression to dissection and rupture. Circulation. 2003; 107:1158-1163.
7. Uchida K, Imoto K, Takahashi M, Suzuki S, Isoda S, Sugiyama M, Kondo J, Takanashi Y. Pathologic characteristics and surgical indications of superacute type A intramural hematoma. Ann Thorac Surg. 2005; 79:1518-1521.
8. Harris KM, Braverman AC, Eagle KA, *et al*. Acute aortic intramural hematoma: An analysis from the International Registry of Acute Aortic Dissection. Circulation. 2012; 126:S91-S96.
9. Song JK. Aortic intramural hematoma: Aspects of pathogenesis 2011. Herz. 2011; 36:488-497.
10. Keren A, Kim CB, Hu BS, Eyngorina I, Billingham ME, Mitchell RS, Miller DC, Popp RL, Schnittger I. Accuracy of biplane and multiplane transesophageal echocardiography in diagnosis of typical acute aortic dissection and intramural hematoma. J Am Coll Cardiol. 1996; 28:627-636.
11. Motoyoshi N, Moizumi Y, Komatsu T, Tabayashi K. Intramural hematoma and dissection involving ascending aorta: The clinical features and prognosis. Eur J Cardiothorac Surg. 2003; 24:237-242; discussion 242.
12. Kang DH, Song JK, Song MG, Lee IS, Song H, Lee JW, Park SW, Kim YH, Lim TH, Park SJ. Clinical and echocardiographic outcomes of aortic intramural hemorrhage compared with acute aortic dissection. Am J Cardiol. 1998; 81:202-206.
13. Li DL, Zhang HK, Cai YY, Jin W, Chen XD, Tian L, Li M. Acute type B aortic intramural hematoma: Treatment strategy and the role of endovascular repair. J Endovasc Ther. 2010; 17:617-621.
14. Zhang G, Feng Q, Zheng D, Ma L, Li R, Jiang J, Ni Y. Early aggressive medical treatment associated with selective prophylactic aortic stent-grafting for aortic intramural hematoma. Thorac Cardiovasc Surg. 2011; 59:342-348.
15. Nienaber CA, Sievers HH. Intramural hematoma in acute aortic syndrome: More than one variant of dissection? Circulation. 2002; 106:284-285.
16. Mussa FF, Horton JD, Moridzadeh R, Nicholson J, Trimarchi S, Eagle KA. Acute aortic dissection and intramural hematoma: A systematic review. JAMA. 2016; 316:754-763.
17. Harris KM, Pelzel JM, Braverman AC. Letter by Harris *et al*. regarding article, "Outcomes of patients with acute type a aortic intramural hematoma". Circulation. 2010; 121:e456; author reply e457.
18. Song JK, Kim HS, Kang DH, Lim TH, Song MG, Park SW, Park SJ. Different clinical features of aortic intramural hematoma versus dissection involving the ascending aorta. J Am Coll Cardiol. 2001; 37:1604-1610.
19. Song JK, Kang DH, Lim TH, Song MG, Kim JJ, Park SW, Park SJ. Different remodeling of descending thoracic aorta after acute event in aortic intramural hemorrhage versus aortic dissection. Am J Cardiol. 1999; 83:937-941.
20. Nienaber CA, Powell JT. Management of acute aortic syndromes. Eur Heart J. 2012; 33:26-35b.
21. Evangelista A, Mukherjee D, Mehta RH, O'Gara PT, Fattori R, Cooper JV, Smith DE, Oh JK, Hutchison S, Sechtem U, Isselbacher EM, Nienaber CA, Pape LA, Eagle KA. Acute intramural hematoma of the aorta: A mystery in evolution. Circulation. 2005; 111:1063-1070.
22. Goldstein SA, Evangelista A, Abbara S, *et al*. Multimodality imaging of diseases of the thoracic aorta in adults: From the American society of echocardiography and the european association of cardiovascular imaging: Endorsed by the society of cardiovascular computed tomography and society for cardiovascular magnetic resonance. J Am Soc Echocardiogr. 2015; 28:119-182.
23. Shimizu H, Yoshino H, Udagawa H, Watanuki A, Yano K, Ide H, Sudo K, Ishikawa K. Prognosis of aortic intramural hemorrhage compared with classic aortic dissection. Am J Cardiol. 2000; 85:792-795, A10.
24. Yamada T, Tada S, Harada J. Aortic dissection without intimal rupture: Diagnosis with MR imaging and CT. Radiology. 1988; 168:347-352.
25. Li Y, Yang N, Duan W, Liu S, Yu S, Yi D. Acute aortic dissection in China. Am J Cardiol. 2012; 110:1056-1061.
26. Cho JR, Shin S, Kim JS, *et al*. Clinical characteristics of acute aortic syndrome in Korean patients: From the Korean multi-center registry of acute aortic syndrome. Korean Circ J. 2012; 42:528-537.
27. Mohr-Kahaly S, Erbel R, Kearney P, Puth M, Meyer J. Aortic intramural hemorrhage visualized by transesophageal echocardiography: Findings and prognostic implications. J Am Coll Cardiol. 1994; 23:658-664.
28. Gore I. Pathogenesis of dissecting aneurysm of the aorta. AMA Arch Pathol. 1952; 53:142-153.
29. Vilacosta I, de Dios RM, Pinto AG. Aortic intramural hematoma during coronary angioplasty: Insights into the pathogenesis of intramedial hemorrhage. J Am Soc Echocardiogr. 2000; 13:403-406.
30. Vilacosta I, Castillo JA, Peral V, Batlle E, Rollán MJ, Sánchez-Harguindey L. Intramural aortic haematoma following intra-aortic balloon counterpulsation. Documentation by transoesophageal echocardiography. Eur Heart J. 1995; 16:2015-2016.
31. Uchida K, Imoto K, Karube N, Minami T, Cho T, Goda M, Suzuki S, Masuda M. Intramural haematoma should be referred to as thrombosed-type aortic dissection. Eur J Cardiothorac Surg. 2013; 44:366-369; discussion 369.
32. Grimm M, Loewe C, Gottardi R, Funovics M, Zimpfer D, Rodler S, Dorfmeister M, Schoder M, Lammer

J, Aharinejad S, Czerny M. Novel insights into the mechanisms and treatment of intramural hematoma affecting the entire thoracic aorta. Ann Thorac Surg. 2008; 86:453-456.
33. Park KH, Lim C, Choi JH, Sung K, Kim K, Lee YT, Park PW. Prevalence of aortic intimal defect in surgically treated acute type A intramural hematoma. Ann Thorac Surg. 2008; 86:1494-1500.
34. Kitai T, Kaji S, Yamamuro A, Tani T, Kinoshita M, Ehara N, Kobori A, Kim K, Kita T, Furukawa Y. Detection of intimal defect by 64-row multidetector computed tomography in patients with acute aortic intramural hematoma. Circulation. 2011; 124:S174-S178.
35. Hiratzka LF, Bakris GL, Beckman JA, *et al*. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM Guidelines for the diagnosis and management of patients with thoracic aortic disease. A report of the American college of cardiology foundation/American heart association task force on practice guidelines, American association for thoracic surgery, American college of radiology, American stroke association, society of cardiovascular anesthesiologists, society for cardiovascular angiography and interventions, society of interventional radiology, society of thoracic surgeons, and society for vascular medicine. J Am Coll Cardiol. 2010; 55:e27-e129.
36. Erbel R, Aboyans V, Boileau C, *et al*. 2014 ESC Guidelines on the diagnosis and treatment of aortic diseases: Document covering acute and chronic aortic diseases of the thoracic and abdominal aorta of the adult. The task force for the diagnosis and treatment of aortic diseases of the European society of cardiology (ESC). Eur Heart J. 2014; 35:2873-2926.
37. Hayter RG, Rhea JT, Small A, Tafazoli FS, Novelline RA. Suspected aortic dissection and other aortic disorders: Multi-detector row CT in 373 cases in the emergency setting. Radiology. 2006; 238:841-852.
38. Chao CP, Walker TG, Kalva SP. Natural history and CT appearances of aortic intramural hematoma. Radiographics. 2009; 29:791-804.
39. Abbara S, Kalva S, Cury RC, Isselbacher EM. Thoracic aortic disease: Spectrum of multidetector computed tomography imaging findings. J Cardiovasc Comput Tomogr. 2007; 1:40-54.
40. Valente T, Rossi G, Lassandro F, Rea G, Marino M, Muto M, Molino A, Scaglione M. MDCT evaluation of acute aortic syndrome (AAS). Br J Radiol. 2016; 89:20150825.
41. Schlatter T, Auriol J, Marcheix B, Lebbadi M, Marachet MA, Dang-Tran KD, Tran M, Honton B, Gardette V, Rousseau H. Type B intramural hematoma of the aorta: Evolution and prognostic value of intimal erosion. J Vasc Interv Radiol. 2011; 22:533-541.
42. Ganaha F, Miller DC, Sugimoto K, Do YS, Minamiguchi H, Saito H, Mitchell RS, Dake MD. Prognosis of aortic intramural hematoma with and without penetrating atherosclerotic ulcer: A clinical and radiological analysis. Circulation. 2002; 106:342-348.
43. Sueyoshi E, Matsuoka Y, Sakamoto I, Uetani M, Hayashi K, Narimatsu M. Fate of intramural hematoma of the aorta: CT evaluation. J Comput Assist Tomogr. 1997; 21:931-938.
44. Wu MT, Wang YC, Huang YL, Chang RS, Li SC, Yang P, Wu TH, Chiou KR, Huang JS, Liang HL, Pan HB. Intramural blood pools accompanying aortic intramural hematoma: CT appearance and natural course. Radiology. 2011; 258:705-713.
45. Lee YK, Seo JB, Jang YM, Do KH, Kim SS, Lee JS, Song KS, Song JW, Han H, Kim SS, Lee JY, Lim TH. Acute and chronic complications of aortic intramural hematoma on follow-up computed tomography: Incidence and predictor analysis. J Comput Assist Tomogr. 2007; 31:435-440.
46. Kitai T, Kaji S, Yamamuro A, Tani T, Tamita K, Kinoshita M, Ehara N, Kobori A, Nasu M, Okada Y, Furukawa Y. Clinical outcomes of medical therapy and timely operation in initially diagnosed type a aortic intramural hematoma: A 20-year experience. Circulation. 2009; 120:S292-S298.
47. Sawaki S, Hirate Y, Ashida S, Takanohashi A, Yagami K, Usui M. Clinical outcomes of medical treatment of acute type A intramural hematoma. Asian Cardiovasc Thorac Ann. 2010; 18:354-359.
48. Park GM, Ahn JM, Kim DH, Kang JW, Song JM, Kang DH, Lim TH, Song JK. Distal aortic intramural hematoma: Clinical importance of focal contrast enhancement on CT images. Radiology. 2011; 259:100-108.
49. Tang XK, Xiong J, Xu YL, Guo W, Guo JJ, Liang WX. Acute Stanford B aortic intramural hematoma: Early and midterm follow-ups and morphological changes of computed tomography. Zhonghua Yi Xue Za Zhi. 2013; 93:649-652. (in Chinese)
50. Vilacosta I, San Román JA, Ferreirós J, Aragoncillo P, Méndez R, Castillo JA, Rollán MJ, Batlle E, Peral V, Sánchez-Harguindey L. Natural history and serial morphology of aortic intramural hematoma: A novel variant of aortic dissection. Am Heart J. 1997; 134:495-507.
51. Kakazu C, Augustus J, Paullin C, Julka IS, White RA. Acute ascending aortic intramural hematoma as a complication of the endovascular repair of a Type B aortic dissection. J Anesth. 2012; 26:589-591.
52. Agricola E, Slavich M, Bertoglio L, Fisicaro A, Oppizzi M, Marone E, Melissano G, Tufaro V, Margonato A, Chiesa R. The role of contrast enhanced transesophageal echocardiography in the diagnosis and in the morphological and functional characterization of acute aortic syndromes. Int J Cardiovasc Imaging. 2014; 30:31-38.
53. Baliga RR, Nienaber CA, Bossone E, Oh JK, Isselbacher EM, Sechtem U, Fattori R, Raman SV, Eagle KA. The role of imaging in aortic dissection and related syndromes. JACC Cardiovasc Imaging. 2014; 7:406-424.
54. Augoustides JG, Hosalkar HH, Milas BL, Acker M, Savino JS. Upper gastrointestinal injuries related to perioperative transesophageal echocardiography: Index case, literature review, classification proposal, and call for a registry. J Cardiothorac Vasc Anesth. 2006; 20:379-384.
55. Goldberg JB, Kim JB, Sundt TM. Current understandings and approach to the management of aortic intramural hematomas. Semin Thorac Cardiovasc Surg. 2014; 26:123-131.
56. Murray JG, Manisali M, Flamm SD, VanDyke CW, Lieber ML, Lytle BW, White RD. Intramural hematoma of the thoracic aorta: MR image findings and their prognostic implications. Radiology. 1997; 204:349-355.
57. Ma X, Zhang Z, Fan Z, Zhao L, Yu J. Natural history of spontaneous aortic intramural hematoma progression: Six years follow-up with cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2010; 12:27.
58. von Kodolitsch Y, Nienaber CA. Intramural hemorrhage of the thoracic aorta: Diagnosis, therapy and prognosis of

209 *in vivo* diagnosed cases. Z Kardiol. 1998; 87:797-807. (in German)
59. Lee CW, Kang JW, Lee HJ, Lim TH. MDCT evaluation of intimal defects in intramural hematoma of the aorta: Initial findings and follow-up. Int J Cardiovasc Imaging. 2010; 26:295-302.
60. Lavingia KS, Ahanchi SS, Redlinger RE, Udgiri NR, Panneton JM. Aortic remodeling after thoracic endovascular aortic repair for intramural hematoma. J Vasc Surg. 2014; 60:929-935; discussion 935-936.
61. Sueyoshi E, Nagayama H, Hashizume K, Eishi K, Sakamoto I, Uetani M. Computed tomography evaluation of aortic remodeling after endovascular treatment for complicated ulcer-like projection in patients with type B aortic intramural hematoma. J Vasc Surg. 2014; 59:693-699.
62. JCS Joint Working Group. Guidelines for diagnosis and treatment of aortic aneurysm and aortic dissection (JCS 2011): Digest version. Circ J. 2013; 77:789-828.
63. Evangelista A, Czerny M, Nienaber C, Schepens M, Rousseau H, Cao P, Moral S, Fattori R. Interdisciplinary expert consensus on management of type B intramural haematoma and penetrating aortic ulcer. Eur J Cardiothorac Surg. 2015; 47:209-217.
64. Bischoff MS, Geisbüsch P Peters AS, Hyhlik-Dürr A, Böckler D. Penetrating aortic ulcer: Defining risks and therapeutic strategies. Herz. 2011; 36:498-504.
65. Geisbüsch P, Kotelis D, Weber TF, Hyhlik-Dürr A, Kauczor HU, Böckler D. Early and midterm results after endovascular stent graft repair of penetrating aortic ulcers. J Vasc Surg. 2008; 48:1361-1368.
66. Kan CB, Chang RY, Chang JP. Optimal initial treatment and clinical outcome of type A aortic intramural hematoma: A clinical review. Eur J Cardiothorac Surg. 2008; 33:1002-1006.
67. Uzuka T, Ito T, Hagiwara T, Yanase Y, Koyanagi T, Kurimoto Y, Kawaharada N, Higami T. A treatment strategy for early thrombosed Stanford type A acute aortic dissection. Gen Thorac Cardiovasc Surg. 2013; 61:84-88.
68. Sawhney NS, DeMaria AN, Blanchard DG. Aortic intramural hematoma: An increasingly recognized and potentially fatal entity. Chest. 2001; 120:1340-1346.
69. Ho HH, Cheung CW, Jim MH, Miu KM, Siu CW, Lam YM, Chan HW, Lee WL, Tse HF. Type A aortic intramural hematoma: Clinical features and outcomes in Chinese patients. Clin Cardiol. 2011; 34:E1-E5.
70. Matsushita A, Fukui T, Tabata M, Sato Y, Takanashi S. Preoperative characteristics and surgical outcomes of acute intramural hematoma involving the ascending aorta: A propensity score-matched analysis. J Thorac Cardiovasc Surg. 2016; 151:351-358.
71. Song JK, Kim HS, Song JM, Kang DH, Ha JW, Rim SJ, Chung N, Kim KS, Park SW, Kim YJ, Sohn DW. Outcomes of medically treated patients with aortic intramural hematoma. Am J Med. 2002; 113:181-187.
72. Choi YJ, Son JW, Lee SH, *et al*. Treatment patterns and their outcomes of acute aortic intramural hematoma in real world: Multicenter registry for aortic intramural hematoma. BMC Cardiovasc Disord. 2014; 14:103.
73. Tolenaar JL, Harris KM, Upchurch GR Jr, Evangelista A, Moll FL, di Eusanio M, Eagle K, Trimarchi S; IRAD investigators. The differences and similarities between intramural hematoma of the descending aorta and acute type B dissection. J Vasc Surg. 2013; 58:1498-1504.
74. Monnin-Bares V, Thony F, Rodiere M, Bach V, Hacini R, Blin D, Ferretti G. Endovascular stent-graft management of aortic intramural hematomas. J Vasc Interv Radiol. 2009; 20:713-721.
75. Felisaz A, Dufranc J, Heyndrickx M, Palcau L, Gouicem D, Berger L. Midterm results of type B intramural hematoma endovascular treatment. Ann Vasc Surg. 2015; 29:898-904.
76. Manning BJ, Dias N, Manno M, Ohrlander T, Malina M, Sonesson B, Resch T, Ivancev K. Endovascular treatment of acute complicated type B dissection: Morphological changes at midterm follow-up. J Endovasc Ther. 2009; 16:466-474.
77. Dagenais F, Dumont E, Voisine P. Intramural hematoma of the arch and ascending aorta owing to acute aortic syndrome of the descending aorta: To stent or not to stent? J Endovasc Ther. 2008; 15:544-549.
78. Iyer VS, Mackenzie KS, Tse LW, Abraham CZ, Corriveau MM, Obrand DI, Steinmetz OK. Early outcomes after elective and emergent endovascular repair of the thoracic aorta. J Vasc Surg. 2006; 43:677-683.

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Review

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Heart transplantation in patients with dystrophinopathic cardiomyopathy: Review of the literature and personal series

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Summary **Cardiomyopathy associated with dystrophinopathies [Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), X-linked dilated cardiomyopathy (XL-dCM) and cardiomyopathy of Duchenne/Becker (DMD/BMD) carriers] is an increasing recognized manifestation of these neuromuscular disorders and notably contributes to their morbidity and mortality. Dystrophinopathic cardiomyopathy (DCM) is the result of the dystrophin protein deficiency at the myocardium level, parallel to the deficiency occurring at the skeletal muscle level. It begins as a "presymptomatic" stage in the first decade of life and evolves in a stepwise manner toward pictures of overt cardiomyopathy (hypertrophic stage, arrhythmogenic stage and dilated cardiomyopathy). The final stage caused by the extensive loss of cardiomyocytes results in an irreversible cardiac failure, characterized by frequent episodes of acute congestive heart failure (CHF), despite a correct pharmacological treatment. The picture of a severe dilated cardiomyopathy with intractable heart failure is typical of BMD, XL-dCM and cardiomyopathy of DMD/BMD carriers, while it is less frequently observed in patients with DMD. Heart transplantation (HT) is the only curative therapy for patients with dystrophinopathic end-stage heart failure who remain symptomatic despite an optimal medical therapy. However, no definitive figures exist in literature concerning the number of patients with DCM transplanted, and their outcome. This overview is to summarize the clinical outcomes so far published on the topic, to report the personal series of dystrophinopathic patients receiving heart transplantation and finally to provide evidence that heart transplantation is a safe and effective treatment for selected patients with end-stage DCM.**

Keywords: Cardiomyopathy, Duchenne muscular dystrophy, Becker muscular dystrophy, X-linked dilated cardiomyopathy, Duchenne/Becker carrier's cardiomyopathy

1. Introduction

Dystrophinopathies are X-linked muscle disorders caused by mutations in the dystrophin gene, located at Xp21, that encodes for the sarcolemmal protein dystrophin, virtually present in all tissues, but most abundant in skeletal muscle cells and heart (*1-3*). Dystrophin provides the connection between a large complex of glycoproteins called the dystrophin-associated glycoprotein complex (DAG) on the muscle cell membrane and the intracellular actin filaments, that transmit forces generated by the sarcomere contraction to the extracellular matrix (*4,5*). Absence, reduced levels or abnormal structure of dystrophin lead to membrane fragility, making muscle fibres more prone to injury during contraction (*6*). As muscle disease progresses, muscle repair cannot adequately compensate for damage, leading to necrosis of skeletal and cardiac myocytes and the progressive replacement by fibro-fatty tissue (*7-15*). Dystrophinopathies include four different clinical presentations: Duchenne muscular dystrophy (DMD), the more severe form, Becker muscular dystrophy (BMD), the more benign

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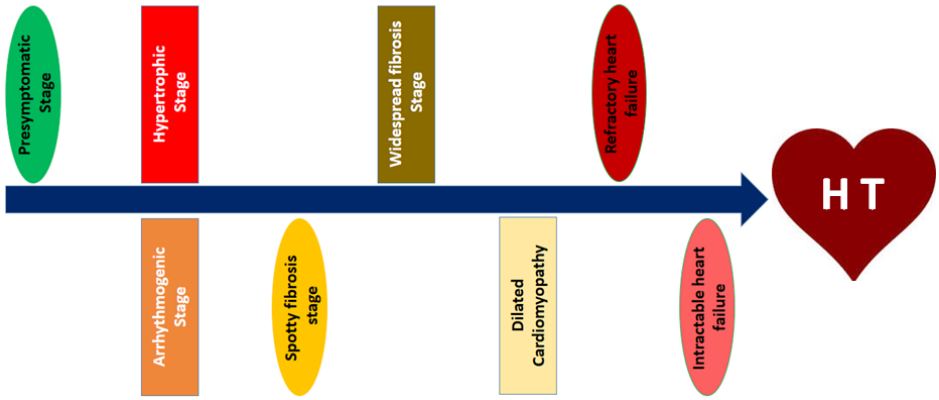

Figure 1. Flow-chart showing the evolution of dystrophinopathic cardiomyopathy.

form, the X-linked dilated cardiomyopathy (XL-dCM) (*8*) and the cardiomyopathy of DMD/BMD carriers.

Duchenne muscular dystrophy (DMD, OMIM 310200) is the most common muscle disorder in infancy, affecting 1 in 3.500 male newborns (*9*). It is the most severe form of dystrophinopathy, caused by the complete absence of the dystrophin protein at both skeletal and cardiac muscle level. Becker muscular dystrophy (BMD, OMIM 300376) is the milder dystrophinopathy affecting 1 in 18.450 males (*9*). Symptoms typically begin between ages 3 and 21, with a mean age of onset of 11 years. The disease is usually caused by "in frame" mutations able to produce a certain amount of dystrophin (*16*). BMD, unlike Duchenne-type muscular dystrophy, is characterized by a slow progression of muscle weakness so that in many patients the independent activity is maintained until late adulthood.

XL-dCM, first described in 1993 by Towbin (*17*), is a rare cardiomyopathy caused by mutations in the 5' end of the dystrophin gene that lead to the absence of the M-isoform of dystrophin only in the heart (*17*). The preserved skeletal muscle strength increases cardiac demands, leading to dilated cardiomyopathy (*18*). The clinical picture is characterized by the onset of congestive heart failure (CHF) in young men with a mean age of 15-30, with a rapid fatal evolution within 2 years from the onset, although older patients can also be affected.

The majority of DMD and BMD female carriers are asymptomatic; however, few of them may show muscle symptoms (*8,16*) such as mild muscle weakness and elevated serum CK, while a larger part present cardiomyopathy (*20-25*). The onset of symptoms in DMD/BMD carriers may have several causes, the most frequent of them represented by a skewed X-chromosome inactivation (XCI) (*26-28*).

The above mentioned clinical presentations arise from different pathogenic conditions with consequent variable degrees of skeletal muscle and cardiac involvement. Cardiac death usually occurs from ventricular dysfunction and dilated cardiomyopathy, that represents the end-stage of dystrophinopathic cardiomyopathy (DCM) (*10-11,29-30*). DCM is constantly progressive and evolves in a stage-wise manner, passing from a presymptomatic condition to dilated cardiomyopathy through a number of pathophysiologically distinct stages (*10,11*) (Figure 1).

In brief, the presymptomatic stage is followed by a stage in which the myocardial fibrosis is limited to few foci (spotty or focal fibrosis), often inducing a compensatory hypertrophy of the surrounding areas still expressing dystrophin, with consequent regional heterogeneity of repolarization that can favor the onset of arrhythmias. Later, the confluence of these areas determines a widespread diffuse fibrosis leading to dilated cardiomyopathy, a stage characterized by ventricle enlargement, tinning of ventricular walls, and reduced left ejection fraction. Dilated cardiomyopathy in turn evolves toward the stage of heart failure (HF). The first episodes of HF are usually responsive to pharmacological treatment; subsequently, HF evolves towards the stage of intractable HF not amenable to adequate drug treatment. Therefore heart failure represents an important contributor to mortality in these patients (*9,10*) for which the heart transplantation (HT) remains the ultimate intervention to preserve life. However, despite the high incidence of end-stage DCM in patients with dystrophinopathies, there is a reluctance to perform heart transplantation in these patients due to the paucity of donors and the concerns that the accompanying myopathy will limit the benefits obtained through the HT.

Furthermore no definitive figures exist in literature about the number of patients with DCM transplanted and their outcome, nor clear indications in the current guidelines considering heart transplantation as an option for dystrophinopathic patients with end-stage heart failure.

This overview aims to *i*) report the so far published frequencies of heart transplantation in patients with DCM and related clinical outcomes; *ii*) report the personal series of dystrophinopathic patients who

received HT and *iii*) provide evidence that HT is a safe and effective treatment for selected patients with end-stage DCM.

2. Heart transplantation in patients with dystrophinopathic cardiomyopathy

2.1. *Review of the literature*

Inherited myopathies in patients with secondary end-stage cardiomyopathies have always been considered a relative contraindication for cardiac transplantation. High operative risk related to muscle impairment and potential graft involvement secondary to the underlying myopathy have been the two main reasons implicated in the poor prognosis. For these reasons, HT has not been considered an appropriate treatment option for DMD patients with drug-resistant dilated cardiomyopathy, because of pulmonary function and skeletal muscles impairment (*31*). The literature contains only two isolated reports of successful heart transplantation in these patients (*31,32*). Cripe *et al*. (*31*) reported the case of a 14-year-old DMD patient with severe dilated cardiomyopathy, but preserved pulmonary function who underwent a successful cardiac transplantation surviving four years later. Rees *et al*. (*32*) described HT in 3 patients with DMD with a mean duration of follow-up of 40 months. All the patients tolerated immunosuppression, had no complications in post-operative intubation and were able to be rehabilitated, suggesting that cardiac transplantation can be successfully performed in DMD patients presenting a severe cardiomyopathy, preserved pulmonary function, and a discrete muscle function. Recently, Amodeo *et al*. (*33*) explored at the Bambino Gesù Children's Hospital in Rome, the use of left ventricular assist devices (LVAD-Jarvik 2000) as destination therapy in 7 DMD patients, as an alternative treatment for selected end-stage heart failure in DMD. All patients survived to hospital discharge and resumed previous activities; however data about their long-term outcomes remain limited.

Likewise, few cases of HT in patients with Becker muscular dystrophy have been so far reported in literature (*34-39*), though heart failure is the most frequent cause of death in these patients. In fact patients with BMD seem to pay the advantage of a prolonged ambulation with a higher occurrence of heart involvement and dilated cardiomyopathy, that represents the major cause of their morbidity and mortality (*19,40-43*). Some dystrophin mutations have been correlated to an increased incidence of severe cardiomyopathy (*3*) in these patients in which the presence of cardiomyopathy dramatically reduces the life expectancy, otherwise only slightly limited compared to normal subjects (*44*). Wu *et al*. (*34*) reported the results of a retrospective review of the Cardiac Transplant Research Database, a multi-institutional registry of 29 transplant centres in the United States, in the period 1990-2005. In their review - the largest study describing the long-term outcomes of cardiac transplantation in patients with BMD with a follow-up period extending more than 10 years - the post-transplant cardiac outcomes of 15 BMD patients were compared with those of 275 non-muscular dystrophy patients with non-ischemic cardiomyopathy, matched for age, body mass index, gender, and race. The survival rate in BMD patients was similar to that of controls at 1 year (89% *vs*. 91%; $p = 0.5$) and 5 years (83% *vs*. 78%; $p = 0.5$). The differences in rates of cumulative infection, rejection, or allograft vasculopathy between the 2 groups were not significant ($p = 0.5$ for all comparisons).

Ruiz-Cano *et al*. (*35*) described a Spanish single-centre experience with HT in 3 BMD patients with a mean follow-up duration of 57.4 months. Both intra-operative and post-operative course of these individuals did not show higher complication rates than other patients. All recipients experienced successful rehabilitation; no evidence of graft dysfunction was detected during the follow-up. All patients were alive at the time of the study, and in good performance. The AA concluded that HT for patients with BMD end-stage cardiomyopathy is not associated with a poorer prognosis when there is a mild degree of muscular impairment.

Casazza *et al*. (*36*), reported the case of a successful cardiac transplantation because of a severe and rapidly progressive dilated cardiomyopathy complicated by terminal heart failure in a BMD patient with a mild muscle impairment. Unfortunately, no data about the long-term prognosis of this patient have been reported. Patanè *et al*. (*37*) described a 27-year-old BMD patient with end-stage dilated cardiomyopathy and CHF who was fairly well, one year after a successful transplantation. Melacini *et al*. (*38*) reported the case of a 24-years-old BMD patient, with end-stage dilated cardiomyopathy and moderate myopathy, who successfully underwent heart transplantation. The patient received triple immunosuppressive therapy consisting of azathioprine, cyclosporineA (CyA) and prednisone in the post-operative period.

Steger *et al*. (*39*) extrapolated 4 patients (1.19%) affected by end-stage cardiomyopathy related to inherited myopathies from a large cohort of 335 patients undergoing HT at the Innsbruck Medical University, between January 1994 and December 2011. Three of them had BMD, and 1- female- was affected by limb-girdle muscular dystrophy; the mean age of patients was 38.5 years (range 16-56) and the post-operative follow-up period was on average 68.5 months (range 16-139). All patients had an uneventful immediate post-operative course. One of them (BMD) died 16 months after the HT because of pulmonary embolism and right heart failure, and one 11 years after HT because of myocardial and cerebral infarction following tricuspid

Table 1. Patients' clinical characteristics

Patient	Diagnosis	Dystrophin Gene Defect	Gender	Age at diagnosis (years)	CK (U/L) pre HT	Age at HT (years)	NYHA class at HT	Follow-up (months)
1	BMD	del ex. 45-49	M	15	1152	34	IV	153
2	BMD	del ex. 45-49	M	17	1263	33	IV	121
3	BMD	del ex 3-4	M	29	856	27	IV	174
4	XL-dCM	del promoter	M	22	320	27	IV	131
Mean ± SD				20 ± 6.2	897.8 ± 421.7	30.2 ± 3.8	IV	144.7 ± 23.6

BMD: Becker's muscular dystrophy; CK: creatinkinase; HT: heart transplantation; M: male; XL-dCM: X-linked dilated cardiomyopathy.

valve replacement. The remaining 2 patients were in good general condition without progression of the muscle disease, for the entire period of the study. The AA concluded that HT may increase the quality of life and life expectancy in selected patients suffering from end-stage heart failure due to inherited benign muscular dystrophies. These data seem to disclaim the long-held belief that patients with muscular dystrophy and advanced stages of cardiomyopathy may have worse outcomes after HT compared with patients without it.

