

ISSN 2186-3644 Online ISSN 2186-361X

# IRDR

## Intractable & Rare Diseases Research

Volume 6, Number 3  
August, 2017



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# IRDR

## Intractable & Rare Diseases Research



ISSN: 2186-3644  
Online ISSN: 2186-361X  
CODEN: IRDRA3  
Issues/Year: 4  
Language: English  
Publisher: IACMHR Co., Ltd.

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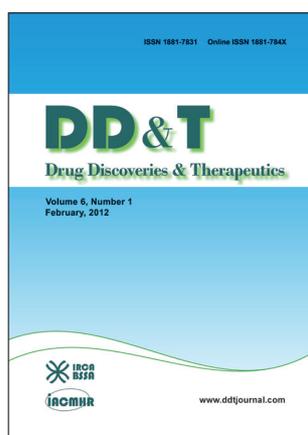
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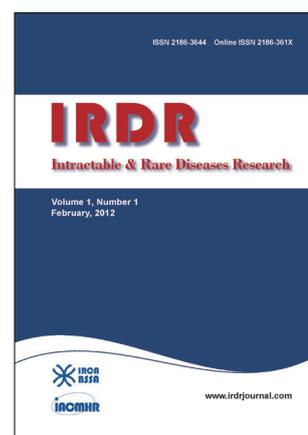
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Online ISSN: 1881-7823  
CODEN: BTIRCZ  
Issues/Year: 6  
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Publisher: IACMHR Co., Ltd.  
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ISSN: 1881-7831  
Online ISSN: 1881-784X  
CODEN: DDTRBX  
Issues/Year: 6  
Language: English  
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ISSN: 2186-3644  
Online ISSN: 2186-361X  
CODEN: IRDRA3  
Issues/Year: 4  
Language: English  
Publisher: IACMHR Co., Ltd.  
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**Correction**

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- E1      **Correction: Novel *SLC16A2* mutations in patients with Allan-Herndon-Dudley syndrome**

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**Guide for Authors**

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# Management of syndromic diarrhea/tricho-hepato-enteric syndrome: A review of the literature

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## Summary

Syndromic diarrhea/tricho-hepato-enteric syndrome (SD/THE) is a rare disease linked to the loss of function of either *TTC37* or *SKIV2L*, two components of the SKI complex. It is characterized by a combination of 9 signs (intractable diarrhea, hair abnormalities, facial dysmorphism, immune abnormalities, IUGR/SGA, liver abnormalities, skin abnormalities, congenital heart defect and platelet abnormalities). We present a comprehensive review of the management of SD/THE and tested therapeutic regimens. A review of the literature was conducted in May 2017: 29 articles and 2 abstracts were included describing a total of 80 patients, of which 40 presented with mutations of *TTC37*, 14 of *SKIV2L*. Parenteral nutrition was used in the management of 83% of the patients and weaned in 44% (mean duration of 14.97 months). Immunoglobulins were used in 33 patients, but data on efficacy was reported for 6 patients with a diminution of infection ( $n = 3$ ) or diarrhea reduction ( $n = 2$ ). Antibiotics ( $n = 11$ ) provided no efficacy. Steroids ( $n = 17$ ) and immunosuppressant drugs ( $n = 13$ ) were used with little efficacy and mostly in patients with IBD-like SD/THE. Hematopoietic stem cell transplantation (HSCT) was performed in 4 patients: 2 died, for one it corrected the immune defects but not the other features and for the last one, it provided only a partial improvement. Finally, no specific diet was effective except for some contradictory reports for elemental formula. In conclusion, the management of SD/THE mainly involves parenteral nutrition and immunoglobulin supplementation. Antibiotics, steroids, immunosuppressants, and HSCT are not recommended as principle treatments since there is no evidence of efficacy.

**Keywords:** *TTC37*, *SKIV2L*, very early onset IBD

## 1. Introduction

Syndromic diarrhea/tricho-hepato-enteric syndrome (SD/THE) is a rare disease linked to an alteration of the human SKI complex by recessive mutations of either *TTC37* or *SKIV2L*. To date, it is characterized by the combination of 9 signs (1). As nearly constant features

are found intractable diarrhea during infancy, hair abnormalities often found with presence of trichorrhexis nodosa, intra-uterine growth restriction or small size at birth for gestational age, facial dysmorphism, immune abnormalities (mostly a lack of immunoglobulin or a lack of antibody response to vaccination). The other signs seen in half of the cases are liver abnormalities, and skin abnormalities but congenital heart defects and platelet abnormalities are not often reported (1-2). To date case management involves parenteral nutrition and in some cases immunoglobulin supplementation (1). However, a lot of empirical treatments have been tried over the past 30 years because of delayed diagnosis. The aim of this review is to assess these treatments and their potential efficacy.

Released online in J-STAGE as advance publication August 19, 2017.

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**Table 1. Summary of clinical signs according to molecular defect**

Items	All (n = 80)	Patient with mutation of TTC37 (n = 40)	Patient with mutation of SKIV2L (n = 14)	Patient not tested (n = 25)	Patient without mutation of SKIV2L or TTC37 (n = 1)
Sex (F/M)	39/35	20/18	8/5	11/11	0/1
Intractable diarrhea	76/77	38/39	14/14	23/23	1/1
Facial dysmorphism	66/67	31/32	10/10	24/24	1/1
Hair abnormalities	71/73	36/36	11/13	23/23	1/1
Trichorrhexis nodosa	46/59	30/32	5/13	11/14	
Immune deficiency	48/67	28/36	5/12	15/18	0/1
IUGR/SGA	48/63	24/32	9/9	15/21	0/1
Liver disease	41/61	18/31	8/10	14/19	1/1
Skin abnormalities	29/48	15/24	6/6	8/17	0/1
Hypo/hyperpigmented	17/29	12/15	4/6	1/8	
Cardiac abnormalities	15/43	10/26	4/4	1/13	
Outcome (Alive/Dead)	56/24	30/10	13/1	12/13	1/0

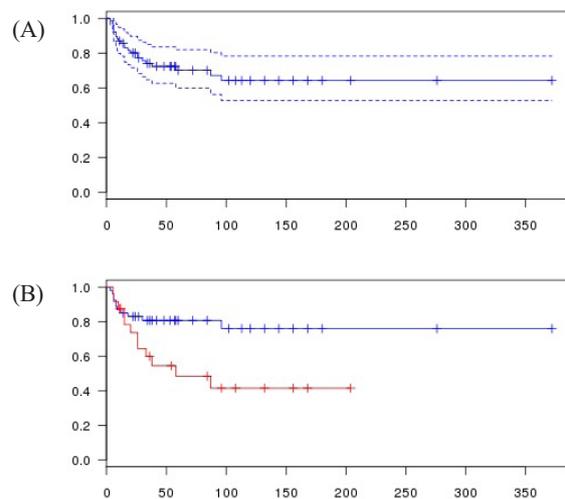
## 2. Methods

A search was done on PubMed ([www.pubmed.com](http://www.pubmed.com)) in May 2017 using "trichohepatoenteric", "trichohepato-enteric", "intractable diarrhea with phenotypic anomalies", "intractable diarrhea and trichorrhexis nodosa", "syndromic diarrhea", "syndromic diarrhoea" and "phenotypic diarrhea", "stankler syndrome", "SKIV2L" and "TTC37". All in all, we retrieved 73 articles of which 27 had individual clinical data (3-29). A second search on Google scholar ([www.scholar.google.fr](http://www.scholar.google.fr)) in the "cited by" article from Girault *et al.* (4) and Verloes *et al.* (5), produced 2 more articles (30-31). A search performed on abstracts from the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) meetings from 2012 to 2017, produced two abstracts with unpublished data (32,33). Finally, a medical thesis provided more complete clinical data from patients that were only briefly described in reference 11 (34). Consequently, 29 articles, 2 abstracts and 1 medical thesis were included. For each case the clinical data (regarding the 9 canonical signs), genetic status, disease evolution, treatments and their efficacy were retrieved. Statistical analyses were performed with biostatgv software.

## 3. Results

### 3.1. Clinical data

Between 1982 and May 2017, 80 patients (sex ratio 35/39 for patients with recorded sex) were described as having SD/THE with some clinical data. 14 presented disease with variants in SKIV2L, 40 in TTC37, 25 were of unknown status and 1 was negative for both TTC37 and SKIV2L. The patients presented classical SD/THE symptomatology with nearly all (> 97%) presenting with intractable diarrhea, facial dysmorphism and hair abnormalities. More than 2/3 were small for gestational age, presented an immune deficiency or liver disease.



**Figure 1. Kaplan-Meier survival curve.** (A), whole cohort (dashed line 95% confidence Interval); (B), According to genetic status (mutated in SKIV2L or TTC37 in blue, Unknown in red);  $p = 0.019$ . Time in month

Skin abnormalities (60%) and cardiac abnormalities (35%) were the least recorded signs. One third of the patients died at a mean age of 23.5 months (3-96), mostly, from infection (7 patients) or hepatic failure (7 patients) for the 18 patients with recorded information. The probability of survival for the whole cohort at 96 months was  $0.64 (\pm 0.06)$  and  $0.76 (\pm 0.07)$  for patients mutated in SKIV2L or TTC37 respectively, or  $0.41 (\pm 0.11)$  for patients of unknown status at 87 months ( $p = 0.019$ ). Table 1 summarizes the clinical data according to molecular defect. Figure 1 shows the Kaplan-Meier survival curve for the whole group of patients and compares patients according to their molecular status.

### 3.2. Therapeutics

Table 2 and 3 summarizes the therapeutic and dietetic management for the 80 patients according to molecular defect. A detailed account is given in the following paragraphs.

3.2.1. Nutritional management

Parenteral nutrition was used in 83% of the patients. Nearly half had been weaned off parenteral nutrition with a mean duration of 14.97 months (1-55). However, non-weaned patients stayed on parenteral nutrition for a long period of time (Figure 2).

3.2.2. Immunoglobulin supplementation

Thirty three patients were given immunoglobulin supplementation, but the effects were described in only 6 cases. Three reported a diminution of infection (4,19,21), 2 a reduction of diarrhea (4,23) and 1 described no effect either on diarrhea or infection (34).

3.2.3. Antibiotherapy

In 11 cases, antibiotics were used to treat SD/THE, mostly in Girault *et al.* (8 patients with Vancomycin, Colistimethate, Tobramycin, and Amphotericin B) but also in Busoni *et al.* (Vancomycin, Amoxiciline, Metronidazole, Quinolone) and in Lee *et al.* 2016 (Ceftriaxone, Amikacine and "aggressive antibiotics")

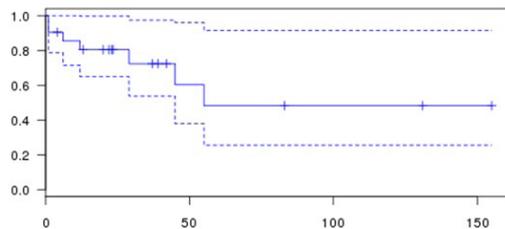


Figure 2. Kaplan-Meier survival curve of parenteral dependency according to the duration of parenteral nutrition (dashed line 95% confidence Interval). Time in month

(4,23,27). In 10 cases (4,23) antibiotics had no effect and only in one case (27) lead to a temporary reduction in diarrhea.

3.2.4. Steroids

Steroids were administered to 17 patients (4,17,23,26,27,30,32-34). No effect was reported in 11 patients and in 5 patients only a partial amelioration was noted. In one patient (33) there were no details and it was before HSCT. It should be noted that the patients with partial effect presented with some aspect of IBD-like SD/THE (17,26,27,32). In some cases steroids were given in combination with immunosuppressant drugs.

3.2.5. Immunosuppressant drugs

Seven drugs were used for a combined total of 24 times in 13 patients (4,17,26,27,30,32,33). Thus, some patients were given multiple drugs, either sequentially or at the same time. Summing up: 5 ASA was used four times with no effect in three patients (17,27) and one case of partial amelioration in combination with steroids (27). Azathioprine was used 5 times, with no effect in 4 patients (4,17,26) and possibly a partial amelioration in one (26). Ciclosporine was used in two patients in combination with steroids: one patient died of infection (30) and the other showed only a mild improvement (4). Methotrexate was used in one patient (17) with no effect. Sirolimus was used in 2 patients without effect, Tacrolimus was used twice in 3 patients without any effect (17,26) and one before HSCT (33). Anti-TNF antibody was used in 7 patients; for one there was no description of outcome (33), for 2 (17) there was no improvement, for 3 there was a partial and inconsistent improvement (26,27,32).

Table 2. Summary of therapeutic management according to molecular defect

Items	All (n = 80)	Patient with mutation of TTC37 (n = 40)	Patient with mutation of SKIV2L (n = 14)	Patient not tested (n = 25)	Patient without mutation of SKIV2L or TTC37 (n = 1)
Parenteral nutrition	59/71	29/34	11/13	18/23	1/1
Weaning of parenteral nutrition	22/50	10/23	5/9	6/17	1/1
Mean duration in month of parenteral nutrition for weaned patients	14.97 (1-55)	12.6 (1-29)	10.62 (4-24)	25 (1-55)	6
Mean duration in month of parenteral nutrition for ongoing patients	57.09 (4-179)	67.94 (13-55)	105.33 (66-179)	32.85 (4-155)	6
Immunoglobulin supplementation	33/40	19/22	3/5	11/12	0/1
Azathioprine	5	2	2	1	0
Methotrexate	1	1	0	0	0
Ciclosporine	2	0	0	2	0
Sirolimus	2	1	1		
Tacrolimus	3	2	1	0	0
Steroids	17	9	2	6	0
TNF blockade	7	5	2	0	0
Antibiotics	11	3	0	8	0
5 ASA	4	3	1	0	0
Hematopoietic stem cell transplantation	4	3	0	1	0

**Table 3. Summary of diet management according to molecular defect**

Items	All (n = 80)	Patient with mutation of TTC37 (n = 40)	Patient with mutation of SKIV2L (n = 14)	Patient not tested (n = 25)	Patient without mutation of SKIV2L or TTC37 (n = 1)
Elemental formula	7	2	2	2	1
Lactose free diet	3	2	1	0	0
Glucose-Galactose free diet	3	1	0	1	1
Gluten free diet	4	1	0	2	1
Hydrolyzed formula	11	4	1	5	1

Eight patients on immunosuppressive therapy were described as having an IBD-like SD/THE. Moreover, patients described in Kammermeier 2014 and 2017 (17,26) were given multiple immunosuppressant drugs (2 patients treated with 2 molecules, and 2 patients with 5). For these patients, reported in a synthetic table, it is rather hard to determine the efficacy of each therapy precisely.

### 3.2.6. Hematopoietic stem cell transplantation (HSCT)

HSCT was performed on 4 patients. The first one was in Girault *et al.*, and the patient underwent two HSCT: the first was a failure and he died from severe interstitial pneumonia after the second attempt (4). Another case was reported in Kammermeier *et al.*: HSCT produced only a moderate improvement, however the case is very slightly reported (26). Two patients were reported in Cleminson *et al.*: one died, 46 days post HSCT, from adenovirus pneumonitis, the second reported successful engraftment and after nearly two years of follow-up, immunology normalized afterwards but diarrhea and failure to thrive persisted (33).

### 3.2.7. Diet management

For 22 patients, some information about diet was available (Table 3). 11 were given hydrolyzed formula, 7 an elemental formula, 4 a gluten free diet and 3 a lactose free diet and 3 a glucose-galactose free diet. Except for elemental formula which led to an amelioration of the diarrhea for 2 patients (21,24), all other types of diet did not improve the diarrhea. For one patient, both soy based formula and artisanal rice water with sugar cane were provided, but did not improve diarrhea (30).

Of the twelve patients who were not given parenteral nutrition, one was reported to have a normal diet (24) and one was given hydrolyzed formula (32). There is no data of the diet for the others. For the 22 patients weaned off parenteral nutrition, data are available about the diet for 11. Five patients were given elemental formula and/or gluten free and cow's-milk-free diets (18). All the others were given a different diet: no gluten or cow's milk (4), hydrolyzed formula (4), elemental formula (24), or a hydrolyzed formula, gluten free diversified diet (11,34), or normal diet with enteral supplement (24) or glucose galactose free formula (31).

## 4. Discussion

As far as we know, this is the first comprehensive review of the management of SD/THE. Up to now, no clinical trials have been organized, only single cases or small series are reported with only a low level of evidence. However, some elements can be highlighted. We confirm that parenteral nutrition is important in the management of SD/THE as already established in previous reviews (1,11). It also plays a vital role in the management of SD/THE, since 83% of the patients need it. However, there is some variability and 17% of the patients did not require parenteral nutrition. Except for some cases (19,24), it is not clear whether the absence of parenteral nutrition was due to non-availability or for some other reason. With parenteral nutrition, weaning can be achieved in nearly 50% of the patients as already reported in the literature (35). One of the limits with this approach is that it can also reflect different practices in different countries. Moreover, it often remains unclear if weaned patients have a good nutritional status or if some level of parenteral nutrition may still be useful. It has already been noted that SD/THE patients are of small stature despite adequate nutrition (11,35) and that in two patients growth hormone administration failed to improve growth (35).

Immunoglobulin supplementation was used in 33 patients, but the effects are rarely and poorly described. Nonetheless, an improvement of either the number of infections or of the diarrhea was noticed for 5/6 patients. Thus, immunoglobulin supplementation could probably be useful in some cases of SD/THE if an immune defect is present (notably low levels of immunoglobulin), but also in case of recurrent infection, as we know that immunoglobulin function is impaired (19). In all cases a discussion with immunologists appears mandatory.

Steroids, antibiotics or immunosuppressant drugs did not seem useful in the management of SD/THE and can even have adverse effects. The only exception is in IDB-Like SD/THE, where these drugs could be useful to some extent. However, as noted in Busoni *et al.*, the effect seems transient or partial (27).

Hematopoietic stem cell transplantation presents only little data with no full reports. However according to the data, HSCT did not cure SD/THE, except possibly for the immune defects, but is associated with high mortality (2/4). Thus SD/THE is clearly different from

defects in intestinal immune-related homeostasis like immunodysregulation polyendocrinopathy enteropathy, X-linked (IPEX) or IPEX-like disorders where either immunosuppressant drugs or HSCT could be useful (36).

For diet management, data are hard to come by and the only regimen that seems to have some effect is the elemental formula, and even then reports are contradictory. All the others did not seem to be effective. The diet of weaned patients is highly diverse thus it is hard to determine whether restrictive diets (like gluten, lactose, cow's milk free) are really useful. No suggestion can be made on the basis of the data and the choice should be made by the medical team in agreement with the patient and the family.

On a more general level, mortality is still high for SD/THE. Whereas, mortality is lower for patients with mutation of TTC37 or SKIV2L than for patients with unknown status. However, these cases could be a bias because most of the patients with unknown status are from older publications.

In conclusion, to date the management of SD/THE is mainly based on parenteral nutrition and immunoglobulin supplementation. Other drugs such as antibiotics, steroids and immunosuppressant drugs have, showed no evidence of efficacy, except in some cases of IBD-like SD/THE. HSCT could potentially treat the immune defects but does not improve the other signs and is associated with high mortality. Finally, diet management data are confusing and no clear conclusion can be made. SD/THE is a rare disease requiring management by an expert team, especially in relation to nutrition and immunity.

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(Received July 18, 2017; Revised July 27, 2017; Accepted August 11, 2017)

# Fetal pleural effusion and Down syndrome

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## Summary

**Fetal pleural effusion is a rare abnormality that results from accumulation of fluid in the chest cavity. It can be classified as primary fetal hydrothorax and secondary fetal hydrothorax. The underlying causes of pleural effusion are still unknown, and the current treatment strategies are mainly based on symptoms. The prognosis of fetal pleural effusion varies significantly, ranging from spontaneous resolution to perinatal death. Recent advances in prenatal diagnostic methods and treatment such as thoracoamniotic shunting have significantly improved the survival rates for patients with or without hydrops.**

**Keywords:** Pleural effusion, Down syndrome, etiology, diagnosis, treatment, prognosis

## 1. Introduction

Fetal pleural effusion is an abnormality resulting from accumulation of fluid in the chest cavity, and the condition was first described by Carroll in 1977 (1). Fetal pleural effusion is a rare condition, with a reported incidence ranging from 1/10,000 to 1/15,000 (2-4). The incidence of fetal pleural effusion in newborns ranges from 2.2 to 5.5 per 1,000 births (5). The underlying causes of fetal pleural effusion are still unclear; it can occur as an initial symptom of hydrops fetalis, but it can also occur in isolation (6).

## 2. Causes and classifications

Fetal pleural effusion can be classified as primary fetal hydrothorax and secondary fetal hydrothorax. Primary

fetal hydrothorax, also known as congenital chylothorax, can result from multiple lymphatic vessel anomalies or thoracic cavity defects caused by external force, a tumor, or cardiovascular diseases (7). It can occur unilaterally or bilaterally and affects males more than females at a ratio of 2:1; primary fetal hydrothorax has a perinatal mortality rate of 22% to 53% (8,9). Secondary fetal hydrothorax is a feature of immune and non-immune hydrops. Autoimmune conditions include Rh or ABO blood type incompatibility; non-immune factors include chromosomal abnormalities, genetic disorders, infections, congenital cardiac anomalies, congenital lung anomalies, hematologic diseases, metabolic diseases, and noncardiac anomalies (10). Hydrops is usually bilateral, and is also often associated with ascites, pericardial effusion, subcutaneous edema, hydramnios, and placental thickening. The most common causes of non-immune hydrops are chromosomal anomalies such as Down syndrome and Turner syndrome, which can also be present with additional structural abnormalities (1,4).

## 3. Diagnosis

The treatment of fetal hydrothorax and patient prognosis are closely associated with its specific causes (11-15); therefore, a detailed prenatal examination and evaluation must be performed. Steps include identification of maternal blood type and screening serum specific antibodies to exclude immune hydrops, detection

Released online in J-STAGE as advance publication July 24, 2017.