To date there is only one report on HT in patients with XL-dCM (*45*). Out of 4 patients (mean age 24, 4 years; range 16-31) who were heart transplanted in the period August 1989 - January 2000, only one died suddenly at 66 months of follow-up. At the time of heart transplantation all patients were in NYHA functional class IV. After 44 months of FU on average (range 22-66) all the patients were fine and in NYHA functional class I, stressing the concept that HT should be the first choice option for patients with XL-dCM and preserved muscle function, for whom myocardial end-stage disease and serious rhythm disturbances are severely disabling and life-threatening (*46*).

Very few cardiac transplantation case reports have been so far reported in cardiological manifesting DMD carriers (*47,48*) for whom once again the only hope for survival remains heart transplantation. Melacini *et al*. (*47*) reported the first case of successful HT in a symptomatic DMD carrier, with severe dilated cardiomyopathy, showing a deletion of exons 50-52. Davies *et al*. (*48*) reported the case of a 25-year-old DMD carrier who during the third trimester of her pregnancy developed a severe cardiac failure and performed successful heart transplantation, after 311 days of mechanical circulatory support.

2.2. *Personal series*

Among 55 patients affected by dystrophin related end-stage cardiomyopathy followed in our division in the last 15 years, 4 of them (7.2%) underwent heart transplantation. Three of them had Becker's muscular dystrophy and the fourth was affected by XL-dCM. The clinical characteristics of the patients are summarised in Table 1.

After transplantation, all patients received triple drug immunosuppressive therapy with cyclosporine, everolimus and steroids. High-dose steroid therapy was withdrawn within 12 months following HT in all patients.

Becker Cases: Cases 1 and 2 were diagnosed as BMD at the age of 15 and 17 years respectively, when they complained of mild muscle weakness, associated with increased serum creatin-kinase (CK) levels (mean values 6.3 times the maximum normal values). Molecular investigation confirmed the diagnosis, showing a deletion of exons 45-49 in dystrophin gene in both cases.

At the age of 31 years, both developed dilated cardiomyopathy that required orthotopic HT in the following few years, performed at the age of 34 years in case 1, and at the age of 33 years in case 2 (Figure 2A).

At the time of transplant pre-evaluation, both patients had very mild muscular impairment and no respiratory involvement. The intra-operative and early post-operative courses did not reveal any complications; mechanical ventilation was withdrawn within 10 hours following surgery. Both patients had a successful functional rehabilitation reaching a good performance status. Case 1, nine months following HT, experienced mild signs of laboratory rejection, promptly resolved by the immunosuppressive therapy optimization. Case 2 showed, in the last 2 years, a slight progressive worsening of kidney function related to immunosuppressive therapy (serum creatinine value of 2.6 mg/dL; CrCl 40.96 mL/min; stage III of renal failure), which required cyclosporine dose reduction. To date, more than 10 years following HT, no evidence of graft dysfunction was detected (Figure 2B), while a very slow progression of the pre-existing muscular impairment was observed. Both patients continue to have a good quality of life.

Case 3 was initially diagnosed as idiopathic dilated cardiomyopathy, complicated by terminal heart failure. He required 2 years later an orthotopic HT. The early post-surgical period was uneventful. Two years after HT, the patient was addressed to our division for muscle weakness at lower limbs and increased serum CK values. Based on clinical evaluation and molecular investigation (presence of a deletion of exons 3 and 4 in dystrophin gene), a diagnosis of BMD was made. Seven years after HT, the patient showed clinical and echocardiographic

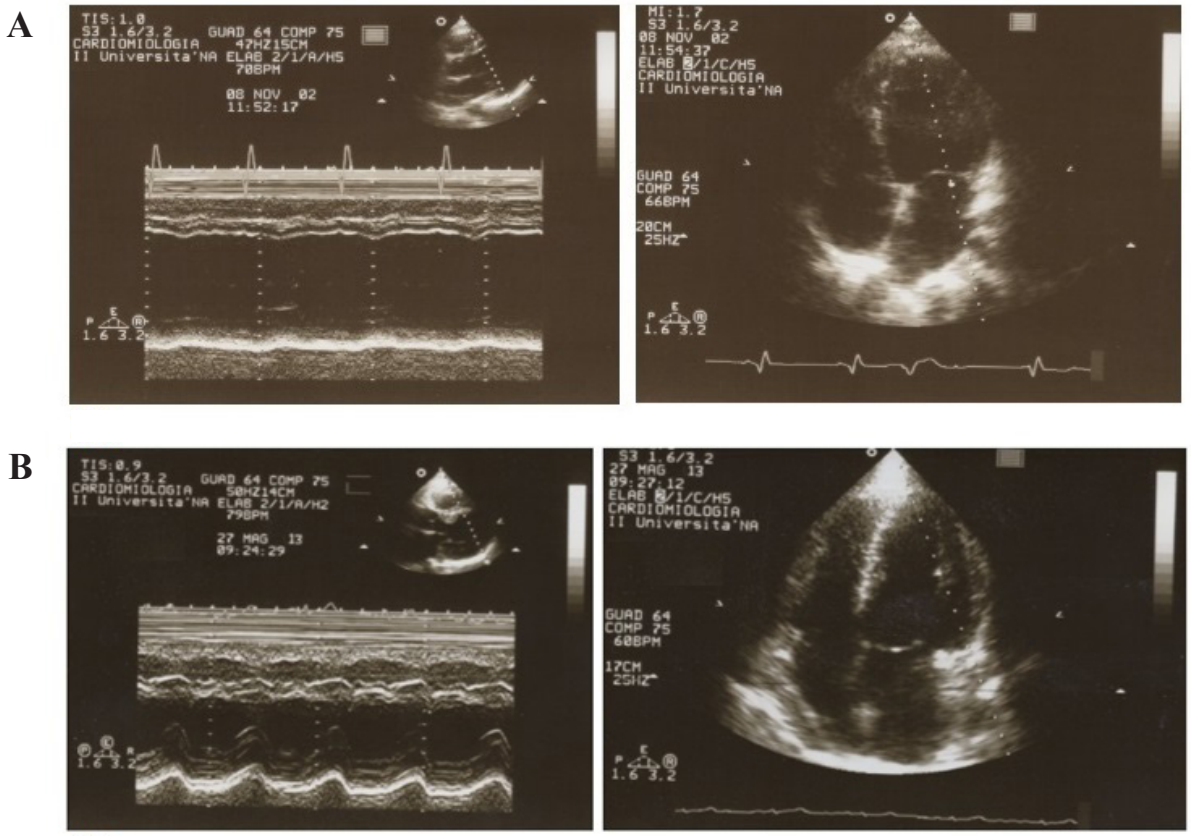

Figure 2. (A) Echocardiography (M-mode scan and apical view of the left ventricle) in the BMD patient (case 2). To be noted: the enlargement of the left atrium (systolic dimension = 48 mm) and the marked left ventricular dilatation (end diastolic diameter = 72 mm); the hypokinesis of the interventricular septum and the virtually absent motion of the left ventricular posterior wall. **(B)** Echocardiography in the same patient 10 years after heart transplantation. The M-mode scan and the apical view of the left ventricle show normal chamber diameters and ventricular systolic function.

evidence of acute graft dysfunction, and a reduction (< 35%) of left ventricular ejection fraction (LVEF). The replacement of everolimus with mycophenolate mofetil produced a progressive improvement of patient's condition and of echocardiographic parameters (LVEF: 50%). Twelve years later, he developed an acute complete (3rd degree) atrio-ventricular block requiring a pacemaker implantation; after two years a new heart transplant was indicated, for evidence of clinical and echocardiographic graft rejection (LVEF 25%). However, the transplant was not performed as the patient died suddenly during sleep, before performing it.

Case 4: The patient was addressed to our department for signs and symptoms of low cardiac output, history of reduced exercise tolerance and exertional dyspnea. At the first examination, no muscle and respiratory weakness were noted, while CK values were increased. The patient underwent both muscle and endomyocardial biopsies, who showed the absence of the dystrophin staining with the antibody anti-NH2 terminal domain only at the cardiac level, not in the muscle. The dystrophin staining was instead normal at both cardiac and muscle level with the antibodies anti-*ROD* and anti-*COOH* domains. A diagnosis of X-linked dilated cardiomyopathy was made and the patient treated accordingly.

During the following years an intractable congestive heart failure required HT, performed at the age of 27 years. The early post-operative course was uncomplicated; mechanical ventilation was withdrawn within the following 24 hours. The patient returned to work and till now he continues to have a good exercise tolerance and quality of life.

3. Discussion

The long-term clinical outcomes of cardiac transplantation in patients with dystrophinopathic cardiomyopathy properly selected, seem to be similar to that of a matched cohort of patients undergoing HT for idiopathic dilated cardiomyopathy, by analysing the few evidence available in literature.

However, special care and consideration are necessary during the peri-operative and post-operative period to avoid life-threatening complications and the progression of the primary disease. Among them, an intensive neuromyological observation is necessary in order to *i*) adequately adapt the dosage of immunosuppressants to avoid the onset of a secondary myopathy and the occurrence of rhabdomyolysis due

to the toxic affect of cyclosporine and *ii*) to limit the post-operative complications and the progression of the primary muscle disease. In fact the combined use of cyclosporine and lipid-lowering agent, such as statins and gemfibrozil, could be toxic for muscle cells (*49*).

Also in our experience, HT remains the treatment of choice for patients with advanced DCM and poor muscle impairment. No other treatment option such as medical therapy or electrical and/or mechanical devices, can compete with HT long-term results, particularly when compared with the natural course of end-stage heart failure. Even the fear of a possible heart rejection is limited - at least for patients suffering from BMD, XL-dCM and relative carriers - because the presence of some dystrophin makes it very unlikely the production of antibodies against the dystrophin-deleted-region. As a consequence, a more favourable prognosis may be expected in these subjects.

Given for that a special attention should be given to the extent of skeletal myopathy, respiratory muscle involvement and survival in these patients, however it's time that cardiac surgeons overcome the reluctance to heart transplant patients with dystrophinopathic cardiomyopathy, due to the supposed reduced life expectancy. The long-term prognosis in these patients in fact is closely related to the possibility to be transplanted.

References

1. Muntoni F, Torelli S, Ferlini A. Dystrophin and mutations: One gene, several proteins, multiple phenotypes. Lancet Neurol. 2003; 2:731-740.
2. Sadoulet-Puccio HM, Kunkel LM. Dystrophin and its isoforms. Brain Pathol. 1996; 6:25-35.
3. Nigro G, Politano L, Nigro V, Petretta VR, Comi LI. Mutation of dystrophin gene and cardiomyopathy. Neuromuscul Disord. 1994; 4:371-379.
4. Rafael JA, Cox GA, Corrado K, Jung D, Campbell KP, Chamberlain JS. Forced expression of dystrophin deletion constructs reveals structure-function correlations. J Cell Biol. 1996; 134:93-102.
5. Corrado K, Rafael JA, Mills PL, Cole NM, Faulkner JA, Wang K, Chamberlain JS. Transgenic mdx mice expressing dystrophin with a deletion in the actin-binding domain display a "mild Becker" phenotype. J Cell Biol. 1996; 134:873-884.
6. Petrof BJ, Shrager JB, Stedman HH, Kelly AM, Sweeney HL. Dystrophin protects the sarcolemma from stresses developed during muscle contraction. Proc Natl Acad Sci U S A. 1993; 90:3710-3714.
7. Wallace GQ, McNally EM. Mechanisms of muscle degeneration, regeneration and repair in the muscular dystrophies. Annu Rev Physiol. 2009; 71:37-57.
8. Obler D, Wu BL, Lip V, Estrella E, Keck S, Haggan C, Semigran M, Smoot LB. Familial dilated cardiomyopathy secondary to dystrophin splice site mutation. J Card Fail. 2010; 16:194-199.
9. Emery AE. Population frequencies of inherited neuromuscular diseases. A world survey. Neuromuscul Disord. 1991; 1:19-29.
10. Nigro G, Comi LI, Politano L, Bain RJ. The incidence and evolution of cardiomyopathy in Duchenne muscular dystrophy. Int J Cardiol. 1990; 26:271-277.
11. Politano L, Nigro G. Managing dystrophinopathic cardiomyopathy. Expert Opin Orphan Drugs. 2016; 4:1159-1178.
12. Passamano L, Taglia A, Palladino A, Viggiano E, D'Ambrosio P, Scutifero M, Rosaria Cecio M, Torre V, DE Luca F, Picillo E, Paciello O, Piluso G, Nigro G, Politano L. Improvement of survival in Duchenne muscular dystrophy: Retrospective analysis of 835 patients. Acta Myol. 2012; 31:121-125.
13. Politano L, Nigro G. Treatment of dystrophinopathic cardiomyopathy: Review of the literature and personal results. Acta Myol. 2012; 31:24-30.
14. Jefferies JL, Eidem BW, Belmont JW, Craigen WJ, Ware SM, Fernbach SD, Neish SR, Smith EO, Towbin JA. Genetic predictors and remodeling of dilated cardiomyopathy in muscular dystrophy. Circulation. 2005; 112:2799-2804.
15. Nigro G, Comi LI, Politano L, Nigro V. Dilated cardiomyopathy of muscular dystrophy: A multifaceted approach to management. Semin Neurol. 1995; 15:90-92.
16. Cohn RD, Campbell KP. Molecular pathways for dilated cardiomyopathy. In: Molecular basis of cardiovascular disease: A companion to Braunwald's heart disease (Chien K, editor). 2nd ed. Saunders Philadelphia, USA, 2004; pp. 306-310.
17. Towbin JA, Hejtmancik JF, Brink P, Gelb B, Zhu XM, Chamberlain JS, McCabe ER, Swift M. X-linked dilated cardiomyopathy. Molecular genetic evidence of linkage to the Duchenne muscular dystrophy (dystrophin) gene at the Xp21 locus. Circulation. 1993; 87:1854-1865.
18. Spurney CF. Cardiomyopathy of Duchenne muscular dystrophy: Current understanding and future directions. Muscle Nerve. 2011; 44:8-19.
19. Kaspar RW, Allen HD, Montanaro F. Current understanding and management of dilated cardiomyopathy in Duchenne and Becker muscular dystrophy. J Am Acad Nurse Pract. 2009; 21:241-249.
20. Nigro G, Di Somma S, Comi LI, Politano L, Papparella S, Restucci B, Petretta VR, Giugliano MA, Carotenuto A, Limongelli FM, DE Divitiis O. Structural basis of cardiomyopathy in Duchenne/Becker carriers. Endomyocardial biopsy evaluation. Ann N Y Acad Sci. 1995; 752:108-110.
21. Politano L, Nigro V, Nigro G, Petretta VR, Passamano L, Papparella S, Di Somma S, Comi LI. Development of cardiomyopathy in female carriers of Duchenne and Becker muscular dystrophies. JAMA. 1996; 275:1335-1338.
22. Comi LI, Nigro G, Politano L, Petretta VR. The cardiomyopathy of Duchenne/Becker consultands. Int J Cardiol. 1992; 34:297-305.
23. Hoogerwaard EM, van der Wouw PA, Wilde AA, Bakker E, Ippel PF, Oosterwijk JC, Majoor-Krakauer DF, van Essen AJ, Leschot NJ, de Visser M. Cardiac involvement in carriers of Duchenne and Becker muscular dystrophy. Neuromuscul Disord. 1999; 9:347-351.
24. Kamakura K, Kawai M, Arahata K, Koizumi H, Watanabe K, Sugita H. A manifesting carrier of Duchenne muscular dystrophy with severe myocardial symptoms. J Neurol. 1990; 237:483-485.
25. Mirabella M, Servidei S, Manfredi G, Ricci E, Frustaci A, Bertini E, Rana M, Tonali P. Cardiomyopathy may be the only clinical manifestation in female carriers of Duchenne

muscular dystrophy. Neurology. 1993; 43:2342-2345.
26. Viggiano E, Picillo E, Cirillo A, Politano L. Comparison of X-chromosome inactivation in Duchenne muscle/myocardium-manifesting carriers, non-manifesting carriers and related daughters. Clin Genet. 2013; 84:265-270.
27. Viggiano E, Ergoli M, Picillo E, Politano L. Determining the role of skewed X-chromosome inactivation in developing muscle symptoms in carriers of Duchenne muscular dystrophy. Hum Genet. 2016; 135:685-698.
28. Viggiano E, Picillo E, Ergoli M, Cirillo A, Del Gaudio S, Politano L. Skewed X-chromosome inactivation plays a crucial role in the onset of symptoms in carriers of Becker muscular dystrophy. J Gene Med. 2017; doi: 10.1002/jgm.2952.
29. Finsterer J, Stöllberger C. The heart in human dystrophinopathies. Cardiology. 2003; 99:1-19.
30. Michele DE, Campbell KP. Cardiomyopathy in muscular dystrophies. In: Molecular mechanisms of cardiac hypertrophy and failure (Walsh RA, editor). Taylor & Francis, London, UK, 2005; pp.541-567.
31. Cripe L, Kinnett K, Uzark K, Eghtesady P, Wong B, Spicer R. P1.14 Cardiac transplantation in Duchenne muscular dystrophy: A case report. Neuromuscul Disord. 2011; 21:645. Doi:10.1016/j.nmd.2011.06.774
32. Rees W, Schüler S, Hummel M, Hetzer R. Heart transplantation in patients with muscular dystrophy associated with end-stage cardiomyopathy. J Heart Lung Transplant. 1993; 12:804-807.
33. Amodeo A, Adorisio R. Left ventricular assist device in Duchenne cardiomyopathy: Can we change the natural history of cardiac disease? Int J Cardiol. 2012; 29:161:e43.
34. Wu RS, Gupta S, Brown RN, Yancy CW, Wald JW, Kaiser P, Kirklin NM, Patel PC, Markham DW, Drazner MH, Garry DJ, Mammen PP. Clinical outcomes after cardiac transplantation in muscular dystrophy patients. J Heart Lung Transplant. 2010; 29:432-438.
35. Ruiz-Cano MJ, Delgado JF, Jiménez C, Jiménez S, Cea-Calvo L, Sánchez V, Escribano P, Gómez MA, Gil-Fraguas L, Sáenz de la Calzada C. Successful heart transplantation in patients with inherited myopathies associated with end-stage cardiomyopathy. Transplant Proc. 2003; 35:1513-1515.
36. Casazza F, Brambilla G, Salvato A, Morandi L, Gronda E, Bonacina E. Cardiac transplantation in Becker muscular dystrophy. J Neurol. 1988; 235:496-498.
37. Patanè F, Zingarelli E, Attisani M, Sansone F. Successful heart transplantation in Becker's muscular dystrophy. Eur J Cardiothorac Surg. 2006; 29:250.
38. Melacini P, Gambino A, Caforio A, Barchitta A, Valente ML, Angelini A, Fanin M, Thiene G, Angelini C, Casarotto D, Danieli GA, Dalla-Volta S. Heart transplantation in patients with inherited myopathies associated with end-stage cardiomyopathy: Molecular and biochemical defects on cardiac and skeletal muscle. Transplant Proc. 2001; 33:1596-1599.
39. Steger CM, Höfer D, Antretter H. Cardiac manifestation in muscular dystrophies leading to heart transplantation. European Surgery. 2013; 45:245-250.
40. Yilmaz A, Gdynia HJ, Baccouche H, Mahrholdt H, Meinhardt G, Basso C, Thiene G, Sperfeld AD, Ludolph AC, Sechtem U. Cardiac involvement in patients with Becker muscular dystrophy: New diagnostic and pathophysiological insights by a CMR approach. J Cardiovasc Magn Reson. 2008; 10:50.
41. Nigro G, Comi LI, Politano L, *et al*. Evaluation of the cardiomyopathy in Becker muscular dystrophy. Muscle Nerve. 1995; 18:283-291.
42. Politano L, Palladino A, Nigro G, Scutifero M, Cozza V. Usefulness of heart rate variability as a predictor of sudden cardiac death in muscular dystrophies. Acta Myol. 2008 ;27:114-122.
43. Ducceschi V, Nigro G, Sarubbi B, Comi LI, Politano L, Petretta VR, Nardi S, Briglia N, Santangelo L, Nigro G, Iacono A. Autonomic nervous system imbalance and left ventricular systolic dysfunction as potential candidates for arrhythmogenesis in Becker muscular dystrophy. Int J Cardiol. 1997 May 23;59:275-279.
44. Fayssoil A, Abasse S. Cardiac resynchronization therapy in Becker muscular dystrophy: For which patients? Hellenic J Cardiol. 2010; 51:377-378.
45. Grande AM, Rinaldi M, Pasquino S, D'Armini AM, Viganò M. Heart transplantation in X-linked dilated cardiomyopathy. Ital Heart J. 2002; 3:476-478.
46. Diegoli M, Grasso M, Favalli V, Serio A, Gambarin FI, Klersy C, Pasotti M, Agozzino E, Scelsi L, Ferlini A, Febo O, Piccolo G, Tavazzi L, Narula J, Arbustini E. Diagnostic work-up and risk stratification in X-linked dilated cardiomyopathies caused by dystrophin defects. J Am Coll Cardiol. 2011; 58:925-934.
47. Melacini P, Fanin M, Angelini A, Pegoraro E, Livi U, Danieli GA, Hoffman EP, Thiene G, Dalla Volta S, Angelini C. Cardiac transplantation in a Duchenne muscular dystrophy carrier. Neuromuscul Disord. 1998; 8:585-590.
48. Davies JE, Winokur TS, Aaron MF, Benza RL, Foley BA, Holman WL. Cardiomyopathy in a carrier of Duchenne's muscular dystrophy. J Heart Lung Transplant. 2001; 20:781-784.
49. Ketelsen UP, Trenk D, Eschenbruch EM, Tollenaere PJ. Myopathy/rhabdomyolisis in patients after heart transplantation by presurgical treatment with lipid-lowering drugs? Interaction of cyclosporine and HMG-CoA reductase inhibitor therapy? Neuromuscul Disord.1997; 7:446.

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Mini-Review

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Surgical management of hepatolithiasis: A minireview

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Summary **Hepatolithiasis is highly prevalent in Asia but rare in Western countries. However, the incidence of hepatolithiasis may be increasing in Western countries due to the increased rate of immigration from areas where hepatolithiasis is prevalent. There are many non-surgical treatments for hepatolithiasis, but surgical management remains the best curative treatment for some cases of hepatolithiasis. Surgical treatments can remove biliary stones and relieve stricture of the bile ducts. This review describes the indications for and the outcomes of surgical treatment of hepatolithiasis, including liver resection and liver transplantation.**

Keywords: Liver resection, liver transplantation, hepatolithiasis

1. Introduction

The incidence of hepatolithiasis varies. It is highly prevalent in parts of Asia, such as China, Japan, and South Korea, with a reported incidence between 3.1% and 21.2% (*1-3*). However, hepatolithiasis is rare in Western countries, with an incidence of about 1% (*2,4*). The mechanism by which hepatolithiasis develops has yet to be fully elucidated. Studies have suggested that cholestasis, cholangitis, an anatomical abnormality of the bile ducts, abnormal bile metabolism, malnutrition, and low socio-economic status are risk factors for hepatolithiasis (*5,6*). The incidence of hepatolithiasis may decrease in areas where the condition is prevalent because people in those areas have adopted a Western diet. A Japanese nationwide survey reported that the relative prevalence of hepatolithiasis was 4.1% in the years from 1970-1977, 3.0% in the years from 1975-1984, 2.3% in the years from 1985-1988, 2.2% in the years from 1989-1992, and 1.7% in the years from 1993-1995 (*6,7*). However, the incidence of hepatolithiasis may be increasing in Western countries due to the increasing rate of immigration from areas where hepatolithiasis is prevalent.

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2. Surgical treatments

According to Japanese nationwide surveys over a period of 40 years, the primary treatments of hepatolithiasis in the 1970s were cholecystectomy with stone removal and insertion of a T-tube. Surgery was used for primary management of hepatolithiasis until 1998. However, its use later decreased (76.6% in 1998; 52.4% in 2006; 33.3% in 2011, including surgical and non-surgical treatment) (*7*). In contrast, use of nonsurgical treatments, including endoscopic retrograde cholangiography and percutaneous transhepatic cholangioscopic lithotomy, has markedly increased. However, endoscopic retrograde cholangiography with stone removal results in more residual or recurrent stones than surgery does (*7*).

3. Indications for hepatectomy

Studies have reported that hepatectomy is indicated for treatment of hepatolithiasis in the following instances: (*i*) unilobar hepatolithiasis, and particularly that on the left; (*ii*) atrophy or severe fibrosis of the affected liver segments or lobe; (*iii*) presence of a liver abscess; (*iv*) cholangiocarcinoma; and (*v*) multiple intrahepatic stones causing marked biliary stricture or dilation (*8-12*).

In China, Dong proposed a system for classification (Table 1) of hepatolithiasis (*13*). Dong also proposed corresponding treatments for different classes of hepatolithiasis (*13*). Dong suggested that patients with type I or type IIb hepatolithiasis are the best candidates for hepatectomy, whereas patients with type

Table 1. Dong's classification of hepatolithiasis

Type	Definition or content
Type I	Localized stones: unilobar or bilobar
Type II	Diffusely distributed stones
IIa	Without hepatic atrophy; no stricture of the intrahepatic bile ducts
IIb	Atrophy limited in segment or/and stricture of the intrahepatic bile ducts.
IIc	With biliary cirrhosis and portal hypertension
Additional Type E	Extrahepatic stones
Ea	Normal sphincter of Oddi
Eb	Relaxation of the sphincter of Oddi.
Ec	Stricture of the sphincter of Oddi.

IIc hepatolithiasis should undergo liver transplantation (*13*). Moreover, a hepaticojejunostomy should also be performed to treat "Eb" and "Ec" hepatolithiasis (*13*).

4. Outcomes of hepatectomy for treatment of hepatolithiasis

Chen *et al.* (*10*) reported outcomes for 103 consecutive patients with hepatolithiasis who underwent a hepatectomy between 1989 and 2001. Ninety percent of those patients had immediate stone clearance, and 98% had final stone clearance after subsequent choledochoscopic lithotomy via a T-tube tract or cutaneous stoma (*10*). Twenty-eight percent of the 103 patients suffered postoperative complications and 2% died (*10*). Uenishi *et al*. (*14*) reported outcomes for 86 patients who underwent a hepatectomy from 1998 to 2012. Seventy-six patients (88%) had immediate stone clearance whereas 82 patients (95%) had final stone clearance (*14*). Li *et al*. (*15*) reported outcomes for 718 patients who underwent a hepatectomy. The immediate stone clearance rate was 93.5% for patients with unilateral hepatolithiasis and 71.1% for patients with bilateral hepatolithiasis. The final clearance rate was 99.3% for patients with unilateral hepatolithiasis and 90.2% for patients with bilateral hepatolithiasis (*15*). The mortality rate for hepatectomy was 0.4% (*15*). Yang *et al*. reported that the immediate stone clearance rate was 81.5% after bilateral hepatectomy and 65.9% after unilateral hepatectomy while the final clearance rate was 85.2% after bilateral hepatectomy and 81.7% after unilateral hepatectomy (*16*). Liver resection can also be performed on some patients with complex hepatolithiasis. Dong *et al*. reported outcomes for 12 patients with complex hepatolithiasis who underwent a subtotal hepatectomy (*17*). After surgery, one patient died from acute purulent cholangitis (*17*). The remaining 11 patients recovered without recurrent cholangitis (*17*).

Some techniques may improve the effectiveness with which hepatectomy treats hepatolithiasis. Fang *et al*. (*18*) suggested that hepatectomy for bilateral hepatolithiasis based on three-dimensional reconstruction resulted in a higher rate of stone clearance than conventional treatment. Both immediate (96.1% versus 81%) and final clearance rates (100% versus 90.5%) were improved by use of three-dimensional reconstruction (*18*). Guan *et al*. also reported similar results (*19*). Jarufe *et al*. (*20*) suggested that anatomical hepatectomy may reduce the incidence of postoperative complications and postoperative recurrence for patients with hepatolithiasis. A study by Jiang *et al*. (*21*) suggested that anatomical hepatectomy for treatment of hepatolithiasis was associated with fewer residual stones, fewer infections, and a lower incidence of bile leakage.

5. Laparoscopic hepatectomy for treatment of hepatolithiasis

Laparoscopic hepatectomy is a minimally invasive treatment for hepatolithiasis. Laparoscopic treatments may result in satisfactory outcomes for select patients with hepatolithiasis. Lai *et al*. (*22*) reported outcomes for 55 consecutive patients with hepatolithiasis who underwent a laparoscopic hepatectomy or bile duct exploration. The immediate stone clearance rate was 90.9% and the final stone clearance rate was 94.5% (*22*). During the follow-up period, only 3 patients suffered from recurrence (*22*). Ye *et al*. (*23*) reported outcomes for 36 patients who underwent a purely laparoscopic left hemihepatectomy for hepatolithiasis. Residual stones were noted in only 2 patients (*23*). Stones recurred after surgery in 2 patients (*23*). Namgoong *et al*. (*24*) compared the outcomes of laparoscopic versus open left hemihepatectomy for treatment of hepatolithiasis. Laparoscopic hepatectomy was associated with a longer operating time, briefer hospitalization, a lower postoperative morbidity, and a higher stone clearance rate (*24*). Ye *et al*. also noted similar results (*25*). However, Jin *et al*. (*26*) suggested that laparoscopic and open hepatectomy resulted in a similar operating time, duration of postoperative hospitalization, postoperative morbidity, rate of residual stones, and rate of recurrent stones. Recently, a systemic review and meta-analysis by Peng *et al*. (*27*) suggested that patients who underwent

a laparoscopic hepatectomy had significantly fewer intraoperative transfusions and overall complications and a significantly briefer duration of hospitalization. However, laparoscopic and open hepatectomy had a similar operating time and resulted in a similar rate of residual and recurrent stones.

6. Factors associated with postoperative recurrence

Many risk factors are associated with the postoperative recurrence of stones. Li *et al*. (*15*) found that bilateral stones and limited resection of the liver (*i.e*. not all segments affected by stones were resected) were two independent risk factors for the postoperative recurrence of stones. A study of surgical and non-surgical treatment of hepatolithiasis suggested that non-surgical treatment, biliary cirrhosis, residual stones, and strictures were risk factors for the recurrence of stones (*28*). In a study of patients with hepatolithiasis in whom stones were merely removed, Cheung *et al*. suggested that bilateral stones, strictures, and atrophy were related to the postoperative recurrence of stones (*29*).

7. Liver transplantation for treatment of hepatolithiasis

Liver transplantation is an effective treatment for terminal hepatolithiasis. According to Dong's classification, liver transplantation should be undergone by patients with type IIc hepatolithiasis. In 2002, Strong *et al*. reported 4 liver transplants to treat hepatolithiasis (*30*). All patients had satisfactory outcomes after liver transplantation. Tang *et al*. reported a case of a patient with hepatolithiasis and situs inversus who underwent liver transplantation (*31*). The patient survived as of a 40-month follow-up. Chen *et al*. reported outcomes for 15 patients with hepatolithiasis who underwent liver transplantation (*32*). Two of those patients had cholangiocarcinoma. After liver transplantation, the 1-year survival rate was 100% and the 5-year survival rate was 73%. Although studies have suggested that liver transplantation results in satisfactory outcomes for patients with hepatolithiasis, no study has examined this point in a large sample.

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References

1. Nakayama F, Soloway RD, Nakama T, *et al*. Hepatolithiasis in East Asia. Retrospective study. Dig Dis Sci. 1986; 31:21-26.
2. Shoda J, Tanaka N, Osuga T. Hepatolithiasis – Epidemiology and pathogenesis update. Front Biosci. 2003; 8:e398-409.
3. Su CH, Lui WY, P'Eng FK. Relative prevalence of gallstone diseases in Taiwan. A nationwide cooperative study. Dig Dis Sci. 1992; 37:764-768.
4. Pausawasdi A, Watanapa P. Hepatolithiasis: Epidemiology and classification. Hepatogastroenterology. 1997; 44:314-316.
5. Ran X, Yin B, Ma B. Four Major Factors Contributing to Intrahepatic Stones. Gastroenterol Res Pract. 2017; 2017:7213043.
6. Kim HJ, Kim JS, Joo MK, Lee BJ, Kim JH, Yeon JE, Park JJ, Byun KS, Bak YT. Hepatolithiasis and intrahepatic cholangiocarcinoma: A review. World J Gastroenterol. 2015; 21:13418-13431.
7. Suzuki Y, Mori T, Yokoyama M, Nakazato T, Abe N, Nakanuma Y, Tsubouchi H, Sugiyama M. Hepatolithiasis: Analysis of Japanese nationwide surveys over a period of 40 years. J Hepatobiliary Pancreat Sci. 2014; 21:617-622.
8. Chijiiwa K, Yamashita H, Yoshida J, Kuroki S, Tanaka M. Current management and long-term prognosis of hepatolithiasis. Arch Surg. 1995; 130:194-197.
9. Uchiyama K, Onishi H, Tani M, Kinoshita H, Ueno M, Yamaue H. Indication and procedure for treatment of hepatolithiasis. Arch Surg. 2002; 137:149-153.
10. Chen DW, Tung-Ping Poon R, Liu CL, Fan ST, Wong J. Immediate and long-term outcomes of hepatectomy for hepatolithiasis. Surgery. 2004; 135:386-393.
11. Sakpal SV, Babel N, Chamberlain RS. Surgical management of hepatolithiasis. HPB (Oxford). 2009; 11:194-202.
12. Tabrizian P, Jibara G, Shrager B, Schwartz ME, Roayaie S. Hepatic resection for primary hepatolithiasis: A single-center Western experience. J Am Coll Surg. 2012; 215:622-626.
13. Feng X, Zheng S, Xia F, Ma K, Wang S, Bie P, Dong J. Classification and management of hepatolithiasis: A high-volume, single-center's experience. Intractable Rare Dis Res. 2012; 1:151-156.
14. Uenishi T, Hamba H, Takemura S, Oba K, Ogawa M, Yamamoto T, Tanaka S, Kubo S. Outcomes of hepatic resection for hepatolithiasis. Am J Surg. 2009; 198:199-202.
15. Li SQ, Liang LJ, Peng BG, Hua YP, Lv MD, Fu SJ, Chen D. Outcomes of liver resection for intrahepatic stones: A comparative study of unilateral versus bilateral disease. Ann Surg. 2012; 255:946-953.
16. Yang T, Lau WY, Lai EC, Yang LQ, Zhang J, Yang GS, Lu JH, Wu MC. Hepatectomy for bilateral primary hepatolithiasis: A cohort study. Ann Surg. 2010; 251:84-90.
17. Dong J, Lau WY, Lu W, Zhang W, Wang J, Ji W. Caudate lobe-sparing subtotal hepatectomy for primary hepatolithiasis. Br J Surg. 2012; 99:1423-1428.
18. Fang CH, Liu J, Fan YF, Yang J, Xiang N, Zeng N. Outcomes of hepatectomy for hepatolithiasis based on 3-dimensional reconstruction technique. J Am Coll Surg. 2013; 217:280-288.
19. Guan T, Fang C, Mo Z, Xiang N, Yang J, Zeng N. Long-Term Outcomes of Hepatectomy for Bilateral Hepatolithiasis with Three-Dimensional Reconstruction: A Propensity Score Matching Analysis. J Laparoendosc Adv Surg Tech A. 2016; 26:680-688.
20. Jarufe N, Figueroa E, Munoz C, Moisan F, Varas J, Valbuena JR, Bambs C, Martinez J, Pimentel F. Anatomic hepatectomy as a definitive treatment for hepatolithiasis:

A cohort study. HPB (Oxford). 2012; 14:604-610.
21. Jiang H, Wu H, Xu YL, Wang JZ, Zeng Y. An appraisal of anatomical and limited hepatectomy for regional hepatolithiasis. HPB Surg. 2010; 2010:791625.
22. Lai EC, Ngai TC, Yang GP, Li MK. Laparoscopic approach of surgical treatment for primary hepatolithiasis: A cohort study. Am J Surg. 2010; 199:716-721.
23. Ye X, Ni K, Zhou X, Xie K, Hong X. Laparoscopic Left Hemihepatectomy for Left-sided Hepatolithiasis. Surg Laparosc Endosc Percutan Tech. 2015; 25:347-350.
24. Namgoong JM, Kim KH, Park GC, Jung DH, Song GW, Ha TY, Moon DB, Ahn CS, Hwang S, Lee SG. Comparison of laparoscopic versus open left hemihepatectomy for left-sided hepatolithiasis. Int J Med Sci. 2014; 11:127-133.
25. Ye X, Ni K, Zhou X, Xie K, Hong X. Laparoscopic versus open left hemihepatectomy for hepatolithiasis. J Surg Res. 2015; 199:402-406.
26. Jin RA, Wang Y, Yu H, Liang X, Cai XJ. Total laparoscopic left hepatectomy for primary hepatolithiasis: Eight-year experience in a single center. Surgery. 2016; 159:834-841.
27. Peng L, Xiao J, Liu Z, Li Y, Xiao W. Laparoscopic Versus Open Left-Sided Hepatectomy for Hepatolithiasis: A Systematic Review and Meta-Analysis. J Laparoendosc Adv Surg Tech A. 2016.
28. Cheon YK, Cho YD, Moon JH, Lee JS, Shim CS. Evaluation of long-term results and recurrent factors after operative and nonoperative treatment for hepatolithiasis. Surgery. 2009; 146:843-853.
29. Cheung MT, Kwok PC. Liver resection for intrahepatic stones. Arch Surg. 2005; 140:993-997.
30. Strong RW, Chew SP, Wall DR, Fawcett J, Lynch SV. Liver transplantation for hepatolithiasis. Asian J Surg. 2002; 25:180-183.
31. Tang DN, Wei JM, Liu YN, Qiao JC, Zhu MW, He XW. Liver transplantation in an adult patient with situs inversus: A case report and overview of the literature. Transplant Proc. 2008; 40:1792-1795.
32. Chen ZY, Yan LN, Zeng Y, Wen TF, Li B, Zhao JC, Wang WT, Yang JY, Xu MQ, Ma YK, Wu H. Preliminary experience with indications for liver transplantation for hepatolithiasis. Transplant Proc. 2008; 40:3517-3522.

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Original Article

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Immunosuppressive medication is not associated with surgical site infection after surgery for intractable ulcerative colitis in children

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Summary **Pediatric ulcerative colitis (UC) sometimes progresses to an intractable condition for medical therapy. The surgical management of UC is challenging because of difficult procedures and frequent infectious complications. The aim of this study was to survey surgical procedures and infectious complications in pediatric patients with UC in Japan and to assess the relationship between preoperatively administered immunosuppressive drugs and postoperative surgical site infection (SSI). A survey of pediatric patients treated from 2000 to 2012 was sent to 683 facilities nationwide. Secondary questionnaires were sent to physicians who followed up patients with UC who had undergone surgery with the aim of assessing the relationships between postoperative SSI and selected preoperative patient characteristics, disease severity, medications, and operative procedures. Data for 136 patients (77 boys and 59 girls) were assessed. Median age at surgery was 14.1 years (range: 2.4-18.9 years). Surgery was performed in one stage in 35 cases, two stages in 57 cases, and three stages in 44 cases. SSI occurred in 36/136 patients (26%). According to multiple logistic regression analysis, there were statistically significant associations between SSI and staged surgery (three/one, OR: 6.7, 95% CI: 2.1-25.5, $p = 0.0007$; three/two, OR: 3.4, 95% CI: 1.4-8.6, $p = 0.0069$) and female sex (OR: 2.3, 95% CI: 1.0-5.4, $p = 0.0434$). Preoperative medications and incidence of SSI were not significantly associated. Preoperative immunosuppressive medication does not affect the incidence of SSI. Three-stage surgery and female sex are independent predictors of development of postoperative SSIs in pediatric patients with UC.**

Keywords: Ulcerative colitis, children, colectomy, complication

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1. Introduction

Inflammatory bowel diseases (IBDs), a group of chronic inflammatory gastrointestinal disorders, include Crohn disease and ulcerative colitis (UC). In recent years, the incidence of IBD has increased markedly in both adults and children. IBD's onset during childhood is in 20-30% of patients and this condition is characterized by extensive intestinal involvement and rapid early progression. IBD is more severe in children than in adults (*1*).

Colectomy is uncommonly performed for pediatric UC; rates have remained stable from 1983 to 2009. However, the rates of in-hospital postoperative complications have increased, particularly in patients undergoing emergency colectomy (*2,3*). Such complications reportedly occur in 25% of cases, gastro-intestinal (13%) and infectious (9.3%) complications being the most common (*2*). Independent predictors of postoperative complications were identified in these studies and it was concluded that optimizing timing of colectomy may reduce postoperative complications in pediatric patients with UC. Knod *et al*. (*4*) have reported that colectomy with ileoanal anastomosis does not have a greater incidence of complications in young children (≤ 11-years-old) with UC than in older patients and achieves a good postoperative quality of life and stool patterns.

Generally, patients with UC undergoing elective colectomy have high rates of surgical site infection (SSI), specifically deep and organ/space infections (*5*). Uchino *et al*. (*6*) have also demonstrated that, in surgical patients with clean-contaminated wounds, IBD is an independent risk factor for incisional SSI.

Recently, immunomodulators and biologics that affect patients' immune systems have been increasingly used to treat steroid-dependent and -resistant pediatric UC. A European Crohn's and Colitis Organisation paper has stated that preoperative thiopurines do not increase the risk of postoperative complications in adult patients (*7*). Additionally, there does not appear to be a higher rate of postoperative complications after colectomy for UC performed immediately following or in the medium term after the use of cyclosporine. Two studies have demonstrated that previous exposure to azathioprine/6-mercaptopurine does not affect the rates of short term and late postoperative complications after pouch surgery for UC (*8,9*). However, there are few studies concerning the relationship between preoperative drugs and post-colectomy infectious complications in children.

The aim of this study was to survey surgical procedures and postoperative infectious complications in patients with pediatric UC in Japan and to assess the relationship between preoperatively administered drugs and postoperative SSI.

2. Materials and Methods

This study was a retrospective analysis of data from multiple institutions in Japan. A national survey of immunomodulators and biologics used in pediatric patients with IBD (aged < 17 years at diagnosis) between 2000 and 2012 was sent to 683 facilities treating pediatric patients with IBD in Japan from December 2012 to March 2013 (*10*). All participating physicians followed the Japanese diagnostic criteria for Crohn disease and UC (*11*). These diagnostic criteria are substantially the same as the Port criteria published by the IBD working group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (*12*). This primary survey assessed the total number of pediatric patients with IBD, the number treated with immunomodulators and biologics, and the number who underwent surgery.

Secondary questionnaires were sent to physicians who followed up patients with UC who had undergone surgery, the purpose being to assess the following factors: selected patient characteristics, severity of UC, preoperative medications, operative procedure, and SSI. Patient characteristics assessed included sex, age at disease onset, age at surgery, preoperative duration of disease, indications for surgery, and preoperative blood analysis data including serum albumin, hemoglobin and C-reactive protein (CRP) concentrations.

Clinical severity of disease was evaluated according to the Pediatric Ulcerative Colitis Activity Index (PUCAI) (*13*) and Japanese disease severity criteria as established by the Research Committee on Inflammatory Bowel Disease of the Ministry of Health and Welfare in Japan in 1994 (*11*). Extent of disease at surgery was evaluated according to the Montreal classification of UC as E1 (ulcerative proctitis), E2 (left-sided UC), and E3 (extensive UC) (*14*).

Preoperative medical therapy assessed included systemic prednisolone (PDN), 5-aminosalicylates, azathioprine (AZA), cyclosporine A (CYA), tacrolimus (TAC), infliximab (IFX), and apheresis therapy. Patients' records were reviewed if any of these therapies had been used within 90 and 30 days prior to the first-stage operation. The dosage of PDN administered during the month before surgery was also calculated. Serum trough levels of TAC, CYA, and IFX and 6-thioguanine nucleotide concentrations in patients receiving AZA were not monitored.

Generally, operative procedures for UC both adults and children were classified as one-, two-, or three-stage procedures to complete total proctocolectomy with ileal J-pouch anal anastomosis (IPAA). The one-stage procedure is total proctocolectomy with IPAA without a diversion stoma. The two-stage procedure includes total proctocolectomy with IPAA and creation of diversion ileostomy in the first stage and closure of the ileostomy in the second stage. The three-stage procedure includes subtotal colectomy with creation of a diversion ileostomy and mucus fistula of the sigmoid colon or rectal stump in the first stage. In the second stage, remnant proctocolectomy and IPAA with recreation of diversion

ileostomy are performed. Finally, the ileostomy is closed in the third stage.

SSI was defined according to the definition of the Centers for Disease Control and Prevention as infections occurring within 30 days after surgery (*15*). All patients were followed up for a minimum of 30 days after the final stage of their procedures. SSI, including incisional wound infection (I-SSI), and/or organ/space infection (OS-SSI), was evaluated. Anastomotic leaks and abscesses (OS-SSI) were diagnosed by contrast imaging, computed tomography, or intraoperative findings (*16*).

The relationships between preoperative factors, including administered drugs, and incidence of postoperative SSI occurrence after first stage operations on pediatric patients with UC was assessed in this study.

The data were analyzed by the χ^2 and Mann-Whitney U tests and by multiple logistic regression analysis using the JMP version 7 software program (SAS Institute, Cary, NC, USA). A p value of less than 0.05 was considered to be statistically significant.

This study was approved by the institutional ethical committees of Juntendo University and all participating institutions.

3. Results

In the primary survey, 1,617 pediatric patients with UC were enrolled from 1 January 2000 to 31 December 2012. Data for 146 patients who had undergone surgery were collected *via* secondary questionnaires that were sent to the physicians who were following them up. Finally, we were able to assess 136 patients (77 boys and 59 girls), 10 of these patients being excluded because of incomplete data concerning surgery, medications, and SSI.

Table 1 shows the study of patients' baseline characteristics. Median age at disease onset was 12.0 years (range 0.3-17.7 years), median age at surgery 14.1 years (range: 2.4-18.9), and median preoperative duration of disease 679 days (range 16-3,611 days). Median preoperative serum albumin, hemoglobin, and CRP concentrations were 3.2 g/dL (range: 1.4-4.8 g/dL), 10.6 g/dL (range: 4.9-17.5 g/dL), and 0.33 g/dL (range: 0.0-16.11 g/dL), respectively. As to preoperative disease activity, median PUCAI score was 45 (range: 0-85). According to Japanese criteria of disease severity, 12% of patients had mild disease, 40% moderate, and 45% severe. According to the Montreal classification, 96% of patients had E3 disease (extensive UC).

Indications for surgery, which overlapped each other in this study, are listed in Table 2. The most frequent indications for surgery were intractable disease caused by resistance to medications, including steroid dependency and steroid resistance, in 77 % of patients. The next most frequent indications were poor quality of life in 23%, adverse effects of drugs in 12%, massive bleeding in 11%, and growth retardation in 9%.

Table 1. Baseline patients' characteristics

Characteristics	
Gender (Feale/Male)	59/77
Age at disease onset (years old)	12.0 (0.3-17.7)
Age at operation (years old)	14.1 (2.4-18.9)
Preoperative disease duration (days)	679 (16-3611)
Preoperative serum albumin (g/dL)	3.2 (1.4-4.8)
Preoperative serum hemoglobin (g/dL)	10.6 (4.9-17.5)
Preoperative serum CRP (mg/dL)	0.33 (0-16.11)
PUCAI	45 (0-85)
Japanese disease severity criteria	
Mild	16 (12%)
Moderate	55 (40%)
Severe	61 (45%)
unknown	4 (3%)
Montreal classification of UC	
E1 (ulcerative proctitis)	0 (0%)
E2 (left-sided UC)	1 (1%)
E3 (extensive UC)	131 (96%)
unknown	4 (3%)

CRP, creactive protein; PUCAI, pediatric ulcerative colitis activity index; UC, ulcerative colitis. Data was shown by median (range).

Table 2. Operative indications

Indication for operation	*n*	%
Perforation	4	3
Massive bleeding	16	11
Toxic Megacolon	6	4
Colon cancer	0	0
Fluminant disease	4	3
Growth Retardation	13	9
Intractable disease	109	77
Lower QOL	33	23
Drug side effect	17	12
Extra-intestinal complications	2	1

Table 3 shows the surgical procedures and relevant patient characteristics, including preoperative medications and laboratory data. Thirty-five patients had undergone one-stage surgery, 57 two-stage surgery, and 44 three-stage surgery. Patients who had undergone three-stage surgery had a significantly shorter duration of preoperative disease than those in the other groups ($p = 0.0147$); their more severe conditions being reflected by the necessity for more complex surgery ($p < 0.0001$), worse PUCAI scores at surgery ($p < 0.0001$), lower preoperative albumin ($p < 0.0001$) and hemoglobin concentrations ($p = 0.0059$), and higher preoperative CRP concentrations ($p < 0.0001$). Preoperative medications varied by group and institution. In particular, few patients in this study received IFX.

SSI developed in 36/136 patients (26%) (Table 4). I-SSI occurred in 28/136 patients (21%) and OS-SSI in 16/136 patients (12%). SSI occurred in 11% of patients who had undergone one-stage surgery, comprising I-SSI in 9% and OS-SSI in 9%; in 19% patients who had undergone two-stage surgery, comprising I-SSI in 13% and OS-SSI in 12%; and in 48% patients who had

Table 3. Patients' characteristics among operative procedures

Items	One	Two	Three	*p* value
girls : boys	14 : 21	21 : 35	24 : 20	0.2055
Age at disease onset (years old)	11.4 ± 3.6	11.4 ± 3.1	11.1 ± 5.0	0.7996
Severity at onset (mild/moderate/severe)	3/21/8	5/23/20	4/16/20	
PUCAI at disease onset	52.5 ± 25.4	53.4 ± 21.5	62.2 ± 21.2	0.3496
Disease extent at onset (rectum/left side/total colitis)	0/4/30	3/5/41	1/6/35	
Age at operation (years old)	14.0 ± 2.7	13.8 ± 2.6	13.1 ± 3.6	0.8278
Preoperative disease duration (days)	951.6 ± 782	873.2 ± 635.7	657.1 ± 834.1	0.0147
Severity at operation (mild/moderate/severe)	3/22/10	11/26/16	2/8/34	< 0.0001
PUCAI at operation	38.9 ± 20.5	39.5 ± 25.8	69.0 ± 21.6	< 0.0001
Disease extent at operation (E1/E2/E3)	0/1/33	0/1/54	0/1/43	0.5627
Preoperative serum albumin (g/dL)	3.7 ± 0.8	3.7 ± 0.6	2.7 ± 0.8	< 0.0001
Preoperative serum hemoglobin (g/dL)	11.5 ± 2.3	11.2 ± 2.3	9.7 ± 2.2	0.0059
Preoperative serum CRP (mg/dL)	1.9 ± 3.7	0.8 ± 2.2	4.0 ± 4.5	< 0.0001
Preoperative 90 5ASA (y/n)	31/2	53/4	28/11	0.0064
Preoperative 90 PDN (y/n)	32/2	42/15	32/7	0.0344
Preoperative 90 APT (y/n)	13/19	15/42	8/31	0.1665
Preoperative 90 AZA (y/n)	11/21	30/27	8/31	0.0047
Preoperative 90 TAC (y/n)	3/29	17/40	5/34	0.0277
Preoperative 90 CYA (y/n)	3/29	13/44	10/29	0.1589
Preoperative 90 IFX (y/n)	1/31	9/48	1/38	0.0288
Preoperative 30 5ASA (y/n)	30/3	49/8	26/16	0.003
Preoperative 30 PDN (y/n)	31/3	39/18	38/6	0.0131
Preoperative 30 APT (y/n)	9/23	8/49	13/29	0.0935
Preoperative 30 AZA (y/n)	12/20	24/33	7/35	0.0179
Preoperative 30 TAC (y/n)	4/28	13/44	6/36	0.3762
Preoperative 30 CYA (y/n)	3/29	12/45	9/33	0.2805
Preoperative 30 IFX (y/n)	1/31	1/56	1/41	0.9182
EsPDN(g)	633.5 ± 751.0	663.7 ± 1004.7	838.2 ± 839.4	0.87
EsPDN(g)/BW (kg)	14.7 ± 15.9	21.8 ± 34.9	22.0 ± 22.5	0.1586

CRP; creactive protein, PUCAI; pediatric ulcerative colitis activity index, 5ASA; 5-aminosalicylates, PDN; prednisolone, APT; apheresis therapy, AZA; azathioprine, TAC; tacrolimus, CYA; cyclosporine A, IFX; infliximab, y; yes, n; no, EsPDN; estimated PDN administered dose during 1month before operation, BW; body weight. Data was shown by mean ± standard deviation.

Table 4. SSI occurrence among operative procedures

Items	Total (136)	One (35)	Two (57)	Three (44)
SSI	36 (26%)	4 (11%)	11(19%)	21 (48%)
I-SSI	28 (21%)	3 (9%)	8 (13%)	17 (35%)
OS-SSI	16 (12%)	3 (9%)	7 (12%)	6 (14%)

SSI; surgical site infection, I-SSI; incisional wound infection, OS-SSI; organ/space infection.

undergone three-stage surgery, comprising I-SSI in 35% and OS-SSI in 14%.

Table 5 shows the relationships between development of SSI after first stage operations and selected factors. SSI occurred significantly more frequently in girls than boys (p = 0.012). Neither disease severity nor preoperative medications were significantly associated with development of SSI. There was a statistically significant relationship between staged surgery and development of SSI (p = 0.0004).

Table 6 shows the results of multiple logistic regression analysis. Staged surgery (three/one, OR: 6.7, 95% CI: 2.1-25.5, p = 0.0007; three/two, OR: 3.4, 95% CI: 1.4-8.6, p = 0.0069) and female sex (OR: 2.3, 95% CI: 1.0-5.4, p = 0.0434) were statistically significant predictors of development of SSI in pediatric patients with UC. Staged surgery was the sole statistically significant risk factor for I-SSI, (three/one, OR: 6.5, 95% CI: 1.9-30.2, p = 0.0021; three/two, OR: 3.6, 95% CI: 1.4-9.9, p = 0.0091); however, no predictors of OS-SSI, such as anastomotic leakage or intraperitoneal abscess, were identified in this study.

4. Discussion

The major findings of this study were that female sex and three-stage surgery are independent predictors of development of SSI after surgery for UC in pediatric patients. Three-stage surgery was an independent predictor of development of I-SSI occurrence; female sex was not. No predictors of OS-SSI were identified. Preoperative medications, including 5-ASA, AZA, TAC, and CYA, did not significantly affect the incidence of SSI.

SSI is the most frequent complication of colorectal surgery. In adult colorectal surgery, dirty or contaminated surgery, open ileostomy or colostomy placement, emergency surgery, and multiple procedures are reportedly associated with increased risk of SSI (*17,18*). Segal *et al*. (*19*) reported that risk factors for I-SSI and OS-SSI differ in patients who undergo colon resection.

The surgical management for UC is challenging because the procedure of IPAA is difficult; additionally,

Table 5. The relationship between SSI occurrence and several factors in patients who received first stage operation

Items	SSI (+)	SSI (-)	*p* value
girls : boys	22 : 14	37 : 63	0.0125
Age at disease onset (years old)	11.4 ± 4.3	11.3 ± 3.7	0.6570
Severity at onset (mild/moderate/severe)	2/15/16	6/49/32	0.5065
PUCAI at disease onset	58.3 ± 26.2	54.9 ± 20.3	0.5956
Disease extent at onset (rectum/left side/total colitis)	36674	4/10/78	0.2459
Age at operation (years old)	13.9 ± 2.2	13.5 ± 3.2	0.7962
Preoperative disease duration (days)	864.9 ± 980.6	811.0 ± 642.9	0.4544
Severity at operation (mild/moderate/severe)	5/14/16	12/41/44	0.9492
PUCAI at operation	53.7 ± 28.6	45.0 ± 26.1	0.1828
Disease extent at operation (E1/E2/E3)	0/0/35	1/2/95	0.3952
Preoperative serum albumin (g/dL)	3.2 ± 0.8	3.4 ± 0.9	0.1087
Preoperative serum hemoglobin (g/dL)	10.4 ± 2.7	11.0 ± 2.2	0.1998
Preoperative serum CRP (mg/dL)	2.4 ± 4.0	1.9 ± 3.5	0.0927
Preoperative 90 5ASA (y/n)	27/6	85/12	0.4716
Preoperative 90 PDN (y/n)	26/7	80/17	0.6412
Preoperative 90 APT (y/n)	9/24	27/68	0.8992
Preoperative 90 AZA (y/n)	12/21	37/58	0.7921
Preoperative 90 TAC (y/n)	6/27	19/76	0.8194
Preoperative 90 CYA (y/n)	6/27	20/75	0.7216
Preoperative 90 IFX (y/n)	1/32	10/85	0.1453
Preoperative 30 5ASA (y/n)	25/9	80/18	0.3226
Preoperative 30 PDN (y/n)	29/7	79/20	0.9223
Preoperative 30 APT (y/n)	8/26	21/76	0.8211
Preoperative 30 AZA (y/n)	9/25	32/65	0.4761
Preoperative 30 TAC (y/n)	6/28	17/80	0.9872
Preoperative 30 CYA (y/n)	5/29	21/76	0.3706
Preoperative 30 IFX (y/n)	0/34	3/94	0.1764
EsPDN(g)	843.5 ± 869.9	652.0 ± 915.9	0.0917
EsPDN(g) / BW (kg)	24.8 ± 29.4	18.6 ± 27.3	0.1800
Stage operation (one/two/three)	4/11/21	31/46/23	0.0004

SSI; surgical site infection, CRP; creactive protein, PUCAI; pediatric ulcerative colitis activity index, 5ASA; 5-aminosalicylates, PDN; prednisolone, APT; apheresis therapy, AZA; azathioprine, TAC; tacrolimus, CYA; cyclosporine A, IFX; infliximab, y; yes, n; no, EsPDN; estimated PDN administered dose during 1month before operation, BW; body weight. Data was shown by mean ± standard deviation.

Table 6. Multiple logistic regression analysis

Items	Chi square	Multiple logistic regression analysis			
	p value		OR	95% CI	*p* value
SSI					
female	0.0141	two/one	2.3	1.0-5.4	0.0434
staged operation	0.0004	three/one	2	0.6-7.7	0.272
		three/two	6.7	2.1-25.5	0.0007
			3.4	1.4-8.6	0.0069
I-SSI					
female	0.0460				0.132
staged operation	0.0012	two/one	1.8	0.5-8.9	0.3888
		three/one	6.5	1.9-30.2	0.0021
		three/two	3.6	1.4-9.9	0.0091
OS-SSI					
none					

SSI; surgical site infection, I-SSI; incisional wound infection, OS-SSI; organ/space infection.

patients with UC tend to be in poorer condition than patients with other colorectal diseases requiring surgery because of infection or contamination, pro-inflammatory factors characteristic of UC, anemia and malnutrition, and immunosuppression, including that induced by immunosuppressive medication. Patients with UC undergoing elective colectomy reportedly have significantly higher rates of SSI, especially OS-SSI (*5,6*). Araki *et al*. (*20*) have reported that poor general physical status is a significant independent risk factor for OS-SSI in adult patients with UC. In a comparison of colorectal surgery for colorectal cancer versus IBD in patients with clean-contaminated wounds, IBD was shown to be an independent risk factor for I-SSI (*6*).

In this study, surgery for UC was performed in one stage in 35 cases, two stages in 57 cases, and three stages in 44 cases. We found three-stage surgery to be a statistically significant independent predictor of SSI, likely because of risk factors such as dirty or contaminated surgery, creation of ileostomies, and the characteristically poor condition of patients requiring three-stage surgery compared with that of those requiring only one- or two- stage surgery.

We found that female sex is a predictor of SSI, as shown by two other studies (*17,21*) of colorectal surgery for adult patients. In contrast, Morikane *et al*. (*18*) found an association between male sex and increased risk of SSI in adult patients undergoing colorectal surgery. The reasons for SSI being more common in female than male patients in three of these four studies is unclear; however, Pedroso-Fernandez *et al*. (*17*) proposed in a personal communication that the procedure for extracting the resected tissue and size of incision may differ in female patients for cosmetic reasons.

Until ten years ago, high total PDN doses were administered before surgery and this was strongly associated with incidence of SSI in patients with UC, especially in Japanese institutes (*22-25*). Use of non-steroid drugs, including immunomodulators and anti-tumor necrosis factor, has recently been increasing in pediatric patients with a hopeful future, though corticosteroids remain the main drug for inducing remission of UC. If the medications used instead of PDN for treating UC have immunosuppressive effects that may lead to infectious complications, such drugs could increase the incidence of surgical complications. Thus far, several studies have concluded that there is no relationship between preoperatively administered medications and postoperative SSI in pediatric patients with UC (*25-29*). Regarding use of immunomodulators, preoperative exposure to thioprines or calcineurin inhibitors is reportedly not associated with increased postoperative complications in pediatric patients with UC who undergo colectomy (*27,29*). Patton *et al*. (*28*) found that preoperative IFX was not associated with postoperative complications in pediatric patients with UC. However, Kennedy *et al*. (*26*) reported that postoperative small bowel obstruction occurred more frequently in children who had been treated with IFX prior to proctocolectomy than in those who had not; however, this relationship is unclear. Because too few patients in our study had received IFX, our results did not allow us to draw a conclusion about any relationship between preoperative IFX administration and SSI occurrence.