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Table 1. Characteristics and outcomes of Down syndrome cases with fetal or neonatal hydrothorax

Author	Case number	Time of detection	Unilateral or bilateral	Hydramnios (Yes/No)	Structural abnormalities (Yes/No)	Hydrops (Yes/No)	Intervention (Yes/No)	Karyotype	Outcome
Yoss <i>et al.</i> (1977) (1)	1	Neonatal period	Unilateral (right)	No	No	No	No	47,XY,+21	Alive
Footo <i>et al.</i> (1986) (22)	1	Neonatal period	Unilateral (left)	Yes	AVSD	No	No	46,XY,-22,+t(21q:22q)	Alive
	1	34 weeks of gestational age	Unilateral (right)	Yes	No	No	No	46,XY,t(21:21)	Unknown
	1	28 weeks of gestational age	Unilateral (left)	Yes	AVSD	No	No	47,XY,+21	Unknown
Modi <i>et al.</i> (1987) (23)	1	Neonatal period	Unilateral (right)	Unknown	No	No	No	47,XX,+21	Alive
Rodeck <i>et al.</i> (1988) (24)	1	32 weeks of gestational age	Unilateral (right)	Yes	AVSD	No	No	47,XX,+21	Alive
Blott <i>et al.</i> (1988) (25)	1	32 weeks of gestational age	Unilateral (right)	Unknown	ASD	No	Thoracoamniotic shunting	Trisomy 21	Alive
Petrikovsky <i>et al.</i> (1991) (26)	1	34 weeks of gestational age	Bilateral	No	No	No	No	Trisomy 21	Alive
	1	18 weeks of gestational age	Bilateral	No	Fifth digit clinodactyly	No	No	47,XX,+21	TOP
Ilagan <i>et al.</i> (1992) (27)	1	32 weeks of gestational age	Bilateral	Yes	No	Yes	No	47,XX,+21	Neonatal death after 3 days of birth
Hamada <i>et al.</i> (1992) (28)	1	36 weeks of gestational age	Bilateral	No	No	Yes	No	47,XY,+21	Alive
Sherer <i>et al.</i> (1993) (29)	1	18 weeks of gestational age (resolved after 1 week)	Unilateral (left)	No	Fifth digit clinodactyly	No	No	47,XY,+21	TOP
Achiron <i>et al.</i> (1995) (30)	1	18 weeks of gestational age	Unilateral	No	No	No	No	47,XX,+21	Alive
	1	28 weeks of gestational age	Bilateral	No	No	No	No	47,XY,+21	IUD
Yamamoto <i>et al.</i> (1996) (31)	1	32 weeks of gestational age	Bilateral	No	No	Yes	No	47,XX,+21	Alive
Rotmensch <i>et al.</i> (1997) (32)	1	24-28 weeks of gestational age	Unknown	Unknown	Pyelectasis	Yes	No	Trisomy 21	Unknown
Puddy <i>et al.</i> (1999) (33)	1	29 weeks of gestational age	Bilateral	Yes	No	Yes	Thoracocentesis (29 weeks of gestational age); Thoracoamniotic shunting (29 weeks of gestational age)	46,XX/47XX,+21 mosaicism	Alive
Turan <i>et al.</i> (2001) (34)	1	Neonatal period	Bilateral	Unknown	No	No	No	47,XY,+21	Alive
Hwang <i>et al.</i> (2003) (35)	1	Neonatal period	Unilateral (left)	Unknown	No	No	No	47,XY,+21	Alive
Picone <i>et al.</i> (2005) (36)	1	Pregnancy period	Unknown	Unknown	Unknown	Unknown	Thoracoamniotic shunting	Trisomy 21	TOP
	1	Pregnancy period	Unknown	Unknown	Unknown	Unknown	Thoracoamniotic shunting	Trisomy 21	IUD
Kallanagowdar <i>et al.</i> (2006) (37)	1	Pregnancy period	Unilateral (left)	Unknown	AVSD, fifth digit clinodactyly	No	No	47,XX,+21	Alive
Rocha <i>et al.</i> (2006) (38)	1	Neonatal period	Unilateral (left)	Unknown	Unknown	Unknown	No	Trisomy 21	Alive
	1	Neonatal period	Bilateral	Unknown	Unknown	Unknown	No	Trisomy 21	Neonatal death
Albano <i>et al.</i> (2007) (39)	1	32 weeks of gestational age	Unilateral (right)	Yes	Hepatic hemangioma	Yes	No	47,XX,+21	Alive
Kim <i>et al.</i> (2008) (40)	1	32 weeks of gestational age	Bilateral	Yes	Posterior urethral valve	No	Repeated amniocentesis, then thoracoamniotic shunting and vesicoamniotic shunting	47,XY,+21	Alive
Ergaz <i>et al.</i> (2009) (41)	1	Neonatal period	Right	Unknown	AVSD, TOF	No	No	47,XY,+21	Alive
	1	Neonatal period	Bilateral	Unknown	VSD	Yes	No	47,XY,+21	Alive

Abbreviations: ASD, atrial septal defect; AVSD, atrioventricular septal defect; IUD, intrauterine death; TOP, termination of pregnancy; VSD, ventricular septal defect.

**Table 1. Characteristics and outcomes of Down syndrome cases with fetal or neonatal hydrothorax (continued)**

Author	Case number	Time of detection	Unilateral or bilateral	Hydramnios (Yes/No)	Structural abnormalities (Yes/No)	Hydrops (Yes/No)	Intervention (Yes/No)	Karyotype	Outcome
Yinon <i>et al.</i> (2010) (42)	3	Pregnancy period	Unknown	Unknown	Unknown	Unknown	Unknown	Trisomy 21	Unknown
Ugras <i>et al.</i> (2010) (43)	1	Neonatal period	Left	Unknown	Anal atresia, VSD	No	No	47,XY,+21	Alive
Casario <i>et al.</i> (2010) (44)	3	Pregnancy period	Unknown	Unknown	Unknown	Unknown	Unknown	Trisomy 21	Unknown
Ruano <i>et al.</i> (2011) (45)	5	Pregnancy period	Unknown	Unknown	Ventriculomegaly; complex heart defect; absence of nasal bones	Unknown	Unknown	Trisomy 21	Unknown
Karagol <i>et al.</i> (2012) (46)	1	30 weeks of gestational age	Unilateral (right)	Yes	Fifth-finger clinodactyly	No	No	47,XY,+21	Alive
Bialkowski <i>et al.</i> (2015) (47)	1	Newborn	Unknown	Unknown	Unknown	Unknown	Unknown	Trisomy 21	Alive
Mallmann <i>et al.</i> (2016) (48)	14	Pregnancy period	Unknown	Unknown	Unknown	Unknown	Thoracoamniotic shunting	Trisomy 21	Unknown
Li <i>et al.</i> (1998) (49)	1	Pregnancy period	Bilateral	No	No	No	No	46,XY,+21-3	Neonatal death
Guan <i>et al.</i> (1998) (50)	1	Pregnancy period	Unilateral (right)	No	Congenital hypothyroidism	No	No	47,XY,+21	Neonatal death
Wei <i>et al.</i> (2005) (51)	1	Neonatal period	Unilateral (left)	Unknown	Known	No	No	47,XX,+21	Alive
Hu <i>et al.</i> (2007) (52)	1	Pregnancy period	Unilateral (right)	No	ASD	No	No	46,XY,-14,+t(21;14)	Alive
Li <i>et al.</i> (2009) (53)	1	Pregnancy period	Unilateral (left)	No	No	No	No	47,XX,+21	Alive
Zhang <i>et al.</i> (2011) (54)	1	Neonatal period	Bilateral	Yes	No	Yes	No	Trisomy 21	Neonatal death
Zhang <i>et al.</i> (2011) (55)	1	Neonatal period	Left	Unknown	No	No	No	47,XY,+21	Alive
Jiang <i>et al.</i> (2012) (56)	1	Pregnancy period	Unknown	Unknown	Pulmonary defect	Unknown	No	Trisomy 21	TOP
La <i>et al.</i> (2014) (57)	1	23 weeks of gestational age	Unknown	Unknown	Unknown	Unknown	No	Trisomy 21	Neonatal death
Li <i>et al.</i> (2015) (58)	1	Neonatal period	Unknown	Unknown	Unknown	Unknown	No	Trisomy 21	Neonatal death

Abbreviations: ASD, atrial septal defect; ABS, atrioventricular septal defect; IUD, intrauterine death; TOP, termination of pregnancy; VSD, ventricular septal defect.

of infectious factors including TORCH, syphilis, and parvovirus B19, and performing a K-B test to exclude fetal maternal transfusion syndrome. A careful ultrasound examination should also be performed to observe the placenta, amniotic fluid, and fetal structure (and especially the fetal heart), and pulse Doppler should be used to detect the blood flow spectrum of the umbilical artery, middle cerebral artery, and venous system (16,17). Karyotyping or genetic testing is also routinely performed, especially in fetuses in which early pleural effusion has been detected (18-21).

**4. Hydrothorax and chromosomal anomalies**

Studies have shown that chromosomal anomalies are associated with fetal and/or neonatal hydrothorax. Table 1 summarizes the literature regarding characteristics and outcomes of Down syndrome cases involving patients with fetal or neonatal hydrothorax.

**5. Treatment and prognosis**

The prognosis for fetal pleural effusion is highly variable and difficult to predict, ranging from spontaneous resolution to progression to fetal hydrops and eventual perinatal death (59-64). The current strategy for treatment of fetal pleural effusion is based more on symptoms rather than underlying causes. Primary hydrothorax with small volumes of pleural fluid and no hydrops is more likely to resolve or remain stable, so more conservative treatment can be provided. Aubard *et al.* reported that the survival rate for conservative treatment of primary hydrothorax was 24% when hydrops was present and 75% when it was not (60), and Rustico *et al.* noted similar survival rates of 35% and 73%, respectively (4). Survival rates have improved significantly in recent years, and Wada *et al.* (21) reported survival rates of 58% and 97.8%, respectively, that can be largely attributed to improved methods of neonatal treatment. Thoracentesis is easy to perform and can reduce distress and improve fetal pulmonary development, but the procedure must be repeated after 24-48 hours in many patients, so thoracoamniotic shunting is usually recommended for fetuses with hydrops (65). Recent studies have indicated that the survival rate for congenital hydrothorax with hydrops is around 60% for patients treated with thoracoamniotic shunting, approximately 50% for those treated with thoracentesis, and from 35% to about 60% for those receiving conservative treatment (4,21).

**Acknowledgements**

This work was supported by the National Natural Science Foundation of China (Grant No. 31571196 to Ling Wang), the 2015 Program to Guide Medicine ("Yixueyindao") of the Shanghai Municipal Science and

Technology Commission (Grant No. 15401932200 to Ling Wang), the FY2008 JSPS Postdoctoral Fellowship for Foreign Researchers (P08471, Ling Wang), the National Natural Science Foundation of China (Grant No. 30801502 to Ling Wang), the Shanghai Pujiang Program (Project No. 11PJ1401900, Ling Wang), and the Shanghai Program for Support of Leading Disciplines-Integrated Chinese and Western Medicine (Project No. 20150407).

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(Received May 10, 2017; Revised May 22, 2017; Accepted May 27, 2017)

## Pulmonary hypertension associated with antiphospholipid antibody: Call for a screening tool?

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### Summary

Antiphospholipid (aPL) antibodies are antibodies specific for anionic phospholipids. They are immunoglobulins that attack phospholipids, phospholipid-binding proteins, or phospholipid-protein complexes and are detected in anticardiolipin and lupus anticoagulant assays. aPL antibodies are often associated with antiphospholipid syndrome (APS) which can be idiopathic or from secondary causes such as systemic lupus erythematosus (SLE), infection or drugs. They have also been shown to be associated with Pulmonary Hypertension. We conducted a review of the literature that included all articles on PubMed with keywords 'antiphospholipid antibody' and 'pulmonary hypertension' between January 1980 and July 2017 and identified 217 articles. A total of 47 articles were found to be relevant to the topic and included as references. We ascertained that aPL antibodies have been implicated in the development of both idiopathic pulmonary arterial hypertension (PAH) and PAH associated with connective tissue disease (CTD). aPL antibodies were also noted to be associated with left-sided valvular heart disease that can lead to pulmonary venous hypertension (PVH). Patients with antiphospholipid antibody syndrome (Diagnostic criteria includes +aPL antibodies) were noted to have a high risk of developing chronic thromboembolic pulmonary hypertension (CTEPH). A recent study also found a positive association of aPL antibodies with ILD and PH in patients with systemic sclerosis. While association between autoimmune thyroid disease and PH (Group V PH), and autoimmune thyroid disease and aPL antibodies is established, no studies linked these three phenomena together. Thus, aPL antibodies had an association with all WHO groups of Pulmonary hypertension (PH). In this review article, we study the association and discuss the need for screening for PH in patients with positive aPL antibodies.

**Keywords:** Pulmonary hypertension, antiphospholipid antibodies, antiphospholipid antibody syndrome, chronic thromboembolic pulmonary hypertension, systemic lupus erythematosus

### 1. Introduction

Antiphospholipid antibodies (aPL) are antibodies specific for anionic phospholipids. They are immunoglobulins that attack phospholipids, phospholipid-binding proteins,

or phospholipid-protein complexes (Figure 1) (1). They are usually detected in anticardiolipin assays. Due to their ability to prolong coagulation tests they are also identified in lupus anticoagulant assays (2). aPL antibodies are usually associated with antiphospholipid syndrome (APS). APS is an autoimmune disorder that manifests clinically as recurrent venous or arterial thrombosis and/or fetal loss associated with persistently detected aPL antibodies on two or more occasions at least 12 weeks apart. It can be idiopathic or from

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secondary causes such as systemic lupus erythematosus (SLE), infection and drugs. The criteria for definite APS classification were first proposed in 1999 and updated in 2005 (Figure 2) (3). APS is usually detected for the first time as a blood clot in an artery or vein, or as recurrent pregnancy loss. Catastrophic antiphospholipid syndrome (CAPS) is a rare, serious, and often fatal type of APS characterized by multi-organ failure in a span of a few days to weeks.

Pulmonary manifestations of APS with positive aPL antibodies include pulmonary embolism and infarction, acute respiratory distress syndrome (ARDS), intra-alveolar bleeding, primary thrombosis of lung vessels,

pulmonary capillaritis or fibrosing alveolitis (4-6). aPL antibodies have also been shown to be associated with pulmonary hypertension (Figure 3). In this article we review the existing literature regarding the association of aPL antibodies with various groups of PH and discuss the possible implications in clinical practice.

## 2. Methodology

We conducted a review of the literature that included all articles on PubMed with keywords "antiphospholipid antibody" and "pulmonary hypertension" between January 1980 and July 2017. These articles were independently examined by 2 reviewers AA and RP. We largely selected publications from the last 20 years, but did not exclude any older publications that were widely referenced and highly regarded. A total of 217 articles were identified from PubMed for review by this strategy. All pertinent reports were retrieved and the relative reference lists were systematically searched in order to identify any potential additional studies that could be included. For less researched topics, we included case reports for purposes of our review. For topics with available retrospective and prospective studies and metanalysis, we excluded case series and case reports. Thus a total of 47 articles were found to be relevant to the topic with pertinent information and were included as references in this review.

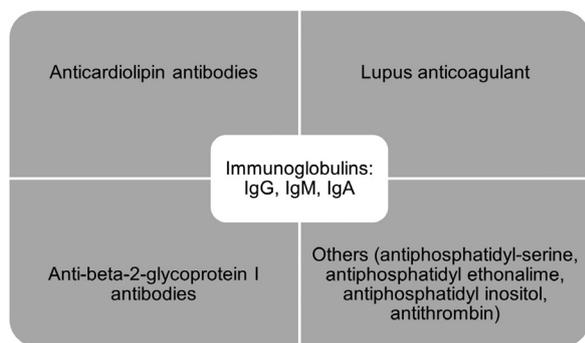


Figure 1. Different types of antiphospholipid antibodies.

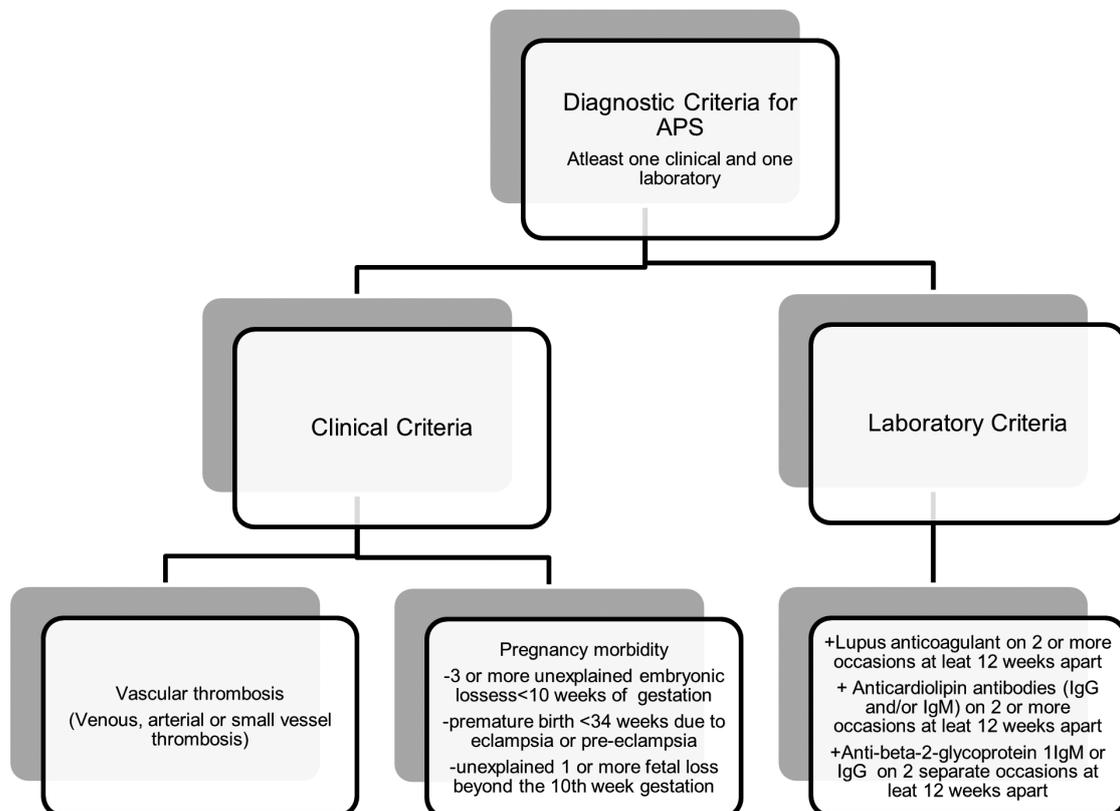
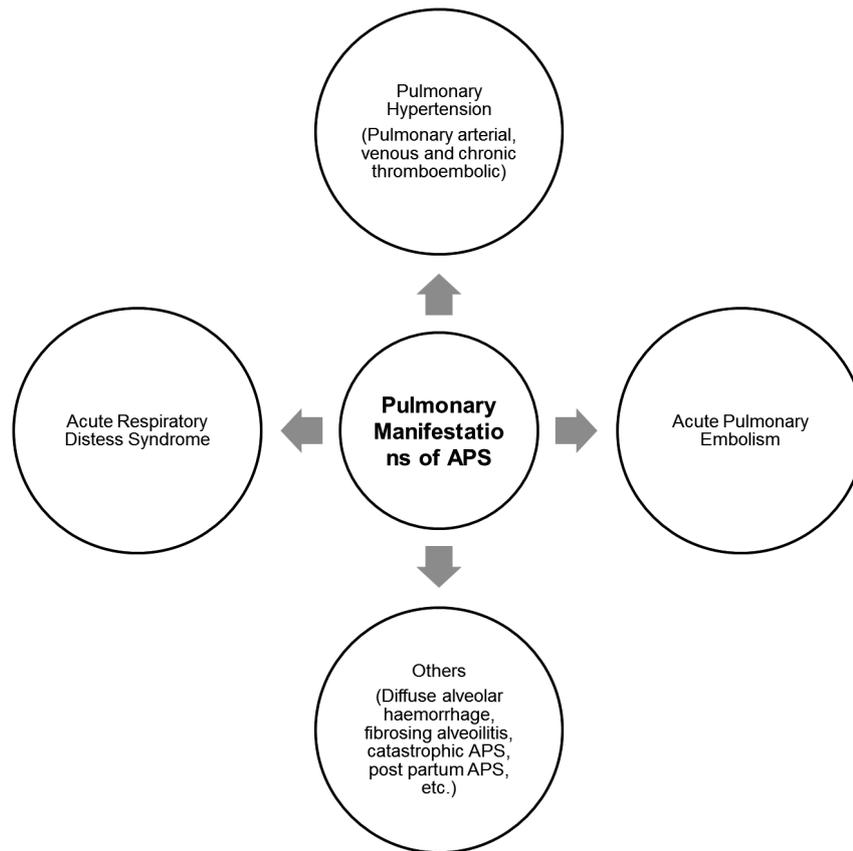


Figure 2. Diagnostic criteria of antiphospholipid syndrome (APS).



**Figure 3. Pulmonary manifestations of antiphospholipid syndrome (APS).**

### 3. aPL antibodies and pulmonary hypertension

Pulmonary hypertension is a chronic and progressive condition defined by a mean pulmonary artery pressure > 25 mm Hg at rest (7,8). The World Health Organization (WHO) currently classifies PH into 5 groups (Table 1) (9). Recent evidence suggests the association of PH with the presence of aPL antibodies (10). Hypotheses regarding the pathophysiology include large vessel and small vessel thrombosis, chronic thromboemboli and associated endothelial remodeling (10). Recent findings of exuberant inflammation and lymphoid neogenesis in remodeled vessels have suggested a role of inflammation in PH, especially pulmonary arterial hypertension (PAH) (11,12). Pioneering work from Soon *et al.* demonstrated significantly increased levels of a broad range of inflammatory cytokines in patients with PAH. It has been well established that aPL antibodies have proinflammatory cellular effects. Thus we hypothesize that pro-inflammatory effects of aPL antibodies may also be contributing to the pathogenesis of PH in such patients.

Asherson *et al.* first reported the association of aPL antibodies with PH in 1985 (13). Karmochkine *et al.* went on to document a high prevalence of aPL antibodies in pre-capillary PH. In this study of 38

patients with PH, 4/9 (44%) patients with primary pulmonary hypertension (PPH) and 7/29 (24%) patients with secondary pulmonary hypertension (SPH) were found to have positive aPL antibodies. Most patients had IgG alone (22/33). Multiple studies since then have studied the association of aPL antibodies with PH. A thorough review of the literature suggested that aPL antibodies are associated with PH across all five WHO groups. aPL antibodies have been noted in patients with WHO Group I PH including idiopathic pulmonary arterial hypertension, pulmonary arterial hypertension associated with connective tissue disorders and rarely PH associated with pulmonary veno-occlusive disease. aPL antibodies can be associated with cardiac valvular disease and thus can lead to pulmonary venous hypertension (WHO Group II PH). These antibodies have also been noted in patients with interstitial lung disease associated with connective tissue disorders like SLE and scleroderma (WHO Group III PH). It is well known that patients with positive aPL antibodies and APS have an increased incidence of recurrent venous thromboembolism leading to CTEPH (Group IV PH). While association between autoimmune thyroid disease and PH (Group V PH), and autoimmune thyroid disease and aPL antibodies, no studies have yet linked these three phenomenon together. Studies with association

**Table 1. Classification of pulmonary hypertension based on etiology (9)**

Group 1	- Idiopathic - Heritable - Drug induced - Associated with conditions like connective tissue disease, HIV, congenital heart disease, schistosomiasis or chronic hemolytic anemia - Pulmonary veno occlusive disease (PVOD) and pulmonary capillary hemangiomatosis
Group 2	- Left heart diseases - Heart failure (systolic or diastolic) - Valvular heart disease
Group 3	- Underlying lung diseases like chronic obstructive lung disease, interstitial lung disease (ILD) and restrictive lung disease - Induced hypoxia due to sleep disorder, alveolar hypoventilation or chronic exposure to high altitude
Group 4	- Pulmonary hypertension due to chronic thromboembolic events also known as chronic thromboembolic pulmonary hypertension (CTEPH)

of aPL antibodies and various WHO groups of PH are listed in Table 2.

#### 4. Group I PH – pulmonary arterial hypertension

##### 4.1. Idiopathic pulmonary arterial hypertension

Idiopathic pulmonary arterial hypertension (IPAH) is defined as the presence of pulmonary arterial hypertension in the absence of another underlying disease. The presence of aPL antibodies has been reported in patients with IPAH (14). While the causative role of aPL antibodies in these patients is unclear, possible mechanisms include both platelet and endothelin-1 activation. Endothelin-1 (ET-1) is a potent vasoconstrictor and its role in PAH is well established (15). Platelet and endothelin activation can also lead to pulmonary vascular remodeling eventually leading to elevated pulmonary arterial pressures. This hypothesis is supported by the fact that increased levels of circulating ET-1 have been observed in patients with aPL antibodies present (16).

##### 4.2. Pulmonary hypertension associated with connective tissue disease

PH associated with connective tissue disorders (CTD) is classified as Group I PH. aPL antibodies have been identified in patients with CTD's such as SLE and scleroderma. A study compared the presence of aPL antibodies in patients with CTD and found the prevalence of aPL antibodies in SLE patients to be roughly six times more than those with scleroderma (17). Tanaseanu *et al.* studied the prevalence of aPL antibodies and its relationship with PAH in 30 patients with SLE. Presence of aPL antibodies was noted in all patients with SLE and PAH (15/15) as compared to only 5/15 in patients with SLE without PAH. Multiple studies have noted the presence of positive anticardiolipin antibodies (aCL) has a significant association with PAH

and SLE patients (18-21). Similarly Houman *et al.* found out that the frequency of PAH in SLE was higher in patients with antibodies against B2Glycoprotein I (B2GPI) than those without these antibodies (22).

Most patients with aPL antibodies do not have APS, but have symptoms similar to IPAH thereby suggesting a role of aPL antibodies in pulmonary vasculopathy. The role of aPL antibodies in CTD-PAH development is unknown. Zuily *et al.* did a meta-analysis of 31 studies with about 4,480 patients with SLE and showed that people with positive aPL antibodies had a higher prevalence of PH (12.3%) as compared with aPL antibody negative SLE patients (7.3%). The overall OR for PH in aPL-positive versus aPL-negative SLE patients was 2.28 (95% CI, 1.65 to 3.15;  $p < 0.0001$ ).