This study has some limitations. Three-stage surgery was selected for pediatric patients with perforation, toxic megacolon, and IBD-unclassified whereas selection of one- or two-stage surgery was dependent on the policy and judgement within each surgical institute and the obtaining of informed consent for proposed procedures. Additionally, precautions taken to prevent SSI, surgical indications and procedures, and length and positions of incisions differed between the surgical institutes. Concerning the relationship between preoperatively administered medications and SSI, we did not monitor 6-thioguanine nucleotide concentrations in patients receiving AZA (*30*) or trough serum con/ss in those receiving TAC (*31*), CYA (*32*), or IFX (*33*) in this study; we therefore could not investigate the relationship between serum concentrations of drugs and postoperative infectious complications.

In conclusion, three-stage surgery is an independent predictor of development of SSI after surgery in pediatric patients with UC. In this study, preoperative medication, including 5-ASA, AZA, TAC, and CYA, did not affect the overall incidence of SSI, or that of I-SSI or OS-SSI. An immediate correct diagnosis, appropriate medical therapy without using high total doses of PDN, optimal timing of surgery, and avoidance of three-stage surgery may result in better surgical outcomes and quality of life.

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References

1. Ishige T, Tomomasa T, Takebayashi T, Asakura K, Watanabe M, Suzuki T, Miyazawa R, Arakawa H. Inflammatory bowel disease in children: Epidemiological analysis of the nationwide IBD registry in Japan. J Gastroenterol. 2010; 45:911-917.
2. Soon IS, deBruyn JC, Hubbard J, Wrobel I, Sauve R, Sigalet DL, Kaplan GG. Rising post-colectomy complications in children with ulcerative colitis despite stable colectomy rates in United States. J Crohns Colitis. 2014; 8:1417-1426.
3. Soon IS, Wrobel I, deBruyn JC, Sauve R, Sigalet DL, Kaplan BS, Proulx MC, Kaplan GG. Postoperative complications following colectomy for ulcerative colitis in

children. J Pediatr Gastroenterol Nutr. 2012; 54:763-768.
4. Knod JL, Holder M, Cortez AR, Martinez-Leo B, Kern P, Saeed S, Warner B, Dickie B, Falcone RA, von Allmen D, Frischer JS. Surgical outcomes, bowel habits and quality of life in young patients after ileoanal anastomosis for ulcerative colitis. J Pediatr Surg. 2016; 51:1246-1250.
5. Bhakta A, Tafen M, Glotzer O, Ata A, Chismark AD, Valerian BT, Stain SC, Lee EC. Increased Incidence of Surgical Site Infection in IBD Patients. Dis Colon Rectum. 2016; 59:316-322.
6. Uchino M, Ikeuchi H, Tsuchida T, Nakajima K, Tomita N, Takesue Y. Surgical site infection following surgery for inflammatory bowel disease in patients with clean-contaminated wounds. World J Surg. 2009; 33:1042-1048.
7. Øresland T, Bemelman WA, Sampietro GM, *et al*. European Crohn's and Colitis Organisation (ECCO). European evidence based consensus on surgery for ulcerative colitis. J Crohns Colitis. 2015; 9:4-25.
8. Mahadevan U, Loftus EV Jr, Tremaine WJ, Pemberton JH, Harmsen WS, Schleck CD, Zinsmeister AR, Sandborn WJ. Azathioprine or 6-mercaptopurine before colectomy for ulcerative colitis is not associated with increased postoperative complications. Inflamm Bowel Dis. 2002; 8:311-316.
9. Aberra FN, Lewis JD, Hass D, Rombeau JL, Osborne B, Lichtenstein GR. Corticosteroids and immunomodulators: Postoperative infectious complication risk in inflammatory bowel disease patients. Gastroenterology. 2003; 125:320-327.
10. Hosoi K, Ohtsuka Y, Fujii T, *et al*. The treatment with infliximab for pediatric Crohn's disease: Nationwide survey of Japan. J Gastroenterol Hepatol. 2017; 32:114-119.
11. IBD Working Group of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. Inflammatory bowel disease in children and adolescents: Recommendations for diagnosis – the Porto criteria. J Pediatr Gastroenterol Nutr. 2005; 41:1-7.
12. Turner D, Otley AR, Mack D, Hyams J, de Bruijne J, Uusoue K, Walters TD, Zachos M, Mamula P, Beaton DE, Steinhart AH, Griffiths AM. Development, validation, and evaluation of a Pediatric Ulcerative Colitis Activity Index: A prospective multicenter study. Gastroenterology. 2007; 133:423-432.
13. Hiwatashi N. The diagnostic criteria for determination of ulcerative colitis severity. In: The 1993 Annual Report of the Research Committee of Inflammatory Bowel Disease. Ministry of Health and Welfare of Japan, Tokyo, Japan, 1994; pp. 90-92.
14. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: Controversies, consensus, and implications. Gut. 2006; 55:749-53.
15. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999 Centers for Disease Control and Prevention (CDC) Hospital Infection Control Practices Advisory Committee. Am J Infect Control. 1999; 27:97-132.
16. Pendlimari R, Cima RR, Wolff BG, Pemberton JH, Huebner M. Diagnoses influence surgical site infections (SSI) in colorectal surgery: A must consideration for SSI reporting programs? J Am Coll Surg. 2012; 214:574-580.
17. Pedroso-Fernandez Y, Aguirre-Jaime A, Ramos MJ, Hernández M, Cuervo M, Bravo A, Carrillo A. Prediction of surgical site infection after colorectal surgery. Am J Infect Control. 2016; 1:450-454.
18. Morikane K, Honda H, Yamagishi T, Suzuki S, Aminaka M. Factors associated with surgical site infection in colorectal surgery: The Japan nosocomial infections surveillance. Infect Control Hosp Epidemiol. 2014; 35:660-666.
19. Segal CG, Waller DK, Tilley B, Piller L, Bilimoria K. An evaluation of differences in risk factors for individual types of surgical site infections after colon surgery. Surgery. 2014; 156:1253-1260.
20. Araki T, Okita Y, Uchino M, *et al*. Risk factors for surgical site infection in Japanese patients with ulcerative colitis: A multicenter prospective study. Surg Today. 2014; 44:1072-1078.
21. Blitzer DN, Davis JM, Ahmed N, Kuo YH, Kuo YL. Impact of procedure on the post-operative infection risk of patients after elective colon surgery. Surg Infect (Larchmt). 2014; 15:721-725.
22. Kusunoki M, Möeslein G, Shoji Y, Fujita S, Yanagi H, Sakanoue Y, Saito N, Utsunomiya J. Steroid complications in patients with ulcerative colitis. Dis Colon Rectum. 1992; 35:1003-1009.
23. Shinozaki M, Suzuki K, Sawada T, Tsuno N, Higuchi Y, Muto T. Steroid complications and surgery in intractable ulcerative colitis. J Gastroenterol. 1998; 33:196-200.
24. Uchida K, Araki T, Toiyama Y, Yoshiyama S, Inoue M, Ikeuchi H, Yanagi H, Miki C, Yamamura T, Kusunoki M. Preoperative steroid-related complications in Japanese pediatric patients with ulcerative colitis. Dis Colon Rectum. 2006; 49:74-79.
25. Markel TA, Lou DC, Pfefferkorn M, Scherer LR 3rd, West K, Rouse T, Engum S, Ladd A, Rescorla FJ, Billmire DF. Steroids and poor nutrition are associated with infectious wound complications in children undergoing first stage procedures for ulcerative colitis. Surgery. 2008; 144:540-545.
26. Kennedy R, Potter DD, Moir C, Zarroug AE, Faubion W, Tung J. Pediatric chronic ulcerative colitis: Does infliximab increase post-ileal pouch anal anastomosis complications? J Pediatr Surg. 2012; 47:199-203.
27. Schaufler C, Lerer T, Campbell B, Weiss R, Cohen J, Sayej W, Hyams J. Preoperative immunosuppression is not associated with increased postoperative complications following colectomy in children with colitis. J Pediatr Gastroenterol Nutr. 2012; 55:421-424.
28. Patton D, Gupta N, Wojcicki JM, Garnett EA, Nobuhara K, Heyman MB. Postoperative outcome of colectomy for pediatric patients with ulcerative colitis. J Pediatr Gastroenterol Nutr. 2010; 51:151-154.
29. Hait EJ, Bousvaros A, Schuman M, Shamberger RC, Lillehei CW. Pouch outcomes among children with ulcerative colitis treated with calcineurin inhibitors before ileal pouch anal anastomosis surgery. J Pediatr Surg. 2007; 42:31-34.
30. Ohtsuka Y, Arai K, Aoyagi Y, Fujii T, Yamakawa Y, Ohtani K, Ikuse T, Baba Y, Inage E, Kudo T, Suzuki R, Nagata S, Shimizu T. Monitoring 6-thioguanine nucleotide concentrations in Japanese children and adolescents with inflammatory bowel disease. J Gastroenterol Hepatol. 2010; 25:1626-1630.
31. Navas-López VM, Blasco Alonso J, Serrano Nieto MJ, Girón Fernández-Crehuet F, Argos Rodriguez MD, Sierra Salinas C. Oral tacrolimus for pediatric steroid-resistant ulcerative colitis. J Crohns Colitis. 2014; 8:64-69.
32. Benkov KJ, Rosh JR, Schwersenz AH, Janowitz HD,

LeLeiko NS. Cyclosporine as an alternative to surgery in children with inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 1994; 19:290-294.
33. Singh N, Rosenthal CJ, Melmed GY, Mirocha J, Farrior S, Callejas S, Tripuraneni B, Rabizadeh S, Dubinsky MC. Early infliximab trough levels are associated with persistent remission in pediatric patients with inflammatory bowel disease. Inflamm Bowel Dis. 2014; 20:1708-1713.

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Brief Report

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Dent disease: Same *CLCN5* mutation but different phenotypes in two brothers in China

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Summary **Dent disease is an X-linked recessive proximal tubular disorder that affects mostly male patients in childhood or early adult life, caused by mutations in *CLCN5* (Dent disease 1) or *OCRL* (Dent disease 2) genes, respectively. It presents mainly with hypercalciuria, low-molecular-weight proteinuria, nephrocalcinosis and progressive renal failure. We report here the same *CLCN5* mutation but different phenotypes in two Chinese brothers, and speculate on the possible reasons for the variability of the genotype-phenotype correlations.**

Keywords: Dent disease, *CLCN5*, phenotpye, China

1. Introduction

Dent disease (OMIM #300009) is a rare X-linked renal proximal tubulopathy clinically defined by low molecular weight proteinuria (LMWP) associated with hypercalciuria and/or its complications (nephrocalcinosis or nephrolithiasis) and progressive renal failure. Dent disease may also be associated with aminoaciduria, phosphaturia, glycosuria, uricosuria, kaliuresis, hematuria, impaired urinary acidification, and rickets or osteomalacia (*1,2*). According to the differences in phenotype, it is divided into two groups, Dent disease 1 (OMIM #300008), which is caused by mutations of the *CLCN5* genes located in chromosome Xp 11.22, and Dent disease 2 (OMIM#300555), which is caused by mutations in *OCRL* genes localized on chromosome Xq 25 (*3*). Overall, 60% of individuals with Dent disease are found to have a *CLCN5* mutation, 15% have an OCRL mutation, and in the remaining 25% a mutation cannot be identified (*4*). The prevalence of Dent disease is unknown and it may be underdiagnosed. Dent disease mainly affects males, whereas female carriers may show a milder phenotype (*5,6*). Patients are usually diagnosed in childhood or in young adult years. LMWP is the most consistent feature, occurring in 99% of affected male patients. Proteinuria is usually subnephrotic but may reach a nephrotic level (*2,7,8*).

Fanconi syndrome (FS) is a generalized dysfunction of the renal proximal tubules leading to excessive urinary wasting of amino acids, glucose, phosphate, uric acid, bicarbonate, and other solutes. The patients develop failure to thrive, polyuria, polydipsia, dehydration, and rickets in children, and osteoporosis and osteomalacia in adults. The patients also manifest renal salt wasting, hypokalemia, metabolic acidosis, hypercalciuria, and LMWP. The causes of FS are divided into three main categories; hereditary, acquired, and exogenous substances (*9,10*).

The effects of Dent disease 1 are variable both within and between affected families (*11*). It is well known that a *CLCN5* mutation could be a rare cause of inherited forms of induced Fanconi syndrome (*12-16*). Moreover, a considerable intra and between familial variability in disease severity has been observed and no genotype-phenotype correlation has been described (*11,17*).

We report here two brothers with the same *CLCN5* mutation but different phenotypes, the elder was consistent with Fanconi syndrome while the younger was consistent with Dent disease. We speculate on the possible reasons for the variability of genotype-phenotype correlations.

2. Materials and Methods

2.1. *Participants*

This work was carried out with human research ethics approval from the Peking University First Hospital

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and followed the guidelines of the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008. All patients and their family members gave their consent for inclusion in this study.

2.2. *Methods*

Genomic DNA was extracted from the peripheral blood of the patients and their parents. Genetic analysis was performed in the Genetics laboratory of Biotechnology companies in China, using "the hereditary renal tubular disease" which covers about 50 genes strongly correlated with this disorder.

The expected segregation of putative mutations was confirmed in families, whenever possible, and their absence was confirmed in SNPs databases of common benign variants (*http://exac.broadinstitute.org/*, *http://www.ncbi.nlm.nih.gov/projects/SNP/*), and *http://www.1000genomes.org/*. Human Splicing Finder (*http://www.umd.be/HSF/*) and Mutation tasting (*http://mutationtaster.org/*) were used for analysis on splicing mutations and other mutations, respectively.

2.3. *Case presentation*

Case 1: A 10.9-years boy (Birth date 29/10, 2004) was hospitalized to our hospital (14/08, 2015) with a complaint of proteinuria for 5.3 years, polydipsia and polyuria for 4 years. He was found to present with proteinuria (78 mg/kg) but without edema, hypoalbuminemia and hypercholesterolemia 5.3 years ago (28/05, 2010), renal biopsy was done in a local hospital. Immunofluorescence (IM) was negative. The light microscope (LM) showed 13 glomeruli with podocyte swelling, minimal proliferation of mescenteric cells and matrix, segmental glomeruli base membrane thickening, tubular atrophy (15%) and interstitial fibrosis. Electron microscopy (EM) found no obvious abnormal podocytes, except for tubular atrophy and interstitial fibrosis. With a presumptive diagnosis of minimal change disease (MCD), he was treated as nephrotic syndrome with a full steroid dose for 4 weeks, then combined with cyclophosphamide (CTX) for 7 doses (one dose per month), but no apparent change of his proteinuria (55~83 mg/kg) was seen. Further investigation revealed a normal creatine (29.4 umol/L), an estimated glomerular filtration rate (eGFR) of 125 mL/min/1.73 m^2, serum albumin level 46 mg/L, cholesterol 4.18 mmol/L, autoimmune profile including C3 and C4 compliments within normal range; Urine protein 64 mg/kg, urine α1-microglobin 161 mg/L, urine microalbumin 64 mg/L, LMWP 61.3%, hypophosphatemia (0.62~0.89 mmol/L), hypokalemia (2.9~3.2 mmol/L), acidosis (HCO_3- 15.6~20.7 mmol/L), hypomagnesemia (0.75~0.84 mmol/L), hypochloremia (91.8~95.4 mmol/L), aminoaciduria and rickets, but no hypercalciuria (0.05~0.08 mmol/kg/24 h). Renal ultra-sonography showed no obvious abnormality. He was diagnosed with Fanconi syndrome, treated initially with oral potassium citrate (2 mmol/kg/d) and sodium bicarbonate (3 mmol/kg/d), but the therapy was discontinued and no regular monitoring was carried out. Polydipsia and polyuria appeared 4 years ago, and intermittent apyretic tetanus appeared two years ago.

At admission, his height was 110 cm, weight was 21 kg, both below the 1st centile. Investigation in our hospital revealed a normal creatine (54.2 umol/L) and estimated glomerular filtration rate (eGFR) of 102 mL/min/1.73 m^2, serum albumin level 46 mg/L, cholesterol 4.12 mmol/L, urine protein 44~94 mg/kg, urine α1-microglobin 161~186 mg/L, urine microalbumin 70~124 mg/L, LMWP 63.7%, hypokalemia (1.76 mmol/L), hypomagnesemia (0.76 mmol/L), hypophosphatemia (0.57 mmol/L), hypochloremia (91.3 mmol/L), hyposthenuria (specific gravity 1.005~1.008), aminoaciduria and rickets, but without acidosis (HCO_3- 23.6 mmol/L) and still no hypercalciuria (0.11 mmol/kg/24h). Renal ultra-sonography showed nephrocalcinosis. He fulfilled the criteria for Fanconi syndrome. Because his brother also had low-molecular-weight proteinuria (see Case 2), hereditary renal tubular disease was suspected. See Table 1, 2, and 3.

Case 2: A 2.3-years boy (Birth date 13/04, 2013) was hospitalized in our hospital (17/08, 2015) with a complaint of proteinuria for 8 months. He was found to present with proteinuria (2+ ~ 3+) but without edema, hypoalbuminemia and hypercholesterolemia about 8 months ago (28/12, 2014). There was no proteinuria change and other signs after observation for 8 months.

At admission, his height was 88 cm, weight was 12 kg. Investigation in our hospital revealed a normal creatine (25.8 umol/L) and estimated glomerular filtration rate (eGFR) of 116 mL/min/1.73 m^2, serum albumin level 50 mg/L, cholesterol 3.26 mmol/L, urine protein 85~98 mg/kg, urine α1-microglobin 413~708 mg/L, urine microalbumin 318~470 mg/L, LMWP 56%, hypercalciuria (0.23~0.25 mmol/kg/24 h, urine calcium to creatine = 0.26 g/g) and aminoaciduria, but without any other electrolyte disturbances and rickets. Renal ultra-sonography showed no abnormality. He was suspected for Dent disease, and also hereditary renal tubular disease same as his elder brother (see Case 1). See Table 1, 2, and 3.

3. Results and Discussion

Our two brothers both presented with a nephrotic level of LMWP and aminoaciduria, the elder brother also had polydipsia, polyuria, nephrocalcinosis, hypophosphatemia, hypokalemia, acidosis, hyposthenuria and rickets but without hypercalciuria, while the younger brother also had hypercalciuria but without other symptoms, they fulfilled the diagnostic criteria of Fanconi syndrome and Dent disease, respectively. Genetic analysis showed they both carried

Table 1. General information of the two brothers

Items	Case 1	Case 2
Gender	Male	Male
Onset age	5.6 years	1.7 years
Diagnosis age	10.9 years	2.3 years
Body weight	21 kg	12 kg
Body height	110 cm	88 cm
Chief complaint	Proteinuria, polydipsia and polyuria	Proteinuria

Table 2. Clinical characters of the two brothers

Items	Case 1	Case 2
Proteinuria	Yes	Yes
Hypercalciuria	No	Yes
Hematuria	No	No
Aminoaciduria	Yes	Yes
Nephrocalcinosis	Yes	No
Renal function	Normal	Normal
Renal biopsy	MCD	Not Done
CLCN5 mutation	c.731C>T, p.S244L	c.731C>T, p.S244L
Others	Hypophosphatemia, hypokalemia, acidosis, hyposthenuria and rickets	None

Table 3. Laboratory data of the two brothers

Items	Case 1	Case 2
Alb (g/L)	50 ± 2	50 ± 1
UPE (mg/kg/24h)	68 ± 16	99 ± 10
α1MG (mg/L)	174 ± 13	535 ± 98
MA (mg/L)	101 ± 21	394 ± 86
α1MG/MA	1.7 ± 0.6	1.4 ± 0.1
LMWP (%)	52.3 ± 9.1	55.9 ± 4.5
UC/Ucr	0.11 ± 0.05	0.26 ± 0.08
UCE (mmol/kg/24h)	0.07 ± 0.02	0.25 ± 0.02

Alb: Albumin; UPE: Urinary protein excretion; α1MG: α1-microglobinuria; MA: Microalbuminuria; LMWP: Low molecular weight proteinuria; UC: Urine calcium; Ucr: Urine creatinine; UCE: Urine calcium excretion.

the homozygous mutation c.731C>T (p.S244L) in exon 7 in the *CLCN5* gene, transmitted from their mother. The mother carried the same mutation but father was normal. Mother showed no proteinuria, hematuria, hypercalciuria, nephrolithiasis or nephrocalcinosis, and her renal function was normal. No variations were found in inherited Fanconi syndrome related genes in both cases, such as *OCRL1*, *COQ2*, *CNTS*, *GALT1*, *ALDOB*, *SLC2A2*, *SLC5A2*, *ATP7B*, or *NDUFAF6*. The mutation has been reported (*20,21*).

Case 1 was treated continuously with potassium citrate (2~3 mmol/kg/d) and a mixture of phosphate (disodium hydrogen phosphate and sodium dihydrogen phosphate, 3~5 mmol/kg/d), with a follow up of 10 months, his electrolyte disturbance recovered and remained normal. Case 2 was treated with hydrochlorothiazide (1 mg/kg/d) and potassium citrate (2~3 mmol/kg/d), with a follow up of 10 months, his urine calcium decreased and remained 0.08~0.09 mmol/kg/d, but with no change of urine protein (58~89 mg/kg/d).

Fanconi syndrome represents a generalized dysfunction of the proximal tubular resorption of glucose, phosphate, bicarbonate and amino acids in the kidney. Dent disease is one form of inherited renal Fanconi syndrome (*14*). It was confusing why the same *CLCN5* mutation in our two brothers induced different phenotypes.

First, the course of disease maybe had some effects. The elder brother was 5.6 years old while the younger brother was only 1.7 years old when proteinuria was found. As a genetic disease, the LMWP of Dent disease should be presented after birth, it is well known that persistent proteinuria itself is a damaging factor for the renal system. For example, polydipsia, polyuria and hyposthenuria appeared in the younger brother a year after the finding of proteinuria. It was similar to another report of Dent disease in China. Jian *et al*. reported four cases of Dent disease, three of which were diagnosed Fanconi syndrome aged 12, 10 and 8 years old, respectively (*15*). We suggested that the renal tubulopathy might be worsening as the course progresses, but this needs further observation, especially for our younger brother. However, Hodgin *et al*. also reported two Dent disease brothers both presenting as partial Fanconi syndrome, 6 years old and 4 years old, respectively (*12*), which suggested excepting the course of disease, there might be other factors influencing the phenotype of Dent disease.

Second, the genotype maybe had some effects on phenotype. As a rare hereditary renal tubular disorder, there are no genotype-phenotype correlations because various mutations are associated with different clinical phenotypes, even within the same family (*22*). The mutation c.731C>T (p.S244L) has been previously reported, the missense mutation occurs within transmembrane domains, and none of the cases presented

as Fanconi syndrome (*20,21*). Our two brothers have the same mutation but presented a different phenotype, one Dent disease while the other Fanconi syndrome. There was no mutation found on other Fanconi syndrome related genes in the elder brother, and it seemed that the genotype could not explain the variance. However, because the younger brother is perhaps too young, his phenotype should be monitored to see if any changes take place when he reaches his elder brother's age. A considerable intrafamilial variability in disease severity has been observed. Dent disease patients have a variable phenotype ranging from renal Fanconi syndrome with or without rickets to the association of LMWP and hypercalciuria (with or without nephrocalcinosis or nephrolithiasis) (*11*). The severity of renal disease varies greatly among individuals within a family, and there is no relationship between the degree of LMW proteinuria and the severity of chronic kidney disease (CKD) or hypercalciuria (*17*).

Third, the renal pathology may have some effects on the phenotype. Renal biopsy of our elder brother showed no obvious abnormality on podocytes, except tubular atrophy (15%) and interstitial fibrosis. Also, he had no history of drugs used which might damage the renal tubule and interstitium. However, it was a pity that our younger brother did not have a renal biopsy, and therefore the severity of his tubular atrophy was unknown. There was a report on renal pathology and renal function, which showed that higher percentages of globally sclerotic glomeruli, foot process effacement, and interstitial inflammation were associated with a lower estimate of glomerular filtration rate (eGFR) at biopsy, whereas foot process effacement was associated with steeper annual eGFR decline (*23*). However, no correlation between phenotype and tubular atrophy has been reported in Dent disease.

Finally, how *CLCN5* mutations can cause a lot of clinical manifestations is not very clear. As a vesicular chloride/proton exchanger, it has been proposed that CLC-5 allows maximal acidification of the endosome by providing an electrical shunt to dissipate the positive charge created by the proton-ATPase pump. LMWP are freely filtered by the glomerulus and reabsorbed by the proximal tubular epithelium. Thus, the LMWP seen with CLC-5 dysfunction, results from impaired proximal tubular endocytosis. Consequent abnormalities in membrane recycling could explain other defects in proximal tubular function such as phosphaturia, aminoaciduria, and glycosuria. However, the mechanisms of hypercalciuria and nephrocalcinosis remain unclear. It is known that in patients with proven *CLCN5* mutations, as many as 30% do not demonstrate hypercalciuria on repeated urine analysis, even though some of these do exhibit nephrocalcinosis, such as our elder brother, who had nephrocalcinosis but without hypercalciuria (*24*). It is hypothesized that the functional loss of ClC-5 is essentially reflected by manifestations of proximal tubular dysfunction and may contribute to the genesis of kidney stones in different ways, reflecting its involvement in specific tubular functions (*25*).

In conclusion, we report two brothers in China with the same *CLCN5* mutation of S244L but different phenotypes, one presented with a nephrotic level of LMWP, aminoaciduria, polydipsia, polyuria, nephrocalcinosis, hypophosphatemia, hypokalemia, acidosis, hyposthenuria and rickets but without hypercalciuria, while the other presented with a nephrotic level of LMWP, hypercalciuria and aminoaciduria, they fulfilled the diagnosis criteria of Fanconi syndrome and Dent disease, respectively. The possible reasons for the variability of genotype-phenotype correlations remain unclear.

References

1. Wrong OM, Norden AG, Feest TG. Dent's disease; a familial proximal renal tubular syndrome with low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease, progressive renal failure and a marked male predominance. QJM. 1994; 87:473-493.
2. Frymoyer PA, Scheinman SJ, Dunham PB, Jones DB, Hueber P, Schroeder ET. X-linked recessive nephrolithiasis with renal failure. N Engl J Med. 1991; 325:681-686.
3. Szczepanska M, Zaniew M, Recker F, Mizerska-Wasiak M, Zaluska-Lesniewska I, Kilis-Pstrusinska K, Adamczyk P, Zawadzki J, Pawlaczyk K, Ludwig M, Sikora P. Dent disease in children: Diagnostic and therapeutic considerations. Clin Nephrol. 2015; 84:222-230.
4. Anglani F, D'Angelo A, Bertizzolo LM, Tosetto E, Ceol M, Cremasco D, Bonfante L, Addis MA, Del Prete D, Dent Disease Italian N. Nephrolithiasis, kidney failure and bone disorders in Dent disease patients with and without CLCN5 mutations. Springerplus. 2015; 4:492.
5. Reinhart SC, Norden AG, Lapsley M, Thakker RV, Pang J, Moses AM, Frymoyer PA, Favus MJ, Hoepner JA, Scheinman SJ. Characterization of carrier females and affected males with X-linked recessive nephrolithiasis. J Am Soc Nephrol. 1995; 5:1451-1461.
6. Wu F, Reed AA, Williams SE, *et al*. Mutational analysis of CLC-5, cofilin and CLC-4 in patients with Dent's disease. Nephron Physiol. 2009; 112:p53-p62.
7. Frishberg Y, Dinour D, Belostotsky R, Becker-Cohen R, Rinat C, Feinstein S, Navon-Elkan P, Ben-Shalom E. Dent's disease manifesting as focal glomerulosclerosis: Is it the tip of the iceberg? Pediatr Nephrol. 2009; 24:2369-2373.
8. Valina MR, Larsen CP, Kanosky S, Suchy SF, Nield LS, Onder AM. A novel CLCN5 mutation in a boy with asymptomatic proteinuria and focal global glomerulosclerosis. Clin Nephrol. 2013; 80:377-384.
9. Maher OM, Moonat HR. Fanconi Anemia and fanconi syndrome: Time to correct the misnomers. J Pediatr Hematol Oncol. 2016; 38:585.
10. Klootwijk ED, Reichold M, Unwin RJ, Kleta R, Warth R, Bockenhauer D. Renal Fanconi syndrome: Taking a proximal look at the nephron. Nephrol Dial Transplant. 2015; 30:1456-1460.
11. Mansour-Hendili L, Blanchard A, Le Pottier N, *et al*.

Mutation update of the *CLCN5* gene responsible for dent disease 1. Hum Mutat. 2015; 36:743-752.
12. Hodgin JB, Corey HE, Kaplan BS, D'Agati VD. Dent disease presenting as partial Fanconi syndrome and hypercalciuria. Kidney Int. 2008; 73:1320-1323.
13. Pook MA, Wrong O, Wooding C, Norden AG, Feest TG, Thakker RV. Dent's disease, a renal Fanconi syndrome with nephrocalcinosis and kidney stones, is associated with a microdeletion involving DXS255 and maps to Xp11.22. Hum Mol Genet. 1993; 2:2129-2134.
14. Solano A, Lew SQ, Ing TS. Dent-Wrong disease and other rare causes of the Fanconi syndrome. Clin Kidney J. 7:344-347.
15. Jian S, Wei M, He YY, Wang W, Kang YL, Sun ZX. Clinical and genetic analysis of Dent disease in 4 Chinese children. Zhongguo dang dai er ke za zhi. 2015; 17:1261-1266. (in Chinese)
16. Bhardwaj S, Thergaonkar R, Sinha A, Hari P, Hi C, Bagga A. Phenotype of dent disease in a cohort of Indian children. Indian Pediatr. 2016; 53:977-982.
17. Wong W, Poke G, Stack M, Kara T, Prestidge C, Flintoff K. Phenotypic variability of Dent disease in a large New Zealand kindred. Pediatr Nephrol. 2017; 32:365-369.
18. Claverie-Martin F, Ramos-Trujillo E, Garcia-Nieto V. Dent's disease: Clinical features and molecular basis. Pediatr Nephrol. 2011; 26:693-704.
19. Devuyst O. Dent's disease: Chloride-proton exchange controls proximal tubule endocytosis. Nephrol Dial Transplant. 2010; 25:3832-3835.
20. Lloyd SE, Pearce SH, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ, Harding B, Bolino A, Devoto M, Goodyer P, Rigden SP, Wrong O, Jentsch TJ, Craig IW, Thakker RV. A common molecular basis for three inherited kidney stone diseases. Nature. 1996; 379:445-449.
21. Wu F, Reed AA, Williams SE, *et al*. Mutational analysis of CLC-5, cofilin and CLC-4 in patients with Dent's disease. Nephron Physiol. 2009; 112:p53-62.
22. Ludwig M, Utsch B, Monnens LA. Recent advances in understanding the clinical and genetic heterogeneity of Dent's disease. Nephrol Dial Transplant. 2006; 21:2708-2717.
23. Wang X, Anglani F, Beara-Lasic L, *et al*. Glomerular pathology in dent disease and its association with kidney function. Clin J Am Soc Nephrol. 2016; 11:2168-2176.
24. Ludwig M, Utsch B, Balluch B, Fründ S, Kuwertz-Bröking E, Bökenkamp A. Hypercalciuria in patients with CLCN5 mutations. Pediatr Nephrol. 2006; 21:1241-1250.
25. Devuyst O, Luciani A. Chloride transporters and receptor-mediated endocytosis in the renal proximal tubule. J Physiol. 2015; 593:4151-4164.

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Brief Report

Diagnosis of Morquio-A patients in Mexico: How far are we from prompt diagnosis?

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Summary **Mucopolysaccharidosis IV A, better known as Morquio-A syndrome, is a rare condition with severe skeletal and multiorgan involvement. Sometimes is not easy to differentiate from other skeletal dysplasias. Prior to definitive diagnosis, patients have been delayed or misdiagnosis due to lack of knowledge of local physicians about this disease. The aim of this study is to compare the age of onset of clinical manifestations, age of diagnosis, as seen by the parent or primary caregiver and compare this age with other population reports worldwide. Self-administered questionnaires were conducted to the primary caregiver of confirmed patients, collecting information about the onset of symptoms, age, previous diagnoses and biological variables (age, gender, sex). Data from 50 patients, 23 men and 27 women was obtained. Mean age at definitive diagnosis was 5.6 years, age at onset of signs or symptoms was 4.14 years starting with pigeon chest deformity, valgus knees at 4.5 years, stiff hands and increasing mobility of wrists to the 5.8 years, followed by limitation to lift shoulders to 7.1 years. In 78% of patients the diagnosis was by a geneticist. First and subsequent observed clinical changes were orthopedic, starting as early as 4.4 years as noted by parents. Rise of suspicious may delay 16 months' average to definitive diagnosis based on other multi-systemic findings. The most frequent specialist aid in diagnosis is a clinical geneticist followed by orthopedic surgeon. The diagnosis of Morquio-A disease in Mexico is as early as reports from other centers.**

Keywords: Morquio-A syndrome, diagnosis, Mexico, delay diagnosis, height, dwarphism, skeletal dysplasia, orphan disease

1. Introduction

Mucopolysaccharidoses IV A, better known as Morquio-A syndrome, is a rare disease that represents one of the multi-organic and progressive diseases with the most severe musculoskeletal system involvement (*1,2*). It has a wide clinical spectrum, that may not be easy to recognize from other non-systemic skeletal dysplasias, which represents a challenge in diagnosis in young patients (*3,4*). This condition has progressive multisystem dysfunction and impaired functional capacity. Skeletal abnormalities may be first line clinical findings to aid in diagnosis (*1,5,6*).

Since Morquio-A patients have not primary neurological involvement (*1,2,4,5*), an early detection, as well as early treatment could prevent the severe functional impairment seen on these patients, improving outcomes and extending their autonomy (*2,7-9*). Generally, before definitive diagnosis, patients are delayed or misdiagnosed due the lack of knowledge about the disease (*10,11*).

International series describe a widespread amount of skeletal manifestations such as short trunk and neck, hip and knee dysplasia, spinal abnormalities, pigeon chest, among other less frequent characteristics (*12,13*). Early onset findings are kyphosis, scoliosis, growth retardation and altered gait (*4,14*). The vast majority of papers detail clinical features but only few mention the age of onset.

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Knowing average time of features may help raise clinical suspicion of the disorder at early time (*5,15,16*).

We made this research trying to compare age of diagnosis versus age of onset of evident clinical signs as observed from the parents of Morquio-A patients in Mexico. To describe time delay in diagnosis since the first manifestation until definite diagnosis. This work may help guide the intervention in improving suspicion and early detection of Morquio-A syndrome in our population as well as in other similar Hispanic communities.

2. Materials and Methods

During the first Mexican Morquio-A meeting, a self-administered questionnaire was given to caregivers and patients with this syndrome. All of them have had diagnosis confirmation through determination of deficient enzymatic activity in leukocytes or fibroblasts. The patients were enrolled by accepting an informed consent and agreement of confidentiality. They were all informed about the reaching of the present study.

The questionnaire consisted in open questions about biological variables (age, gender, height) as well as age at diagnosis, concomitant and familial diseases. Questions about first symptoms and clinical features of the disease were dichotomic in order to avoid to forget any major or minor joint, followed to blank spaces to write down kind of alteration and the age it was noticed. Other questions referred with time of suspicion, health care provider in charge of suspicious and age at diagnosis were open blank spaces.

All data were collect in electronic datasheets to group similar answers and findings. All answers and results are described and analyzed with measures of central tendency.

3. Results and Discussion

We received full answered questionnaires from 50 patients or first relatives, all of them had enzymatic activity confirmation of Morquio-A syndrome; 23 were of male gender (46%) and 27 females (54%). Phenotypically all correspond to non-attenuated form of the disease. Mutational analyses of all patients were not possible, so it was not included on this study.

3.1. *Height and weight*

Mean age of the patients in present study was 15.3 years (range 3.1 to 41 years). The mean height of patients studied is 99 cm (ranging from 60 to 140 cm; n = 47), and mean weight of 20.4 kg (10 to 49 kg; n = 48).

Excluding the younger patients, the final height of adult Morquio-A patients is 87 cm (94 to 125 cm) and final adult weight of 24.5 kg (10 to 49 kg) with a body mass index average of 22.08 (range 10.0 to 34.02 kg/m^2). This data is only from patients older than 18 years and excluding both the tallest and shortest patients (n = 15) (Figure 1).

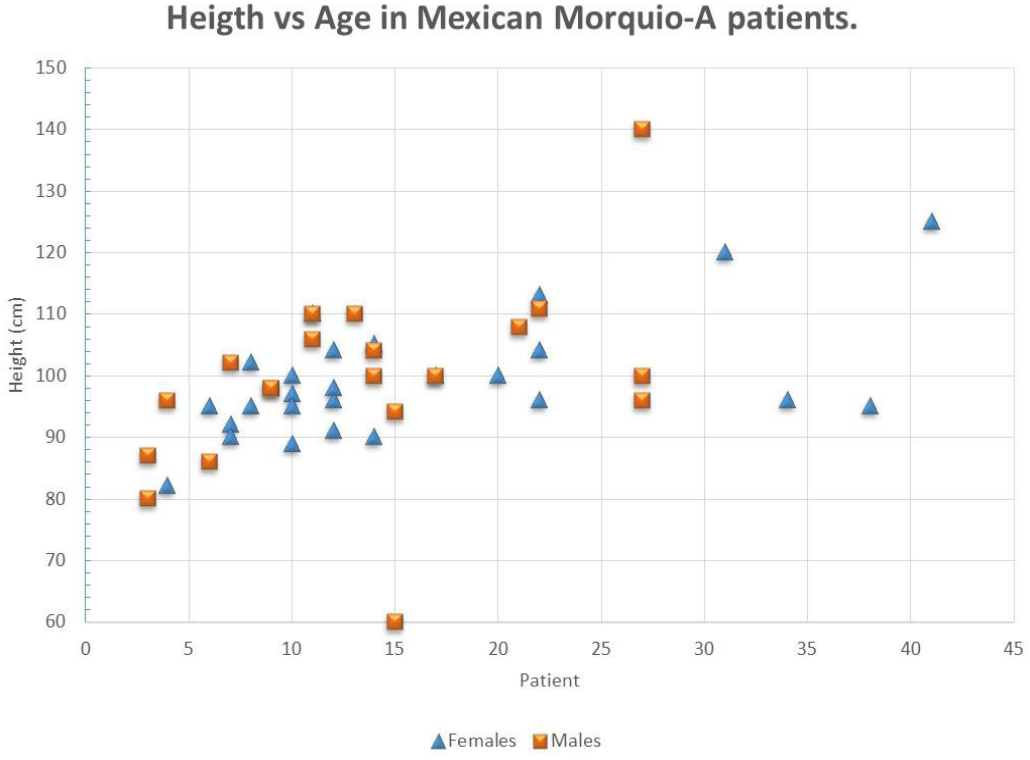

Figure 1. Height according age in Morquio-A patients. Notice all of them are between 90 and 110 cm. Most patients reach final height during second decade of life.

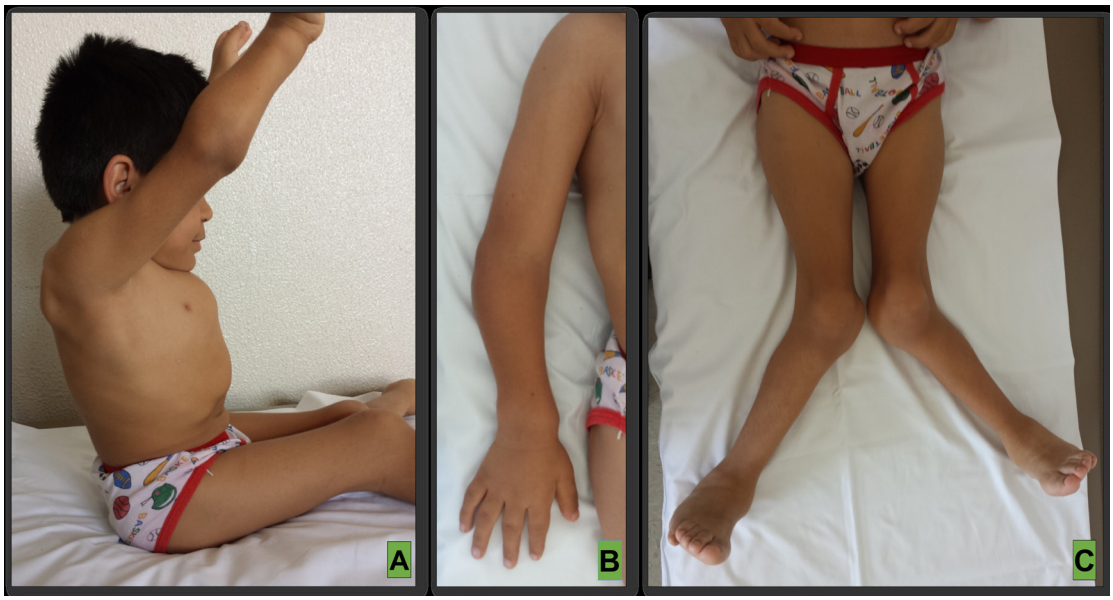

Figure 2. Clinical features of Morquio-A patients. (A), Progressive inability to raise arms upperhead and full extend elbows, pigeon chest and relative short neck; **(B)**, Wrist, elbow and hand deformations; **(C)**, Severe genu valgus. Personal archive files. Patient and guardian consent was obtained prior attachment of present clinical images.

3.2. *Definitive diagnosis*

Mean age at definite diagnosis was 5.6 years, ranging from 1 month to 15 years in all patients in this cohort (*n* = 50). Eighteen patients had a positive familiar history of Morquio-A syndrome (9 siblings and 9 cousins or other relatives). In patients with brothers affected with Morquio-A, the diagnosis was made at mean 5.5 years old (from one month to 15 years); and in patients with relatives other than sibs, diagnosis was made at mean 5.8 years (from 1 year to 15 years). In the remain (*n* = 32) patients without family history, diagnosis was made between 18 months and 14 years (mean 4.6 years).

For earliest diagnosis, two patients were diagnosed before one year of life: at age of one and nine months, respectively. Both had one sibling affected as a source of clinical suspicion.

Ten patients had different previous misdiagnoses before Morquio-A confirmation: among them skeletal dysplasia (*n* = 4), achondroplasia (*n* = 2), Ehlers-Danlos syndrome (*n* = 1), Sotos syndrome (*n* = 1), scoliosis (*n* = 1) and chronic bronchitis (*n* = 1). Eleven patients did not respond (22%) and more than half of the patients did not have a previous presumptive diagnosis (58%, *n* = 29) (Figure 2).

Definitive diagnosis of Morquio-A was achieved by a genetic specialist in 78% of patients (*n* = 39), 8% by an orthopedic surgeon (*n* = 4), by the neurologist (*n* = 1) and by endocrinologist (*n* = 1). Five patients (10%) did not remember the doctor who gave them the accurate diagnosis. Most of the patients (28%, *n* = 14) were not referred for assessment by any specialist, 32% (*n* = 16) did not answer and 40% (*n* = 20) had a referral for specialized assessment from pediatrics, physical therapist or family doctor. We did not have the mutational analysis of these patients, so clinico-mutational correlation was not possible to achieve.

3.3. *Clinical manifestations*

First clinical syndromic manifestation observed by parents and patients was progressive deformity in the chest (pigeon chest) starting from 8 months to 9 years (mean of 4.14 years; *n* = 34), followed by valgus knees between 1.6 and 13 years (mean 4.5 years; *n* = 37). Progressive deformity, weakness or functional limitation in wrists ranged from 1 to 13 years (average 5.8 years; *n* = 29), limitation or progressive inability to raise overhead shoulders was noticed since the 2 until 20 years (mean 7.1 years; *n* = 16).

Before a known accurate diagnosis, seven patients had surgical procedure history (14%), three for umbilical hernia repair, two for neurosurgical stabilization in cervical spine, one for ear nose and throat and one for orthopedic knee procedure. Other 19 patients had surgery after the Morquio-A diagnosis. The remaining 24 patients (48%) had no history of surgery.

Since this syndrome has not any primary neurological involvement (*17*), the earlier abnormal signs in our population were seen around 4 years old and described as pigeon chest or thoracic deformities (including triangular cifosis, widened chest and thoracic asymmetry), however this is not corresponding with Bhattacharya, who describes this first manifestation evident as early as 6 to 12 months (*11*).

The second relevant finding to make parents search for medical advice was mayor joint involvement as seen

in knee valgus and limitation to lift shoulders upper head and finally, small joint affection with wrist deformity and functional worsen hand function towards 5 or 6 years.

All patients in this population belong to the so-called severe phenotype, with all similar physical features that make an early diagnosis possible. unlike patients with the attenuated phenotype, in whom the diagnosis is more difficult and thus delayed.

None of the parents mentioned as an early sign the growth delay, although this is showed in all patients since the 4 years old (*18*), but as shown on the record, this was the parent or patient perspective, not the medical course, and all patients showed short stature at mature age.

The most evident clinical features of this syndrome are orthopedic type (*14*), nonetheless in only one patient the diagnosis was suspected due a non-skeletal feature (chronic bronchitis).

Diagnosis was made almost one year earlier on patients without family history of the disease, compared to those with positive familiar history (4.6 years *vs*. 5.5 years, respectively). We don't have a clear explanation for this, but the hypothesis is that similar phenotype observed among family members is responsible for not raising suspect of the syndrome in affected individuals.

These early signs found in our population, are considered the most common in all population reports, regardless of the type of mutation of the patient, and this is consistent with world literature (*19*). All these Hispanic patients share ethnic characteristics which may be regionally oriented than other populations around the globe. Is the need to early diagnose these patients in order to avoid clinical deterioration and worst surgery outcomes (*20*).

In conclusion, according to our results, the main suspected data for this disease are of the orthopedic type, drawing attention before the first four years, with thoracic deformity and knee valgus, followed for limitation to raise the arms above the shoulder level. Any of these alterations will merit assessment by the physician, who needs to have the suspected diagnosis or reference to the specialist to assist in the earliest detection. We consider that diagnosis of Morquio-A syndrome in Mexico is as early in childhood as in other world centers. Further studies are needed to determine the most frequent errors as well as delays in diagnosis, which help diagnose patients and prevent complications arising from delayed treatment.

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References

1. Harmatz P, Mengel KE, Giugliani R, *et al*. The Morquio-A: Clinical Assessment Program: Baseline Results Illustrating Progressive, Multisystemic Clinical Impairments in Morquio-A Subjects. Mol Genet Metab. 2013; 109:54-61.
2. Hendriksz CJ, Berger KI, Giugliani R, Harmatz P, Kampmann C, Mackenzie WG, Raiman J, Villarreal MS, Savarirayan R. International Guidelines for the Management and Treatment of Morquio-A Syndrome. Am J Med Genet A. 2015; 167:11-25.
3. Rush ET. Atypical Presentation of Mucopolysaccharidosis Type IVA. Mol Genet Metab Rep. 2016; 8:8-12.
4. Wood TC, Harvey K, Beck M, *et al*. Diagnosing Mucopolysaccharidosis IVA. J Inherit Metab Dis. 2013; 36:293-307.
5. Hendriksz CJ, Harmatz P, Beck M, Jones S, Wood T, Lachman R, Gravance CG, Orii T, Tomatsu S. Review of clinical presentation and diagnosis of mucopolysaccharidosis IVA. Mol Genet Metab. 2013; 110:54-64.
6. Clarke LA, Harmatz P, Fong EW. Implementing Evidence-Driven Individualized Treatment Plans within Morquio A Syndrome. Mol Genet Metab. 2016; 117:217.
7. Regier DS, Tanpaiboon P. Role of Elosulfase Alfa in Mucopolysaccharidosis IV A. Appl Clin Genet. 2016; 9:67-74.
8. Lavery C, Hendriksz C. Mortality in Patients with Morquio Syndrome A. JIMD Reports. 2015; 15:59-66.
9. Tomatsu S, Sawamoto K, Alméciga-Díaz CJ, *et al*. Impact of Enzyme Replacement Therapy and Hematopoietic Stem Cell Transplantation in Patients with Morquio A Syndrome. Drug Des Devel Ther. 2015; 9:1937-1953.
10. Solanki GA, Martin KW, Theroux MC, Lampe C, White KK, Shediac R, Lampe CG, Beck M, Mackenzie WG, Hendriksz CJ, Harmatz PR. Spinal involvement in mucopolysaccharidosis IVA (Morquio-Brailsford or Morquio A syndrome): Presentation, diagnosis and management. J Inher Metab Dis. 2013; 36:339-355.
11. Bhattacharya K, Balasubramaniam S, Choy YS, *et al*. Overcoming the Barriers to Diagnosis of Morquio A Syndrome. Orphanet J Rare Dis. 2014; 9:1-11.
12. Yabe H, Tanaka A, Chinen Y, Kato S, Sawamoto K, Yasuda E, Shintaku H, Suzuki Y, Orii T, Tomatsu S. Hematopoietic stem cell transplantation for Morquio A syndrome. Mol Genet Metab. 2016; 117:84-94.
13. Wang Z, Zhang W, Wang Y, Meng Y, Su L, Shi H, Huang S. Mucopolysaccharidosis IVA Mutations in Chinese Patients: 16 Novel Mutations. J Hum. Genet. 2010; 55:534-540.
14. Yasuda E, Fushimi K, Suzuki Y, *et al*. Pathogenesis of Morquio A syndrome: An autopsied case reveals systemic storage disorder. Mol Genet Metab. 2013; 109:301-311.
15. Suarez-Guerrero JL, Gómez HP, Arias FJ, Contreras-García GA. Mucopolysaccharidosis: Clinical features, diagnosis and management. Rev chil Pediatr. 2015; 87:295-304.
16. Montaño AM, Tomatsu S, Gottesman GS, Smith M, Orii T. International Morquio A Registry: Clinical manifestation and natural course of Morquio A disease. J Inherit Metab Dis. 2007; 30:165-174.
17. Davison JE, Kearney S, Horton J, Foster K, Peet AC, Hendriksz CJ. Intellectual and neurological functioning

in Morquio syndrome (MPS IV A) J Inherit Metab Dis. 2013; 36:323-328.
18. Montaño AM, Tomatsu S, Brusius A, Smith M, Orii T. Growth charts for patients affected with Morquio A disease. Am J Med Genet A. 2008;146A:1286-1295.
19. Lachman RS, Burton BK, Clarke LA, Hoffinger S, Ikegawa S, Jin DK, Kano H, Kim OH, Lampe C, Mendelsohn NJ, Shediac R, Tanpaiboon P, White KK. Mucopolysaccharidosis IVA (Morquio A syndrome) and VI (Maroteaux-Lamy syndrome): Under-recognized and challenging to diagnose. Skeletal Radiol. 2014; 43:359-369.
20. Colmenares-Bonilla D, Vasconcelos-Martinez M, Guerra-Jasso J, Ocampo-Perez L. Guided growth may not be the best option for knee valgus deformity in adolescent patients with Morquio-A. Musculoskelet Surg. 2016; DOI: 10.1007/s12306-016-0441-0

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Case Report

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A fatal case of herpes simplex virus hepatitis in a pregnant patient

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Summary **We present a middle aged pregnant woman who developed signs and symptoms of acute liver failure and was found to have herpes simplex virus hepatitis. Patient had an emergent delivery and was started on antiviral therapy, but unfortunately due to the severity of her liver failure, she passed away. The importance of reporting this case is to emphasize on the importance of considering herpes simplex infection in pregnant women who present with acute liver failure, and the importance of early administration of antiviral therapy.**

Keywords: Herpes simplex hepatitis, acute liver failure, hepatitis in pregnancy

1. Introduction

Pregnant women are at increased risk for herpes simplex virus (HSV) hepatitis, particularly in the third trimester of pregnancy (*1*). Hepatitis can result from acute or latent infection with either HSV serotype 1 or serotype 2. Approximately half of cases lack typical HSV mucocutaneous lesions and the disease can arise in patients with no known history of genital or oral HSV infections. The differential diagnosis for HSV hepatitis is quite broad and includes acute fatty liver of pregnancy, severe preeclampsia/Hemolysis Elevated Liver enzymes and Low Platelets (HELLP) syndrome, other viral infections, and exogenous exposures, including drug reactions (*2*). Possible distinguishing clinical characteristics include markedly elevated liver enzymes with normal bilirubin and coagulopathy (*2-4*). The first adult case of hepatic dysfunction due to herpes virus was reported in 1969 by Flewett *et al*. (*5*). Although considered rare, herpes hepatitis is a fatal condition if not considered early with a mortality rate of 74.4% in the general population (*6*). To the best of our knowledge, 36 cases of HSV hepatitis during pregnancy have been reported, although those with autopsy examination remains outstanding (*2,3,5-14*) . We present a case with autopsy examination findings of fatal herpes simplex hepatitis.

2. Case Report

A 41-year old woman with no significant past medical history was 30-weeks pregnant when she presented to an outside hospital with a two-day history of cough, nausea and vomiting. She sought medical attention (day 1) and her urinalysis showed trace leukocyte esterase so she was treated initially for possible urinary tract infection versus upper respiratory infection with amoxicillin. Laboratory testing also revealed mildly elevated aspartate aminotransaminase (AST) and alanine aminotransaminas (ALT) (90 and 61 U/L, respectively). By day 5 the patient's AST and ALT increased to 5,652 and 1,559 U/L, respectively. Additionally, her serum creatinine increased to 3.2 mg/dL, hemoglobin decreased to 8.9 gm/dL, platelets decreased to 129,000/mm^3, and International Normalized Ratio (INR) increased to 2.3, all from normal baseline.

On day 6, she became acutely encephalopathic (grade 3) with tachycardia (120 beats per minute) and tachypnea (30 breaths per minute). Plasma ammonia was elevated at 102 μmol/L. Workup for acute liver failure included laboratory testing for hepatitis A (IgM and RNA), B (surface antigen, core antibody IgM and IgG and DNA), and C (surface antibody and RNA), Epstein-Barr virus (EBV), cytomegalovirus

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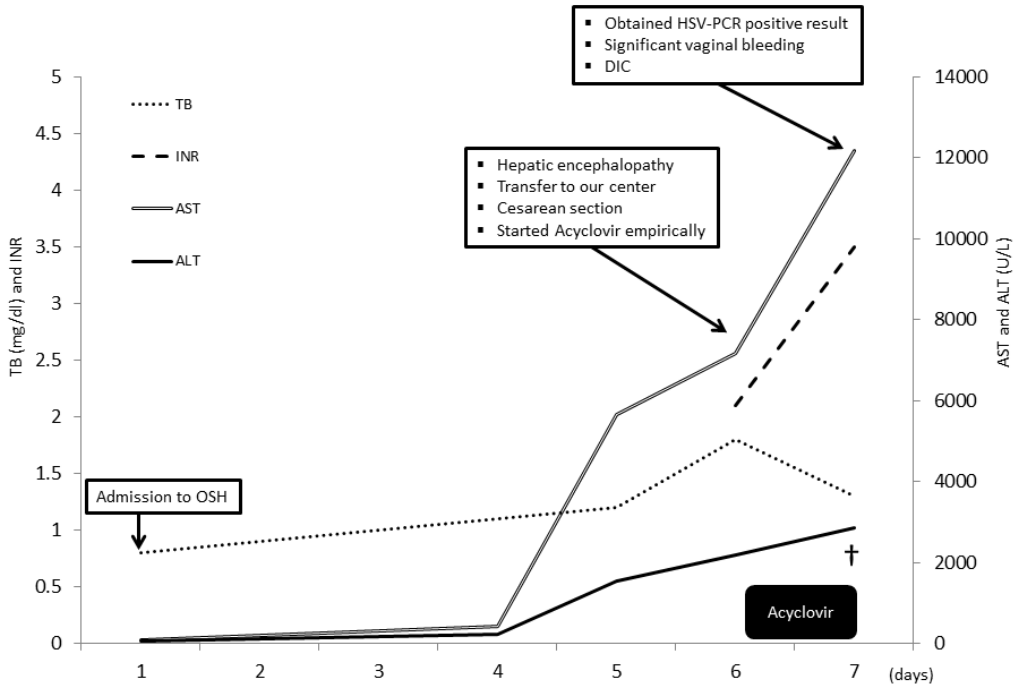

Figure 1. The clinical course of the case. The case with acute liver failure due to HSV developed rapidly progressive clinical deterioration despite empiric administration of acyclovir and passed away (†: time of death).

(CMV), and parvovirus serologies, all of which were negative. Therefore, she was transferred to a tertiary center for further management on that day. She was taken on arrival for emergent Cesarean section with no intraoperative complications noted and she delivered a male infant. She remained intubated postoperatively; continuous renal replacement (CRRT) was initiated for acidosis, hyperkalemia, and other electrolyte abnormalities.

Laboratory testing were repeated and revealed markedly elevated AST and ALT (7,161 U/L and 2,176 U/L, respectively), as well as elevated bilirubin (1.8 mg/dL total bilirubin, 1.7 mg/dL direct bilirubin), alkaline phosphatase (ALP, 370 U/L) and gamma-glutamyltransferase (GGT, 46 U/L). Serum creatinine was elevated at 3.4 mg/dL. Differential diagnosis of acute liver failure were reconsidered and included: Acute fatty liver of pregnancy, acute viral hepatitis, autoimmune hepatitis, Wilson's disease and Hemolysis Elevated Liver Enzymes and Low platelets (HELLP) syndrome. Serologies for infectious etiologies were again obtained, this time including HEV and HSV. Patient was started on empiric acyclovir (450 mg q12hours, intravenously).

On Day 7, HSV RNA was detected in blood obtained on day 6. Her postoperative course was complicated by significant vaginal bleeding with hemodynamic instability requiring pressor support. Bleeding could not be controlled by tamponade of the cervix and massive blood transfusion protocol was started. She underwent bilateral uterine artery embolization by interventional radiology but bleeding continued. Liver transplantation (LT) and hysterectomy were considered but unfortunately due to rapidly progressive clinical deterioration with a clear evidence of disseminated intravascular coagulopathy (DIC) from liver failure, she had cardiac arrest. Cardiopulmonary resuscitation was started but despite maximal resuscitative efforts, restoration of spontaneous circulation was never achieved. After discussion with family, resuscitation was stopped and patient was declared dead. The clinical course of the case is summeraized in Figure 1.

Autopsy examination of the liver revealed pale, punctate lesions diffusely scattered throughout the parenchyma. Microscopically, these lesions corresponded to non-zonal foci of hepatocyte necrosis with minimal associated inflammation. Ringing the necrotic areas were hepatocytes showing distinct HSV viral cytopathic effect. Immunohistochemistry performed on liver sections for HSV-1 was positive. The adrenal glands also showed scattered foci of punctate necrosis and HSV-1 positivity by immunohistochemistry. No mucocutaneous lesions were identified, and there was no other evidence of visceral organ involvement (Figures 2A, B and C).

3. Discussion

HSV hepatitis is a rare, frequently fulminant disease that typically affects immunocompromised patients including pregnant women (*15*). Clinical presentation of fever with abdominal pain and hepatic dysfunction in a pregnant woman should prompt immediate consideration of herpes hepatitis and work up should be initiated to exclude other common causes like HELLP

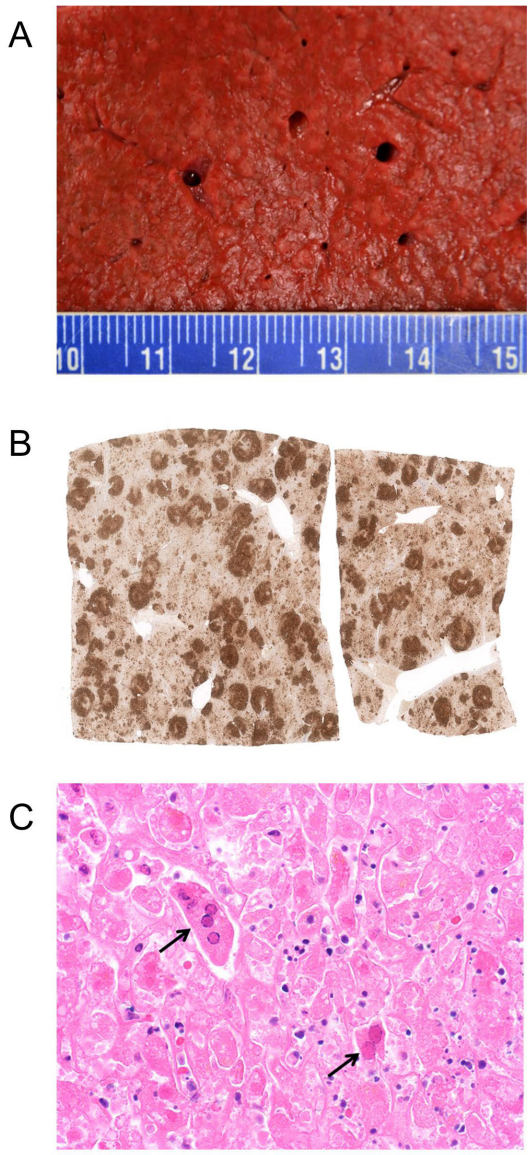

Figure 2. The autopsy findings. (A) The cut surface of the liver demonstrated innumerable 2-4 mm cream colored, soft nodules; **(B)** On histopathology, these proved to represent foci of geographic necrosis, highlighted here with an HSV immunostain; **(C)** High power photomicrograph demonstrates two hepatocytes with typical HSV cytopathic effect (arrows) characterized by multinucleation, margination (*i.e.*, peripheral displacement) of chromatin, and ground-glass nuclei.

and acute fatty liver of pregnancy (*3*). Unfortunately signs and symptoms are non-specific and can be vague, and the gold standard for diagnosis is liver biopsy with histopathology, electron microscopy, and viral culture. Pathognomic findings include: hemorrhagic necrosis and enlarged ground glass nuclei with marginalized chromatin (*15*). However, liver biopsy in this setting (especially due to maternal coagulopathy) is a significant risk of adverse events related to bleeding. Thus the histopathological findings obtained from autopsy examinations still remain outstanding. Development of alternative diagnostic modalities are desired (*3*).