The treatment of these patients is similar to other patients with pulmonary arterial hypertension with special focus on anticoagulation due to higher risk of VTE in patients with aPL antibodies (1). Current guidelines suggest the use of oral anticoagulants in patients with idiopathic PAH and PH associated with CTD on an individual basis and based upon the presence of thrombophilic predisposition (Grade II b, Level C) (23). The COMPERA study conformed the benefit of anticoagulation in IPAH. The number of SLE or other CTD patients was too small to draw any inferences (24). Thus it is unknown whether anticoagulation is of benefit in aPL antibody positive SLE patients with PH without thrombosis. In thrombotic APS patients with PH, anticoagulation is recommended (25). The presence of PH in SLE patients markedly worsens prognosis (26,27). It is unclear if aPL antibody positivity is a prognostic marker for survival or lung transplantation in these patients (10,27).

Pulmonary veno-occlusive disease (PVOD) is another subset of group I PH that is seen in patients with CTD, HIV infection, bone marrow transplantation, sarcoidosis and pulmonary langerhans cell granulomatosis (28). The authors are aware of only 1 case reporting the possible association of aPL antibodies to PVOD (29).

**Table 2. Studies on Association of antiphospholipid antibody with pulmonary hypertension**

Author/Year	Type of Study	Number of subjects	Results
Karmochkine <i>et al.</i> (55) 1996	Prospective	<i>n</i> = 38 PH (9PPH+29 SPH) aPL	(11/30) +aPL in pre capillary PAH (4/9) +aPL in PPH (44%) (7/29) +aPL in SPH (24%)
Wolf <i>et al.</i> (45) 2000	Prospective	<i>n</i> = 116: CTEPH <i>n</i> = 83 : PPH	(25/116) CTEPH + APA (8/83) PPH +APA Among 33 CTEPH or PPH patients: (22) + IgG (7) + IgG+IgM (4) + IgM, High titers of antibody 20% in CTEPH & 10% in PPH
Tanaseanu <i>et al.</i> (56) 2007	Prospective	30 SLE (15PH+/15PH-)  30 COPD (15 PH+/15PH-)	Frequency of aPL antibody: SLE: (15/15(100%) in SLE+/PH) & (33% in SLE-/PH) COPD: (8/15(53%) in COPD+/PH) & (13% IN COPD-/PH)
Fois <i>et al.</i> (19) 2010	Retrospective	12 patients: SLE+/PH 9 SLE/+ isolated PH (Group1); 1 SLE/+ PH due to left heart disease(group2); 2 SLE+/PH related to lung disease (group3)	(9/12) SLE+/PH had a + aCL antibody (frequency 75%)  2 SLE+/PH related due to lung disease: +aCL antibody 2/2 in this group
Kamel <i>et al.</i> (57) 2011	Cross-sectional	Total SLE: 74 SLE+/PH: 8	7/8 (88%): SLE with PH/ + (aPL IgG), <i>p</i> = 0.08 Linear regression analysis: + aCL were significantly associated with PAH occurrence in SLE with <i>p</i> value of 0.006
Cefle <i>et al.</i> (18) 2011	Retrospective	10: SLE+/PH 97: SLE-/PH	SLE+/PH: Total 10 2: + aCL IgG 4: + both aCL IgG & + aCL IgM 1: + aCL IgG, + aCL IgM, + LAC 1: + aCL IgM, + LAC aPL positivity: SLE+/PH (80%) vs. SLE-/PH (36%), <i>p</i> < 0.005.
Ware <i>et al.</i> (58) 2015	Cross-sectional, observational	<i>n</i> = 50 SLE 23/50: aPL + 11/23: aPL+/+PH	(11/23) aPL+/+PH (14/23) + aCL (3/23) + LAC (16/23) + Anti-beta-2-glycoprotein I antibodies (11) + 2 or more antibodies (PHT) 11/23 APA+ & 2/27 APA- Moderate to severe PH ( 7/ 11) APA +
Zuily S <i>et al.</i> (59) 2017	Meta analysis	4,480 SLE	Prevalence of PH: + aPL(12.3%) vs. - aPL (7.3%) The highest risk for PH: +LAC & + IgG aCL Low risk: IgM aCL & anti-β-2-glycoprotein I antibodies

aCL: anticardiolipin antibodies; aPL: antiphospholipid antibodies; COPD: chronic obstructive pulmonary disease; CTEPH: Chronic thromboembolic pulmonary hypertension; LAC: lupus anticoagulant; PAH: pulmonary arterial hypertension; PH: Pulmonary hypertension; PPH: primary pulmonary hypertension; SLE: systemic lupus erythematosus; SPH: secondary pulmonary hypertension.

## 5. Group 2 PH – Pulmonary venous hypertension associated with heart valve disease

Cardiac valvular disease is the most frequent cardiac manifestation in patients with APS, with a prevalence of 30%. Heart valve lesions (vegetation, thickening and dysfunction) are usually reported in patients with APS with and without SLE, and in those with aPL antibodies alone. Several autoantibodies can directly affect the heart tissue. For example, aPL antibodies can affect the heart by enhancing atherosclerosis, causing thrombosis of coronary arteries or via means of an immune-complexes-mediated reaction. They lead to immune complex formation and deposition which acts

as the initial triggers for valvular endothelial activation. This leads to the thickening of the valves or formation of sterile vegetations on these valves (mainly mitral and aortic) (30). This is known as Libman-Sacks endocarditis *i.e.* verrucous endocarditis of valve leaflets, papillary muscles and the mural endocardium. This endocarditis is present in about 20% to 30% of patients with SLE and about a third of patients with primary APS (31-33). These lead to a significant amount of valvular regurgitation and can lead to left heart failure and associated pulmonary venous hypertension (PVH) (34). The risk of valvular heart disease is highest for patients with lupus anticoagulant and anti-cardiolipin antibodies (IgG) (35).

The presence of aPL antibodies in SLE patients is associated with a threefold greater risk of cardiac valvular disease than those without aPL antibodies, leading to thrombotic manifestations on valves because of hypercoagulability. APS patients undergoing valve-replacement surgery are at high risk of thrombotic and bleeding complications. Thus aPL antibody-related cardiac valvular disease affects APS management.

### 6. Group 3 PH – Pulmonary hypertension associated with interstitial lung disease

Patients with interstitial lung disease (ILD) present with onset of chronic cough, dyspnea, and decreased exercise tolerance. PH-ILD is secondary to parenchymal and vascular remodeling in the lungs. Its prevalence in ILD is estimated at 30-40% and causes severe exercise limitation and dismal prognosis for ILD patients (36). Mejia M *et al.* (37) in 2017 have found several auto-antibodies associated with ILD. Many studies have shown association of ILD with SLE, but there is a paucity of literature showing a specific relationship of aPL antibodies with PH in this group (38). Similarly the association of other CTD's with ILD is well established (39). As discussed earlier, there is an increased incidence of PH in patients with CTD and the presence of aPL antibodies. Thus by association, it is likely that aPL antibodies are present in patients with both ILD and PH, especially in those with underlying CTD.

Morrisroe *et al.* studied 940 patients with systemic sclerosis and found the presence of aPL antibodies in 226 patients. Elevated titres of both anticardiolipin antibody IgM (OR 2.04, 95% CI: 1.4-3.0,  $p < 0.0001$ ) and IgG (OR 1.84, 95% CI: 1.2-2.8,  $p = 0.005$ ) were associated with an increased likelihood of ILD in these patients. Positive anticardiolipin IgG was also noted to be a marker of co-existent ILD and PH (OR 2.10, 95% CI: 1.1-4.2,  $p = 0.036$ ) (40).

### 7. Group 4 PH – Chronic thromboembolic pulmonary hypertension

Chronic thromboembolic pulmonary hypertension (CTEPH) is the form of pulmonary hypertension defined as pre-capillary hypertension with at least one segmental perfusion defect at scintigraphy and typical findings at conventional or computed tomographic pulmonary angiography, after at least 3 months of anticoagulation (41). It occurs usually as a consequence of incomplete resolution of acute pulmonary emboli, progressively organized into fibrotic material obstructing large pulmonary arteries, which leads to elevated pulmonary artery pressures (42). There has been an increased awareness regarding the pathogenesis of CTEPH in the last two decades with several reports of association of aPL antibodies and CTEPH. Colorio *et al.* noted a high proportion of positive aPL antibodies (50%) in 24 patients

with CTEPH (43). In a retrospective study, D'Armini *et al.* detected aPL antibodies in 15% of patients (28/184) who underwent pulmonary endarterectomy for CTEPH (44). Wolf *et al.* studied the presence of thrombotic risk factors in patients with CTEPH and PPH. The study showed 20% positive aPL antibodies in patients with CTEPH and 10% positive aPL antibodies in patients with PPH (45). Similarly Martinuzzo *et al.* found a higher prevalence of aPL antibodies in patients with CTEPH as compared to patients with primary or secondary PH (46).

Patients with positive aPL antibodies especially in APS are prone to venous thrombosis and may have a recurrent pulmonary embolism leading to CTEPH. APS is the most common acquired cause of venous thromboembolism (VTE). The curative treatment for CTEPH is pulmonary thromboendarterectomy (PTE), a surgical procedure in which the blood vessels of the lungs are cleared of clot and scar material. But about 20-40% CTEPH patients are not operable either due to distal and inaccessible nature of the lesions or comorbid conditions, and in up to 35% of patients, the disease persists or reoccurs after PTE (47). Although several vasodilators are in the market for the treatment of PAH, none have been shown to be invariably effective in the treatment of CTEPH. Patients that are ineligible for surgery have been treated with Riociguat, a soluble guanylate cyclase stimulator (48). Lifelong anticoagulation is recommended for all patients with CTEPH in order to prevent recurrent VTE and progressive PH. Vitamin K antagonists (*e. g.* warfarin) are used for anticoagulation. Since the underlying mechanism of aPL antibodies causing CTEPH is immunological, the addition of immunosuppressive agents to the therapeutic regimen may be of benefit. It is therefore important to consider CTEPH in all patients with CTDs and PH as the management of CTEPH is significantly different from other forms of PH.

### 8. Group 5 PH

#### 8.1. PH and autoimmune thyroid disease

WHO group 5 PH includes patients with thyroid disorders, especially hyperthyroidism. It is unclear whether the association between pulmonary hypertension (PH) and hyperthyroidism is an incidental one or if there is an unexplained pathological mechanism (49). The autoimmune hypothesis can possibly help answer this question. Sugiura *et al.* found a direct linear correlation between pulmonary artery pressure's and thyroid stimulating hormone receptor antibodies (TRAb) ( $r = 0.74$ ,  $p < 0.001$ ) (50). This hypothesis is based upon a possible indirect influence of TRAb in inducing immune-mediated damage of the endothelium that could be able to promote endothelial dysfunction leading to PH. Pagi *et al.* and Marongiu *et al.* noted increased incidence of aPL antibodies in patients with autoimmune thyroid

disease, especially Grave's disease. Nabriski *et al.* similarly demonstrated the prevalence of aPL antibodies is increased in patients with autoimmune thyroid disorders. They suggested that non-specific autoantibody production may accompany the synthesis of tissue-specific immunoglobulin in autoimmune disorders (51,52). Thus while there might be an association between autoimmune thyroid disease, aPL antibodies and PH, this has not yet been studied and requires further research.

## 8.2. PH and Sarcoidosis

Ina *et al.* studied the correlation between aPL antibodies and sarcoidosis. While no correlation was observed between the occurrence of aPL antibodies and disease activity of sarcoidosis, the presence of aPL antibodies in sarcoidosis was found to be a useful index to judge the prolongation of the disease (53). Recently Pathak *et al.* performed a systematic review to study the association of APS and sarcoidosis and identified 4 cases of sarcoidosis in patients with APS. All patients were noted to have a one of the thrombotic manifestations of APS. None of the studies recorded the incidence of association with PH (54). Thus similar to autoimmune thyroid disease the possible association of PH with sarcoidosis and aPL antibodies needs future studies.

## 9. Conclusion

The association of aPL antibodies with VTE and PE is well established. Now we know that the effects of aPL antibodies on the pulmonary vasculature extend beyond CTEPH with manifestations that are similar to pulmonary arterial hypertension. There is emerging literature studying the association of aPL antibodies with cardiac valvular disease which can eventually lead to pulmonary venous hypertension. Thus the presence of aPL antibodies will make clinicians think about PH across all WHO groups. Our hypothesis is that aPL antibodies are a harbinger of pulmonary vasculopathy and thus the presence of aPL antibodies can be possibly used as a screening tool for PH patients. Further research is needed to clarify the pathogenesis of aPL antibodies and PH, and to lay guidelines regarding the routine screening of these patients.

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*(Received July 26, 2017; Revised August 7, 2017; Accepted August 21, 2017)*

# Surgical treatments for patients with recurrent bile duct stones and Oddis sphincter laxity

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**Summary** Recurrent bile duct stones is the most common complication after gallstone surgery and the incidence is about 4-24%. Sphincter of Oddi laxity will lead to duodenal content flow into the bile or pancreatic duct. Patients with recurrent bile duct stones and Oddis sphincter laxity were intractable. Here we sought to present the possible and helpful surgical treatments for such patients. Prospective randomized clinical trial are needed for evaluating the outcome of surgical treatments.

**Keywords:** Recurrent bile duct stones, Odds sphincter laxity, surgical treatments

## 1. Introduction

Recurrent bile duct stones usually refer to bile ducts stones that reappeared 2 years after bile duct exploration operation, and is the most common long-term complication after gallstone surgery (1,2). Because there are still no effective therapies for preventing this complication, some patients have experienced bile duct stone recurrence for many times. According to literatures, the incidence of recurrent bile duct stones is about 4-24% (3-5).

Gallstones are distinguished into the following two types: cholesterol gallstones that contain more than 50% of cholesterol (nearly 75-80% of gallstones) and pigment gallstones that contain less than 30% of cholesterol by weight, which can be subdivided into black pigment gallstones (10-15%) and brown pigment gallstones (5-10%) (6-9). Cholesterol gallstones are associated with risk factors such as dyslipidemia, total parenteral nutrition, gallbladder hypomotility, gender (female), and old age. Black pigment gallstones are associated with typical hyperbilirubinemia factors such as hemolysis, liver cirrhosis, and pathologic enterohepatic cycling of unconjugated bilirubin. Brown pigment gallstones are associated with biliary infection (10-12). Recurrent bile

duct stones mostly belong to pigment gallstones. Patients with recurrent bile duct stones with Oddis sphincter laxity were intractable; here we sought to present the possible and helpful surgical treatments for such patients.

## 2. Causes of pigment gallstone of bile duct

### 2.1. Bile infection

Pigment gallstones were often associated with severe bile duct inflammation and biliary tract infection. During the operation of bile duct lithotomy, surgical instruments can damage the bile duct wall, and the compression of the T tube can also damage the bile duct wall, these can destroy bile duct mucous membrane, further cause bile infection.

By electron microscopy, it was found that bacteria were present in the core of the gallstone, suggesting that bacteria plays a major role in the formation of bile pigment gallstones (13,14). Through the bacterial culture of bile, the highest positive rate is *Escherichia coli* (15). The mechanism of pigment gallstone to *Escherichia coli*, can be understood as the bile bacteria producing beta glucuronidase, it can be combined with bilirubin hydrolysis into unconjugated bilirubin and glucuronic acid, unconjugated bilirubin and calcium binding to bilirubin precipitated calcium, thereby promoting pigment gallstone formation (16-20).

### 2.2. The obstruction of biliary tract

Biliary tract exploration surgery can often lead to

Released online in J-STAGE as advance publication August 25, 2017.

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obstruction of the biliary tract, It was often occurred in the operation of exploratory rough, postoperative T tube compression, postoperative Oddi sphincter edema (21,22). These biliary lesions can cause biliary strictures during the healing process, and then cause a series of pathological changes. When bile duct obstruction occurs, the concentrated bile was strongly stimulated by the bile duct mucosa, which stimulates the exfoliated bile duct epithelial cells as well as the aggregated bacteria to precipitate more easily, resulting in bile pigment stones (23).

### 2.3. Duodenal diverticulum

Duodenal diverticulum (DD) was originally described by Chomel in 1710, and got more detailed appraisal by Morgagni in 1762 (24-26). It was difficult to evaluate the exact incidence of DD. According to published data, DD were seen in 15-22% of post mortem studies (27-30). Most diverticula are located in the second part of the duodenum, commonly within 2.5 cm of the ampulla.

It was reported that 88.9% of patients with DD were associated with bile duct stones. In Manometric's study, duodenal diverticulum could lead to the formation of pigment stones. They reported that the pressure in the sphincter of Oddi may be decreased. The decreased pressure might allow reflux of intestinal microfloras for a stagnant diverticulum, and then  $\beta$ -glucuronidase derived from bacterial would deconjugate bile pigments. It would explain why patients with duodenal diverticula are more likely to suffer from pigment stones.

### 2.4. Sphincter of Oddi Laxity

The sphincter of oddi (SO), a smooth muscle, was initially described by Ruggero Oddi in 1887 (31). Regarding to the function of SO, there are three main functions: regulation the discharge of bile and pancreatic juice, resist reflux from duodenum juice to the bile and pancreatic duct, and make the gallbladder filling.

Normally, the diameter of the duodenum papilla orifice is no more than 2-3 mm, but in some patients, the sphincter of Oddis is completely relaxed, it was called sphincter of Oddi laxity (SOL) (32). Primary or secondary SOL mainly depends on patients whether have a history of biliary tract surgery.

In SOL patients, the sphincter of Oddi lose the ability of reflux prevention, and will lead to duodenal content flow into the bile or pancreatic duct (33). It might result in bacterial infection and imbalance of biliary PH, and then  $\beta$ -glucuronidase generated by *E.cilo* lead to the formation of pigment stones in the biliary tract.

### 3. Causes of Sphincter of Oddi Laxity

Currently, it is not clear for the specific causes of SOL. These causes could disrupt the normal structure

of SO, which results in altered function of SO, and eventually SO can not act as "gate" function. According to published data (32,33), SOL are classified as primary and secondary based on the causes. Primary sphincter of Oddi Laxity is mainly due to congenital abnormality, congenital malformation of smooth muscle and absence of some neurotransmitter receptors can result in SOL.

Secondary SOL (SSOL), the most common, was attributed to at least two major factors. The first cause of SSOL was mechanical injury by stones that repeatedly induced large amounts of inflammation mediators. Regular contraction and relaxation of SO response to neurotransmission is impaired, which may contribute to abnormal SO relaxation. The second cause of SSOL was iatrogenic injury, which include endoscopic retrograde cholangiopancreatography (ERCP), endoscopic sphincterotomy (EST), and Biliary tract exploration surgery using a Bakes dilator. Those above injuries would induce inflammation of SO over times, and then result in SSOL.

The discharge of bile into the duodenum involves both neural and hormonal pathways. The sphincter of Oddi relaxes while gallbladder contracts, the bile is discharged into the duodenum (34,35). Neural and hormonal regulation of Sphincter of Oddi loses work when there is Oddis sphincter laxity, the basal pressure of biliary tract decreases, then resulting in gallbladder excessive filling and possible decreased discharge of bile flow into duodenum, and subsequently biliary tract cholestasis develops.

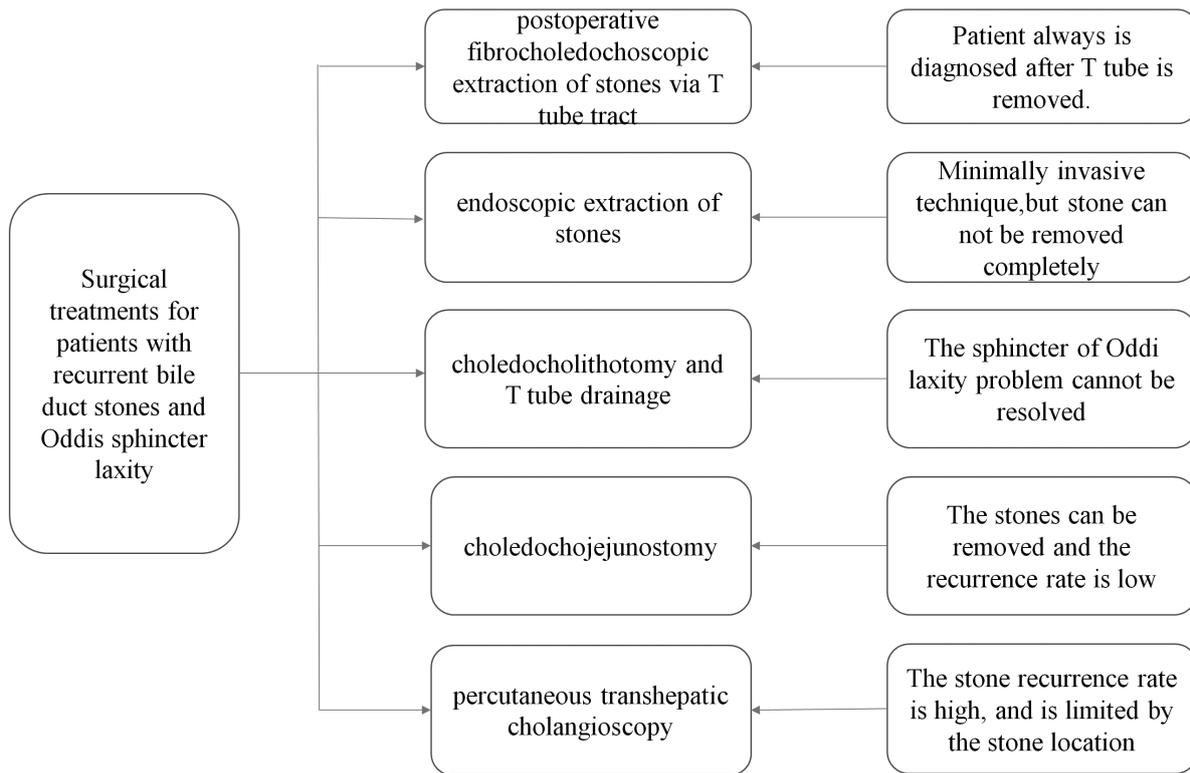
### 4. Surgical treatments for patients with recurrent bile duct stones and Oddis sphincter laxity

Recurrent bile duct stone, as an intractable disease, is prevalent in China. The incidence was estimated as high as 24%. In some clinical practice, recurrent bile duct stone had a tendency of Oddis sphincter laxity and always needed reoperation. However, the current methods of reoperation have not been unified, and there are few clinical comparative studies showing which surgical procedure is better.

Many methods of surgical treatments can be considered for patients with recurrent bile duct stones. These are: a) postoperative fibrocholedochoscopic extraction of stones; b) endoscopic extraction of stones; c) choledocholithotomy and T tube drainage; d) choledochojejunostomy; e) percutaneous transhepatic cholangioscopy (Figure 1).

Postoperatively, while T-tube is still in the common bile duct, a firm fibrous tract formed, and it is feasible to extract the recurrent stones through fibrocholedochoscopy (36,37). However, most of patients with recurrent bile duct stones were diagnosed after the removal of T-tube.

Since 1974, endoscopic sphincterotomy (EST) has been used for removal of recurrent common bile



**Figure 1. Surgical treatments for patients with recurrent bile duct stones and Oddis sphincter laxity.**

duct stones. After a successful EST, the stones can be extracted with Dormia basket (38,39). EST is generally considered both safe and effective, but the possibility of complications can not be ignored. Among the complications, post-procedural impaired Oddis sphincter is thought to be the major cause of Oddis sphincter laxity and recurrence of bile stones.

Percutaneous transhepatic cholangioscopy (PTCS), advocated in 1974, is a minimally invasive technique for removal of biliary stones. When patients with stones in more than two lobes of the liver, PTCS is a reasonable choice. However, PTCS is ineffective in treatment of stenosis of bile duct and Oddis sphincter laxity (40,41). Hence, PTCS is only a temporary method of stone removal, and it can not be effective in preventing recurrence of bile duct stones.

If the stone is large and impacted in the bile duct, the above treatments may face failure. Under these circumstances, reoperation continues to be indicated for recurrent bile duct stones. Choledocholithotomy with T tube drainage and Choledochojejunostomy are the basic method in the treatment of recurrent bile duct stones. According to a retrospective study from China, when recurrent bile duct stones with Oddis sphincter laxity, the recurrence rate of bile duct stone after choledochojejunostomy was lower than that of Choledocholithotomy with T tube drainage (42). The main reason is that choledochojejunostomy can make the bile duct drainage unobstructed. In addition, choledocholithotomy did not solve the problem of Oddis

sphincter laxity, duodenal content can still flow into the bile or pancreatic duct.