Although the efficacy of antiviral therapy has not been proven in randomized controlled trial, intravenous acyclovir – a synthetic guanosine analogue- has been used for treating herpesviruses. Those have different degrees of susceptibility to acyclovir, with HSV type 1 (HSV-1) being most susceptible, followed by HSV type 2 (HSV-2) and VZV, and to a lesser extent Epstein-Barr virus (EBV). In cases of resistance, the use of foscarnet had been advised (*16*).

Despite supportive care and administration of antiviral therapy to the most susceptible genotype (HSV-1), our patient's condition continued to deteriorate with multi-organ failure that led to her death. The immunosuppressive effects of pregnancy make pregnant women infected by HSV at risk of disseminated disease (*4*). The prognosis was initially reported to be poor (39% mortality rate) in 1999, but the more recently, from 2000, it seemed to be improving (9% mortality rate amoung 11 cases) (*3*). Treatment of acute liver failure due to HSV infection include antiviral therapy and LT, and in one report therapeutic plasma exchange (*17*). Norvell *et al*. (*6*) conducted a retrospective analysis on 134 patients with HSV hepatitis including 32 (23%) pregnant women. The following variables were statistically associated with need for LT or death compared to spontaneous recovery: age > 40 year old, male gender, coagulopathy, any immunocompromised state, encephalopathy, ALT > 5,000 U/L, platelet count < 75,000 U and lack of acyclovir treatment. A total of 7 patients underwent LT and 3 of them survived at one year. In our case, despite the effort of a multidisciplinary team, the fast progression of events and rapid worsening of clinical status in the presence of dissiminated intravascular coagulopathy made transplant a difficult option to pursue. Acyclovir was given in our case, but only after her presentation to our center, which was 6 days after the onset of symptoms.

In conclusion, herpes hepatitis is a fatal condition, and should be considered in all pregnant patients with concern for evolving hepatic failure and early administration of antiviral therapy should be considered.

References

1. Kourtis AP, Read JS, Jamieson DJ. Pregnancy and infection. N Engl J Med. 2014; 370:2211-2218.
2. Allen RH, Tuomala RE. Herpes simplex virus hepatitis causing acute liver dysfunction and thrombocytopenia in pregnancy. Obstet Gynecol. 2005; 106:1187-1189.
3. Herrera CA, Eichelberger KY, Chescheir NC. Antiviral-resistant fulminant herpes hepatitis in pregnancy. AJP Rep. 2013; 3:87-90.
4. Kang AH, Graves CR. Herpes simplex hepatitis in pregnancy: A case report and review of the literature. Obstet Gynecol Surv. 1999; 54:463-468.
5. Flewett TH, Parker RG, Philip WM. Acute hepatitis due to Herpes simplex virus in an adult. J Clin Pathol. 1969;

22:60-66.
6. Norvell JP, Blei AT, Jovanovic BD, Levitsky J. Herpes simplex virus hepatitis: An analysis of the published literature and institutional cases. Liver Transpl. 2007; 13:1428-1434.
7. Mortelé KJ, Barish MA, Yucel KE. Fulminant herpes hepatitis in an immunocompetent pregnant woman: CT imaging features. Abdom Imaging. 2004; 29:682-684.
8. Nagappan R, Parkin G, Simpson I, Sievert W. Fulminant hepatic failure from herpes simplex in pregnancy. Med J Aust. 2002; 176:595-596.
9. Lazar P. An interesting herpes simplex infection. Arch Dermatol. 1977; 113:145-149.
10. Frederick DM, Bland D, Gollin Y. Fatal disseminated herpes simplex virus infection in a previously healthy pregnant woman. A case report. J Reprod Med. 2002; 47:591-596.
11. Halliday J, Lokan J, Angus PW, Gow P. A case of fulminant hepatic failure in pregnancy. Hepatology. 2010; 51:341-342.
12. Thurman RH, König K, Watkins A, Weerasiri T, Permezel M. Fulminant herpes simplex virus hepatic failure in pregnancy requiring liver transplantation. Aust N Z J Obstet Gynaecol. 2010; 50:492-494.
13. Navaneethan U, Lancaster E, Venkatesh PG, Wang J, Neff GW. Herpes simplex virus hepatitis - it's high time we consider empiric treatment. J Gastrointestin Liver Dis. 2011; 20:93-96.
14. Nienaber JH, McNamara DR, Banerjee R, Pritt BS, Karre T, Sohail MR. Fulminant gestational hepatitis due to primary herpes simplex type 2 infection: Use of serum HSV polymerase chain reaction for noninvasive diagnosis. Diagn Microbiol Infect Dis. 2012; 72:181-184.
15. Kaufman B, Gandhi SA, Louie E, Rizzi R, Illei P. Herpes simplex virus hepatitis: Case report and review. Clin Infect Dis. 1997; 24:334-338.
16. Razonable RR. Antiviral drugs for viruses other than human immunodeficiency virus. Mayo Clin Proc. 2011; 86:1009-1026.
17. Holt EW, Guy J, Gordon SM, Hofmann JC, Garcia-Kennedy R, Steady SL, Bzowej NH, Frederick RT. Acute liver failure caused by herpes simplex virus in a pregnant patient: Is there a potential role for therapeutic plasma exchange? J Clin Apher. 2013; 28:426-429.

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Case Report

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Anaplastic myxopapillary ependymoma in an infant: Case report and literature review

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Summary **A 7-month-old boy presented with gastrointestinal disturbance, mild neurologic deficit of the left lower extremity and levo-scoliosis of the thoracic spine. Magnetic resonance imaging demonstrated a large intramedullary lesion involving the thoracic spine, from level T1 to T11. Histologic analysis showed a glial tumor with fibrillary processes arranged in radial pattern around mucoid fibrovascular cores with a high proliferative index (focally up to 80%) and prominent vascular endothelial hyperplasia. These findings were consistent with an anaplastic myxopapillary ependymoma. Subtotal resection was performed *via* a T3-T10 laminoplasty. A ventricular shunt was placed, and the patient subsequently received chemoradiation therapy. To date, this is the second case of a myxopapillary ependymoma with high-grade anaplastic features and the first case in an infant reported in the literature.**

Keywords: Myxopapillary ependymoma, anaplasia, infant

1. Introduction

Myxopapillary ependymoma is considered a lower-grade variant of ependymoma with fibrillary cells arranged in a papillary configuration around a mucoid fibro-vascular cores (*1*). Of those patients affected by this lesion, typically 66% are males, with an average age of incidence of 36 years, although the age range of presentation can be between 6 and 82 years. Patients usually present with low-back pain, often with a long progressive course. Nerve impingement is rare and can be indolent due to the lesion's typically slow growth (*2*). Myxopapillary ependymomas mostly occur in the area of the conus medullaris, cauda equina and filum terminale of the spinal cord. They are the most common intramedullary lesion of this region (*1-3*). Imaging typically shows a well-circumscribed neoplasm that may have cystic or hemorrhagic features. In the instance of hemorrhagic change, cauda equina syndrome may be the presenting feature (*4*).

Although the World Health Organization classifies conventional ependymomas as grade II lesions due to the obstructive symptoms that manifest from occlusion of cerebrospinal fluid flow in the ventricles leading to hydrocephalus with injury to the peri-ventricular brain parenchyma, myxopapillary ependymomas are considered as grade I lesions with their slow growth, high rate of total resections and the relatively low rate of serious sequelae. The survival rate is 98.4% for those patients who have had either a total or subtotal resection. Adjuvant radiotherapy improves progression-free survival (*3,5*). Anaplastic myxopapillary ependymomas are featured by high grade histopathological changes such as increased mitotic activity, necrosis, vascular endothelial hyperplasia. These tumors are clinically more aggressive.

2. Case Report

2.1. *Clinical history*

A 7-month-old boy was initially seen by his primary care provider for concern of stool impaction. X-ray at that time showed levo-scoliosis (curvature of the spine to the left) of the thoracic spine. The patient was treated symptomatically and discharged home. Two months later, his mother noticed decrease movement, stiffness,

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asymmetry and episodes of discoloration of the left lower extremity. MRI showed an intramedullary solid and cystic tumor extending from T1-T11 (Figure 1),

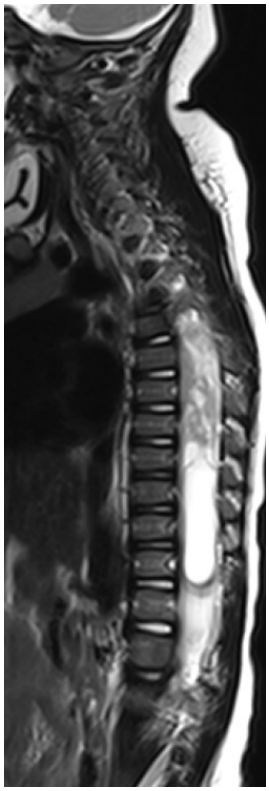

Figure 1. Magnetic Resonance Imaging, T2-weighted, of the thoracic spine lesion extending from level T1 to T11.

with associated edema involving the cervico-medullary junction to L1. The patient's condition rapidly worsened with bilateral paralysis and loss of sensation of the lower extremities. A subtotal resection was performed with laminoplasty of T3-T10. Ventricular drainage was initially placed to treat hydrocephalus. This was later revised to a ventriculo-peritoneal shunt. The patient's subsequent course was complicated only by a self-resolved episode of metapneumonia infection. The patient was discharged after one month in the pediatric intensive care unit. At that time, he had regained only limited movement and sensation of the lower extremities. Chemotherapy and radiation therapy were initiated at an outside hospital.

2.2. *Histopathology*

Examination of the lesion using hematoxylin and eosin staining showed a well-vascularized glial lesion with fibrillary processes. There was mucoid degeneration around the vasculature. The fibrillary processes were seen extending through the peri-vascular mucoid matrix and contacting the outer wall of the blood vessels. Vascular endothelial proliferation was observed (Figure 2A). Immunohistologically, the tumor was positive for glial fibrillary acid protein (GFAP) indicative of a glial origin (Figure 2B). Epithelial membrane antigen (EMA) was focally positive, highlighting ependymal cells ringing vasculature. These neoplastic cells also focally expressed pan-cytokeratin, which is a common feature of myxopapillary ependymomas

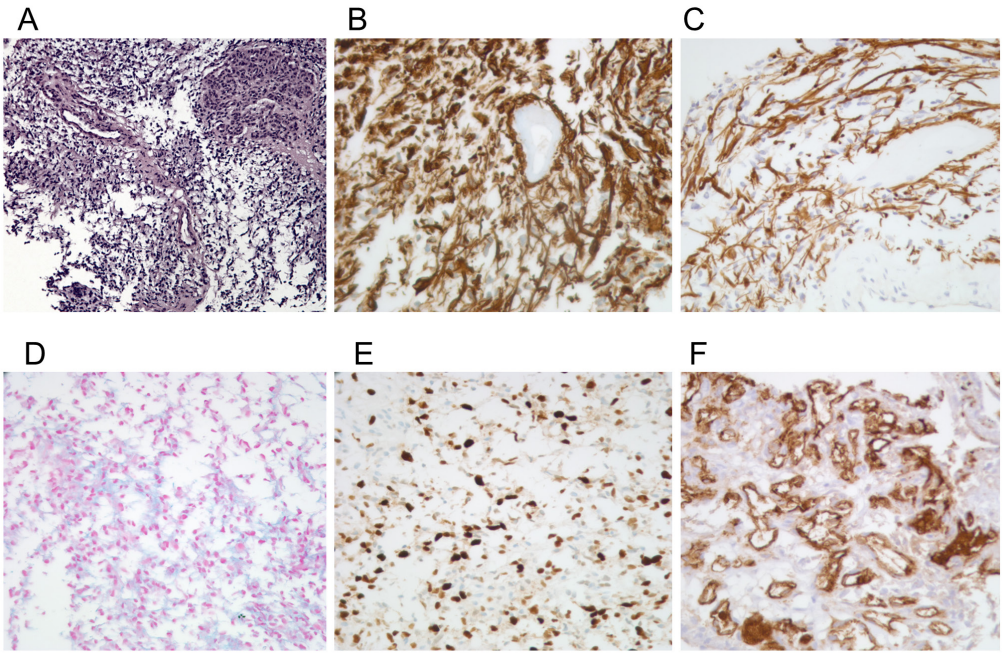

Figure 2. The morphological pattern and immunohistochemical profiles. (A) The lesion demonstrated regions of mucoid degeneration with fibrillary glial processes extending from vascular walls. Focal vascular endothelial proliferation was seen. H&E, 200×. **(B)** The lesion demonstrated glial fibrillary acid protein (GFAP) positivity. **(C)** The lesion demonstrated pan-cytokeratin (PAN-CK) staining**. (D)** Mucoid matrix was highlighted by Alcian Blu**e. (E)** Ki-67 immunostaining demonstrated an increased proliferative index. **(F)** CD31 immunostaining demonstrated endothelial proliferation.

(Figure 2C). The mucoid component of the lesion was clearly visualized using both alcian blue and periodic acid Schiff staining (Figure 2D). Proliferative index measured by Ki-67 immunohistochemistry was very high and focally reached 80% (Figure 2E). CD31 immunohistochemistry confirmed the vascular endothelial proliferation (Figure 2F). The morphological pattern and immunohistochemical profile are consistent with anaplastic myxopapillary ependymoma (*6*).

3. Discussion

Anaplastic features include increased mitosis, necrosis and vascular endothelial hyperplasia (*1*). Up to this time, there has only been one prior report of these findings in a myxopapillary ependymoma (*7*). That prior case occurred in a 15-year-old boy and extended from T12 to L2. The tumor had extensive necrosis and glomeruloid vascular proliferation, the later characteristic being consistent with the case presented in our report. The proliferative index was lower (10.1%) than our case, but significantly higher than would be expected in a conventional myxopapillary ependymoma. It is worth to note that myxopapillary ependymoma is typically seen in the region of conus medullaris, cauda equina and filum terminale. Interestingly, both tumors in the previously reported case and our case are located in the thoracic and lumbar regions of spinal cord. Both patients are children. Whether this higher spinal cord location and the young age of patients are the unique features of anaplastic myxopapillary ependymoma remains unclear and requires more cases for further study.

Recent genetic analysis of myxopapillary ependymomas found that these lesions are characterized by genome-wide polyploidy, often among several chromosomes (*1,8*). The excellent outcomes of subtotal resection are often attributed to this significant genetic instability. The specific familial, epigenetic or environmental cause predisposing to anaplastic transformation of this lesion has not been identified. Genetic studies could not be fully carried out in this case due to financial concerns of the family.

Myxopapillary ependymomas can present with spine deformation clinically. Levo-scoliosis is common in the lumbar spine, but its occurrence in the thoracic spine is an early indicator of a thoracic spinal cord neoplasm (*9*). Early recognition of levo-scoliosis in the thoracic region could potential spare patients from severe neurological sequelae, particularly for pediatric patients.

In general, gross total resection is the best predictive factor of outcomes for conventional myxopapillary ependymoma. These lesions can be difficult to resect completely and recurrences are common following subtotal resection. Distant spinal metastases were found in 9.3% of patients and brain metastases in 6% of patients (*10-13*). Patient age (increased risk with younger age), lack of adjuvant radiotherapy and subtotal resection were the strongest factors predisposing to spread of the lesion (*5*). Expression of endothelial growth factor receptor (EGFR) has been cited as a possible biomarker of recurrence (*14*).

This report documents a case of anaplastic myxopapillary ependymoma and is contributory to understanding this rare neoplasm.

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References

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Ellison DW, Figarella-Branger D, Perry A, Reifenberger G, von Deimling A. WHO Classification of Tumours of the Central Nervous System (4th edition). IARC, Lyons, France, 2016.
2. Cervoni L, Celli P, Caruso R., Gagliardi FM, Cantore GP. Neurinomas and ependymomas of the cauda equine. A review of the clinical characteristics. Minerva Chir. 1997; 52:629-633.
3. Kurt E, Zheng PP, Hop WC, van der Weiden M, Bol M, van der Bent MJ, Avezaat CJ, Kros JM. Identification of relevant prognostic histopathologic features in 69 intracranial ependymomas, excluding myxopapillary ependymomas and subependymomas. Cancer. 2006; 106:388-395.
4. Tait MJ, Chelvarajah R, Garvan N, Bavetta. Spontaneous hemorrhage of a spinal ependymoma: A rare cause of cauda equina syndrome: A case report. Spine. 2004; 29:E502-505.
5. Weber DC, Wang Y, Miller R, Villà S, Zaucha R, Pica A, Poortmans P, Anacak Y, Ozygit G, Baumert B, Haller G, Preusser M, Li J. Long-term outcome of patients with spinal myxopapillary ependymoma: Treatment results from the MD Anderson Cancer Center and instutions from the Rare Cancer Network. Neuro Oncol. 2015; 17:588-595.
6. Ang LC, Taylor AR, Bergin D, Kaufmann JC. An immunohistochemical study of papillary tumors in the central nervous system. Cancer. 1990; 65:2712-2719.
7. Awaya H, Kaneko M, Amatya VJ, Takeshima Y, Oka S, Inai K. Myxopapillary ependymoma with anaplastic features. Pathology Int. 2003; 53:700-703.
8. Pajtler KW, Witt H, Sill M, *et al*. Molecular classification of ependymal tumors across all CNS compartments, Histopathological grades, and age groups. Cancer Cell. 2015; 27:728-743.
9. Bouaziz MC, Daghfous S, Ladeb MF. Childhood scoliosis revealing spinal tumors. Euro J Ortho Surg & Trauma. 2006; 16:318-321.
10. Libório R, Pais RF, Soares GB, Rocha A, Ferreira F, Garcia T, Pais FF. Medullary and intracranial metastases of myxopapillary ependymomas. Acta Med Port. 2001; 14:133-138.
11. Mavroudis C, Townsend JJ, Wilson CB. A metastasizing ependymoma of the cauda equine: Case report. J Neurosurg. 1977; 47:771-775.

12. Woesler B, Moskopp D, Kuchelmeister K, Schul C, Wassmann H. Intracranial metastasis of a spinal myxopapillary ependymoma: A case report. Neurosurg Rev. 1998; 21:62-65.
13. Yücesoy K, Ozer E, Koyuncuoglu M. Parenchymal brain metastasis of a spinal myxopapillary ependymoma after extradural manipulation. Acta Neurochir (Wein). 2001; 143:1071-1072.
14. Verma A, Zhou H, Chin S, Bruggers C, Kestle J, Khatua S. EGFR as a predictor of relapse in myxopapillary ependymoma. Pediatr Blood Cancer. 2012; 59:746-748.

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Case Report

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A novel *PGK1* mutation associated with neurological dysfunction and the absence of episodes of hemolytic anemia or myoglobinuria

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Summary **Phosphoglycerate kinase (PGK) deficiency affects three different organs: red blood cells (RBC), the central nervous system, and muscles. Next-generation sequencing identified a hemizygous *PGK1* mutation (p.V217I) in a 16-year-old Japanese male patient presenting with intellectual disability and episodes of muscle weakness of unknown etiology. Enzymatic analysis demonstrated slightly lower RBC-PGK activity and compensatory increases of other glycolysis enzymes. This is the first *PGK1* mutation found through next-generation sequencing.**

Keywords: Phosphoglycerate kinase 1 gene (*PGK1*), novel mutation, PGK deficiency, intellectual disability, muscle involvement

1. Introduction

Phosphoglycerate kinase (PGK) deficiency is a rare cause of congenital hemolytic anemia (*1*). Mutations in the phosphoglycerate kinase 1 gene (*PGK1*) result in an X-linked recessive disorder (MIM #311800) involving three tissues: red blood cells (RBC), the central nervous system (CNS), and muscles (*2*). Variable symptoms have been observed, for example, chronic anemia, exercise-intolerant myopathy, muscle weakness, cramping, myalgia, myoglobinuria, and intellectual disability (*3*).

Here, we report a rare patient with a *PGK1* mutation associated with only CNS and muscular symptoms.

2. Case Report

A 16-year-old Japanese male patient with no family history of neuromuscular or blood-cell disorders was born uneventfully at 41 weeks of gestation, weighing 3,460 g. Since early infancy, his development was delayed: head control at 7 months, sitting at 13 months, crawling and standing with support at 17 months, and walking independently at 29 months of age. Hence, he was referred to our hospital. Neurological examination showed no finding of muscular involvement, and routine laboratory examinations revealed no abnormalities.

He suffered epileptic seizures starting at 20 months of age. By 35 months, he exhibited recurrent attacks, 2-3 times per month, of transient hemiplegia, with or without tonic stiffness of the unilateral extremity, and nystagmus occurring during sleep. These attacks alternated between the two sides of the extremities. Interictal electroencephalogram (EEG) showed only mild, diffuse, background abnormality. Combinatorial use of antiepileptic drugs has controlled the attacks since 6 years of age.

At 7 years, the patient was examined by the modified Binet Intelligence Scales test, revealing an intelligence quotient (IQ) of 30. Brain magnetic resonance imaging showed nonspecific, mild, cerebral and cerebellar atrophy.

At 16 years, the patient developed recurrent peculiar episodes, characterized by sudden, early morning onset of muscle weakness lasting 1-2 hours. There were no trigger events, such as exercise, before the episodes. During the episodes, he was unable to sit or stand and

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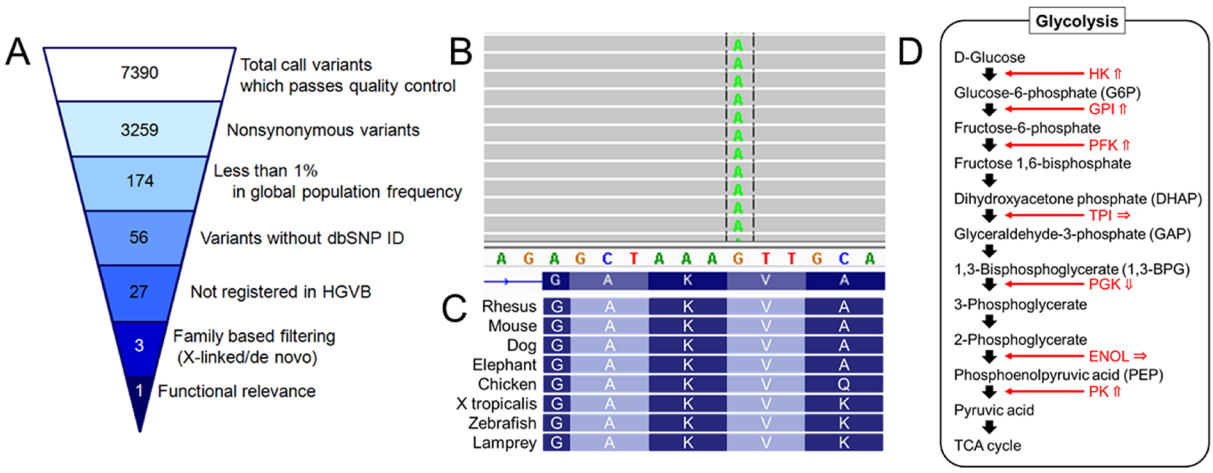

Figure 1. Results of next-generation sequencing. (A) Filtering steps and the number of filtered variants are shown in inverted pyramids. **(B)** The Integrative Genomics Viewer (IGV) shows the identified *PGK1* variant in 100% of reads. **(C)** The affected codon is conserved among species. **(D)** A schematic representation of glycolysis and the results of the enzymatic activities in this patient. Many enzymes other than PGK show increased levels.

Table 1. Summary of the previously reported mutations and its organ involvements

Items	Nucleotide change	Protein change	Anemia	CNS symptoms	Muscle involvement	RBC residual activity
Noel *et al*. (2006)	c.140T>A	p.I47N	+	+	-	5.9-10.4%
Ramirez-Bajo *et al*. (2011)	c.140T>A	p.I47N	+	+	-	20%
Maeda and Yoshida (1991)	c.266T>C	p.L89P	+	+	-	
Tsujino *et al*. (1994)	c.417+1G>T	splicing	-	+	+	
Fujii *et al*. (1992)	c.473G>T	p.G158V	+	-	+	0.7%
Cohen-Sola *et al*. (1994)	c.491A>T	p.D164V	+	+	-	
Turner *et al*. (1995)	c.491A>T	p.D164V	+	-	-	
Flanagan *et al*. (2006)	c.491A>T	p.D164V	+	+	-	<5%
Yoshida *et al*. (1995)	c.571_573del	p.K191del	+	-	-	4%
Fujii and Yoshida (1980)	c.617G>C	p.R206P	+	+	-	20-30%
Svaasandet *et al*. (2007)	c.639C>T	p.G213G (splicing?)	-	-	+	2-3%
Present patient	**c.649G>A**	**p.V217I**	**-**	**+**	**+**	**78-91%**
Hamano *et al*. (2000)	c706_709del	frameshift	-	-	+	2.9%
Ookawara *et al*. (1996)	c.755A>C	p.E252A	-	-	+	8%
Coppens *et al*. (2016)	c.756+3A>G	splicing	-	+	+	11-18%
Shirakawa *et al*. (2006)	c.756+5G>A	splicing	-	+	+	8.9%
Sugie *et al*. (1989)	c.758T>C	p.I253T	-	+	+	44%
Fujii *et al*. (1981)	c.796G>A	p.V266I*	+	+	-	16%
Fujii *et al*. (1980)	c.802G>A	p.D268N	-	-	-	20%
Valentin *et al*. (1998)	c.854A>T	p.D285V	+	-	-	49%
Rosa *et al*. (1982)	c.943G>A	p.D315N	-	-	+	21-37%
Cohen-Sola *et al*. (1994)	c.943G>A	p.D315N	-	-	+	
Maeda *et al*. (1992)	c.946T>C	p.C316R	+	+	-	5%
Noel *et al*. (2006)	c.959G>A	p.S320N	+	+	-	28-49%
Yoshida *et al*. (1972)	c.1055C>A	p.T352N	-	-	-	
Morimoto *et al*. (2003)	c.1060G>C	p.A354P	+	+	+	4.9-6.3%
Fermo *et al*. (2012)	c.1112T>A	p.I371K	+	+	+	
Sotiriou *et al*. (2010)	c.1132A>C	p.T378P	-	+	+	1.1%
Spiegel *et al*. (2009)	c.1132A>C	p.T378P	-	-	+	1.6%
Tamai *et al*. (2014)	c.1180A>G	p.T394A	+	-	-	11.2-13.9%

*; It may be a misdescription in original manuscript, suggested by Beutler (2006).

he had difficulty responding to our verbal commands. He had a dull facial appearance with ptosis. EEG taken during the episodes indicated a slight slowing of background activity, similar to that in periodic paralysis; however, levels of creatine kinase and electrolyte were unremarkable.

This study was performed in accordance with the declaration of Helsinki and was approved by the ethics committee of Tokyo Women's Medical University. After receiving informed consent, we obtained blood samples from the patient and his parents and extracted genomic DNA for sequence analysis. Next-generation

sequencing (NGS) was performed using the TruSight One v1.0 sequencing panel (Illumina, San Diego, CA) (*4*). After annotation using GATK, 7390 variants were obtained. Those variants were filtered by Variant Studio software (Illumina) (Figure 1A). Next, synonymous variants, variants more than 1% in global population frequency, variants registered in the dsSNP database, and variants registered in the Human Genetic Variation Database (HGVD) (*http://www.genome.med.kyoto-u.ac.jp/SnpDB*), which is the database provided from Kyoto University in Japan (*5*), were removed. Finally, variants with *de novo* origin or inherited in accordance with a Mendelian inheritance trait were selected. As a result, a hemizygous single nucleotide variant, NM_000291.3(PGK1):c.649G>A [NP_000282.1:p.Val217Ile] on the X-chromosome, was retained in association with X-linked recessive inheritance. This variant has never been reported previously (Table 1). The predicted functional importance scores were calculated using wANNOVAR (*http://wannovar.usc.edu/*). As shown in Table 2, many scores indicated that this variant would be damaging to the encoded protein. The identified variant was checked visually by Integrative Genomics Viewer (IGV; *http://www.broadinstitute.org/igv/*) (Figure 1B) and was verified by Sanger sequencing (data not shown). The mother of the patient was confirmed as an obligate carrier. The affected codon was conserved among species (Figure 1C).

Table 2. Results of prediction scores

SIFT_score	0.08
SIFT_pred	T
Polyphen2_HDIV_score	0.61
Polyphen2_HDIV_pred	P *
Polyphen2_HVAR_score	0.347
Polyphen2_HVAR_pred	B
LRT_score	0
LRT_pred	D *
MutationTaster_score	1
MutationTaster_pred	D *
MutationAssessor_score	1.67
MutationAssessor_pred	L
FATHMM_score	-3.28
FATHMM_pred	D *
RadialSVM_score	0.462
RadialSVM_pred	D *
LR_score	0.743
LR_pred	D *
VEST3_score	0.571
CADD_raw	3.712
CADD_phred	18.85 *
GERP++_RS	4.32
phyloP46way_placental	1.064
phyloP100way_vertebrate	9.756
SiPhy_29way_logOdds	13.55

T, tolerate; P, possibly damaging; B, benign; L, low; *, damaging is suggested.

Routine laboratory test values from the proband did not indicate hemolysis: hemoglobin, 16.7 g/dL; reticulocyte, 0.7%; total bilirubin, 0.2 mg/dL; haptoglobin, 180 mg/dL. However, the screening test for hemolytic anemia revealed RBC-PGK activity as 194 IU/gHb (normal range: 214-249 IU/gHb [mean ± SD]), suggesting slightly lower activity in this patient. In comparison with previously reported patients with PGK deficiency, the decreased level of PGK activity in this patient was not so severe (Table 1). Unexpectedly, other enzymatic activities related to glycolysis were mildly increased (Table 3). For clarity, these findings are depicted in a schematic representation of glycolysis (Figure 1D). From these results, we considered that mildly elevated activities of glycolytic enzymes other than PGK might suggest compensation for the decreased PGK activity in this patient.

3. Discussion

Generally, patients with PGK deficiency show clinical symptoms in any of three organs including RBC, muscles, and the CNS. Major symptoms are chronic

Table 3. Results of the enzymatic activities

Items	Standard (mean ± SD)	Reference	Patient	Evaluation
Hexokinase (HK)	1.08~1.46	1.44	2.08	↑
Glucose phosphate isomerase (GPI)	57.2~70.3	65.1	70.7	↑
Phosphofructokinase (PFK)	14.1~20.0	20.0	22.9	↑
Aldolase (ALD)	2.62~6.30	3.08	3.89	
Triosephosphate isomerase (TPI)	1,052~1,567	1,353	1,238	
Phosphoglycerate kinase (PGK)	214~249	246	194	↓
Enolase (ENOL)	3.89~6.30	5.62	6.01	
Pyruvate kinase (PK)	13.0~19.8	14.4	24	↑
Glucose-6-phosphate dehydrogenase (G6PD)	7.61~9.81	7.00	8.88	
6-Phosphogluconate dehydrogenase (6PGD)	9.00~10.70	8.92	9.78	
Glutathione peroxidase (GSH-Px)	37.2~51.4	40.3	42.1	
Adenylate kinase (AK)	165~307	285	319	↑
Adenosine deaminase (ADA)	0.87~1.59	0.59	0.94	
Acetylcholineesterase (Ach-E)	28.6~42.7	33.7	36.1	
Pyrimidine 5'-nucleotidase (P5N) (CMPase)*	6.90~10.8	11.9	11.7	↑
Pyrimidine 5'-nucleotidase (P5N) (UMPase)*	9.75~15.5	15.4	15.0	

units = IU/gHb (*, μmole Pi liberated/hr/gHb)

anemia (followed by recurrent hemoglobinuria caused by rhabdomyolysis), intellectual disability, and seizures (*1*). In Table 1, previously reported *PGK1* mutations are summarized (*1-3,6-27*). Some patients exhibited symptoms only in the CNS and the muscles, but not in the RBC (*24*). In particular, a few patients showed neurological symptoms similar to those in the present patient, such as hemiplegic migraines (*20,27,28*), although the details of the clinical manifestations appear to be different. Dysfunction of some glycolytic enzymes other than PGK impairs not only for RBC but also for the CNS (*29,30*). The CNS may not tolerate minor glycolysis dysfunction better than the RBC, because the CNS requires substantial energy compared to RBC.

Usually, patients with anemia or myoglobinuria are suspected to exhibit PGK deficiency, and are referred for examination of PGK activities and *PGK1* mutations. However, this patient had no clinical symptoms to suggest PGK deficiency; instead, he presented with neurological symptoms, mimicking alternating hemiplegia and later periodic paralysis. Episodes of dullness were considered the consequence of muscular involvement. Hence, this patient was referred for exome sequencing based on neurological impairments rather than for hemolytic anemia, one of the key symptoms of PGK deficiency. Therefore, similar patients with mildly impaired PGK activities, who show no sign of hemolysis and presenting only CNS and muscle symptoms, might be underdiagnosed.

Recently, a PGK heterozygous carrier mother was reported to show parkinsonism, although she showed normal PKG activity (*31*). In the future, the disease characteristics associated with *PGK1* mutations may expand as more patients with similar CNS symptoms are identified. On the other hand, this patient may still possess an unidentified etiology for his pathology. To confirm the association between mild reduction of PGK activity and neurological impairment, more patients need to be identified.

In this study, a new *PGK1* variant was identified. This is the first case of a *PGK1* variant discovery through next-generation sequencing.

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References

1. Beutler E. PGK deficiency. Br J Haematol. 2007; 136:3-11.
2. Fujii H, Kanno H, Hirono A, Shiomura T, Miwa S. A single amino acid substitution (157 Gly----Val) in a phosphoglycerate kinase variant (PGK Shizuoka) associated with chronic hemolysis and myoglobinuria. Blood. 1992; 79:1582-1585.
3. Tamai M, Kawano T, Saito R, Sakurai K, Saito Y, Yamada H, Ida H, Akiyama M. Phosphoglycerate kinase deficiency due to a novel mutation (c. 1180A>G) manifesting as chronic hemolytic anemia in a Japanese boy. Int J Hematol. 2014;100:393-397.
4. Shimojima K, Okamoto N, Yamamoto T. A novel *TUBB3* mutation in a sporadic patient with asymmetric cortical dysplasia. Am J Med Genet A. 2016; 170A:1076-1079.
5. Higasa K, Miyake N, Yoshimura J. *et al*. Human genetic variation database, a reference database of genetic variations in the Japanese population. J Hum Genet. 2016; 61:547-553.
6. Yoshida A, Watanabe S, Chen SH, Giblet ER, Malcolm LA. Human phosphoglycerate kinase. II. Structure of a variant enzyme. J Biol Chem. 1972; 247:446-449.
7. Fujii H, Krietsch WK, Yoshida A. A single amino acid substitution (Asp leads to Asn) in a phosphoglycerate kinase variant (PGK Munchen) associated with enzyme deficiency. J Biol Chem. 1980; 255:6421-6423.
8. Fujii H, Yoshida A. Molecular abnormality of phosphoglycerate kinase-Uppsala associated with chronic nonspherocytic hemolytic anemia. Proc Natl Acad Sci U S A. 1980; 77:5461-5465.
9. Rosa R, George C, Fardeau M, Calvin MC, Rapin M, Rosa J. A new case of phosphoglycerate kinase deficiency: PGK Creteil associated with rhabdomyolysis and lacking hemolytic anemia. Blood. 1982;60:84-91.
10. Sugie H, Sugie Y, Nishida M, Ito M, Tsurui S, Suzuki M, Miyamoto R, Igarashi Y. Recurrent myoglobinuria in a child with mental retardation: Phosphoglycerate kinase deficiency. J Child Neurol. 1989; 4:95-99.
11. Maeda M, Yoshida A. Molecular defect of a phosphoglycerate kinase variant (PGK-Matsue) associated with hemolytic anemia: Leu----Pro substitution caused by T/A----C/G transition in exon 3. Blood. 1991; 77:1348-1352.
12. Cohen-Solal M, Valentin C, Plassa F, Guillemin G, Danze F, Jaisson F, Rosa R. Identification of new mutations in two phosphoglycerate kinase (PGK) variants expressing different clinical syndromes: PGK Creteil and PGK Amiens. Blood. 1994; 84:898-903.
13. Tsujino S, Tonin P, Shanske S, Nohria V, Boustany RM, Lewis D, Chen YT, DiMauro S. A splice junction mutation in a new myopathic variant of phosphoglycerate kinase deficiency (PGK North Carolina). Ann Neurol. 1994; 35:349-353.
14. Turner G, Fletcher J, Elber J, Yanagawa Y, Davé V, Yoshida A. Molecular defect of a phosphoglycerate kinase variant associated with haemolytic anaemia and neurological disorders in a large kindred. Br J Haematol. 1995; 91:60-65.
15. Yoshida A, Twele TW, Davé V, Beutler E. Molecular abnormality of a phosphoglycerate kinase variant (PGK-Alabama). Blood Cells Mol Dis. 1995; 21:179-181.
16. Ookawara T1, Davé V, Willems P, Martin JJ, de Barsy T, Matthys E, Yoshida A. Retarded and aberrant splicings

caused by single exon mutation in a phosphoglycerate kinase variant. Arch Biochem Biophys. 1996; 327:35-40.
17. Valentin C, Birgens H, Craescu CT, Brødum-Nielsen K, Cohen-Solal M. A phosphoglycerate kinase mutant (PGK Herlev; D285V) in a Danish patient with isolated chronic hemolytic anemia: Mechanism of mutation and structure-function relationships. Hum Mutat. 1998; 12:280-287.
18. Hamano T, Mutoh T, Sugie H, Koga H, Kuriyama M. Phosphoglycerate kinase deficiency: An adult myopathic form with a novel mutation. Neurology. 2000; 54:1188-1190.
19. Morimoto A, Ueda I, Hirashima Y, Sawai Y, Usuku T, Kano G, Kuriyama K, Todo S, Sugimoto T, Kanno H, Fujii H, Imashuku S. A novel missense mutation (1060G --> C) in the phosphoglycerate kinase gene in a Japanese boy with chronic haemolytic anaemia, developmental delay and rhabdomyolysis. Br J Haematol. 2003; 122:1009-1013.
20. Flanagan JM, Rhodes M, Wilson M, Beutler E. The identification of a recurrent phosphoglycerate kinase mutation associated with chronic haemolytic anaemia and neurological dysfunction in a family from USA. Br J Haematol. 2006; 134:233-237.
21. Noel N, Flanagan JM, Ramirez Bajo MJ, Kalko SG, Mañú Mdel M, Garcia Fuster JL, Perez de la Ossa P, Carreras J, Beutler E, Vives Corrons JL. Two new phosphoglycerate kinase mutations associated with chronic haemolytic anaemia and neurological dysfunction in two patients from Spain. Br J Haematol. 2006; 132:523-529.
22. Shirakawa K, Takahashi Y, Miyajima H. Intronic mutation in the *PGK1* gene may cause recurrent myoglobinuria by aberrant splicing. Neurology. 2006; 66:925-927.
23. Svaasand EK, Aasly J, Landsem VM, Klungland H. Altered expression of *PGK1* in a family with phosphoglycerate kinase deficiency. Muscle Nerve. 2007; 36:679-684.
24. Sotiriou E, Greene P, Krishna S, Hirano M, DiMauro S. Myopathy and parkinsonism in phosphoglycerate kinase deficiency. Muscle Nerve. 2010; 41:707-710.
25. Ramírez-Bajo MJ, Repiso A, la Ossa PP, Bañón-Maneus E, de Atauri P, Climent F, Corrons JL, Cascante M, Carreras J. Enzymatic and metabolic characterization of the phosphoglycerate kinase deficiency associated with chronic hemolytic anemia caused by the PGK-Barcelona mutation. Blood Cells Mol Dis. 2011; 46:206-211.
26. Fermo E, Bianchi P, Chiarelli LR, Maggi M, Mandarà GM, Vercellati C, Marcello AP, Barcellini W, Cortelezzi A, Valentini G, Zanella A. A new variant of phosphoglycerate kinase deficiency (p.I371K) with multiple tissue involvement: Molecular and functional characterization. Mol Genet Metab. 2012; 106:455-461.
27. Coppens S, Koralkova P, Aeby A, Mojzikova R, Deconinck N, Kadhim H, van Wijk R. Recurrent episodes of myoglobinuria, mental retardation and seizures but no hemolysis in two brothers with phosphoglycerate kinase deficiency. Neuromuscul Disord. 2016; 26:207-210.
28. Rhodes M, Ashford L, Manes B, Calder C, Domm J, Frangoul H. Bone marrow transplantation in phosphoglycerate kinase (PGK) deficiency. Br J Haematol. 2011; 152:500-502.
29. Schröter W, Eber SW, Bardosi A, Gahr M, Gabriel M, Sitzmann FC. Generalised glucosephosphate isomerase (GPI) deficiency causing haemolytic anaemia, neuromuscular symptoms and impairment of granulocytic function: A new syndrome due to a new stable GPI variant with diminished specific activity (GPI Homburg). Eur J Pediatr. 1985; 144:301-305.
30. Aissa K, Kamoun F, Sfaihi L, Ghedira ES, Aloulou H, Kamoun T, Pissard S, Hachicha M. Hemolytic anemia and progressive neurologic impairment: Think about triosephosphate isomerase deficiency. Fetal Pediatr Pathol. 2014; 33:234-238
31. Sakaue S, Kasai T, Mizuta I, Suematsu M, Osone S, Azuma Y, Imamura T, Tokuda T, Kanno H, El-Agnaf OMA, Morimoto M, Nakagawa M, Hosoi H, Mizuno T. Early-onset parkinsonism in a pedigree with phosphoglycerate kinase deficiency and a heterozygous carrier: Do *PGK-1* mutations contribute to vulnerability to parkinsonism? NPJ Parkinson's Disease. 2017; 3:13.

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Case Report

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Septic thrombophlebitis of the internal jugular vein, a case of Lemierre's syndrome

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Summary **An 18-year-old gentleman with a history of recurrent tonsillitis presented to the emergency room complaining of worsening sore throat. He was found to have a peritonisillar abscess, and imaging revealed a non-occlusive left internal jugular vein thrombosis. Lemierre's syndrome is a rare, potentially fatal condition characterized by internal jugular vein thrombosis with septicemia following an acute oropharyngeal infection. While anticoagulation is the mainstay of treatment of deep venous thromboembolism (DVT) and pulmonary embolism (PE), the use of therapy is controversial in septic thrombophlebitis. This is counterintuitive since a common reported complication is pulmonary emboli. Early in the course of thrombophlebitis, while the thrombus is firmly attached, antibiotics may be all that is necessary to treat the condition.**

Keywords: Lemierre's syndrome, *Fusobacterium necrophorum*, postanginal septicemia, septic thrombophlebitis

1. Introduction

Lemierre's syndrome is a rare, potentially fatal condition characterized by internal jugular vein (IJV) thrombosis with septicemia following an acute oropharyngeal infection. Lemierre's syndrome (LS) has been termed the "forgotten disease" due to the unfamiliarity of many physicians with this syndrome because of its rarity, with less than one case per million occurring in the general population (*1*). LS is typically associated with a gram-negative retropharyngeal abscesses leading to septic metastasis to the vasculature of the head and neck (*2*). The most commonly associated organism is *Fusobacterium necrophorum*, a gram-negative, anaerobic rod-shaped bacterium that is part of the normal oral flora (*3*). Other associated bacterium include Streptococcal species such as *Streptococcus pyogenes* (*1*), *Eikenella corrodens* (*4*), and *Bacteroides species* (*5*). The venous thrombosis associated with this syndrome is secondary to endothelial dysfunction caused by inflammatory factors from the local infection and can be treated with antibiotics alone, often without the need for anticoagulation therapy. We report a case of Lemierre's syndrome in an 18-year-old male adolescent who developed a left internal jugular vein thrombosis following an acute oropharyngeal infection. The patient was successfully managed with incision and drainage (I&D) of the developing peritonsillar abscess in conjunction with intravenous (IV) antibiotics.

2. Case Report

An 18-year-old gentleman with a history of recurrent tonsillitis presented to the emergency room complaining of worsening sore throat. Three days prior he was diagnosed with pharyngitis in the outpatient setting where he was prescribed oral amoxicillin and clindamycin. However, his symptoms continued to progress, including dysphagia, odynophagia, change of voice, and difficulty managing secretions, though without concurrent dyspnea.

The patient had no known drug allergies and was not taking any other home medications. His past

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medical history was significant for recurrent tonsillitis, tonsilloliths, and a left sided peritonsillar abscess treated with I&D approximately 5 years prior.

On physical exam, vital signs were temperature 98.7°F, blood pressure 122/73 mm Hg, heart rate 111 bpm, respiratory rate 18 bpm, and 98% oxygen saturation on room air. He was in no acute distress without wheezing, stridor or signs of respiratory distress. There was left peritonsillar swelling, fullness and mild tenderness with right shift of the uvula. The hypopharynx and larynx could not be visualized. He also had mild tenderness near the left jaw angle that was exacerbated by turning his head to the left. The neck was nontender to palpation without lymphadenopathy. The remainder of the exam was otherwise benign.

Initial labs were significant for a leukocytosis of 16.6×10^3 cell/μL. A rapid strep and monospot test were both negative. A computed tomography (CT) scan of the neck revealed a left-sided peritonisillar phelgmon with a 9 mm fluid collection suspicious for a developing abscess. It also demonstrated a left-sided internal jugular vein abnormality, concerning for thrombosis (Figure 1). Doppler ultrasound of the neck confirmed a nonocclusive thrombus in the left internal jugular vein (IJV). (Figures 2A and 2B)

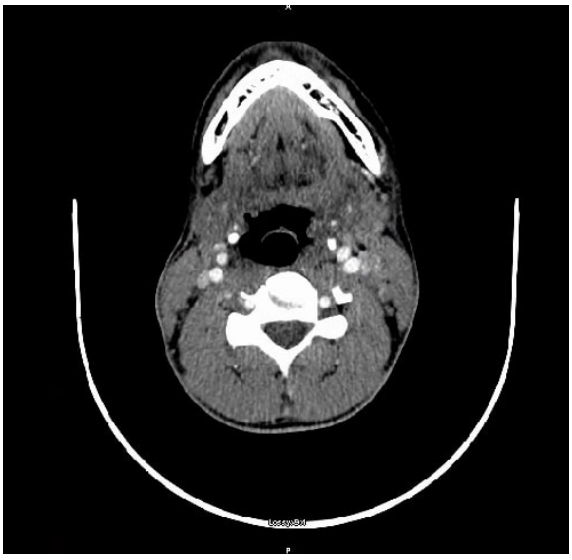

Figure 1. Computed tomography image of the neck: Left-sided peritonsillar phelgmon with associated reactive adenopathy. Note the left internal jugular vein, with an area of limited opacification, concerning for possible thrombosis.

The patient was admitted with the diagnosis of sepsis secondary to left sided peritonsillar abscess complicated with left IJV thrombosis, with microorganism not yet determined. Cultures were drawn and he was started empirically on broad spectrum IV antibiotics with vancomycin and ampicillin/sulbactam. In addition, solumedrol was also administered and ENT was consulted. At that time the question arose whether to start the patient on anticoagulation therapy for the thrombus. The decision was made to hold off on anticoagulation therapy and re-evaluate the thrombus following surgery.

The patient was taken to the operating room for I&D of the peritonsillar abscess and approximately 10 cc of pus was aspirated. He was continued on broad spectrum IV antibiotics and reported immediate clinical improvement postoperatively. On hospital day three his leukocytosis was improving and a repeat ultrasound of the neck showed very minimal residual thrombosis of the left internal jugular vein. Gram stain of the aspirated fluid demonstrated 2+ WBCs, 2+ gram positive cocci in pairs and chains, and 1+ gram positive rods, with few mixed anaerobic flora. Although speciation and sensitivities were unavailable, the antibiotics were de-escalated to ceftriaxone and metronidazole. A peripherally inserted central venous catheter (PICC) line was placed for the patient to continue IV antibiotics in the outpatient setting in order to complete a 2-week course. He was advised to follow up with ENT for interval tonsillectomy in 4-6 weeks to prevent recurrence.

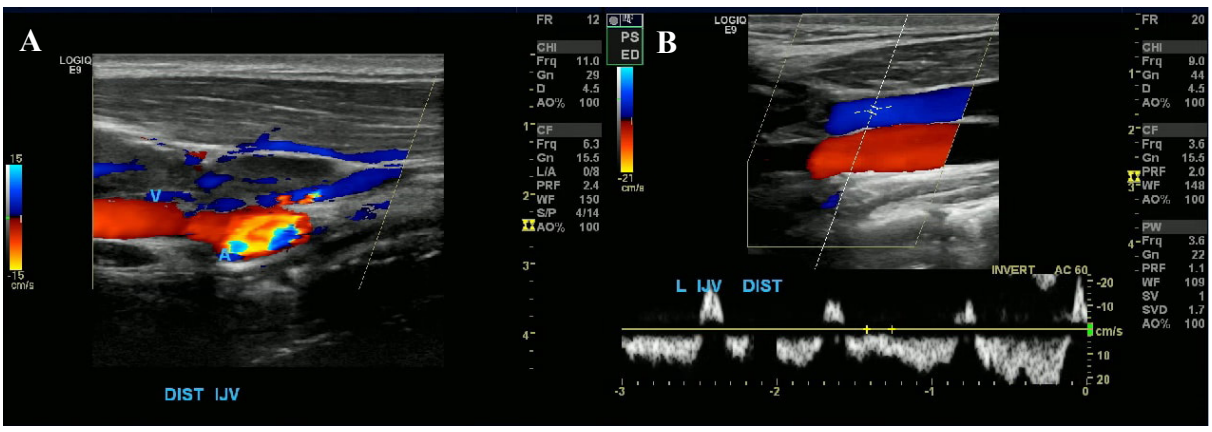

Figure 2. Ultrasound of the neck: (A), intraluminal echogenic filling defect within the distal left jugular vein which was non-compressible, consistent with a non-occlusive thrombus. **(B)**, repeat ultrasound after the initiation of therapy demonstrating very minimal residual thrombosis of the distal IJV.

3. Discussion

Suppurative thrombophlebitis of the internal jugular vein (IJV) is a venous thrombosis due to the infectious involvement of the carotid sheath vessels with bacteria and is seen in association with intravenous catheters or with certain deep neck infections (*5*). It was initially described in 1936 by Andre Lemierre as postanginal septicemia but has also been referred to as Lemierre's Syndrome. Lemierre's syndrome is described as a rare, potentially fatal condition characterized by internal jugular vein thrombosis with septicemia following an acute oropharyngeal infection. The infectious involvement of the carotid sheath vessels can arise from the normal oropharyngeal flora, most notably *Fusobacterium necrophorum*, but other organisms have also been implicated. It has a propensity to affect young healthy adults, with a mean age of 20 years, often occurring within one week of onset of pharyngitis (*5*). Incidence is between 0.6 and 2.3 per million with a mortality rate between 4% and 18% (*6*). Septic pulmonary emboli are a common sequelea, reported in as high as 97% of cases (*5,7,8*). This may lead to hypoxemia and empyema among other potential consequences.

Lemierre's syndrome was once fatal, but the incidence has drastically decreased due to the wide spread use of antibiotics. This decrease has led to Lemierre's syndrome often being referred to as "the forgotten disease" due to its rarity and unfamiliarity among physicians. However, there may be a resurgence in Lemierre's related to recent restriction of antibiotic use for upper respiratory tract infections which are frequently presumed to be viral in etiology (*9*).

One should suspect IJV thrombophlebitis in a patient with pharyngitis whose symptoms do not resolve within 3-5 days, a rapidly worsening course, unilateral neck swelling, and systemic symptoms with persistent fever or bacteremia. Early recognition is vital to prevent sepsis and death, yet the diagnosis is often delayed because of the indolent course and obscurity of the syndrome. The diagnosis was previously made with blood cultures showing F. necrophorum. However, appropriate imaging findings utilizing CT can precede blood culture results (*10*). A CT scan of the neck with contrast is the most useful study to detect internal jugular vein thrombosis (*7*). Due to the frequency of pulmonary emboli that accompanies this condition, a CT scan of the chest is often performed. Furthermore, ultrasonography is also useful to evaluate for jugular vein thrombosis and can be used to assess for thrombus extension (*7*). In addition, microbiologic diagnosis may be performed with blood cultures or cultures from purulent material expressed from the site.

Prior to antibiotics, the treatment for Lemierre's was primarily surgical ligation and excision. Today, the treatment, similar to other forms of septic thrombophlebitis, involves removing the foci of infection, such as with catheter removal or incision and drainage of an abscess, prompt antibiotic administration, and a consideration for possible anticoagulation (*7*). Since beta lactamase production has been identified in some *Fusobacterium* species, empiric antibiotic selection should include a beta lactamase resistant beta lactam, such as ampicillin-sulbactam (*11*).

While anticoagulation is the mainstay of treatment of deep venous thromboembolism (DVT) and pulmonary embolism (PE), the use of therapy is controversial in septic thrombophlebitis. Despite the potential life threatening complications mentioned in the literature, the role of anticoagulation is not recommended in the absence of extension of the thrombus (*12*). In general, the mechanism of the circulatory disturbance in a particular thrombotic disease process should be understood prior to the initiation of anticoagulation therapy. We know that Virchow's triad describes that a thrombus formation can be due to venous stasis, a hypercoagulable state, and endothelial injury. The accepted mechanism of Lemierre's is that certain deep neck infections in predisposed individuals by a strain of bacteria producing endotoxins and hemagglutinin leading to further invasion, endothelial inflammation, platelet aggregation, and a suppurative thrombophlebitis. Early in the course of thrombophlebitis, while the thrombus is firmly attached, antibiotics may be all that is necessary to treat the condition.

There are various complex mechanisms leading to such thrombotic events. Perhaps further studies can elucidate the most appropriate management of these thrombotic events, possibly not requiring anticoagulation to improve clinical outcomes in patients. Clinicians should suspect jugular vein suppurative thrombophlebitis in patients with pharyngitis, septic pulmonary emboli, and persistent fever despite antimicrobial therapy.

References

1. Chamseddin KH, Kirkwood ML. Lemierre's syndrome associated mycotic aneurysm of the external carotid artery with primary internal carotid artery occlusion in a previously healthy 18-year-old female. Ann Vasc Surg. 2016; 36:291.e11-291.e14.
2. Karkos PD, Asrani S, Karkos CD, Leong SC, Theochari EG, Alexopoulou TD, Assimakopoulos AD. Lemierre's syndrome: A systematic review. The Laryngoscope. 2009; 119:1552-1559.
3. Celikel TH, Muthuswamy PP. Septic pulmonary emboli secondary to internal jugular vein phlebitis (postanginal sepsis) caused by Eikenella corrodens. Am Rev Respir Dis. 1984;130:510-513.
4. Sinave CP, Hardy GJ, Fardy PW. The Lemierre syndrome: Suppurative thrombophlebitis of the internal jugular vein secondary to oropharyngeal infection. Medicine. 1989; 68:85-94.
5. Vargiami EG, Zafeiriou DI. Eponym: The Lemierre syndrome. Eur J Pediatr. 2010; 169:411-414.

6. Golpe R, Marín B, Alonso M. Lemierre's syndrome (necrobacillosis). Postgrad Med J. 1999; 75:141-144.
7. Kobayashi Y, Takayanagi N, Sugita Y. A case of Lemierre's syndrome with septic pulmonary embolisms. Kansenshogaku Zasshi. 2014; 88:695-699. (in Japanese)
8. Ramirez S, Hild TG, Rudolph CN, Sty JR, Kehl SC, Havens P, Henrickson K, Chusid MJ. Increased diagnosis of Lemierre syndrome and other *Fusobacterium necrophorum* infections at a Children's Hospital. Pediatrics. 2003; 112:e380.
9. Screaton NJ, Ravenel JG, Lehner PJ, Heitzman ER, Flower CD. Lemierre syndrome: Forgotten but not extinct – report of four cases. Radiology. 1999; 213:369-374.
10. Lin D, Reeck JB, Murr AH. Internal jugular vein thrombosis and deep neck infection from intravenous drug use: Management strategy. Laryngoscope. 2004; 114:56-60.
11. Prandoni P, Tormene D, Pesavento R; Vesalio Investigators Group. High *vs*. low doses of low-molecular-weight heparin for the treatment of superficial vein thrombosis of the legs: A double-blind, randomized trial. J Thromb Haemost. 2005; 3:1152-1157.
12. Phua CK, Chadachan VM, Acharya R. Lemierre syndrome – should we anticoagulate? A case report and review of the literature. Int J Angiol. 2013; 22:137-142.

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Case Report

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Silence pancreatitis in systemic lupus erythematosus

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Summary **We present here a systemic lupus erythematosus (SLE) related biochemically silent pancreatitis which was assessed *via* computed tomography in a 35-year-old woman. A patient with a twelve-year history of SLE presented with exacerbation of symptoms of the basic disease, with SLE Disease Activity Index > 15. She was referred to inpatient care. Dosage of corticosteroid and azathioprine for SLE was increased; subclinically and biochemically silent pancreatitis had developed, and was not diagnosed within an appropriate time. On the 15th hospital day, the patient died due to multisystem organ failure, which was defined as a consequence of clinically and biochemically silent pancreatitis in systemic lupus erythematosus.**

Keywords: Pancreatitis, systemic lupus erythematosus, pancreatic enzymes, computed tomography

1. Introduction

Systemic lupus erythematosus (also known as lupus or SLE) is a chronic inflammatory disease that can affect various parts of the body. The cause of lupus is not well understood; it is an autoimmune condition, meaning that the body's immune system attacks its own tissues as if they are foreign. Virtually every system and organ can be affected by SLE. The gastrointestinal tract is one of the most commonly affected systems in SLE, and the incidence of gastrointestinal manifestations may be underestimated clinically because some of these are indistinct and may not have abdominal symptoms (*1,2*).

About 160 cases of SLE related acute pancreatitis have been reported in the literature (*2*). The main causes of pancreatitis are mechanical obstructions of the pancreatic duct and toxic metabolites such as alcohol intake and certain drugs. However, a number of patients develop "idiopathic" pancreatitis, in which no aetiology other than SLE itself can be identified. The involvement of the pancreas in SLE is rare; elevated serum amylase and lipase are the most commonly detected biochemical abnormalities. Other biochemical abnormalities include hypertriglyceridemia, hypocalcaemia, hypoalbuminemia, abnormal liver function tests and elevated serum creatinine. The exact pathogenic mechanisms of SLE related pancreatitis are not clear, but are probably mediated by immune complex-induced microangiitis (*2-4*).

The aim of this work is to report on an SLE related clinically and biochemically silent pancreatitis, which was assessed *via* diagnostic imaging in a 35-year-old woman.

2. Case Report

A 35-year-old woman with a 12-year history of SLE presented because of exacerbation of symptoms of the basic disease such us joint pain, headache, myositis, rash, *etc*. (SLE Disease Activity Index > 15). She was referred to inpatient care.

She had no history of other disease or surgery. In the last twelve years treatment had been based mostly on azathioprine (50 mg/day) and corticosteroids (20 mg/day).

The patient had been well until seven days before admission, when fatigue and malaise developed. On

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Table 1. Complete blood count, laboratory analysis and serology during hospital stay

Items	On admission	4th day	9th day	13th day
Hemoglobin 12-15 g/dL	8.1	7.4	9.1	8.3
Hematocrit 36-47%	28	26	33	24
Mean corpuscular volume 80-100 fL	71	67	76	68
Red Blood Cells 4.2 -5.4 million/mcL	3.1	2.7	3.8	2.8
White blood cells 4-10 × 10^9/L	12.6	13.4	15.9	15.4
Neutrophils (%)	76	79	84	86
Lymphocytes (%)	14	12	11	11
Monocytes (%)	7.5	6.9	4	2
Platelets 150-400 × 10^9/L	112	99	88	75
Erythrocyte sedimentation rate in first hour	60		105	120
C-reactive protein < 0.5 mg/dL	75	98	120	177
International normalizedratio 0.9-1.2		1.1		
Glucose 4.1-6.1 mmol/L	5.5	5.9	6.3	7.6
Urea nitrogen 2.9-7.1 mmol/L	10.30	12.90	14.8	10.5
Creatinine 61.9-115 µmol/L	290	330.7	462	295
Triglycerides < 2.82 mmol/L	1.7		1.6	1.5
Total cholesterol 3-5.5 mmol/L	5.1		5.0	5.2
Sodium 135-145 mmol/L	144		149	140
Potassium 3.5-5 mmol/L	5.4	5.7	6.6	5.5
Total calcium 2-2.6 mmol/L	2.26	2.12	1.79	1.37
Phosphate 0.8-1.5 mmol/L			1.2	
pH 7.35-7.45	7.39	7.41	7.41	7.44
Bicarbonate 18-22 mmol/L	34	40	41	49
Total protein 60-80 g/L	64			55
Albumin 35-50 g/L	31			24
Aspartateaminotransferase 5-30 U/L	38		60	110
Alanineaminotransferase 5-30 U/L	61		75	158
Gammaglutamyltransferase 6-50 U/L	35		49	68
Alkalinephosphatase 50-100 U/L	68		94	115
Amylase 30-125 U/L		70	95	120
Lipase 10-150 U/L		90		148
Total bilirubin: 2-20 µmol/L		17.8		25
Direct bilirubin: 0-6 µmol/L		5		9
Antinuclear antibodies			positive	
Lactatedehydrogenase 60-160 U/L	390	378	420	385
Urine mL/day		900	800	450

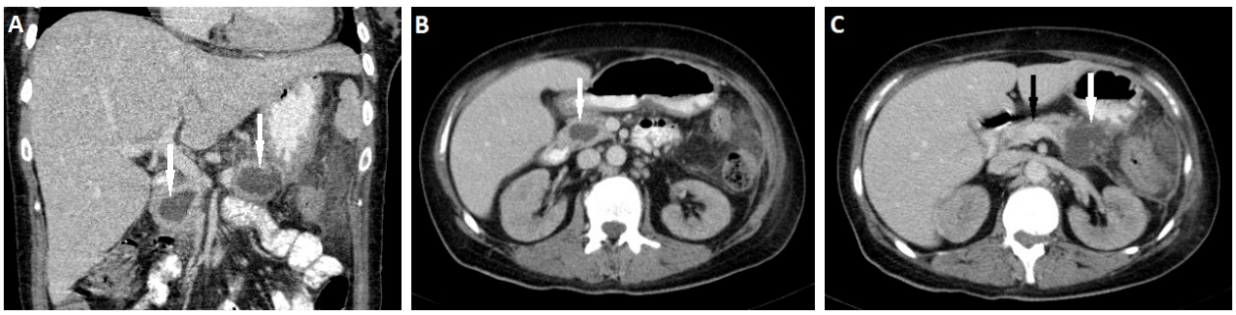

Figure 1. Computed tomography scans (A), (B), (C) show enlarged pancreas with irregular echogenicity and fluid collection (white arrows), (C) scan shows slightly dilated pancreatic duct (black arrow).

examination she felt feverish. Temperature was 37.8°C, blood pressure 110/70 mmHg, pulse 90 beats per minute and respiratory rate 16 breaths per minute. She was alert and oriented.