## 5. Prospects for the future

It is generally known that recurrent bile duct stones are very common in Southeast Asian countries with high recurrence rate. Studies have shown that bile duct drainage was not unobstructed, however, there was still bile duct stones recurrence. The concurrent Oddis sphincter laxity maybe related to the recurrent bile duct stones.

By reviewing some clinical studies, we found that patients with Oddis sphincter laxity are more likely to suffer from bile duct stones and often need reoperation (43). However, there is no consensus on the surgical treatments of recurrent bile duct stones with concurrent Oddis sphincter laxity.

Oddi sphincter laxity will lead to intestinal juice reflux, biliary tract cholestasis, and further biliary tract infection, resulting in recurrence of bile duct stones. Therefore, we could conclude that Oddi sphincter laxity is one of the indirect cause of recurrent bile duct stones.

Choledochojejunostomy for the treatment obstruction of distal common bile duct have been better defined. And it was suggested that recurrent bile duct stones with Oddis sphincter laxity is also one indication of choledochojejunostomy (44). Roux-en-y Choledochojejunostomy can avoid stenosis of bile duct and make the bile drainage unobstructed.

Then the high risk factor of recurrence of bile duct stones will be eliminated. Moreover, Oddi sphincter laxity often accompanied with intestinal reflux, Roux-en-y choledochojejunostomy is with ability of antireflux for some extent, which is another reason, that choledochojejunostomy can reduce or even eliminate the incidence of recurrent bile duct stones with Oddi sphincter laxity.

To our knowledge, there are no guidelines for when to perform Roux-en-y choledochojejunostomy for the treatment of patients with recurrent bile duct stones and Oddi sphincter laxity. Prospective randomized clinical trial is needed for evaluating the outcome of such surgical treatment.

### Acknowledgement

This work was supported by National Natural Science Fund of China (81470860, Yuesi Zhong).

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(Received July 14, 2017; Revised August 13, 2017; Accepted August 15, 2017)

## A novel *CASK* mutation identified in siblings exhibiting developmental disorders with/without microcephaly

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### Summary

The calcium/calmodulin-dependent serine protein kinase gene (*CASK*) mutations are associated with various neurological disorders; a syndrome of intellectual disability (ID) and microcephaly with pontine and cerebellar hypoplasia (MICPCH), FG syndrome, X-linked ID with/without nystagmus, epileptic encephalopathy, and autistic spectrum disorder (ASD). Next generation sequencing was performed to elucidate genetic causes in siblings exhibiting developmental disorders, and a novel *CASK* mutation, c.1424G>T (p.Ser475Ile), was detected in a male patient with ID, ASD, and microcephaly. Radiological examination of his brain showed no structural abnormality. The identified mutation was shared with the healthy mother and a younger sister exhibiting ASD. Although the mother showed a skewed X-chromosome inactivation (XCI) pattern, the sister showed a paradoxical XCI pattern. This would explain why this sister possessed a normal intellectual level, but showed the same ASD symptoms as the affected brother. A novel *CASK* mutation was identified in two siblings with ID and/or ASD, suggesting a relationship between the *CASK* mutation and ASD. Recently performed large molecular cohorts for patients with developmental disorders suggest that *CASK* is one of the genes related to developmental disorders. For better understanding of genotype-phenotype correlation in ASD cases with *CASK* mutations, more information should be accumulated.

**Keywords:** Autism, X-chromosome inactivation, next generation sequencing, obligate carrier, manifesting carrier

### 1. Introduction

Approximately 1.5-2% and 1-0.7% of children show developmental disorders (DD), including intellectual disability (ID) and autism spectrum disorder (ASD), respectively (1). Etiologies of these disorders are heterogeneous and recent developments in genetic analysis have provided evidence that various types of genomic alterations are related to DD. Especially, many large cohort studies have been performed to elucidate

the molecular basis of DD. As a result, the calcium/calmodulin-dependent serine protein kinase gene (*CASK*) was reported as one of the genes responsible for developmental disorders (2,3).

Human *CASK* mutations were first identified in female patients with a syndrome of ID and microcephaly with pontine and cerebellar hypoplasia (MICPCH) (4). *CASK* encodes a calcium/calmodulin-dependent serine protein kinase that belongs to the membrane-associated guanylate kinase (MAGUK) family (4). The proteins included in MAGUK family are scaffold proteins and are located at synapses in the brain. Various neurological disorders have been associated with *CASK*; FG syndrome (5,6), X-linked ID with/without nystagmus (7), and epileptic encephalopathy (8-10). In this study, a novel *CASK* mutation, c.1424G>T (p.Ser475Ile), was identified in siblings exhibiting DD with ID and/or ASD.

Released online in J-STAGE as advance publication July 31, 2017.

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## 2. Materials and Methods

This study, which aimed to identify genetic etiologies in patients with DD, was performed in accordance with the Helsinki declaration. We obtained permission from the ethical committee of the institution. Patients were enrolled in this study after receiving written informed consent and thorough genetic counseling regarding appropriate dealing with genetic information and possible incidental findings.

Blood samples were obtained from the patients and their parents. DNA was then extracted using the QIAamp DNA extraction kit (QIAGEN, Hilden, Germany). Next-generation sequencing (NGS) was performed to screen single-nucleotide variants using TruSight One v1.0 sequencing panel (Illumina, San Diego, CA, USA), in accordance with the previously described protocol (11). To confirm SNVs, Sanger sequencing was performed on all samples. The X-chromosome inactivation (XCI) pattern was determined by quantitation of the methylation of the polymorphic human androgen receptor (HUMARA) locus (12).

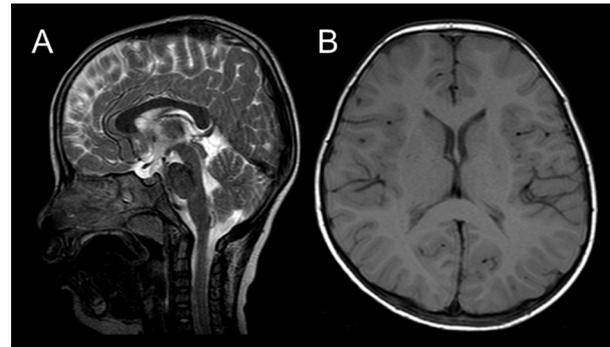
## 3. Results

### 3.1. Patients report

There are two siblings in the indicated family. Both of the siblings, born to healthy parents, are affected. The mother had two spontaneous abortions with unknown etiologies.

A 5-year-old boy (patient 1) was born at 39 weeks of gestational age, with a weight of 2,810 g. There were no remarkable complications during his pregnancy. In early infancy, his mother did not notice any developmental problems. His umbilical herniation was surgically repaired during infancy. He made several motor developmental milestones, with head control at 3 months, crawling at 14 months, and standing with support at 16 months. At 18 months, he could not express speech or walk unassisted. Because of these developmental delays, he was referred to our hospital. There was no history of seizures.

At 26 months, a behavioral assessment indicated the following symptoms of ASD: *i*) poor verbal communication (lack of vocabulary, poor pronunciation, and dysarthria), *ii*) qualitative impairment in social communication (he is not empathetic and only shows a superficial interest in things), and *iii*) limited stereotypical interests and behaviors (when distressed, he strikes his head on the wall repeatedly or will break magazines). He shows a special interest in some marks and signs. The pervasive developmental disorders autism society Japan rating scale (PARS) was scaled at 9, indicating a high possibility of ASD (13). Brain magnetic resonance imaging showed no structural abnormality (Figure 1). Conventional chromosomal



**Figure 1. Results of brain magnetic resonance imaging.** There is no abnormality, including the volume of cerebellum. T2-sagittal (A) and T1-axial images (B) examined at 5 years of age.

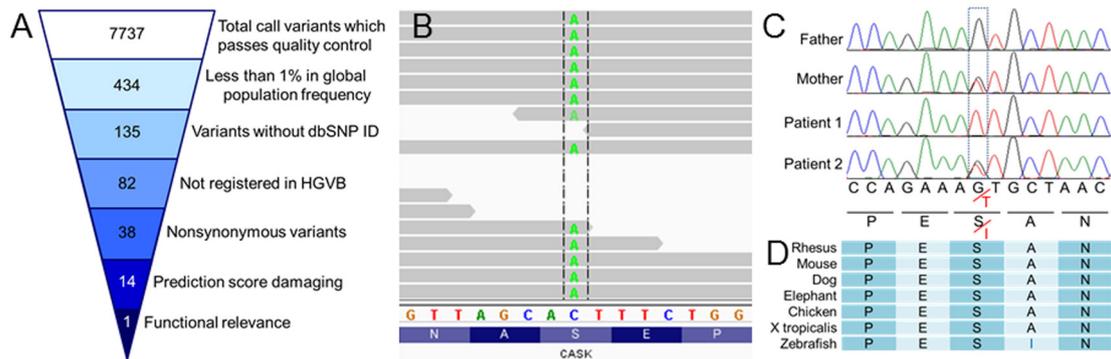
analysis showed a normal male karyotype of 46, XY and chromosomal microarray testing showed no abnormality.

At present, his height is 99.5 cm (3rd-10th centile), weight is 13.4 kg (< 3rd centile), and occipitofrontal circumference (OFC) is 47 cm (< 3rd centile), indicating microcephaly. He requires assistance in eating, bathing, and getting dressed. There is no history of sleep disturbance. The Kyoto Scale of Psychological Development (KSPD) indicated an intelligence quotient (IQ) of 61, indicating mild developmental delay (14).

A younger sister of patient 1 (patient 2) also visited our hospital. She is currently 3 years old. She was crawling at 8 months and walking unassisted at 18 months, indicating mild motor developmental delay. She misbehaves/acts out when a situation does not go her way. Based on further inputs regarding her behavior and developmental history, she was diagnosed with ASD. She showed typical autistic behavior, like her brother (patient 1). However, she did not demonstrate short stature or microcephaly (OFC 46 cm). The KSPD indicated an IQ of 84, which is a normal level. The PARS score was 9, which was the same as her elder brother (patient 1), indicating a high possibility of ASD.

### 3.2. Genetic analysis

NGS for patient 1 extracted 2,561 Gb of targeted aligned sequences and a mean coverage depth of 105.3 was obtained (target coverage at 20× was 95.8%). After filtering, only a missense variant (chrX:41437672C>A, NM\_003688.3(CASK):c.1424G>T [NP\_003679.2:p.Ser475Ile]) remained (Figure 2A). Integrative Genomics Viewer (IGV; <http://www.broadinstitute.org/igv/>) showed the same alteration in all reads mapped at this position, indicating a 100% detection ratio and denying mosaicism (Figure 2B). This codon is conserved among species (Figure 2D). The prediction scores were calculated through wANNOVAR (<http://wannovar.wglab.org/>) (Table 1) and the scores by LRT\_pred, MutationTaster\_pred, and CADD\_phred suggested "damaging". Previously reported *CASK*



**Figure 2. Results of genetic studies.** (A) Filtering steps and the number of filtered variants are shown in inverted pyramids. The Human Genetic Variation Database (HGVD) (<http://www.genome.med.kyoto-u.ac.jp/SnpDB>) provided from Kyoto University was used for the filtering. (B) The Integrative Genomics Viewer (IGV) shows the identified *CASK* variant in 100% of reads. (C) Electropherograms of Sanger sequencing. The father shows the wild type sequence, whereas the mother and patient 2 exhibit heterozygous status. Patient 1 is completely hemizygous for the mutation. (D) The neighboring amino-acid sequences are compared among species. The affected codon "S" is conserved among species.

**Table 1. Results of prediction scores**

SIFT_score	0.19
SIFT_pred	T
Polyphen2_HDIV_score	0.914
Polyphen2_HDIV_pred	P
Polyphen2_HVAR_score	0.887
Polyphen2_HVAR_pred	P
LRT_score	0
LRT_pred	D*
MutationTaster_score	1
MutationTaster_pred	D*
MutationAssessor_score	0.255
MutationAssessor_pred	N
FATHMM_score	0.64
FATHMM_pred	T
RadialSVM_score	-0.843
RadialSVM_pred	T
LR_score	0.162
LR_pred	T
VEST3_score	0.936
CADD_raw	3.177
CADD_phred	16.63*
GERP++_RS	5.44
phyloP46way_placental	2.41
phyloP100way_vertebrate	7.429
SiPhy_29way_logOdds	18.55

T, tolerate; P, possibly damaging; \*, damaging is suggested.

variants are summarized in Table 2. As shown, p.Ser475Ile has never been reported previously and is supposedly a novel mutation. This variant was reconfirmed by Sanger sequencing and identified in the mother and patient 2 (the younger sister of patient 1) (Figure 2C), suggesting segregation by the X-linked recessive trait. Although patient 2 did not show ID, she shared ASD symptoms with her brother (patient 1). Because *CASK* is located on the X chromosome, XCI patterns were analyzed in the mother and patient 2. The mother showed an almost completely skewed XCI pattern; however, patient 2 showed a paradoxical XCI pattern, i.e., the paternally derived allele (supposed normal allele) was predominantly inactivated (Figure 3).

This may indicate that the mother is an obligate carrier and patient 2 is a manifesting carrier.

#### 4. Discussion

Most of the *CASK* mutations found in patients with MICPCH showed loss-of-function effects and were related to an X-linked dominant trait with reduced male viability or even in utero lethality; however, mild and hypomorphic mutations such as missense mutations would be compatible with live birth in affected males (4). Thus, *CASK* mutations found in male patients with clinically mild neurological impairments (compared to MICPCH) would be related to an X-linked recessive trait. Although phenotypic features were recognized as a clinical continuum, Moog *et al.* reviewed *CASK* mutations in males and classified them into three categories (15); *i*) MICPCH with severe epileptic encephalopathy, associated with loss-of-function mutation, *ii*) MICPCH associated with inactivating alterations in the mosaic state, and *iii*) syndromic/nonsyndromic mild to severe ID, with or without nystagmus caused by *CASK* missense/splicing mutations that leave the *CASK* protein intact but likely alter its function.

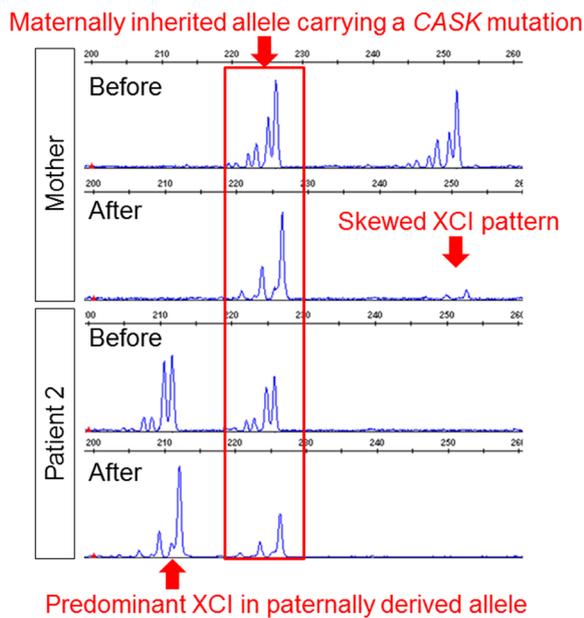
In this study, we identified a novel *CASK* mutation in a family associated with clinical characteristics of ASD/DD and microcephaly. Recently published large cohort studies for ASD or DD suggested *CASK* as one of the responsible genes for ASD/DD (3,16). Thus, it is reasonable to conclude that the identified *CASK* variant is related to ASD/DD in this family.

In this family, the mother, exhibiting a skewed XCI pattern, was considered an obligate carrier. On the contrary, the younger sister of the proband (patient 2) having the same *CASK* mutation showed a paradoxical XCI pattern (the maternally inherited allele was predominantly activated). This would explain why the sister (patient 2), a female carrier, showed a mild ASD

**Table 2. Summary of the intragenic nucleotide changes in *CASK* and phenotypic features of the patients**

Domain	Position	Nucleotide change	Amino-acid alteration	Phenotype	Gender	Inheritance	Reference	Published year
CaMK-like domain	ex1	c.1A>G	M1V	Ohtahara syndrome	M	DN	Saito <i>et al.</i>	2012
	ex1	c.1A>G	M1V	MICPCH	M	DN	Moog <i>et al.</i>	2015
	ex2	c.68delT	frameshift	MICPCH	F	DN	Moog <i>et al.</i>	2011
	ex2	c.79C>T	R27*	MICPCH	F	NT	Hayashi <i>et al.</i>	2012
	ex2	c.79C>T	R27*	MICPCH	M	DN	Moog <i>et al.</i>	2015
	ex2	c.82C>T	R28*	ID, microcephaly, infantile spasms	F	DN	Michaud <i>et al.</i>	2014
	ex2	c.83G>T	R28L	FG syndrome	M	XLR	Piluso <i>et al.</i>	2009
	IVS2	c.173-2A>C	splicing	MICPCH	F	DN	Moog <i>et al.</i>	2011
	ex3	c.174T>A	D58E	MICPCH	F	NT	Moog <i>et al.</i>	2011
	ex3	c.227_228del	frameshift	MICPCH	M	DN	Nakamura <i>et al.</i>	2014
	ex3	c.243_244del	Y81*	MICPCH	F	NT	Hayashi <i>et al.</i>	2012
	IVS3	c.278+1G>A	splicing	MICPCH	F	DN	Burglen <i>et al.</i>	2012
	ex4	c.316C>T	R106*	MICPCH	F	DN	Burglen <i>et al.</i>	2012
	ex4	c.316C>T	R106*	MICPCH	F	NT	Moog <i>et al.</i>	2011
	ex4	c.316C>T	R106*	MICPCH	F	DN	Hayashi <i>et al.</i>	2012
	IVS4	c.357-1G>A	splicing	MICPCH	F	DN	Hayashi <i>et al.</i>	2012
	ex5	c.379C>T	E127*	MICPCH	F	NT	Moog <i>et al.</i>	2011
	IVS5	c.430-2A>T	splicing	MICPCH	F	NT	Moog <i>et al.</i>	2011
	ex7	c.589G>A	G197R	DD	NA	NA	Moog <i>et al.</i>	2014
	ex7	c.704_708del	frameshift	DD	M	DN	Moog <i>et al.</i>	2015
	ex8	c.763C>T	R255C	MICPCH	M	DN	DDDS	2017
	ex8	c.802T>C	Y268H	DD, ASD, hypotonia	F	DN	Hackett <i>et al.</i>	2010
	IVS8	c.831+1G>A	splicing	ID-nystagmus	M	XLR	DDDS	2017
	IVS8	c.831+2T>G	splicing	DD, microcephaly	F	DN	DDDS	2017
	ex9	c.915G>A	splicing	MICPCH	F	DN	Moog <i>et al.</i>	2011
	ex9	c.915G>A	P396S	MICPCH	M	XLR	Najm <i>et al.</i>	2008
	ex13	c.1186C>T	S475I	ID-nystagmus	M	XLR	Hackett <i>et al.</i>	2010
ex15	c.1424G>T	frameshift	DD, ASD, microcephaly	M	XLR	Present patient	2017	
ex15	c.1501dup	frameshift	MICPCH	F	DN	Burglen <i>et al.</i>	2012	
ex16	c.1577del	frameshift	MICPCH	F	DN	Valayannopoulos <i>et al.</i>	2012	
ex17	c.1609C>T	R537*	MICPCH	F	DN	DDDS	2017	
ex17	c.1639C>T	Q547*	MICPCH	F	NT	Moog <i>et al.</i>	2011	
IVS17	c.1668+1G>A	splicing	MICPCH	F	DN	Moog <i>et al.</i>	2011	
ex20	c.1837C>T	R613*	MICPCH	F	NT	DDDS	2017	
ex21	c.1896dup	frameshift	MCA, epilepsy, hypotonia, microcephaly	M	XLR	Nakajiri <i>et al.</i>	2015	
ex21	c.1874C>T	P625L	MCA, DD, microcephaly	M	DN	DDDS	2017	
ex21	c.1915C>T	R639*	MICPCH	F	DN	DDDS	2008	
ex21	c.1968G>A	W656*	MICPCH	F	DN	Najm <i>et al.</i>	2012	
ex21	c.1968G>A	W656*	MICPCH	F	DN	Burglen <i>et al.</i>	2012	
ex21	c.1970G>A	W657*	MICPCH	F	DN	Valayannopoulos <i>et al.</i>	2012	
ex21	c.1970G>A	W657*	MICPCH	F	DN	Burglen <i>et al.</i>	2012	
ex21	c.1970G>A	W657*	MICPCH	F	DN	Valayannopoulos <i>et al.</i>	2012	
IVS21	c.2039+1G>T	splicing	MICPCH	F	DN	Burglen <i>et al.</i>	2012	
IVS21	c.2040-1G>C	splicing	MICPCH	F	DN	Hayashi <i>et al.</i>	2012	
IVS21	c.2040-2A>G	splicing	MICPCH	F	DN	Burglen <i>et al.</i>	2012	
ex22	c.2074C>T	Q692*	MICPCH	F	DN	Burglen <i>et al.</i>	2012	
ex22	c.2080C>T	Q694*	MICPCH	F	DN	Burglen <i>et al.</i>	2012	
ex22	c.2129A>G	D710G	MICPCH	M	DN	Hackett <i>et al.</i>	2010	
ex23	c.2168A>G	Y723C	ID-nystagmus	M	XLR	Hackett <i>et al.</i>	2010	
ex23	c.2302+5G>A	splicing	ID-nystagmus	M	XLR	Hackett <i>et al.</i>	2010	
IVS24	c.2506-2A>G	splicing	MICPCH	F	DN	Burglen <i>et al.</i>	2012	
IVS25	c.2506-2A>G	splicing	FG syndrome	M	DN	Dunn <i>et al.</i>	2016	
IVS25	c.2506-2A>T	splicing	ID-nystagmus	M	XLR	Hackett <i>et al.</i>	2010	
ex27	c.2632C>T	Q878*	MICPCH	F	DN	Hayashi <i>et al.</i>	2012	
ex27	c.2755T>C	W919R	ID-nystagmus	M	XLR	Hackett <i>et al.</i>	2010	

M; male, F; female, NA; not available, DN; de novo, XLR; X-linked recessive trait, MICPCH; microcephaly with pontine and cerebellar hypoplasia, MCA; multiple congenital anomalies, DD; developmental disorder, ASD; autism spectrum disorder, ID; intellectual disability, DDDS; Deciphering Developmental Disorders Study.



**Figure 3. Results of X-chromosome inactivation (XCI) patterns.** The mother shows a skewed XCI pattern, because one of the X-chromosomes (not shared with patient 2) is almost completely missing after digestion by a methylation sensitive restriction enzyme. Patient 2 shows a paradoxical pattern with predominant XCI in the paternally derived allele.

phenotype.

The previously reported *CASK* mutations are summarized in Table 2 (17-20). The mutation identified in this study was the first missense mutation located on the PDZ domain. This may be related to the phenotypic consequence. Owing to identification of *CASK* mutations in patients with atypical clinical features, the clinical entity of *CASK* related disorder is expanding beyond the typical and classical three categories. It is important to accumulate individual cases with *CASK* mutations that have been analyzed using multidisciplinary approaches including genetic analysis and periodic developmental assessments and neuroimaging.

### Acknowledgements

We would like to express our gratitude to the patients and their families for their cooperation.

### Funding

This research was supported by the Practical Research Project for Rare/Intractable Diseases from Japan Agency for Medical Research and Development (AMED) and Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 15K09631 (TY).

### Disclosure

We certify that we have read the journal's position

regarding issues pertaining to ethical publication, and affirm that this report is consistent with the guidelines. The authors have no conflicts of interest to declare.