Physical examination showed swollen joints (elbow, wrist, knee), facies and skin rash. There were no meaningful symptoms from the chest and abdominal organs. Complete blood count (CBC), laboratory analysis and serology markers on admission and during hospital stays are shown in Table 1.

Initially, imaging assessment *via* real-time sonography of abdominal organs (pancreas, liver, gallbladder, spleen, both kidneys) and chest X-ray showed normal appearance.

Within the next few days, besides increased doses of azathioprine (150 mg/day) and corticosteroids (60 mg/day) for SLE, the patient underwent ciprofloxacin (1 g/day), pantoprazole (40 mg/day), NSAID and physiological solution (500 mL/day for the first three days). On the eighth day two units of packed red cells

were transfused. Over this time, she had oral intake of food and drugs.

During inpatient care, the general aspect of the patient worsened, and renal failure significantly increased with mild general swelling. On the 11th hospital day, due to renal failure, the patient was referred to haemodialysis *via* central venous catheter (CVK), and a control chest X-ray showed the appropriate position of the catheter in the superior cava vein. On the same day, real-time sonography showed an enlarged pancreas with irregular morphology and dilated pancreatic duct, which was additionally confirmed by computed tomography (CT) - Figure 1. A day later, pancreatic enzymes remained in the referral range.

On the 15th hospital day, the patient died due to multisystem organ failure, defined as a consequence of clinically and biochemically silent pancreatitis in SLE.

3. Discussion

The diagnosis of acute pancreatitis is based on clinical presentation, laboratory tests where serum amylase and/or lipase are more than three times the upper limit of normal, and CT scan findings. It is difficult to assess the disease due to the lack of accurate and uniformly accepted definitions of disease severity and commonly encountered complications of acute pancreatitis (*5*).

The incidence of SLE-related pancreatitis may be underestimated, because cases of subclinical pancreatitis with elevated pancreatic enzymes but no symptoms are not diagnosed or reported (*2*).

In SLE-related pancreatitis, approximately 90% have abdominal pain, almost 75% of patients have nausea and vomiting, and about 50% have fever (*2,4,6*).

During 12 days of inpatient care, our patient did not have or did not report abdominal or epigastric pain; there was no vomiting, and throughout this time she had an oral intake.

In SLE related pancreatitis, increased values for serum amylase and lipase are the most commonly detected (*2,6*). Normal serum amylase levels have been reported in some cases of acute pancreatitis, but serum lipase levels are usually elevated. Normal serum lipase in the setting of acute pancreatitis is an extremely rare occurrence. A literature review by Shah *et al*. found only two case reports of clinical and radiological evidence of acute pancreatitis with a normal serum lipase level (*7*).

In our case, both pancreatic enzymes, amylase and lipase, remained within normal limits during the hospital stay.

Additional biochemical abnormalities in SLE related pancreatitis include hypoalbuminemia in 78%, abnormal liver function tests in 65% and elevated serum creatinine 44% (*2,4*). Biochemical values for albumin, serum creatinine, liver function tests, *etc*. are presented in Table 1.

Pascual-Ramos *et al*. (*8*) reported that the SLE disease activity index was significantly increased in patients with idiopathic pancreatitis. Their study ruled out the assumption that corticosteroids and azathioprine could cause SLE related pancreatitis. Our patient was admitted in the active phase of the basic disease, and the dosage of corticosteroid and azathioprine for SLE was increased. Abnormalities in CBC are also common: anaemia, leukopenia, and thrombocytopenia are relatively common (81%, 59%, and 48% respectively), while leukocytosis is infrequent (only 15%) (*4*). In our case she had a CBC all the time with severe anemia and thrombocytopenia; leukocytosis existed during inpatient care, and non-specific inflammatory parameters such as C-reactive protein and erythrocyte sedimentation rate were also elevated. Based on imaging assessment of the pancreatic disorder and high SLE disease activity in addition to anemia, thrombocytopenia, hypoalbuminemia, abnormal liver function test and elevated serum creatinine; SLE related pancreatitis had developed, and unfortunately was not diagnosed within an appropriate time.

The high mortality rate in SLE pancreatitis is associated with severe disease activity, thrombocytopenia and acute renal failure (*3,4,6*). Due to clinically significant renal failure, our patient was referred to haemodialysis treatment *via* CVK. On the same day, CT revealed enlarged and irregular morphology of the pancreas, and four days later she died due to multisystem organ damage.

Our case showed an SLE related biochemically silent pancreatitis with normal serum lipase and amylase, which was mainly diagnosed *via* contrast enhanced CT. There are no cases in the recent literature which describe SLE related pancreatitis with normal levels of serum lipase and amylase.

In conclusion, although uncommon, acute pancreatitis should be considered in the differential diagnosis of abdominal pain in SLE patients. Mechanical obstruction (most frequently a result of choledocholithiasis) and toxic-metabolic aetiologies (secondary to alcohol intake, certain drugs, hypocalcaemia or hypertriglyceridemia) should be ruled out. The early diagnosis of acute pancreatitis in SLE patients, especially those with abdominal pain, and appropriate treatment, is beneficial for a better therapeutic outcome in the majority of patients. Acute pancreatitis can have a variable presentation, and physicians caring for patients who are presented to the hospital with epigastric pain should be aware despite normal amylase and lipase levels. In appropriate clinical conditions, further imaging modalities such as CT scans may be helpful.

References

1. Vitali C, Bencivelli W, Isenberg DA, Smolen JS, Snaith ML, Sciuto M, d'Ascanio A, Bombardieri S. Disease activity in systemic lupus erythematosus: Report of the

Consensus Study Group of the European Workshop for Rheumatology Research. I. A descriptive analysis of 704 European lupus patients. European consensus study group for disease activity in SLE. Clin Exp Rheumatol. 1992; 10:527-539.
2. Tian XP, Zhang X. Gastrointestinal involvement in systemic lupus erythematosus: Insight into pathogenesis, diagnosis and treatment. World J Gastroenterol. 2010; 16:2971-2977.
3. Wang Q, Shen M, Leng X, Zeng X, Zhang F, Qian J. Prevalence, severity, and clinical features of acute and chronic pancreatitis in patients with systemic lupus erythematosus. Rheumatol Int. 2016; 36:1413-1419.
4. Breuer GS, Baer A, Dahan D, Nesher G. Lupus-associated pancreatitis. Autoimmun Rev. 2006; 5:314-318.
5. Zerem E. Treatment of severe acute pancreatitis and its complications. World J Gastroenterol. 2014; 20:13879-13892.
6. Makol A, Petri M. Pancreatitis in systemic lupus erythematosus: Frequency and associated factors - a review of the Hopkins Lupus Cohort. J Rheumatol. 2010; 37:341-345.
7. Shah AM, Eddi R, Kothari ST, Maksoud C, DiGiacomo WS, Baddoura W. Acute pancreatitis with normal serum lipase: A case series. JOP. 2010; 11:369-372.
8. Pascual-Ramos V, Duarte-Rojo A, Villa AR, Hernández-Cruz B, Alarcón-Segovia D, Alcocer-Varela J, Robles-Díaz G. Systemic lupus erythematosus as a cause and prognostic factor of acute pancreatitis. J Rheumatol. 2004; 31:707-712.

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Case Report

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Cryotherapy as a conservative treatment modality for gingival enlargement in a patient with Sturge-Weber Syndrome

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Summary **This case report describes a case of Sturge-Weber syndrome reported for unilateral gingival enlargement and bleeding from gingiva in maxillary left region. Initial treatment in the form of scaling and root planing was done but recurrence was observed after one year of follow up. Instead of performing conventional surgery, an alternative conservative treatment was planned in the form of cryotherapy with the help of closed nitrous oxide probe. Seeing the satisfactory results obtained, cryotherapy can be suggested as an atraumatic, bloodless and effective chair side procedure for treating vascular gingival enlargement.**

Keywords: Cryotherapy, hemorrhage, nitrous oxide, gingival enlargement, Sturge-Weber syndrome

1. Introduction

Sturge-Weber syndrome, a rare neurocutaneous disorder belongs to the group of neuroectodermal development anomalies called "phakomatosis" and is characterized by venous angiomas of leptomeninges over the cerebral cortex with ipsilateral angiomatous lesions of face, skull, jaws, and oral soft tissues (*1*). Facial lesions known as port wine stains include rosy-purple nevus flammeus lesions that are sharply demarcated, usually flat and occur unilaterally. Intraorally, angiomatosis on buccal mucosa and lips may present as purplish-red discoloration, which may involve soft palate, tongue, floor of mouth, and gingiva. Gingival lesions range from slight vascular enlargement to large growths.

Treatment modalities for vascular gingival enlargements include conscientious observation, radiation therapy, steroids, antimetabolites, sclerosing solutions and surgical removal of gingival overgrowth with electro-surgery or laser (*2*). Cryotherapy has been used in treatment of keratotic, hyperplastic, granulomatous, vascular, pigmented and salivary gland lesions (*3*). However, an established protocol and description of the procedure for diffuse vascular gingival enlargement is lacking in dental literature.

This case report describes the treatment of vascular gingival enlargement with cryotherapy and discusses the intricacies involved in management of oral manifestations of Struge-Weber syndrome.

2. Case Report

A 12-year-old female patient was referred to Department of Periodontics with swelling in left side posterior region and spontaneous bleeding from gums for last three years. Medical history revealed that she was suffering from Sturge-Weber syndrome and was on antiepileptic drug (phenytoin sodium) from age of six months. This drug was changed to sodium valproate since last two years. Extraoral examination revealed presence of port wine stains, facial asymmetry due to hemihypertrophy of left side of face and increase in nasal bridge width (Figure 1A). Intraorally bright red discoloration of alveolar mucosa and enlargement of gingiva was observed in maxillary left region from

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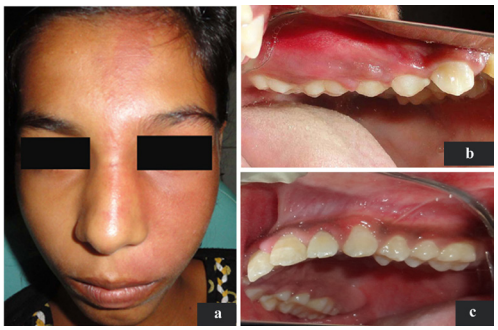

Figure 1. Clinical photographs. (A) Extraoral photograph showing facial asymmetry, portwine stains on the left side of face and increase in nasal bridge width; **(B)** Preoperative intraoral view showing gingival enlargement in maxillary left region and bright red discoloration of alveolar mucosa; **(C)** Normal contralateral side.

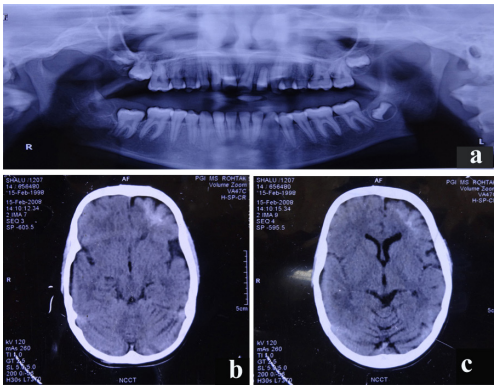

Figure 2. Panoramic radiographic and computed tomographic images. (A) Panormic radiograph showing no alveolar bone loss with respect to involved segment; **(B)** Computed tomographic scan showing hemiatropy of left cerebral hemisphere along with calcifications; **(C)** Normal tomographic findings on right side.

central incisor to second molar (Figure 1B). Enlarged gingiva covering more than one third of tooth structure extending from first premolar to second molar teeth was reddish pink, soft in consistency, non-tender and blanched on pressure. Gingiva and alveolar mucosa was normal on contralateral side (Figure 1C).

Radiographs showed no alveolar bone loss with respect to involved segment (Figure 2A). The complete hemogram was within normal limits. Computed tomography scan done in early childhood revealed subcortical gyriform calcification in left frontal lobe with prominent adjacent sulci representing mild cortical atrophy (Figures 2B and 2C).

A thorough plaque control regimen (scaling, root planing, oral hygiene instructions) was initiated to minimize gingival inflammation. After one month, gingival enlargement had decreased marginally and no bleeding was reported. After one year recurrence of gingival enlargement was observed in maxillary left region (Figure 3A). Cryotherapy, a non-invasive treatment option was planned as the conventional surgical intervention could not be carried out because

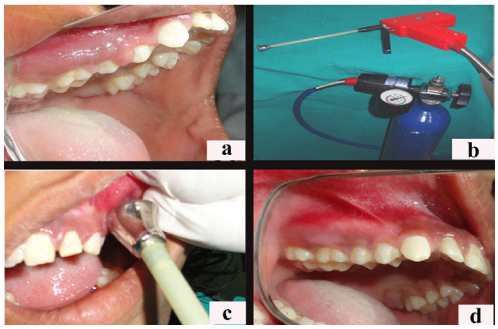

Figure 3: Clinical photographs and Cryotherapy apparatus. (A) Recurrence of gingival enlargement after one year of oral prophylaxis; **(B)** Cryotherapy apparatus showing nitrous oxide cryogun and cylinder; **(C)** Use of nitrous oxide cryoprobe to treat the gingival enlargement; **(D)** Complete resolution of gingival enlargement after one year of cryotherapy.

of risk of hemorrhage and patient's reluctance. The procedure was carried out, under topical anesthesia (2% lignocaine hydrochloride gel), using a closed nitrous oxide probe (Figure 3B) with a freezing cycle of 30 seconds and then thawing for 1 minute. This process was repeated twice with overlapping freezes (Figure 3C).

No postoperative pain and complications were seen on second day. Re-epithelialization of the lesion occurred at seventh day of follow up with remarkable regression of gingival enlargement. Complete regeneration and regression of enlargement was observed after one month. Resolution of pseudo-pockets was noticed and no recurrence of gingival enlargement was found after one year of follow up (Figure 3D).

3. Discussion

Dental rehabilitation of patients with Sturge-Weber syndrome is a complex process requiring initial conservative management and later surgical intervention. However, this has to be exercised with caution because the tissues may bleed profusely intraoperatively and postoperatively.

Cryotherapy is a method of lesion destruction through ischemic necrosis of the target tissue induced by rapid freezing in situ. This type of tissue destruction helps in bloodless field of surgery and is useful in treating vascular gingival enlargement. The basic technique involves rapid cooling, slow thawing and repetition of the freezing process to maximize tissue destruction (*4*). Most tissues freeze at -2.2°C, and tissue death occurs at a temperature of -20°C (*5*). Liquid nitrogen (-191°C), nitrous oxide (-80°C), and carbon dioxide (-79°C) are commonly used cryogens. The available cryotherapy apparatus is classified into open and closed systems. Open systems involve direct application of cryogenic fluid (usually liquid nitrogen) to the lesion with a cotton swab or spray. In closed systems the tissue is frozen by a cryoprobe

(*6-10*). Closed probe technique is useful as direct contact between cryogen and the tissue allows a more controlled and profound depth of freezing.

These probes follow the principles of Joule-Thompson expansion which enable substances to undergo a drop in temperature when moved from high pressure area to low pressure area. When nitrous oxide is released from high pressure inside the cryoprobe to the lower pressure cryotip, the drop in temperature allows freezing of the tissues to occur (*6,10*). Current protocols suggest for most benign mucosal lesions a 1-2 minute freeze/thaw cycle, for premalignant/malignant lesions three 2 minute freeze/thaw cycles and for smaller lesions, shorter freeze cycles (20-30 seconds) using a cryoprobe are adequate (*6,10*).

Ichingolo *et al*. compared traditional surgery, electro surgery, CO_2 and neodymium-doped yttrium aluminium garnet (Nd: YAG) laser in treating gingival hyperplasia in Sturge-Weber syndrome and found most encouraging results with Nd:Yag laser (*11*). In present case, cryotherapy was chosen because of its advantage of providing better hemostatic control which considerably reduced the surgical time along with faster and easier tissue healing as opposed to slow and complex healing in case of traditional scalpel surgery. After therapy, no pain was reported because of blockage of neural transmission to the area and there was absence of secondary infection. Through our observations, we have found cryotherapy as an effective conservative method of management of vascular malformations. In conclusion seeing the satisfactory results obtained, cryotherapy can be suggested as an atraumatic, bloodless, economical and effective chair side procedure for treating gingival enlargement without resorting to knife surgery in patients suffering from Sturge-Weber syndrome.

References

1. Shafer WG, Hine WK, Levy BM. Textbook of oral pathology, 4th edition Philadelphia, W.B Saunders, USA, 1983; pp. 157-158.
2. Hylton RP. Use of CO_2 laser for gingivectomy in a patient with Sturge-Weber disease complicated by dilantin hyperplasia. J Oral Maxillofac Surg. 1986; 44:646-648.
3. Toida M, Ishimaru JI, Hobo N. A simple cryosurgical method for treatment of oral mucous cysts. Int J Oral Maxillofac Surg. 1993; 22:353-355.
4. Salmassy DA, Pogrel MA. Liquid nitrogen cryosurgery and immediate bone grafting in the management of aggressive primary jaw lesions. J Oral Maxillofac Surg. 1995; 53:784-790.
5. Fraser J, Gill W. Observations on ultra-frozen tissue. Br J Surg. 1967; 54:770-776.
6. Farah CS, Savage NW. Cryotherapy for treatment of oral lesions. Aust Dent J. 2006; 51:2-5.
7. Gongloff RK, Gage AA. Cryosurgical treatment of oral lesions: Report of cases. J Am Dent Assoc. 1983; 106:47-51.
8. Leopard PJ, Poswillo DE. Practical cryosurgery for oral lesions. Br Dent J. 1974; 136:185-196.
9. Leopard PJ. Cryosurgery and its application to oral surgery. Br J Oral Surg. 1975; 13:128-152.
10. Nogueira VK, Fernandes D, Navarro CM, Giro EM, de Almeida LY, León JE, Bufalino A. Cryotherapy for localized juvenile spongiotic gingival hyperplasia: Preliminary findings on two cases. Int J Paediatr Dent. 2017; 27:231-235.
11. Inchingolo F, Tatullo M, Abenavoli FM, Marrelli M, Inchingolo AD, Inchingolo AM, Dipalma G. Comparison between traditional surgery, CO_2 and Nd:YAG laser treatment for generalized gingival hyperplasia in Sturge-Weber syndrome: A retrospective study. J Investig Clin Dent. 2010; 1:85-89.

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Commentary

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Defining rare diseases in China

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Summary **China has the world's largest population of people with rare diseases. However, defining rare diseases remains a challenge in China. Over the past few decades, several definitions have been proposed but they have yet to be agreed to by all stakeholders. To overcome this impasse, a list of several rare diseases has recently been created. This rare disease list might be used in place of a prevalence-based definition, especially in healthcare policy-making.**

Keywords: China, rare diseases, definition

China's concept of rare diseases was first sketched out by the radiologists Drs. Gui Lin and Chenglin Wang in the early 1980s, at nearly the same time that the Orphan Drug Act was passed in the United States (*1*). In the 1980s, advanced medical equipment, and computed tomography (CT) and magnetic resonance imaging (MRI) in particular, began to arrive in China, and many cases of rare diseases were identified and reported for the first time by hospitals across the country. However, the definition of rare diseases was not considered at the time.

With China's rapid social and economic development over the past decade, rare diseases have become a major issue once again. Given China's massive population, the widespread view is that China has the largest population of people affected by rare diseases, and now is the time to look for healthcare solutions to rare diseases. A growing chorus of voices is calling for legislation on rare diseases and orphan drugs in line with the model in the United States and elsewhere (*2*). Since legislation would need to specifically identify its scope, the definition of rare diseases has surfaced once again.

Over the past decade, the most widely used definition for rare diseases in China has been the WHO's definition of a rare disease, *i.e*. a disease with an incidence of 0.65-1‰. However, the validity of the WHO's definition has recently been called into question. Definitions of rare disease from other countries and organization such as the United States and Europe Union have consistently been used as a reference. However, these definitions have a relatively broad scope. For example, some cancers with a low prevalence such as ovarian cancer and some rare diseases listed by Orphanet were not regarded as rare diseases by most doctors in Shandong Province, China, according to a previous survey by the current authors (*3*).

At a seminar conducted by the Genetics Branch of the Chinese Medical Association on May 17, 2010, experts mainly in the field of medical genetics suggested that rare diseases in China be defined as "disorders with a prevalence less than 1/500,000 or with an incidence less than 1/10,000 among newborns" (*4*). This definition sets a threshold lower than all currently established definitions in use worldwide, thus excluding most recognized rare disorders. As a result, this definition of rare diseases has not been agreed to by all stakeholders, and especially patient organizations.

Most definitions of rare diseases are prevalence-based. Because of the lack of epidemiological data on rare diseases in China, deducing the population threshold that defines rare diseases is difficult. In 2015, the current authors proposed a bottom-up approach to define rare diseases in China. This strategy depends not on prevalence but on the minimum number of patients needed for industry to make a reasonable profit on an innovative drug (*5*). The current authors proffered 300,000 to 500,000 cases as a reference threshold with which to define rare diseases in China. This proposal

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linked the concept of rare diseases with orphan drugs, so it is highly useful in terms of Chinese policy-making on rare diseases. However, this definition has several limitations since the level of Chinese R&D on innovative drugs cannot be accurately determined.

Supporting evidence for both international and domestic definitions of rare diseases is lacking in China, so reaching a consensus has been difficult. Most recently, some stakeholders began to deviate from a prevalence-based definition and listing rare diseases instead. A year ago, the City of Shanghai drafted a list of rare diseases that include 58 typical rare disorders; most are extremely rare but can be treated with corresponding orphan drugs. In September 2016, a patients' organization proposed another list of rare diseases that included 147 disorders. These efforts stay away from the prevalence threshold and the concept of defining all rare diseases at one time but prioritize the need to identify treatable disorders, which are more amenable to policy-making.

Improvement in healthcare for rare diseases has become a national effort. Last year, China established a committee to formulate medical strategies for rare diseases. An official definition of rare disease will presumably be discussed and proposed in the near future.

Acknowledgements

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References

1. Han J, Cui Y, Zhou X. Rare diseases research in China: Opportunities, challenges, and solutions. Intractable Rare Dis Res. 2012; 1:10-12.
2. Wang JB, Guo JJ, Yang L, Zhang YD, Sun ZQ, Zhang YJ. Rare diseases and legislation in China. Lancet. 2010; 375:708-709.
3. Zhao H, Cui Y, Zhou X, Pang J, Zhang X, Xu S, Han J. Study and analysis of the state of rare disease research in Shandong Province, China. Intractable Rare Dis Res. 2012; 1:161-166.
4. Song P, Gao J, Inagaki Y, Kokudo N, Tang W. Rare diseases, orphan drugs, and their regulation in Asia: Current status and future perspectives. Intractable Rare Dis Res. 2012; 1:3-9.
5. Cui Y, Han J. A proposed definition of rare diseases for China: From the perspective of return on investment in new orphan drugs. Orphanet J Rare Dis. 2015; 10:28.

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Letter

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Pancreatic lipomatosis in cystic fibrosis: Rare manifestation of an uncommon disease

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Summary **Cystic fibrosis is deemed to be uncommon in India. The presentation is usually in the childhood although more cases are now being recognized in adolescence and adulthood. We report a case of an adolescent male who had been treated for recurrent pulmonary infections and received anti-tubercular therapy for a possible diagnosis of sputum negative pulmonary tuberculosis and was evaluated for steatorrhea. The presence of pancreatic exocrine insufficiency along with pancreatic lipomatosis suggested the diagnosis of cystic fibrosis.**

Keywords: Cystic fibrosis, bronchiectasis, pancreatic exocrine insufficiency, steatorrhea, endoscopic ultrasound

Cystic fibrosis is a multisystem disorder characterized by recurrent pulmonary infections, pancreatic exocrine insufficiency and is usually diagnosed in young age. It is an inherited disorder and the age and manner of presentation vary depending on the severity of defect in the cystic fibrosis gene. Since a large number of mutations have been reported the clinical presentation is extremely variable (*1*). We report about an 18 year old male who had been treated as sputum negative tuberculosis elsewhere and presented to us with steatorrhea prompting evaluation for cystic fibrosis.

An 18-year old male had been symptomatic for recurrent episodes of cough associated with mucoid expectoration and occasional hemoptysis for 7 years. He had been treated with multiple courses of antibiotics and had received 6-month therapy for a suspected diagnosis of sputum negative pulmonary tuberculosis elsewhere. For the past 6 months, he had noticed an increased bulk of his stools and passage of oily stools. This was associated with significant loss of weight (6 kg) and had developed temporal hollowing suggesting fat malabsorption. He denied any history of abdominal pain. To avoid steatorrhea, he had reduced the intake of fat in his meals. His body mass index was 15.6 kg/m^2. The patient's younger sibling had died of an undiagnosed respiratory illness at 4 years of age. His parents were asymptomatic and did not report any respiratory illness. His fecal elastase levels were done for the possibility of pancreatic exocrine insufficiency and were 53 (normal > 200 μg/g of stool). His abdominal and chest computed tomography revealed a hypo-attenuating pancreas (Figure 1A) consistent with complete pancreatic lipomatosis and evidence of bronchiectasis (Figure 1B). Endoscopic ultrasound confirmed the presence of diffusely hyperechoic pancreas consistent with pancreatic lipomatosis (Figure 1C). The sweat chloride levels were elevated at 79 mmol/L. This was repeated

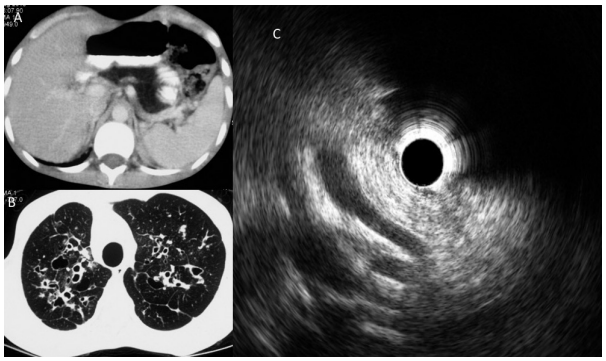

Figure 1. (A), CT abdomen showing pancreatic lipomatosis; (B), CT chest showing bronchiectasis; (C), Endoscopic ultrasound showing hyperechoic pancreas.

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Table 1. Showing results of diagnostic investigations

Feature	Investigation	Findings
Pancreatic involvement	Computed tomography	Pancreatic hypoattenuation
	Endoscopic ultrasound	Hyperechoic pancreas
	Fecal elastase	Low levels
Pulmonary Involvement	Chest X Ray	Bronchiectasis
	Computed tomography	Bronchiectasis
Basis of diagnosis	Bronchiectasis	
	Pancreatic exocrine insufficiency	
	Elevated sweat chloride	
	Pulmonary disease in sibling	

after one month and the levels were still elevated. Therefore, a diagnosis of cystic fibrosis with pulmonary and pancreatic involvement with pancreatic lipomatosis was made. The patient was started on pancreatic enzyme supplementation along with twice daily proton pump inhibitor. The patient reported improvement in steatorrhea and at 3 months of follow-up reported a gain of 4 kg of his weight. He has been advised to continue follow-up for his pancreatic insufficiency

Cystic fibrosis, although reported as case reports, is believed to be uncommon in India (*2*). Given the high prevalence of pulmonary tuberculosis, it is not unexpected that cystic fibrosis may be confused with pulmonary tuberculosis especially with an adolescent presentation and absence of other organ system involvement as in our case. The appearance of steatorrhea directed the evaluation to pancreatic pathology and brought to light the diagnosis in a patient who had evidence of underlying bronchiectasis (Table 1).

Interestingly, the patient also had complete pancreatic lipomatosis which by itself is uncommon and is variously described as pancreatic lipomatosis or steatosis or adipose atrophy. The fatty replacement may involve a part of the pancreas or may be total, as in the present case (*3*). While in adults the causes may include diabetes mellitus, insulin resistance, obesity, chronic pancreatitis, pancreatic ductal obstruction due to stone or malignancy, in younger age group certain hereditary conditions like cystic fibrosis and Shwachman-Diamond syndrome may be responsible (*4*). Although the entity is uncommon, in patients with cystic fibrosis the presence of complete pancreatic lipomatosis and partial lipomatosis has been reported to be as high as 41% and 19% in one report (*5*). To conclude, the presence of pulmonary symptoms and bronchiectasis along with pancreatic exocrine insufficiency must prompt evaluation for cystic fibrosis.

References

1. Fajac I, De Boeck K. New horizons for cystic fibrosis treatment. Pharmacol Ther. 2017; 170:205-211.
2. Ashavaid TF, Raghavan R, Dhairyawan P, Bhawalkar S. Cystic fibrosis in India: A systematic review. J Assoc Physicians India. 2012; 60:39-41.
3. Ambesh P, Lal H. Pancreatic lipomatosis: Complete replacement of pancreas by fat. J Clin Diagn Res. 2015; 9:OL01.
4. Anand R, Narula MK, Chaudhary V, Agrawal R. Total pancreatic lipomatosis with malabsorption syndrome. Indian J Endocrinol Metab. 2011; 15:51-53.
5. Feigelson J, Pécau Y, Poquet M, Terdjman P, Carrère J, Chazalette JP, Ferec C. Imaging changes in the pancreas in cystic fibrosis: A retrospective evaluation of 55 cases seen over a period of 9 years. J Pediatr Gastroenterol Nutr. 2000; 30:145-151.

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