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(Received May 30, 2017; Revised July 14, 2017; Accepted July 18, 2017)

# Mucopolysaccharidosis type I, II and VI and response to enzyme replacement therapy: Results from a single-center case series study

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## Summary

Mucopolysaccharidoses (MPS) types I, II and VI are associated with deficiencies in alpha-L-iduronidase, iduronate-2-sulfatase and N-acetylgalactosamine-4-sulfatase, respectively, and generally involve progressive and multi-systemic clinical manifestations. Enzyme replacement therapy (ERT) appears to be reasonably well tolerated. The aim of this study was to examine clinical and diagnostic findings of a series of pediatric and adult MPS patients, and assess the safety and efficacy of ERT in children and adults with MPS type I, II and VI. Pediatric and adult patients were treated weekly with 1 mg/kg recombinant human N-acetylgalactosamine-4-sulphatase (rhASB), 0.45 mg/kg alpha-L-iduronidase, or 0.5 mg/kg iduronate-2-sulfatase. Clinical and biochemical parameters with ERT were evaluated for a mean duration of 5 years. Mantel-Haenszel risk ratios and associated 95% confidence intervals (CIs) were calculated for rates of death among different types of enzyme replacement therapies (ERTs). Twenty-seven patients (mean ages – pediatric: 6.8 years; adult: 29 years) were included. ERT was found to be consistently well tolerated and effective in attenuating symptoms, but did not prevent the progression of the disease or reduce mortality rates. Our findings demonstrated that early diagnosis and initiation of ERT are critical for improvements in patient-important outcomes and quality of life, although disease progression and mortality rates remain high.

**Keywords:** Lysosomal storage disorders, glycosaminoglycans, treatment, prognosis

## 1. Introduction

Mucopolysaccharidoses (MPS) are lysosomal storage disorders caused by glycosaminoglycan (GAG) enzymatic catabolism deficiencies, leading to organ and tissue deposition. The clinical manifestations of

MPS are generally progressive and multisystemic, and signs and symptoms are variable and may progress rapidly or more slowly after birth. Clinical alterations such as facial infiltration, thick lips, opacity of the cornea, umbilical and/or inguinal hernias, cardiac disease, hepatosplenomegaly, articular stiffness, short stature, intellectual disability and skeletal changes, also known as multiple dysostosis, make this diagnosis challenging (1).

MPS types I, II and VI are associated with deficiencies in alpha-L-iduronidase, iduronate-2-sulfatase and N-acetylgalactosamine-4-sulfatase, respectively (1). Enzyme replacement therapy (ERT) with alpha-L-iduronidase, iduronate-2-sulfatase, and recombinant

Released online in J-STAGE as advance publication August 19, 2017.

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human N-acetylgalactosamine-4-sulphatase (rhASB) have been shown to be effective in several phase 2 and 3 studies (2-4).

The aim of this case series study was to examine clinical and diagnosis findings of pediatric and adult MPS I (Hurler, Hurler-Scheie, Scheie), MPS II (Hunter) and MPS VI (Marateaux Lamy) patients, and to evaluate the long-term safety and efficacy of ERT for these patients.

**2. Materials and Methods**

This study was approved and registered under number 1034/08 by the Ethics Committee for the analysis of Research projects (CAPPesq) of the clinical Medical School of the University of São Paulo. This was an open-label, single-center case series study of 27 patients with confirmed diagnoses of MPS I (n = 13), II (n = 8) and VI (n = 6), managed with ERT in three Brazilian referral centers (Genetic Outpatient Clinic of the Children's Institute of Hospital das Clínicas FMUSP, Chemotherapy Division of PUC Campinas, and the Children's Hospital Candido Fontoura) between October 2012 and July 2015.

MPS diagnosis was confirmed by specific enzymatic activity (nmol/h/mg protein) and elevated urinary glycosaminoglycans (GAGs). All patients were evaluated by one geneticist by means of clinical and laboratory protocols, previously described by Giugliani *et al.* 2007 (5) in their study evaluating the management of patients with MPS with ERT. Laboratory exams included: quantitative and qualitative dosing of urinary GAGs, based on the reference values for age and enzymatic activity in leukocytes; echocardiography; abdominal ultrasonography and/or computed tomography; and magnetic resonance imaging and/or computed tomography of the skull and spine resonance. Genetic mutation research was performed when available. Patients who previously received bone marrow transplantation were excluded.

Treatment consisted of weekly infusions of 0.45 mg/kg alpha-L-iduronidase, 0.5 mg/kg iduronate-2-sulfatase, or 1 mg/kg of rhASB in all patients with MPS I, II, and VI, respectively, except five patients in the alpha-L-iduronidase group who received 0.45 mg/kg every other week.

Primary endpoints were clinical outcomes: coarse facies, cloudy cornea, joint stiffness, claw hands, short neck, macroglossia, macrocephaly, short stature, low weight, hepatosplenomegaly, hernias, intellectual disability, surgery procedures, and clinical and anesthetic complications. Secondary endpoints were laboratory parameters: GAGs, echocardiography, X-ray, MRI of the skull and spine, abdominal ultrasonography, polysomnography, and molecular imaging.

Clinical and biochemical parameters with ERT were evaluated for a mean duration of 5 years. Mantel-

Haenszel risk ratios and associated 95% confidence intervals (CIs) were calculated for rates of death among different types of enzyme replacement therapies (ERTs).

**3. Results**

Twenty-seven patients with confirmed MPS-I (n = 13; 48%), II (n = 8; 30%), and VI (n = 6; 22%) were included (Table 1). No abnormalities were present at birth across included patients; onset of symptoms (*i.e.*, facial anomalies, abdominal volume increase, joint stiffness, growth deficits) were confirmed to have presented from three to six months of age. Frequent findings were joint restraint, claw hands, macrocephaly, short stature, and weight deficit across all types of MPS. Short stature was present in 67% (18/27) of patients: Hurler (1/3), Hurler-Scheie (3/5), Scheie (5/5), MPS II (3/8), and MPS VI (6/6). All MPS patients presented with dysmorphic facial features typical of MPS, with infiltrated face and coarse facies being the most discrete findings in Scheie patients.

In terms of diagnostic findings, initial quantitative GAG was noted to be elevated 2 to 13.3 times the age-specific reference values. As Hunter for GAG chromatography, dermatan sulfate and heparan sulfate were predominant in MPS I and II patients, and dermatan sulfate and chondroitin sulfate in MPS VI (Table 1).

**Table 1. Enzymatic activity according to their respective enzymes of patients with MPS**

MPS	Patients	Enzyme activity
MPS I – Hurler <sup>‡</sup>	1	0,073 (32 a 56)
	2	0,06 (32 a 56)
	3*	0,0 (32 a 56)
MPS I – Hurler-Scheie <sup>‡</sup>	4	0,07 (32 a 56)
	5	0,0 (32 a 56)
	6**	0,06 (32 a 56)
	7	0,1 (32 a 56)
MPS I – Scheie <sup>‡</sup>	8	0,1 (32 a 56)
	9	0,5 (32 a 52)
	10	0,09 (32 a 52)
	11	0,0 (32 a 52)
	12	0,0 (32 a 52)
	13	0,0 (32 a 52)
MPS II – Hunter <sup>§</sup>	14	6 (110-370)
	15	1,1 (31-110)
	16	0,73 (31-110)
	17	0,0 (31-110)
	18	0,35 (122-463)
	19	0,4 (110-370)
	20	0,0 (30-53)
MPS VI – Marateaux Lamy <sup>¶</sup>	21	0,4 (122-463)
	22	4 (72-176)
	23	12 (72-176)
	24	8 (72-176)
	25	0,0 (5,3-21,8)
	26	6 (72-176)
	27	1,39 (5,3-21,8)

<sup>‡</sup>α-L-iduronidase; <sup>§</sup>Iduronate-2-sulfatase; <sup>¶</sup>N-acetylgalactosamine 4-sulfatase; \*Reference value in serum (4,7 a 18,1); \*\*Reference value in leucocytes (32 a 56).

### 3.1. Mucopolysaccharidosis type I

#### 3.1.1. Clinical findings

Thirteen patients ( $n = 4$  female,  $n = 9$  male) belonging to 11 families with MPS I subtypes ( $n = 3$  Hurler,  $n = 5$  Hurler-Scheie,  $n = 5$  Scheie). Consanguinity was observed in 3/11 (27%) families. One family presented two sisters with MPS VI, and another presented two siblings and one cousin with MPS I (Table 2).

The onset of symptoms for Hurler patients varied from 6 to 8 months of age, with a mean of 7 months; for Hurler-Scheie and Scheie, patients ranged from 1 year to 3 years (mean 2 years), and from 5 years to 8 years (mean 6 years 9 months), respectively.

The first symptoms for Hurler patients were spinal deformity, ocular alteration (*i.e.* glaucoma) and infiltrated face, while for Hurler-Scheie, patients presented with increased abdominal volume, infiltrated face and macrocephaly. Joint stiffness and short stature were the only symptoms observed in Scheie patients.

#### 3.1.2. Diagnostic findings

The enzymatic activity of  $\alpha$ -L-iduronidase in leukocytes and/or plasma was undetectable in 1/3, 1/5, and 3/5 of patients with Hurler, Hurler-Scheie, and Scheie,

respectively (Table 1).

### 3.2. Mucopolysaccharidosis type II

#### 3.2.1. Clinical findings

Eight male patients with MPS II, belonging to 8 families, were included. The first symptoms noted were neuropsychomotor development delay, joint restriction and infiltrated face, which appeared between 2 and 5 years of age (mean: 3 years 6 months). Age at diagnosis was 2 to 10 years (mean: 5 years) (Table 3).

#### 3.2.2. Diagnosis findings

The enzymatic activity of  $\alpha$ -L-iduronidase in leukocytes and/or plasma was undetectable in 2/8 patients (Table 1).

### 3.3. Mucopolysaccharidosis type VI

#### 3.3.1. Clinical findings

Six patients ( $n = 4$  female,  $n = 2$  male) with MPS VI, belonging to five families, were included. The first symptoms to present were infiltrated face, spinal deformity and macrocephaly. The onset of symptoms varied from 3 months to 3 years of age (mean 1 year).

**Table 2. Characteristics of mucopolysaccharidosis type I**

Patients	Sex	Cons	Syndromes	Age of Symptoms	Age of Diagnosis	Follow-up	Current age at the time of this publication
1	M	+	Hurler	8m	2y	Death at 5 y and 6 m)	-
2	F	-	Hurler	6m	1y	BMT performed, ongoing	4y 10m
3	F	-	Hurler	8m	1y 8m	Ongoing	6y 6m
4	M	-	Hurler-Scheie	1y	1y 5m	Ongoing	13y 5m
5	M	+	Hurler-Scheie	3y	4y	Death at 13y 10m	-
6	F	-	Hurler-Scheie	2y 6m	7y 9m	Death at 18y 5m	-
7	M	-	Hurler-Scheie	3y	6y	Ongoing	20y 5m
8	M	-	Hurler-Scheie	2y	4y 1m	Ongoing	18y 10m
9	F	-	Scheie	7y	17y	Ongoing	42y 8m
10	M	-	Scheie	5y	24a	Ongoing	41y 6m
11*	M	+	Scheie	6y	12y	Ongoing	40y 2m
12*	M	+	Scheie	8y	7y	Ongoing	35y 4m
13*	M	+	Scheie	8y	8y	Ongoing	37y

\*Same family; Cons: Consanguinity; +: Yes; -: No; m: months; y.: years

**Table 3. Characteristics of mucopolysaccharidosis type II (Hunter)**

Patients	Sex	Cons	Age of Symptoms	Age of Diagnosis	Follow-up	Current age at the time of this publication
14	M	N	2y	4y 6m	Ongoing	23y 9m
15	M	N	2y 6m	3y 3m	Ongoing	7y 10m
16	M	N	4y	6y	Ongoing	15y 5m
17	M	N	5y	5y	Ongoing	18y
18	M	N	2y 6m	5y	Death at 13y 9m	-
19	M	N	3y	10y	Death at 23y	-
20	M	N	2y	5y 6m	Ongoing	10y
21	M	N	2y	2y 6m	Ongoing	11y

M: male; N: no; Cons: Consanguinity; y: years; m: month.

**Table 4. Characteristics of mucopolysaccharidosis type VI (Marateaux Lamy)**

Patients	Sex	Cons	Age of Symptoms	Age of Diagnosis	Follow-up	Current age at the time of this publication
22	F	+	3m	8y	Ongoing	18y
23	F	+	7m	8m	Ongoing	10y 8m
24	M	+	6m	3y 8m	Death at 13y 5m	-
25	F	-	3y	10y	Ongoing	16y 8m
26	M	+	1y	3y	Ongoing	11y 8m
27	F	-	1y	5y	Ongoing	10y 5m

\*Same family; M: Male; F: female; Cons: Consanguinity; y: years; m: months.

Age at diagnosis ranged from 8 months to 10 years (mean 5 years) (Table 4).

3.3.2. *Diagnosis findings*

The enzymatic activity of  $\alpha$ -L-iduronidase in leukocytes and/or plasma was undetectable in only 1 patient (Table 1).

3.4. *Enzyme replacement therapy*

3.4.1. *Initiation of ERT*

The age at onset of ERT ranged from 1 to 31 years (mean 14 years). Duration between time of diagnosis and initiation of ERT ranged from 2 months to 9 years, and was primarily associated with difficulty obtaining the recombinant enzyme. Duration of the infusion ranged from 40 weeks to 556 weeks (mean 259 weeks).

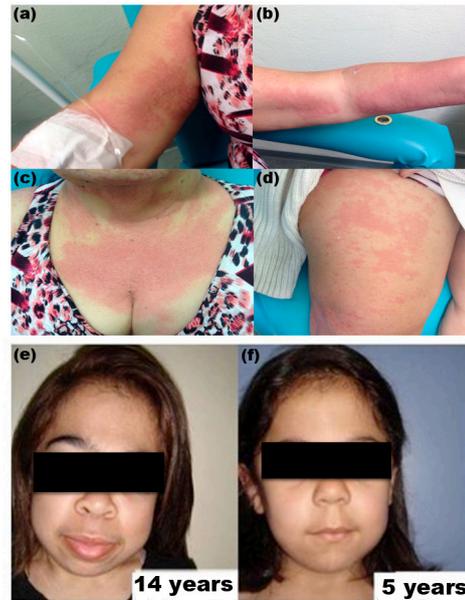
3.4.2. *Non-compliance with ERT*

The proportion of patients lacking infusions ranged from 0% to 32% (mean 17.8%), and were primarily due to the following reasons: lack of enzyme (MPS I, 40%; MPS II, 47%; MPS VI, 29%), holidays (MPS I, 26%; MPS II, 20%; MPS VI 29%), situational reasons such as family illness and public transport strikes (MPS I, 18%; MPS II, 19%; MPS VI 25%), and illnesses (e.g. upper respiratory infections, hospitalizations, surgical procedures) (MPS I, 16%; MPS II, 14%; MPS VI, 17%).

3.4.3. *Adverse reactions to ERT*

Adverse reactions to infusions were observed in 55% (n = 15) of patients, mostly occurring during the first few weeks of treatment. Common adverse reactions included skin rash (Figure 1a-1d), subarachnoid hemorrhage, fever and bronchospasm; symptoms were generally responsive to antihistamines, antipyretics and reduction of infusion speed.

Serious adverse reactions were observed in only 2 (7.4%) patients. One patient with MPS VI, aged 4 years 5 months, presented with shortness of breath, facial flushing, tachypnea, tachycardia and respiratory



**Figure 1. (a-d) Patient with allergic skin reaction during ERT; (e-f) Facial appearance from two sisters with MPS VI.**

failure requiring hospitalization and intensive care unit admission for 30 days under mechanical ventilation, during week 48 of ERT. ERT was suspended and pulmonary hypertension was diagnosed. When ERT was subsequently re-introduced, the patient presented with milder reactions responsive to antihistamines and antipyretics, in which these symptoms lasted 8 months until its resolution. The second patient was a MPS I Scheie patient, aged 29 years, who presented with arterial hypertension at the beginning of the ERT therapy (between weeks 1 and 2); her symptoms were responsive to antihypertensive medications. She subsequently presented with severe shortness of breath, tachycardia, urticarial, reddish and itchy plaques with all infusions from week 320 of her therapy, unresponsive to antihistamines and corticosteroids and requiring the initiation of a rapid desensitization protocol. The protocol consisted of reducing the infusion rate and administering drugs such as diphenhydramine, ranitidine, montelukaste, acetaminophen, methylprednisolone and benzodiazepines. Due to limited infrastructure, the patient was transferred to another private infusion center.

**Table 5. Surgical procedures before (B) and after (A) ERT**

Items	MPS I – Hurler		MPS I – Hurler- Scheie		MPS I – Scheie		MPS II – Hunter		MPS VI – Marateaux Lamy	
	B	A	B	A	B	A	B	A	B	A
Adenotonsillectomy	1/3	1/3	3/5	1/5	1/5	-	6/7	-	-	-
Hernia	1/3	1/3	2/5	1/5	4/5	-	5/7	-	2/6	-
Tympanostomy	-	-	1/5	1/5	-	-	1/7	-	-	-
Glaucoma	1/3	-	-	-	-	-	-	-	-	-
Carpal tunnel	-	-	1/5	-	2/5	-	-	-	-	-
Aquiles tendon	-	-	1/5	-	-	-	-	1/7	-	-
Scoliosis	-	-	1/5	-	-	-	-	-	-	-
Peritoneal Ventricular Bypass	-	2/3	1/5	-	-	-	1/7	-	1/6	-
Cervical Mielopathy	-	-	1/5	-	-	-	-	-	1/6	-
Mitral valve replacement	-	-	-	-	-	-	-	-	1/6	-

#### 3.4.4. Clinical outcomes: Dysmorphic features

Most patients continued to present with infiltrated facies even with ERT. However, we observed a less pronounced facial appearance in one of the two sisters who started ERT earlier (age 1 year 5 months) when compared to her sister, who started ERT at age 8 years 9 months (Figure 1e-1f).

#### 3.4.5. Clinical outcomes: Cardiovascular and respiratory changes

Before ERT, 24/26 (92%) patients presented with echocardiographic alterations, primarily involving thickening and/or insufficiency of the mitral or aortic valve (21/24, 88%) and right and/or left ventricular hypertrophy (14/24, 58%). A Scheie patient presenting with ventricular dysfunction with mild reduction of left ventricular contractile function and mitral and aortic valve alterations showed no improvement with ERT. Two patients ( $n = 1$  Hurler,  $n = 1$  Hurler-Scheie) did not present echocardiographic alterations before ERT, but developed valve alterations and ventricular hypertrophy during therapy.

Pulmonary hypertension was observed before ERT in three patients ( $n = 2$  Hurler-Scheie,  $n = 1$  MPS VI). During ERT, three patients presented with pulmonary hypertension, two of whom died ( $n = 1$  Hurler Scheie,  $n = 1$  MPS VI). Among the 6 patients who presented with pulmonary hypertension across the study, four evolved with ventricular dysfunction ( $n = 1$  Hurler,  $n = 1$  Hurler-Scheie,  $n = 1$  Scheie,  $n = 1$  MPS VI), while two did not develop echocardiographically-diagnosed cardiac abnormalities.

There was a difference in the progression of cardiac lesions in two sisters with MPS VI. The older sister began ERT at 8 years 10 months of age and required surgical repair of the mitral valve three months prior to ERT initiation (8 years 7 months). The younger sister who initiated ERT at 1 year 7 months of age showed a partial reversal of ventricular hypertrophy after 3 years

of therapy, thereby not warranting cardiac surgery.

#### 3.4.6. Clinical outcomes: surgical procedures

Twenty-two patients (81%) from a total of 27 underwent a total of 63 surgical procedures (mean 2.9 surgeries per patient). Inguinal and/or umbilical herniorrhaphy accounted for 50% of the interventions.

Mitral valve replacement with metal prosthesis, patent ductus arteriosus repair and aortic valve repair were conducted in one MPS VI patient at 8 years 7 months of age, prior to ERT. Two Scheie patients underwent carpal tunnel corrective surgery prior to ERT at 22 and 25 years of age. Eight patients (30%) also underwent adenotonsillectomies prior to ERT (Table 5).

One patient with Hurler-Scheie underwent surgical correction of her lumbar kyphoscoliosis at 4 years of age prior to ERT initiation. However, her condition evolved from age 10 years onwards despite ERT therapy, eventually leading to her death at 18 years of age due to pneumonia, pulmonary hypertension, and sepsis (Table 5).

During ERT, one Hurler-Scheie patient underwent bilateral surgical carpal tunnel repair at age 8 years 6 months after 150 weeks of ERT. Two patients ( $n = 1$  Hurler;  $n = 1$  Hurler-Scheie) underwent adenotonsillectomies, and another Hurler-Scheie patient underwent adenotonsillectomy, three tympanotomies, two shunt ventricular peritoneal and surgery for cervical myelopathy and herniorrhaphy. One Hurler-Scheie patient underwent two orthopedic surgeries (Table 5).

#### 3.4.7. Clinical outcomes: mortality

No significant difference in mortality rates was identified across MPS types: MPS I versus MPS II (RR: 0.92, 95% CI: 0.19-4.38;  $p = 0.92$ ); MPS I versus MPS VI (RR: 1.38, 95% CI: 0.18-10.71;  $p = 0.76$ ); and MPS II versus MPS VI (RR: 1.50, 95% CI: 0.17-12.94;  $p = 0.71$ ) (Figure 2, Table S1, <http://www.irdrjournal.com/action/getSupplementalData.php?ID=12>).

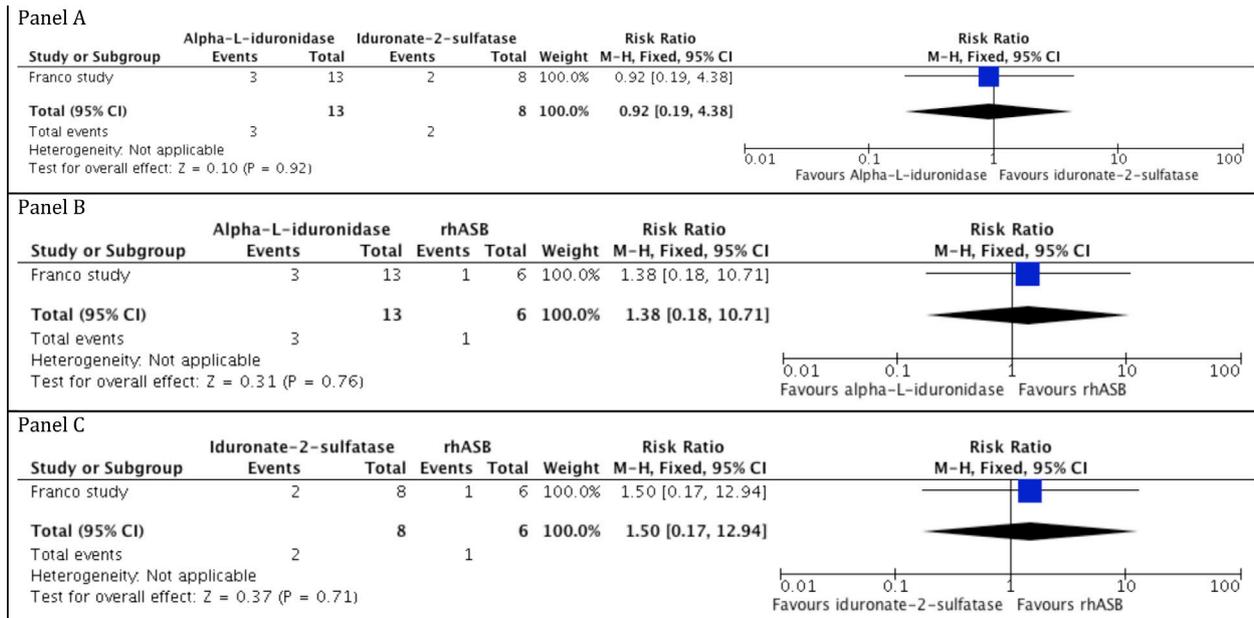


Figure 2. Death rates among studied groups with the use of ERT.

4. Discussion

Our findings regarding age of onset and primary symptoms associated with MPS I, II and VI are consistent with the literature, (1,6-8) except for one MPS I H patient, who presented with glaucoma at 8 months of age. There are few reports of glaucoma with such an early onset (9).

Mean age at time of diagnosis of MPS I in our case series was approximately 7 years of age. In contrast, a multicenter study involving 891 patients with MPS I from 24 countries reported a mean age at time of diagnosis was approximately 5 years (10). Similarly, mean age at time of diagnosis of MPS II in our case series was approximately 5 years of age, while the Hunter Outcome Survey, involving 16 countries with a total of 263 patients, reported a mean age at diagnosis of 3.5 years (11). Mean age at diagnosis for MPS VI patients in our case series was 5 years; the literature generally reports ages of diagnosis ranging from 2 years to 12 years (1,6,12). The discrepancy between age of diagnosis in our study and the literature may be explained by the care of a large number of patients being limited to few specialized in Brazil, delaying time of detection.

In terms of clinical presentations, 6 of 8 MPS I patients (n = 3 Hurler; n = 3 Hurler-Scheie) and 4 of 8 MPS II patients presented with intellectual disability; in contrast, no patients with MPS VI presented with such disabilities. These findings are supported by previous reports in the literature (1,6,13).

The main presenting features such as facial appearance (Figure 1e-1f), joint restriction, and short stature for patients with MPS VI in our case series were similar to those presented in the literature (5,12,14,15).

Current methods for dosing enzyme activity are

not sensitive enough to measure the complete absence or presence of residual enzymatic activity (16). Thus, it is difficult to correlate the undetectable activity of the enzyme to severe phenotypes. In terms of GAG chromatography, dermatan sulfate and heparan sulfate were predominant in MPS I and II patients and dermatan sulfate and chondroitin sulfate in MPS VI.

Regarding the adverse effects, we found that 55% of patients experienced at least one mild adverse effect, most frequently erythema, urticaria (as noted in Figure 1a-1d), and redness/flushing. Such events have been reported in 36% of studied populations as per previous reports in the literature (5,17).

One adult female MPS I Scheie patient in our case series specifically presented with a severe infusion reaction after five years of ERT. She presented with shortness of breath, tachycardia, urticaria, and reddish and itchy plaques in all subsequent infusions. As there was no improvement with standard medication (antihistamines, and corticosteroids), a rapid desensitization adapted protocol was performed, in line with previous reports. The protocol consisted of a reduction of infusion speed (lasting up to 10 hours using three saline bags with different laronidase dilutions) and administration of diphenhydramine, ranitidine, montelukaste, acetaminophen, methylprednisolone and benzodiazepines with infusions, to which the patient was responsive.

Our findings support a strategy that involves the following steps in addition to pre-medication for symptom relief in response to an infusion reaction: i) temporary decrease of the infusion; ii) additional administration of antipyretics and antihistamines; and iii) discontinuation of the infusion and initiation of appropriate supportive measures immediately if a severe hypersensitivity reaction (14).

The most serious adverse reactions observed in our study involved anaphylaxis and impaired respiratory function, warranting further monitoring; these have been previously evidenced in the literature as life-threatening as well (18,19).

Regarding genetic mutations (Table S2, <http://www.irdrjournal.com/action/getSupplementalData.php?ID=12>), our data aligns with the literature for MPS I, showing the most frequent mutations are W402X and P533R (20) and presence of both alleles is linked to a severe phenotype of the disease. Genotypic determination may be useful in providing individual parameters of the patient's therapeutic response to therapies such as ERT and BMT as well. While the literature shows either large or small deletions being associated with MPS II, our study did not identify any of these findings; contrarily, we noticed only punctual deletions (16,21). In terms of MPS VI, the IVS-8T>G intronic mutation was found in two sisters with the condition, both of whom presented with a more serious phenotype of the disease including medullary compression, short stature and need for cardiac surgery. A multicenter study involving 105 patients of different nationalities observed the IVS-8T>G mutation only in Brazilian patients (22).

In 26 MPS patients (I, II, III, IV, VI and VII), Leal *et al.* (23), observed changes in the mitral valve (60.3%); Of the aortic valve (35.8%); ventricular hypertrophy (43%); and pulmonary hypertension (36%).

High mortality rates were found in our patients across all three types of MPS and regardless of ERT use. A study with 24 MPS I patients (24), also reported deaths in 7 patients during ERT. The main causes of death in our case series were respiratory problems such as pneumonia, complications of anesthesia, and pulmonary hypertension, in line with previous reports (23,24).

## 5. Conclusion

Our case series demonstrated that clinical heterogeneity exists across MPS-affected patients by inter- and intrafamilial variability in terms of phenotype. Our findings suggested that while ERT was well tolerated and able to attenuate symptoms effectively, it did not seem to prevent disease progression and reduce mortality. This study emphasizes that early diagnosis and use of ERT are critical for better outcomes and for enhancing the quality of life of these patients, despite mortality and morbidity remaining high. Further well-designed robust studies with larger sample sizes and longer follow-ups are warranted to further examine the effects of ERT in these patient populations in terms of symptom and disease progression control.

## Acknowledgements

José Francisco da Silva Franco received grants from Biomarin for lectures as speaker. Regina El Dib received

a Brazilian Research Council (CNPq) scholarship (CNPq 310953/2015-4).

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(Received July 1, 2017; Revised August 4, 2017; Accepted August 11, 2017)

# Nationwide trends of hospitalizations for cystic fibrosis in the United States from 2003 to 2013

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## Summary

Cystic fibrosis (CF) is a multisystem autosomal recessive genetic disorder with significant advances in early diagnosis and treatment in the last decade. It is important to provide updated information regarding these changing demographics as they also reflect a considerable improvement in survival. We analyzed the National Inpatient Sample Database (NIS) in the United States for all patients in which CF was the primary discharge diagnosis (ICD-9: 277.0-277.09) from 2003 to 2013 to evaluate the rate of hospitalizations and determine the cost and mortality associated with CF along with other epidemiological findings. The statistical significance of the difference in the number of hospital discharges, lengths of stays and associated hospital costs over the study period was calculated. In 2003, there were 8,328 hospital discharges with the principal discharge diagnosis of CF in the United States, which increased to 12,590 discharges in 2013 ( $p < 0.001$ ). The mean hospital charges increased by 57.64% from US\$ 60,051 in 2003 to US\$ 94,664 in 2013. The aggregate cost of hospital visits increased by 138.31% from US\$ 500,105,727 to US\$ 1,191,819,760. In the same time, the mortality decreased by 49.3%. The number of inpatient discharges related to CF has increased from 2003 to 2013. This is due to increased life expectancy of CF patients, resulting in increased disease prevalence. There has been a significant increase in the mean and aggregate cost associated with CF admissions. Over the last decade, many advances have been made in the diagnosis and treatment of CF, consequentially leading to a significant transformation in the epidemiology and demographics of this chronic disease. Rising hospital costs associated with the care of CF patients necessitates future studies analyzing the diagnostic modalities, algorithms and treatment practices of physician's treating CF patients.

**Keywords:** Cystic Fibrosis, epidemiology, length of stay, hospitalizations, mortality, healthcare burden

## 1. Introduction

Cystic fibrosis (CF) is a multisystem genetic disorder that was first formally described in 1938 by Dr. Dorothy Andersen (1). It arises from genetic defects in a single gene on chromosome 7, which encodes the cystic fibrosis transmembrane conductance regulator (CFTR),

a chloride channel that is widely distributed in epithelial surfaces (2). Defects in CFTR function can lead to recurrent lung infections, sinus disease, pancreatic insufficiency, intestinal obstruction, male infertility and liver diseases in some individuals. Recurrent lung infections can lead to frequent pulmonary exacerbations often requiring inpatient care (3).

Around 30,000 individuals with CF currently live in the United States and approximately 70,000 reside worldwide (4). Even though a cure for CF has yet to be developed, patients have benefited from a variety of treatments to improve their symptoms. Over the

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last two decades, many advances have been made in the diagnosis and treatment of CF. Implementation of universal screening in 2010 has led to earlier diagnosis of newborn infants (5). In the United States Cystic Fibrosis Foundation Patient Registry, the number of newly diagnosed patients detected by newborn screening increased from 8% in 2000 to 72% in 2013 (6). This trend towards early diagnosis, specialized CF center care, strategies to avoid cross infection along with the advent of new drugs have contributed to considerable improvement in survival (7). The median predicted survival of patients with CF in the US had improved to 40.7 years by 2013 (6).

The epidemiology of CF has undergone a significant transformation. Information regarding medical care expenditures for CF is needed to evaluate the cost-effectiveness of new medical technology, therapies, and newborn screening techniques for CF. The aim of this study was to assess recent changes in the frequency and cost of inpatient admissions for a principal diagnosis of CF patients in the US from 2003 to 2013. We also sought to examine shifting demographics, in-hospital mortality, and length of hospitalization related to CF in these patients.

## 2. Materials and Methods

The National Inpatient Sample (NIS) database was used to obtain a population-based estimate of national trends for CF. The NIS database is a tool developed as a part of the Healthcare Cost and Utilization Project (HCUP), and is sponsored by the Agency for Healthcare Research and Quality. The NIS database is the largest publicly available all-payer inpatient care database in the United States. It is designed to approximate a 20% sample of United States community (nonfederal) hospitals, and is organized according to geographic region, hospital ownership, location, teaching status, and number of beds, among other characteristics. The 2003 NIS database contains a total of approximately 8 million records. The 2013 NIS database contains a total of about 7.1 million records drawn from 44 states and includes information from 4363 hospitals. A comprehensive synopsis on NIS data is available at <http://www.hcup-us.ahrq.gov>. The immense size of the NIS database provides an exceptional sample representation of the general US population. In order to identify cases of CF, the NIS database was queried for hospital data on all discharges with ICD-9-CM primary diagnosis codes of 277.00-277.09 (CF and manifestations associated with CF) from 2003 to 2013. The NIS database provides administrative data for analysis and does not include patient-specific clinical data.

### 2.1. Variables recorded

Patient demographics included age and gender. Hospital

characteristics included the location (metropolitan vs. non-metropolitan), bed size (small, medium, and large) and region (Northeast, Midwest, South and West). Per HCUP net definitions, metropolitan areas are those with a population of at least 50,000 people. Areas with less than 50,000 people are non-metropolitan areas. Hospital bed size varies depending on the hospital's location and teaching status. Small hospitals range from 1 to 299 beds, medium hospitals range from 50 to 499 beds, and large hospitals range from 100 to 500 or more beds. The payer status for all admissions was also considered and divided into categories of Medicare, Medicaid, private insurance, uninsured, and other. "Hospital Charges" are defined as the amount the hospital charged for the patient's entire hospital stay, not including professional (physician) fees. NIS defines "aggregate charges" or the "national bill" as the sum of all charges for all hospital stays in the United States. "Length of stay" is the number of nights the patient remained in the hospital per stay.

### 2.2. Statistical methods

The trends for average length of stay, mean total charges, and total number of discharges specifically for the diagnosis of CF were plotted and analyzed from 2003 to 2013. The frequency of discharges with CF was calculated by dividing the annual number of discharges with a primary discharge diagnosis of CF by the total number of all discharges listed in the NIS for each year.

The temporal trend in the frequency of discharges, length of stay and mortality was assessed by linear and polynomial regression. The most appropriate functional form for the trend was assessed by examination of regression diagnostic plots. Linear shape was determined for frequency of discharges and mortality.  $P$  value < 0.05 was considered statistically significant. All analyses were performed using SAS (version 9.4, The SAS Institute, Cary, NC).

In addition, the frequency per 10,000 admissions was also calculated for each variable. The numbers represent the density of patients admitted and discharged with the primary diagnosis of CF compared with the total number of hospital discharges per category in that year. Each frequency was calculated by dividing the number of patients discharged with primary diagnosis of CF by the total discharges in the specific categorical variable for the same year and multiplying that number by 10,000. We viewed the counts as arising from a Poisson distribution, yielding Poisson rates that were compared over time using Poisson regression and yielded relative rates (RRs) and 95% confidence intervals (95% CI) that expressed the ratio of rate per 10,000 in 2013 to that of 2003.

## 3. Results

### 3.1. Number and cost of cystic fibrosis discharges

From 2003 to 2013, the total number of hospital discharges with the principal diagnosis of CF increased by 51.2% from 8,328 to 12,590 ( $p < 0.001$ ) (Table 1). The majority of these patients were admitted due to a pulmonary exacerbation (approximately 72% in 2003 vs. 89% in 2013). The number of patients admitted by specific CF related ICD 9 codes are listed in Table 2. The frequency of hospital discharges from CF as the principal diagnosis increased from 2.24 per 10,000 discharges to 3.53 per 10,000 discharges (RR = 1.57, 95% CI: 1.53-1.62;  $p < 0.001$ ). The linear trend of CF discharges also showed a statistically significant increase from 2003 to 2013 ( $R^2 = 0.19666$ ;  $p < 0.01$ ) (Figure 1).

The average length of stay of a CF patient showed a marginal increase from 10.1 days in 2003 to 10.3 days in 2013 (Figure 2). The mean hospital charges per patient increased 57.64% from US\$ 60,051 in 2003 to US\$ 94,664 in 2013 (Adjusted for inflation). The aggregate cost of hospital visits of patients with the principal diagnosis of CF increased 138.31% from US\$ 500,105,727 in 2003 to US\$ 1,191,819,760 in 2013.

The percent mortality of patients admitted with a principal diagnosis of CF decreased from 1.44% in 2003 to 0.71% in 2013 with a statistically significant decrease in the linear trend ( $R^2 = 0.59914$ ;  $p < 0.01$ ) (Figure 3).

### 3.2. Patient characteristic by age

The highest number of patient discharges in both 2003 and 2013 was noted in the 18-44 age group (Table 1). The frequency of discharges was noted to be higher in the 1-17 age group with the rates being 19.3 per 10,000 in 2003 and 32.8 per 10,000 in 2013, respectively (RR = 1.69, 95% CI: 1.62-1.77;  $p < 0.001$ ). The increase in the frequency of discharge rates was most remarkable in age groups 1-17, 18-44, and 45-64. A drop in the rate of discharges was noted in the age group  $< 1$  (RR = 0.81, 95% CI: 0.70-0.95;  $p < 0.05$ ).

### 3.3. Patient characteristics by sex

The absolute number of CF admissions and discharges was noted to be greatest in females both in 2003 and 2013, with the frequency increasing from 2.03 per 10,000 discharges in 2003 to 3.36 per 10,000 discharges in 2013. (RR = 1.65, 95% CI: 1.59-1.72;  $p < 0.001$ ). The increase for males went from 2.5 per 10,000 discharges in 2003 to 3.77 per 10,000 discharges in 2013 (RR = 1.51, 95% CI: 1.45-1.58;  $p < 0.001$ ).

### 3.4. Patient characteristics by payer group

The relative frequency of CF discharges increased for all types of payer groups over this 10-year period. The highest absolute number of CF discharges was

in the Medicaid group in both 2003 and 2013, which increased from 3.54 per 10,000 discharges in 2003 to 5.54 per 10,000 discharges in 2013 (RR = 1.56, 95% CI: 1.49-1.64;  $p < 0.001$ ). While the rate of discharge in patients with private insurance was overall second, a higher increase was seen in this group (84.75%) as compared to patients with Medicaid (56.5%). While there was also an increase in discharges in the Medicare group (RR = 1.66, 95% CI: 1.54-1.80;  $p < 0.001$ ), a drop in the number of discharges was noted in the Uninsured/Other group (RR = 0.05, 95% CI: 0.78-0.94;  $p = 0.001$ ).

### 3.5. Cystic Fibrosis discharges by hospital location, hospital characteristics and region

Southern United States had the highest number of both CF discharges in 2003 as well as 2013. On the contrary, the West had the highest frequency of CF discharges in 2003 with 2.6 per 10,000 discharges and 2013 with 4.0 per 10,000 discharges. The Northeast carried the second highest frequency of discharges (2.4 per 10,000 discharges) in 2003. The frequency of CF discharges doubled in the Midwest from 2003 (1.9 per 10,000 discharges) to 2013 (3.9 per 10,000 discharges) (RR = 1.98, 95% CI: 1.87-2.10;  $p < 0.001$ ). The frequency of discharges in the Northeast (RR = 1.38, 95% CI: 1.36-1.47;  $p < 0.001$ ), South (RR = 1.49, 95% CI: 1.42-1.56,  $p < 0.001$ ) and West (RR = 1.52, 95% CI: 1.43-1.61;  $p < 0.001$ ) also showed a statistically significant increase in 2013 compared to 2003.

In 2003, CF patients were more likely to be admitted to a hospital with a small number of beds (3.05 per 10,000 discharges), whereas by 2013, they were more likely to be admitted to a hospital with a large number of beds (4.1 per 10,000 discharges). It was also noted that in both 2003 and 2013, greater than 95% of CF patients were admitted to a hospital located in a metropolitan area rather than a non-metropolitan area.

## 4. Discussion

The primary finding of this manuscript is that in a large multi-institutional observational cohort within the United States, there was a consistent increase in CF admissions from 2003 to 2013. The majority of these admissions were due to pulmonary exacerbations. There has been an increase in life expectancy of CF patients over the last 2 decades, which has led to an increase in disease prevalence (8). An increased prevalence leads to an increased number of patients with pulmonary or non-pulmonary exacerbations of CF requiring hospitalizations. According to the CF registry, there were 28,103 patients in the registry in 2013 compared to approximately 15,000 in 1986. In today's era, the number of adults living with CF continues to increase, while the number of children has

**Table 1. Trends in characteristics of CF related hospitalizations from 2003 to 2013**

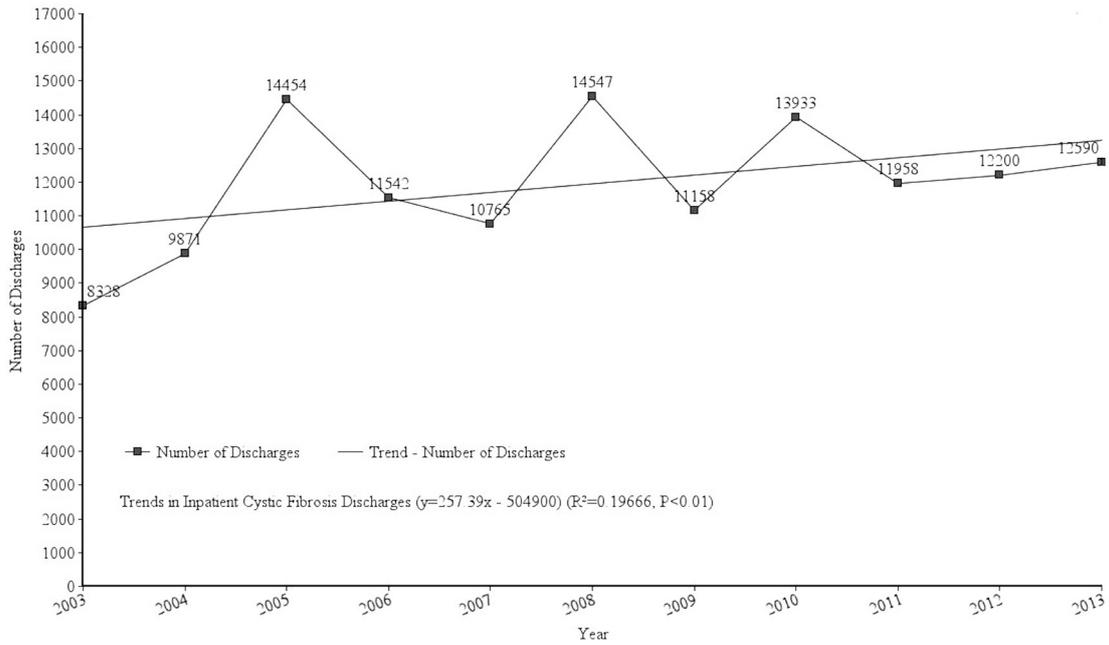
Category	Categorical Variable	2003 Cystic Fibrosis (N, ) C	2013 Cystic Fibrosis (N, ) A	2003 Total (N, ) D	2013 Total (N, ) B	Cystic Fibrosis per 10,000 admissions in 2003	Cystic Fibrosis per 10,000 admissions in 2013	RR (95% CI)	P value
All discharges		8,328 (100.00)	12,590 (100.00)	37,074,605 (100)	35,597,792 (100)	2.24	3.53	1.57 (1.53-1.62)	<0.001
Age group (years)	< 1	379 (4.55)	285 (2.26)	4,581,417 (12.36)	4,232,808 (11.9)	0.82	0.67	0.81 (0.70-0.95)	0.008
	1-17	3,415 (41.01)	4,570 (36.30)	1,762,383 (4.75)	1,393,028 (3.9)	19.3	32.8	1.69 (1.62-1.77)	<0.001
	18-44	4,261 (51.17)	7,165 (56.91)	9,772,014 (26.36)	8,727,809 (24.5)	4.36	8.2	1.88 (1.81-1.95)	<0.001
	45-64	207 (2.48)	520 (4.13)	8,086,876 (21.81)	8,753,270 (24.6)	0.25	0.6	2.32 (1.98-2.73)	<0.001
	65-84	*	*	10,150,753 (27.38)	9,581,434 (26.9)	*	*		
Sex	Male	3,757 (45.11)	5,720 (45.43)	15,064,915 (40.63)	15,154,195 (42.6)	2.5	3.77	1.51 (1.45-1.58)	<0.001
	Female	4,442 (53.34)	6,870 (54.57)	21,861,583 (58.97)	20,436,357 (57.4)	2.03	3.36	1.65 (1.59-1.72)	<0.001
Payer	Medicare	1,015 (12.18)	1,715 (13.62)	13,761,829 (37.12)	13,986,550 (39.4)	0.95	1.22	1.66 (1.54-1.80)	<0.001
	Medicaid	2,420 (29.06)	4,110 (32.64)	6,828,282 (18.42)	7,417,129 (20.9)	3.54	5.54	1.56 (1.49-1.64)	<0.001
	Private Insurance	4,008 (48.12)	5,915 (46.98)	13,555,962 (36.56)	10,851,650 (30.5)	2.95	5.45	1.84 (1.77-1.92)	<0.001
	Uninsured/Other	865 (10.4)	850 (6.75)	2854601 (7.7)	3,287,333 (9.2)	3.03	2.5	0.85 (0.78-0.94)	0.001
Median income for zip code	Low (\$0-\$5,999)	2,124 (25.50)	2,870 (22.80)	10,061,048 (27.14)	10,199,933 (28.65)	2.1	2.8	1.33 (1.26-1.41)	<0.001
	Not low (\$36,000+)	6,029 (72.39)	9,560 (75.93)	26,173,832 (70.60)	24,599,165 (69.10)	2.3	3.8	1.69 (1.63-1.74)	<0.001
Owner	Government	1,137 (13.65)	2,660 (21.13)	5,172,217 (13.95)	4,291,755 (12.06)	2.2	6.2	2.82 (2.63-3.02)	<0.001
	Private, not-for-profit	6,600 (79.25)	9,640 (76.57)	26,964,496 (72.73)	26,111,822 (73.35)	2.4	3.7	1.51 (1.46-1.56)	<0.001
	Private, for-profit	592 (7.11)	290 (2.30)	4,937,891 (13.32)	5,194,215 (14.59)	1.2	0.55	0.47 (0.40-0.54)	<0.001
Location	Non-metropolitan	387 (4.65)	155 (1.23)	5,583,485 (15.06)	3,954,149 (11.1)	0.7	0.4	0.57 (0.47-0.68)	<0.001
	Metropolitan	7,941 (95.35)	12,435 (98.7)	31,471,911 (84.89)	31,643,643 (88.9)	2.5	3.9	1.56 (1.51-1.60)	<0.001
Bed Size	Small	1,321 (15.87)	985 (7.82)	4,327,304 (11.67)	4,884,892 (13.7)	3.05	2.0	0.66 (0.61-0.72)	<0.001
	Medium	1,907 (22.90)	2,780 (22.08)	9,613,451 (25.93)	9,512,936 (26.7)	1.98	2.9	1.47 (1.39-1.56)	<0.001
	Large	5,099 (61.23)	8,825 (70.10)	23,114,641 (62.35)	21,199,964 (59.6)	2.2	4.1	1.89 (1.82-1.95)	<0.001
Region	Northeast	1,765 (21.19)	2,265 (17.99)	7,264,150 (19.59)	6,730,965 (18.9)	2.4	3.3	1.38 (1.36-1.47)	<0.001
	Midwest	1,675 (20.11)	3,120 (24.78)	8,520,023 (22.98)	8,004,912 (22.5)	1.9	3.9	1.98 (1.87-2.10)	<0.001
	South	3,017 (36.22)	4,375 (34.75)	14,205,434 (38.32)	13,818,031 (38.8)	2.1	3.1	1.49 (1.42-1.56)	<0.001
	West	1,871 (22.47)	2,830 (22.48)	7,084,998 (19.11)	7,043,884 (19.8)	2.6	4.0	1.52 (1.43-1.61)	<0.001

CF, cystic fibrosis.

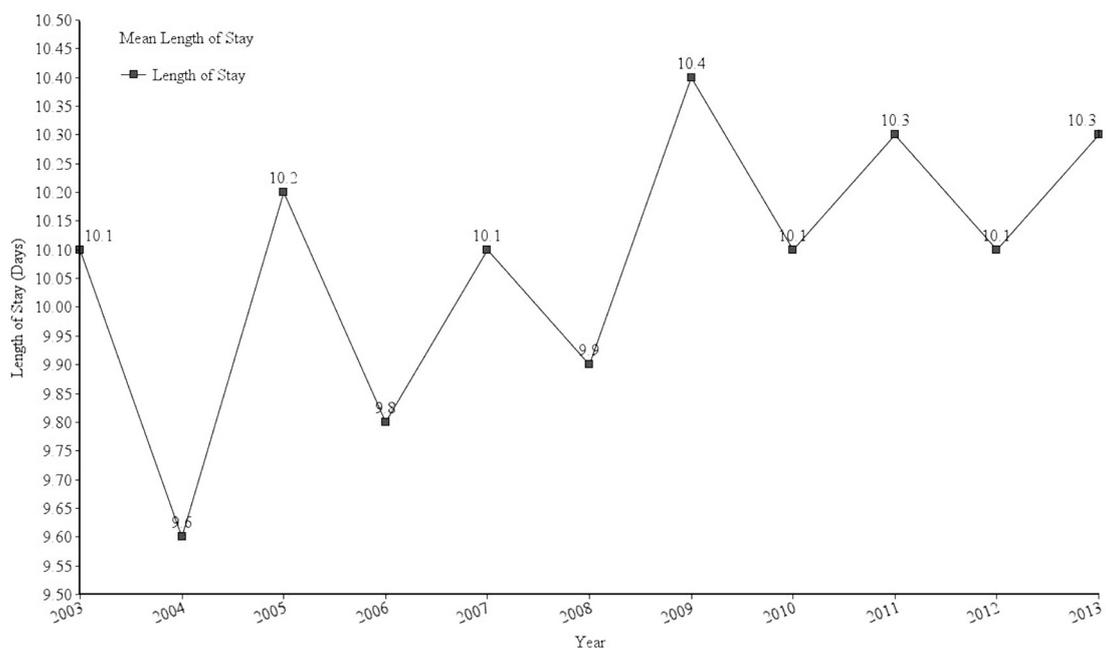
**Table 2. Admission by specific ICD- 9 codes for CF**

ICD – 9 Code	2003 (%)	2013 (%)
277.0 (CF, not otherwise specified)	1,820 (21.85)	710 (5.64)
277.01 (CF with Meconium Ileus)	40 (0.5)	80 (0.6)
277.02 (CF with Pulmonary Manifestations)	5,989 (71.91)	11,235 (89.24)
277.03 (CF with Gastrointestinal Manifestations)	335 (4.02)	380 (3.01)
277.09 (CF with Other Manifestations)	144 (1.73)	185 (1.47)

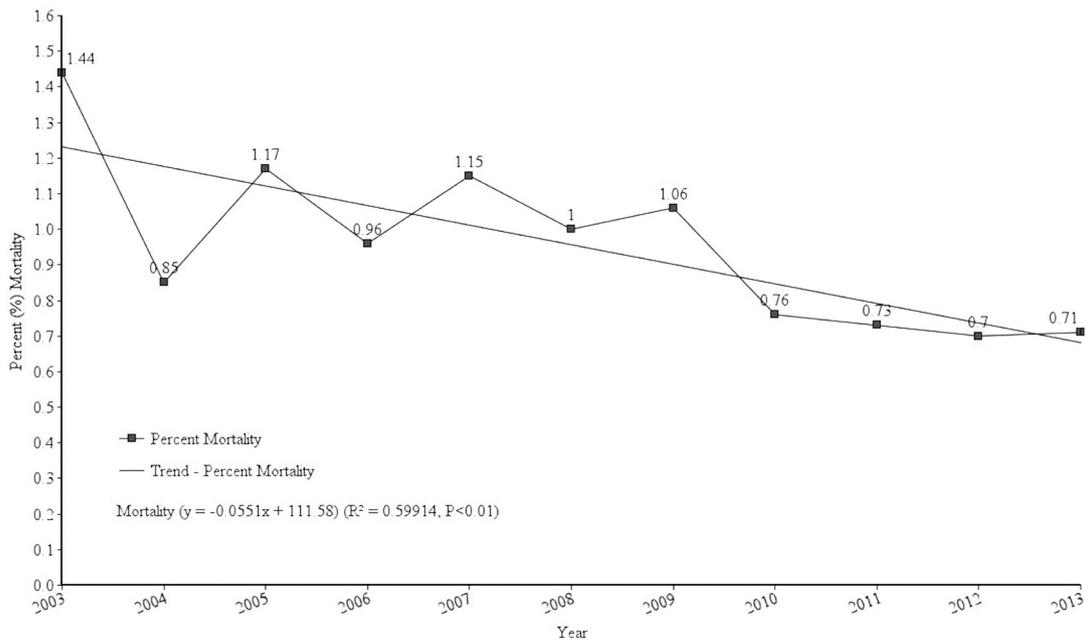
CF, cystic fibrosis.



**Figure 1. Trends of inpatient CF discharges.** CF, cystic fibrosis.



**Figure 2. Trends of mean length of stay of CF patients.** CF, cystic fibrosis.



**Figure 3. Trends in inpatient mortality of CF patients.** CF, cystic fibrosis.

remained relatively stable; adults comprise 51.6 percent of the CF population, compared with 29.2 percent in 1986 (4). Mortality from CF is primarily related to respiratory deterioration, however, newer therapies in chronic inhaled antibiotics, mucociliary clearance, and CFTR modulators are improving treatment for CF. These therapies have reduced the number of pulmonary exacerbations and have led to an improved FEV1 and quality of life. This has translated over the years to a longer life span in the CF patient. The median predicted survival of patients with CF has increased to 40.7 years in the year 2013, compared to only 16 years of age in 1970 (9). Unsurprisingly, as the CF population grows older, there remains a higher chance of recurrent CF exacerbations during one's lifetime, ultimately leading to increasing health-care needs, including hospitalization.

The average length of stay of a CF admission showed a marginal increase from 2003 to 2013. Despite this minimal change in length of stay in the hospital, the aggregate costs of hospital visits for a patient with CF increased by 138% to approximately 1.1 billion USD from 2003 to 2013. Mean hospital costs for each patient also increased by 57.64% (from \$ 60,051 per admission in 2003 to \$ 94,664 per admission in 2013; adjusted for inflation). In the same time, the mortality decreased by 49.3 %. This increase in cost and decrease in mortality is a result of substantial changes in CF care between 2003 and 2013.

Implementation of universal screening in 2010 has led to earlier diagnosis of newborn infants (5). Of the persons diagnosed in 2013, 72.4% were diagnosed by newborn screening compared with only 8.0% of those diagnosed in 2000 (10). Daily regimens in CF patients

include airway clearance therapy, inhaled mucolytic agents, antibiotics and a high calorie, high fat diet (11,12). Based on the CF registry, successive birth cohorts have shown improved pulmonary function. The majority of 18-year-olds now have normal lung function or mild obstruction, and an improved nutritional status with improved weight and height percentiles. Recommended therapies are more widely prescribed in 2013 compared to 2003. For example, the use of hypertonic saline has increased from 24.4 % in 2006 to 63.2 % in 2013 (10). Dornase alfa (also known as Pulmozyme®) was approved by the U.S. Food and Drug Administration (FDA) in 1993; its use increased from 67.6% of patients in 2003 to 85% of patients in 2013 (10). Inhaled aztreonam (also known as Cayston®) was FDA-approved in 2010 and its use increased from 2.5% of patients in 2008 to 41.5% of patients in 2013 (10). Over the past 4 years, the CF community has been introduced to the CFTR modulators, ivacaftor (Kalydeco®) and lumacaftor/ivacaftor (Orkambi®), which address the underlying cause of CF in certain mutations by improving chloride transport at the cellular level.

Improved patient outcomes are attributed to not only new treatment modalities, but also to the practice of the care center model recommended by the Cystic Fibrosis Foundation (CFF). There are more than 120 CF Centers nationwide, accredited by the CFF to deliver comprehensive multidisciplinary specialized care while adhering to clinical practice guidelines. For instance, due to widespread adoption of eradication strategies, prevalence of *P. aeruginosa* infection has decreased (10). Cystic fibrosis-related Diabetes (CFRD) is a highly prevalent complication of CF that increases

morbidity and mortality. Since the publication of the CF Foundation CFRD guidelines in 2010, rates of Oral Glucose Tolerance Test (OGTT) screening have also increased, allowing for closer monitoring and management (13). The CF care team is multidisciplinary and at minimum includes a physician, nurse, dietitian, respiratory therapist, and social worker. Today, the complexity of CF care continues to evolve as the CF patient grows older: pharmaceutical, mental health, cancer screening, and palliative care disciplines are recognized as important issues that must be addressed. Lung transplant remains an option for patients with advanced lung disease despite conventional therapies. The total number of patients receiving lung transplantation has increased from 152 in 2003 to 246 in 2013 (10). All of these care measures are contributing to decreased mortality while inevitably leading to significantly increased costs associated with CF-related hospitalization.

We also analyzed data on the basis of patient characteristics by age, sex and payer status. The frequency of discharges increased for age groups above 1. The highest frequency of hospital discharges was seen in ages 18-44 in both 2003 and 2013, likely because more complications requiring hospitalizations occur in CF patients as they age. Adult CF patients (> 18 years) have higher rates of exacerbations requiring IV antibiotic therapy (14). The median age of CF patients in 2013 was 17.9 years. Prior to initiation of universal newborn screening in 2010, most infants were diagnosed based on clinical symptoms. Following newborn screening implementation, asymptomatic, healthier infants are being diagnosed with CF earlier, which may explain the decreased rate of hospital admissions and discharges in infants. Despite a slightly higher male population among CF patients, more females were hospitalized with CF. This finding is consistent with previous studies, which have demonstrated poorer outcomes among female patients with CF thus leading to more frequent exacerbations and hospitalizations (15,16).

In terms of payer status, the highest number of hospital discharges was seen in the Medicaid group in both 2003 and 2013. This was observed in spite of the fact that in 2013, more patients had private health insurance as compared to any other insurance (10). Medicaid patients may be less likely to seek care at onset of an exacerbation secondary to concerns of limited access to healthcare (17). Delayed care increases risk for worsening exacerbation and need for hospital admission. The difference in socioeconomic factors among patients with insurance can impact care for CF as Medicaid patients have a 3.7-fold higher death risk than CF patients without Medicaid (18). In previous studies, CF patients with Medicaid were found to have worse lung function and need for more IV antibiotics for pulmonary exacerbations which could

explain the increased need for inpatient hospitalizations (19-21). It is also important to remember that Medicare uses a prospective payment system where re-imbbursement is based on a predetermined amount instead of the traditional fee for service model (<https://www.cms.gov/>). Hospitals can be placed at financial risk for increased length of stay in these scenarios. Early discharges in these patients can occasionally lead to an early recurrence of the exacerbation and thus lead to an overall increased number of admissions and discharges. A drop in the number of uninsured patients is encouraging because CF patients without health insurance have been shown to have a higher mortality rate (22).

We also analyzed the data with respect to hospital characteristics such as hospital region (Northeast, Midwest, South and West) and bed size. The South had the highest number of CF discharges in both 2003 and 2013. This may be because the South had the highest number of total hospital discharges in the same time frame. We also found that the Western U.S. had the highest frequency of CF hospital discharges in both 2003 and 2013. Midwest had the highest increase. The frequency of hospital discharges more than doubled in the Midwest from 2003 to 2013. In terms of hospital size, patients with CF were more likely to be admitted to hospitals with large bed size in 2013 compared to hospitals with small bed size in 2003, and to hospitals within a metropolitan area. This is consistent with the CFF accreditation criteria for CF care centers to have the supportive infrastructure necessary to support a multidisciplinary model of care, which is more often available in larger hospitals and in metropolitan areas.

The design of this study and the nature of the NIS database set leads to some important limitations. As this is an administrative data set, it reflects the coding practices of each institution. Thus it is likely that these results underestimate the actual number of patients admitted and discharged with the diagnosis of CF. Discharges may have been coded with an alternative diagnosis such as viral or bacterial pneumonia, hypoxemia and sepsis. This data set also does not control for errors during the entry of the data. Additionally, the NIS data set does not provide details regarding the patient or the hospital, which could help explain the trends in hospital discharges and associated costs. Patient-specific clinical information was not obtainable thereby limiting the demographic data presented in the research. Further studies analyzing hospital coding practices may clarify these concerns.

## 5. Conclusion

Over the last decade, many advances have been made in the diagnosis and treatment of CF, consequentially leading to a significant transformation in the epidemiology and demographics of this chronic disease.

It is important to provide updated information regarding these changing demographics as they also reflect a considerable improvement in survival. Rising hospital costs associated with the care of CF patients necessitates future studies analyzing the diagnostic modalities, algorithms and treatment practices of physician's treating CF patients.

### Acknowledgements

We would like to acknowledge the Division of Pulmonary, Critical Care & Sleep Medicine at Northwell Health, the Cystic Fibrosis Center at Northwell Health and the Cystic Fibrosis Center at Monmouth Medical Center. This study was not funded by any sources.

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(Received July 25, 2017; Revised August 22, 2017; Accepted 26, 2017)

# Theory and practice of laparoscopic surgery against omohyoid muscle syndrome

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**Summary** Omohyoid muscle syndrome (OMS) is a rare disease characterized as a protruding lateral neck mass feature during swallowing. Because there is a 5 cm scar after traditionally surgery, we designed a laparoscopic surgery procedure to meet the cosmetic needs of patients. From the year 2006 to 2016, there were 3 patients diagnosed as omohyoid muscle syndrome that underwent laparoscopic surgery. Operative and postoperative follow-up data were summarized. Average surgery time was  $35 \pm 13$  min. Average blood loss was  $3 \pm 1$  mL. No case converted to open surgery. No major vessel or nerve damage complications occurred. After the surgery, the neck mass completely disappeared during swallowing, and there were no operative scars on the neck. All patients were discharged within 2 days. During the follow-up of a year, no recurrence occurred. In conclusion, the endoscopic procedure is suitable for OMS. It's a safe, effective and cosmetic surgery.

**Keywords:** Omohyoid muscle syndrome, laparoscopic surgery

## 1. Introduction

Omohyoid muscle syndrome (OMS) is presented by a mass without pain on the neck when the patient swallows and disappears after swallowing. The patient often feels discomfort and dysphasia when the mass appears. It's a rare disease. There are only few case-reports published about this disease. It's not able to be diagnosed by esophagus barium radiography or ultrasonography. The fine needle biopsy for the mass often results as muscle fibers atrophy, fibrosis or interstitial inflammation occurs. There is no typical pathology change. It's easily misdiagnosed.

In the normal condition (Figure 1A), the omohyoid muscle (OM) consists of superior and inferior bellies united at an angle by an intermediate tendon (IT) and it passes behind the sternocleidomastoid (SCM) muscle. The inferior belly (InB) inclines forward and slightly

upward and inserts into the IT. The superior belly (SuB) originates from the IT and inserts into the base of the hyoid bone (H). The OM depresses the hyoid bone after it has been elevated during wallowing (1).

In OMS patients (Figure 1B), the most important pathology change is loosening of the IT tendon sheath (2). After that, the OM becomes shortened and fibrosis occurs because of atrophy of disuse and degeneration. When the patient swallows, the OM can't be extended, and the IT moves laterally and superiorly. The posterior clavicle margin of OM replaces IT as a new origin of force. When the patient swallows, the OM shortens like a string, and forms an X-shaped tent to elevate the SCM in the lateral neck during upward movement of the hyoid bone. The elevated SCM forms the mass in the neck.

This theory was acceptable for many scholars (3-5), but we could clearly see the thickened OM and the IT was still at its location contrarily when the patient swallowed. Obviously, the OM did not degenerate, but instead was more sturdy than the normal side. So the etiology responsible for OMS was not known for certain.

Transection of the omohyoid muscle is the standard treatment for this disease due to the pathophysiology change of OMS just described above (6). The prognosis is good except for a 5 cm scar on the neck. Because it's a benign disease, the only reason patients choose to undergo surgery is the cosmetic effect. So we designed

Released online in J-STAGE as advance publication August 25, 2017.

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this laparoscopic surgery procedure to meet the needs of patients.

**2. Materials and Methods**

*2.1. General information*

From the year 2006 to 2016, there were 3 patients diagnosed as omohyoid muscle syndrome and underwent laparoscopic surgery. Among them, 2 cases were male, 1 case was female. Age of the patients were from 26-40 years (35 average). Disease course was from 1 month to 2 years (5 month average). All of them didn't have any congenital cervical disease.

*2.2. Clinical presentation*

In the 3 cases, 2 presented left cervical mass, 1 presented right. Typical complain was a sense of mild

dysphasia or a foreign body sensation in the throat. The history was usually several months. There was also an awareness of a mass appearing in the lower part of the neck when swallowing. Progression of symptoms was noticed. Voice was normal. There was also absence of distinct events that might have precipitated the onset of symptoms. A family history of similar symptoms was absent.

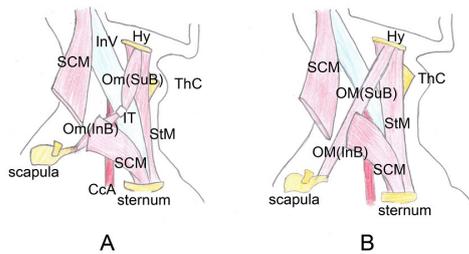
Physical examination showed no positive finding when the patient was not swallowing (Figure 2A). When the patient swallows (Figure 2B), a transient swelling arises up on the neck over the junction of the upper two thirds and the lower third of the sternomastoid muscle. The protruding of the mass coincides with the elevation of the throat, reaching its climax at the moment when the throat is uppermost. With the return of the hyoid, the OM shrinks to their original resting positions, and the mass also disappears. No further trace of the mass could then be discerned until the patient swallows again. Special maneuvers like the Valsalva maneuver or tongue protrusion are unrewarding.

*2.3. Assistant examination*

Blood, Urine tests, liver and kidney function were normal. Upper aerodigestive tract endoscopy, plain radiographs of the neck and thoracic inlet, and routine ultrasound, were normal. Computed tomography (CT) scan of the neck showed the inferior belly of the OM on the diseased side (Figure 3A) was obviously sturdier than that on the normal side (Figure 3B) by 1.15 cm vs. 0.53 cm in diameter. The internal jugular vein of the diseased side was dilated compared with the normal side (Figure 3C) by 1.99 cm vs. 0.98 cm in diameter.

*2.4. Surgical procedure of laparoscopic surgery (Video 1)*

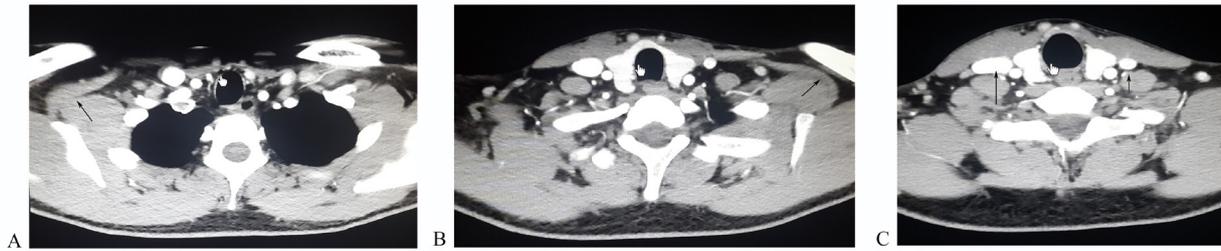
The patient was placed in a supine position with neck slightly extended under general anesthesia. A 10-mm curved skin incision was made at the upper margin of mammary areolas. Diluted adrenalin solution (1:500) was injected into the subcutaneous space in the chest wall and in the subplatysmal space of the neck in order to establish the trocar space and prevent bleeding during



**Figure 1. The anatomy and function of omohyoid muscle in normal and OMS patients. (A).** In normal condition, omohyoid muscle (OM) consists of superior and inferior bellies that are united at an angle by an intermediate tendon (IT) and it passes behind the sternocleidomastoid (SCM) muscle. The inferior belly (InB) inclines forward and slightly upward and is inserted into the IT. The superior belly (SuB) originates from the base of the hyoid bone (H). The OM depresses the hyoid bone after it has been elevated during swallowing. **(B).** In OMS patients, the most important pathology change is loosening of the IT tendon sheath. After that, the OM atrophies from disuse and degeneration happens. The OM becomes short and fibrotic. When the patient swallows, the OM can't be extended, the IT moves laterally and superiorly. The posterior clavicle margin of OM replaces IT as a new origin of force, When the patient swallows, the OM shortens like a string, forming an X-shaped tent to elevate the SCM in the lateral neck during upward movement of the hyoid bone. The elevated SCM forms the mass in the neck.



**Figure 2. The Photograph of the patient resting, swallowing and after surgery.** The features of the patient in the resting (A) and swallowing (B) are shown in the figure. The arrow points to the protruding mass. After surgery (C), the neck mass completely disappeared during swallowing, and there were no operative scars on the neck.



**Figure 3. Computed tomography (CT) scan on the inferior belly of OM and internal jugular vein.** Computed tomography (CT) scan of the neck showed the inferior belly of the OM on the diseased side (Figure 3A) was obviously sturdier than that on the normal side (Figure 3B) by 1.15 cm vs. 0.53 cm in diameter. The arrow points to the inferior belly of the OM. The internal jugular vein of the diseased side was dilated compared with the normal side (Figure 3C) by 1.99 cm vs. 0.98 cm in diameter. The arrow points to the internal jugular vein.

blunt dissection. The subcutaneous space was dissected bluntly using a dissector. Then a 10-mm trocar was inserted. Carbon dioxide was insufflated through the trocar at a pressure of 6-8 mmHg. The endoscope was inserted into the subplatysmal space. Two additional trocars double 5 mm trocars below the axilla were inserted into the space. The remaining connective tissue in the space was dissected endoscopically using an electronic hook, and an adequate operative space above the sternocleidomastoid muscle was created. We isolated the sternocleidomastoid muscle. The color of the omohyoid muscle was pale and dark red, the muscle was obviously fibrotic and tightly adhered to the sternocleidomastoid muscle. The omohyoid muscle was dissected transversely at the upper and lower border of the sternocleidomastoid muscle by harmonic scalpel and electronic hook. Then the surgery was done.

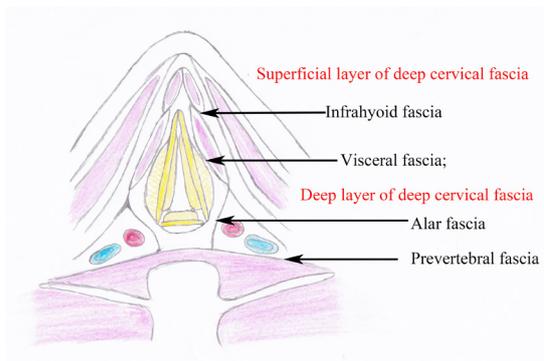
**Video 1 shows the surgical procedure of laparoscopic surgery against OMS** (<http://pan.baidu.com/s/1dELsA33>). The patient was placed in a supine position. At the upper margin of mammary areolas, the endoscope was inserted. Two additional trocars double 5 mm trocars below the axilla were inserted into the subplatysmal space. Adequate operative space above the sternocleidomastoid muscle was created. The sternocleidomastoid muscle was isolated. The omohyoid muscle was dissected transversely at the upper and lower border of the sternocleidomastoid muscle by harmonic scalpel and electronic hook. Then the surgery was done.

### 3. Results

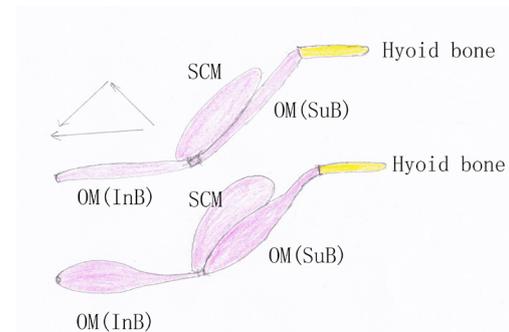
Average surgery time was  $35 \pm 13$  min. Average blood loss was  $3 \pm 1$  mL. and no case converted to open surgery. No major vessel or nerve damage complication occurred. There were no scars on the neck (Figure 2C). All the patients were discharged within 2 days. No recurrence occurred during the follow-up of a year.

### 4. Discussion

OMS, also called omohyoid sling syndrome, is a rare disease. The first report of a patient with OMS was in 1980 (7), a similar terminology, omohyoid syndrome, was first used in a report published in The Lancet in



**Figure 4. The diagram of deep cervical fascia.** The sarcolemma of OM consisted of a superficial layer of deep cervical fascia. At the same time this fascia also contributed to the anterior wall of the internal jugular vein sheath.



**Figure 5. The diagram of the etiology for OMS we thought.** The etiology of OMS should be IT adhesion with the SCM, when swallowing, the OM should counteract part of the SCM forces. This resulted in OM compensatory sturdiness, and finally OM sturdy enough to elevate the SCM.

1969 to describe a case with characteristic symptoms, including pain and tenderness in the neck, voice changes, and swallowing difficulties most likely due to acute spasm or cramping of the omohyoid muscle. However, the patient did not show any mass in the neck during swallowing. Thus, this case is not compatible with the current concept of OMS (8).

From our clinical observation, we found patients who suffered from OMS didn't show OM degeneration and elevated IT as classic etiology. Instead, the OM on the diseased side was sturdier than the normal side.

The sarcolemma of OM consisted of a superficial layer of deep cervical fascia. At the same time this fascia also contributed to the anterior wall of the internal jugular vein sheath (Figure 4). Partial function of the OM was dilating the internal jugular vein. So from the CT scan, we found that the internal jugular vein was obviously dilated compared with the normal side. This phenomenon also identified that the etiology of OMS was not OM degeneration as a classic hypothesis. From our observation, we thought the etiology should be IT adhesion with SCM, when swallowing, the OM should counteract part of the SCM forces (Figure 5). This resulted in OM compensatory sturdiness, and finally OM sturdy enough to elevate the SCM.

The key clinical finding of OMS is the appearance of a transient lower lateral neck mass during swallowing due to dysfunction of the omohyoid muscle. Physical examination characteristically showed no positive finding when the patient was not swallowing. Most patients with OMS were treated by surgical transection of omohyoid muscle. The procedure leaves 5 cm or longer scar on the neck. Botulinum toxin injection to omohyoid muscle under ultrasonography guidance for OMS could offer an effect of omohyoid muscle dilation without operative scars on the neck, but it was not reported whether OMS would recur or if another injection was required (9). Theoretically, the paralysis of the degenerated omohyoid muscle caused by botulinum toxin couldn't be complete and the effect couldn't be long lasting.

The use of endoscopic surgery on the neck is now widely used in thyroid and parathyroid glands (10). It's a safe and effective technique for benign disease with a good cosmetic effect (11). So we tried to use this technique in OMS patients. After surgery, the neck mass completely disappeared during swallowing, and there were no operative scars on the neck. The cosmetic effect was good.

In conclusion, we believe that the endoscopic procedure is suitable for this disease not only because of a safe and effective outcome but also a good cosmetic effect which is the reason why OMS patients underwent surgery.

## Acknowledgements

The research was funded by Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support, code: XMLX201309. We thank the foundations and all the patients studied in this project.

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(Received July 14, 2017; Revised August 9, 2017; Accepted August 15, 2017)

# Laparoscopic resection of ectopic pheochromocytoma

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**Summary** Ectopic pheochromocytoma (EP) is considered as pheochromocytoma located at extra-adrenal site. Surgical removal is believed to be the best choice for treatment of pheochromocytoma. We present a series EP resected by laparoscopic approach (LEP) and confirm its feasibility. We retrospectively reviewed clinical data of 4 patients underwent laparoscopic resection of LEP (periaortocaval EP,  $n = 1$ ; retroperitoneal EP,  $n = 2$ ; bladder EP,  $n = 1$ ), which was collected and analyzed retrospectively in Zhejiang Provincial People's Hospital. The tumors were all successfully resected by laparoscopic approach, and there was no one converted to open surgery or needing blood transfusion. Laparoscopic resection is a feasible and safe choice for EP.

**Keywords:** Ectopic pheochromocytoma, laparoscopic, minimal invasive, hypertension

## 1. Introduction

Pheochromocytoma is a chromaffin cell tumor commonly located at adrenal medulla. Ectopic pheochromocytoma (EP) is considered as pheochromocytoma located at extra-adrenal site, which usually lies at para-aortic region. EP originated from chromaffin cells is a really rare tumor, which is from nerve crest. It has been reported the annual incidence is 0.4/1 million ~ 9.5/1 million (1). The age of onset varies from 30 to 50 years old, commonly sporadic cases. The distribution of EP is very changeable, it could occur from skull base to pelvic cavity where chromaffin cells are. The most common place of EP is paraortic, followed by bladder, mediastinum and head (2).

The clinical presentations of EP include hypertension, palpitations, dizziness and headache, but there are about 24~75% of asymptomatic patients (3). The level of norepinephrine is not high, so the tumor is usually called non-functioning ectopic pheochromocytoma. Clinically,

EP could be diagnosed according to clinical presentation and catecholamines in blood. As for iconography, we can find strengthened vascular shadow on CT scanning due to rich arteries in the neighbor of tumor. Retroperitoneal EP should be differentiated with schwannoma, liposarcoma and hyperplastic lymph gland. EP also should be differentiated with bladder cancer when it locates in bladder.

EP is not sensitive to radiotherapy or chemotherapy, so surgical remove is believed to be the best choice for treatment of pheochromocytoma (4). Laparoscopic approach is widely accepted because of its minimal invasive advantage. However, because of the reality of the EP, only a few publications reported single case reports of laparoscopic resection of EP (5-7). Here we present a series of EP resected by laparoscopic approach to prove the feasibility of the method.

## 2. Patients and Methods

The research admitted by ethics committee is not involved medical ethic. The surgery is rational in our hospital. From 2014 to 2017, the clinical data of 4 consecutive cases underwent laparoscopic resection of LEP (periaortocaval EP,  $n = 1$ ; retroperitoneal EP,  $n = 2$ ; bladder EP,  $n = 3$ ) was collected and analyzed retrospectively in Zhejiang Provincial People's Hospital (Table 1). These are three women and one man. Three patients had hypertension in varying degrees that

Released online in J-STAGE as advance publication August 25, 2017.

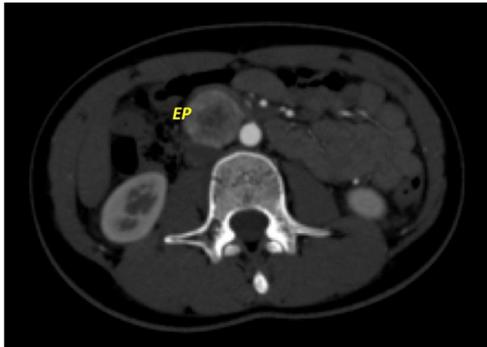
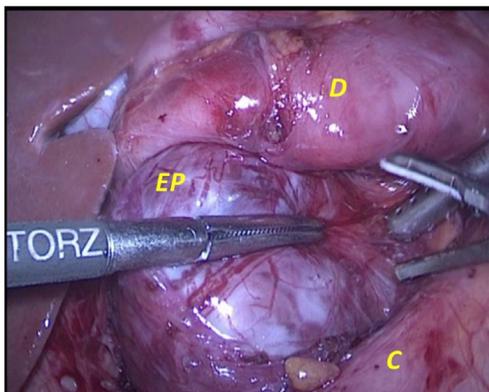
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**Table 1. Patients' characteristics**

Case	Sex	Age	Tumor size (cm)	Preoperative SBP (mmHg)	Preoperative DBP (mmHg)	Preoperative heart rate (bpm)
1	F	27	3.1	136	87	96
2	F	22	5	149	99	130
3	F	46	3.8	130	93	88
4	M	71	1.6	128	74	80

**Figure 1. CT scan shows an enhanced tumor (EP) at periaortocaval area. EP, Ectopic pheochromocytoma.****Figure 2. Intraoperative figure shows an EP at periaortocaval area after Kocher's maneuver was performed. EP, Ectopic pheochromocytoma; C, colon; D, duodenum.**

could be controlled by medicines. Other symptoms include palpitations, dizziness and headache. Besides clinical presentation, patients who underwent LEP were evaluated by biochemical and image test before surgery. Computer tomography (CT) is necessary for preoperative diagnosis and assessment (Figure 1). The tumor size examined before surgery ranged from 1.6 to 5 cm. Preoperative treatments: the patient 3 had Terazosin 2 mg qn; the patient 4 had Nifedipine 30 mg qd.

**Surgical approach** Laparoscopic resection of EP: Under general anesthesia, the patient 1 was placed in supine position. Pneumoperitoneum was established (12 mmHg). Four laparoscopic ports were placed (2 × 10 mm, 2 × 5 mm). Firstly, the Kocher's maneuver was performed to expose tumor (Figure 2). During operation, the blood pressure raised up to 300/110 mmHg when

we touched the tumor. Then we stopped all operation during the surgery and used sodium nitroprusside 15 mg by micro pump lasting for 5 min, the blood pressure dropped to previous level. The tumor was carefully dissociated with surrounding tissue and taken out with an endobag from enlarged umbilical incision. Then the blood pressure dropped to normal range and the surgery was finished. The surgical approach of patient 2 and 3 is similar to the approach mentioned above.

The position of ectopic pheochromocytoma in patient 4 was in bladder. The patient was placed in supine position in the condition of general anesthesia and pneumoperitoneum established at 15 mmHg. Three trocars were placed at left axillary line under navel 3cm, right axillary line under navel 3cm and navel. Firstly, 300 mL water was injected to bladder by catheter, and then the bladder was opened by electrocautery. The tumor was located at the posterior wall of bladder. The SBP went up to 245 mmHg when we touched the tumor. After removing the tumor with electrocautery, we closed the bladder with 2-0 prolene.

### 3. Results and Discussion

The perioperative data of the 4 patients are shown in Table 2. All EPs were resected using transperitoneal laparoscopy, and there was no one converted to open surgery. The operative time ranged from 40 to 95 min. The estimated blood loss ranged from 10 to 100 mL. The HSBP during operation of the 4 patients were higher than normal, and the highest blood pressure even reached to 310 mmHg. When we stopped all operation during the surgery and used sodium nitroprusside or ebrantil, the blood pressure dropped to normal range in a few minutes. Mean LSBP was 109.5, which was higher than normal range. Meanwhile, there was no need of blood transfusion in the 4 patients. All patients started liquid diet after 24 hours and soft diet after 36 hours. There was no complication occurred in the period of hospitalization. The length of postoperative hospital stay ranged from 5 to 10 days. The outcomes of pathology in our study are all benign.

At present, the best treatment approach is surgical removal (4). The incidence of intraoperative bleeding is high due to rich arteries around tumor and the position of tumor, like para-aortic and pare-cava. So the advantages of laparoscopic approach are totally appeared because of

**Table 2. Perioperative results of patients**

Case	Operative time (min)	EBL (mL)	HSBP (mmHg)	LSBP (mmHg)	POHS (day)
1	85	50	310	124	6
2	95	50	168	104	10
3	40	100	180	115	5
4	60	10	170	95	7

EBL, Estimated blood loss; HSBP, Highest SBP; LSBP, Lowest SBP; POHS, Postoperative Hospital stay.

its function of magnification.

It is safer for having a more clear vision for small vessels. If met a tumor extending to big vessels, we should ligate the tumor vessels to prevent catecholamine entering blood. It could lower the risk of postoperative heart failure and pulmonary edema in case of violent drop of blood pressure if giving fluid infusion preoperatively (8).

Compared with traditional surgery, laparoscopic approach will ease the postoperative pain because of 3 or 4 ports rather than a long incision. During the surgery, the operation of laparoscopic approach is more accurate and less damage to vessels or tissues, which contributes to fast postoperative recover and shortens the postoperative hospital stay (9). The appearance of the abdomen is more cosmetic than traditional surgery, which makes patients more satisfied about the surgery. The postoperative hospital stay is about 7 days, reflecting laparoscopic approach minimal invasive advantage.

Most of chromaffin cells are benign, and there is about 2~13% expressing malignant (10). However EP has more chances to mutate to be malignant, some researchers consider the rate is 10%, but someone thinks the rate is higher, even to 50% (10). Once researchers made a study and followed up 7 malignant pheochromocytoma patients and 5 malignant EP patients for 20 years, finding EP having the stronger ability of vascular invasion and lymph node metastasis (1). There is a cohort study based on large samples finding the survival time of malignant EP patients longer than malignant pheochromocytoma patients (11).

The main operation risk of EP is the management of blood pressure during surgery. Alpha-blockage should be used for at least 2 weeks before surgery if the patient has typical clinical symptoms, and when the heart rate is beyond 90 beats per minute,  $\beta$ -blockage should also be given (12). To a great extent, the measures will reduce the risks in surgery. In the period of surgery, blood pressure should be observed closely. If the blood pressure raises up to a abnormal range owing to touching the tumor, the operation should be stopped immediately and medicine, like sodium nitroprusside or ebrantil, could be given if necessary.

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(Received July 3, 2017; Revised August 17, 2017; Accepted August 19, 2017)

# Osteomyelitis due to multiple rare infections in a patient with idiopathic CD4 lymphocytopenia

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## Summary

A 26-year-old male patient presented with features suggestive of osteomyelitis involving the entire left femur, hip joint and knee joint. Culture from the debrided tissue grew *Acinetobacter spp.* and he was treated with sensitivity based antibiotics but the symptoms did not resolve. The synovial biopsy showed multinucleated giant cells and acid fast bacilli on Ziehl Neelsen stain. Cartridge based nucleic acid amplification test (GeneXpert) was negative. The Mycobacteria growth indicator tube culture was found to be positive for *Mycobacterium abscessus*. The patient was started on imipenem, amikacin and macrolide based therapy. There was partial response initially but the patient worsened again. A girdle stone arthroplasty with cemented nail (with tobramycin) insertion after debridement of the infected tissue was done. Potassium hydroxide (KOH) mount from the debridement sample was found to be positive for aseptate hyphae suggestive of mucormycosis. He was treated with liposomal amphotericin B. He was evaluated for immunodeficiency in view of multiple atypical infections and was found to have a low CD4 count. The patient was discharged on amikacin, azithromycin, trimethoprim-sulfamethoxazole and posaconazole. Follow up showed considerable resolution both clinically and radiologically. To our knowledge, this is the first reported case of osteomyelitis with co-infection of *Acinetobacter spp.*, *M. abscessus* and mucormycetes. We report this case to highlight the possibility of multiple rare infections in patients with immunodeficiency. Also, atypical complicated bone infections, such as *Mycobacterium abscessus* and mucormycetes might require combined medical and surgical treatment.

**Keywords:** *Acinetobacter spp.*, *Mycobacterium abscessus*, mucormycetes

## 1. Introduction

Osteomyelitis is a chronic debilitating illness requiring a combination of long term medical and surgical treatment. The most commonly implicated organisms

are gram positive cocci like *Staphylococcus aureus* (1). Atypical organisms can be suspected in immunodeficient patients or those not responding to the initial choice of antimicrobials. Idiopathic CD4 lymphocytopenia (ICL) is defined as CD4 count below 300 cells/uL on two occasions 3 months apart (2). In a review published in 2013, 259 cases of ICL were reported between 1989 and 2012 (3). Infections with nontuberculous mycobacteria (NTM) and fungi are far more common in ICL than in immunocompetent individuals (3). *Mycobacterium abscessus* is a rare cause of osteomyelitis with only few reported cases in the published literature (4). Similarly, bone mucormycosis is an extremely rare entity with

Released online in J-STAGE as advance publication July 28, 2017.

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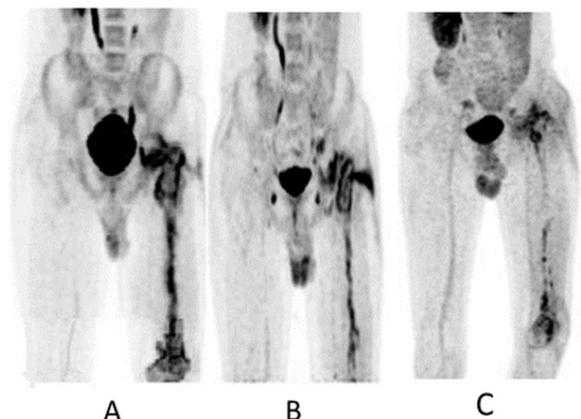
only 34 reported cases according to a systematic review (5). We present a case who was unlucky enough to have multiple rare infections associated with osteomyelitis in a backdrop of idiopathic CD4 lymphocytopenia.

## 2. Case Report

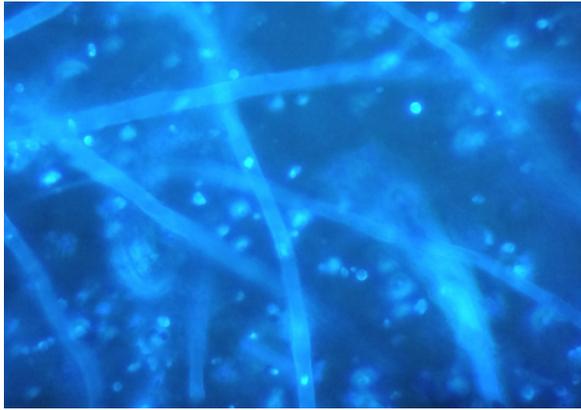
A 26-year-old male patient presented with pain in left thigh and restriction of movement of left hip joint with low grade fever for seven days to a local hospital in March, 2015. There was no antecedent history of trauma, recurrent infections, or intravenous (IV) drug abuse. He had a past history of left middle cerebral artery infarct (possible cause: protein S deficiency/anti-thrombin III deficiency) in 2013 and recovered without residual paralysis. He was immunized with BCG (Bacillus Calmette Guerin) vaccine at birth. On examination, the patient was well built and nourished. His vitals were stable. There was restriction of movement in the left hip joint but the systemic examination was normal. The non-contrast computed tomography (NCCT) scan of thigh showed normal bone anatomy but multiple fluid collections in adjacent muscles. With a provisional diagnosis of septic arthritis, hip arthrotomy and drainage was performed. The synovial fluid examination showed 13,400 cells (97% Neutrophils) and the culture was sterile. He received empirical intravenous antibiotics for one month but his symptoms persisted. He also developed discharging sinus at the surgical site. In April 2015, the patient visited a tertiary care hospital. Debridement of hip joint was done and culture from the debrided tissue grew *Acinetobacter spp.* which was sensitive to only tigecycline, colistin and meropenem. The patient received tigecycline, meropenem and linezolid for one month but symptoms did not resolve. In May 2015, he developed new onset pain and swelling in the left knee joint. Arthrotomy with partial synovectomy was done. The synovial biopsy showed lymphoplasmocytic infiltration with foamy histiocytes along with micro abscess and occasional multinucleated giant cells. The biopsy was positive for acid fast bacilli (AFB) on Ziehl Neelsen (ZN) stain but Gene Xpert (Cartridge based Nucleic acid amplification test) was negative. The patient was discharged on Category 1 Anti-tubercular therapy (Rifampicin, isoniazid, pyrazinamide and ethambutol).

The patient was wheel chair bound when he presented to our hospital in October 2015 with persistent pain in left hip and knee joint and multiple discharging sinuses over the lateral aspect of thigh and knee. On examination, his left hip and knee joint were swollen and tender with partial restriction in range of movement. Multiple discharging sinuses were present in the left hip and knee joints. Complete haemogram, routine blood chemistry and chest X-ray were within normal limits. Tuberculin sensitivity testing was positive. Bone scan showed chronic osteomyelitis involving the entire left

femur. A debridement of hip and knee joint was done. A cavity was seen in the greater trochanter and lateral condyle of femur intraoperatively. The patient underwent multiple debridements and received multiple intravenous antibiotics along with anti-tubercular therapy (ATT) for two months. Repeat bone scan showed partial response. A diagnosis of chronic osteomyelitis refractory to sensitivity based antibiotics and ATT was made. The following possibilities were kept for the etiology: Non tuberculous Mycobacteria (NTM), multi-drug resistant tuberculosis (MDR TB), deep seated bacterial infection and fungal infection. The discharge from the sinuses were sent for bacterial and fungal culture, both of which turned out to be sterile. HBsAg, Anti HCV and HIV serology were nonreactive. Urine and Blood culture were sterile. Histopathological examination of the debrided tissue showed multinucleated giant cells. Both AFB and Gene Xpert were negative this time. A provisional diagnosis of NTM osteomyelitis was made. The patient was discharged in January, 2016 on Category 1 ATT along with clarithromycin. The Mycobacteria growth indicator tube (MGIT 960) culture reports were awaited at the time of discharge. The patient was lost to follow-up and presented to us again in June, 2016 with non-resolving symptoms. MGIT Culture reports were traced to be positive in three of the debridement tissue samples. The MGIT culture was identified as NTM by detecting the MPT 64 antigen using the immunochromatography technique. The 16 S rRNA was sequenced and was identified as *Mycobacterium abscessus* (GenBank: KX343025.1). It was sensitive to both clarithromycin (Minimum inhibitory concentration less than equal to 1) & amikacin (Minimum inhibitory concentration less than equal to 16). Fluorodeoxyglucose positron emission tomography (FDG PET) scan was performed which was consistent with features of Chronic osteomyelitis of left femur (Figure 1A). The patient was started on imipenem (1g IV TDS), amikacin (500 mg IV OD), clarithromycin



**Figure 1. Fluorodeoxyglucose positron emission tomography (FDG PET). (A),** Serial scans showing evidence of osteomyelitis in June, 2016. **(B),** Partial resolution in October, 2016; **(C),** Near complete resolution in April, 2017.



**Figure 2. Calcofluor white – Potassium hydroxide (KOH) mount.** Microscopy at 400× magnification showing broad aseptate hyphae in debridement sample suggestive of invasive mucormycosis.

(500 mg BD oral) and rifabutin (300 mg OD). In October 2016, a repeat FDG PET was performed which showed no resolution (Figure 1B). Clarithromycin was replaced with azithromycin. A girdle stone arthroplasty was performed and an antibiotic impregnated nail (2 gm tobramycin into 40 g of cement.) was inserted intramedullary into the femur. The goal of this surgery was to decrease the disease load, to provide high dose of antibiotics locally, excision of sinus tracts and to get a tissue sample for further investigations. Although, the tissue was negative for bacterial and mycobacterial cultures, KOH turned out to be positive for aseptate hyphae suggestive of mucormycosis (Figure 2). Liposomal amphotericin B (300 mg IV OD) was started. Since the patient was having a multitude of different infections, the patient was evaluated for immunodeficiency. HIV ELISA and HIV RNA were negative. His T cell markers were as follows: CD4+ (20.8%), CD8+ (70.8%) and CD4:CD8 (0.3). Absolute CD4+ count was 113 cells/mm<sup>3</sup> (Normal range: 500-1,500 cells/mm<sup>3</sup>). Serum immunoglobulin levels were normal. Natural Killer (NK) cell activity was normal. The frequency of monocytes expressing the MSMD markers (Mendelian Susceptibility to Mycobacterial diseases) were in normal limits. A final diagnosis of NTM osteomyelitis with secondary *Acinetobacter spp.* infection with secondary mucormycosis in a case of primary isolated CD4 lymphocytopenia was made. The patient was discharged in January, 2017 with the following medications: amikacin (500 mg intramuscular OD), rifabutin (300 mg oral OD), azithromycin (500 mg oral OD), trimethoprim-sulfamethoxazole (160 mg/800 mg oral OD) and posaconazole (200 mg oral QID). A follow up in April 2017 showed marked resolution of symptoms. There was partial return in the range of movement in hip and knee joints. A repeat PET scan showed considerable resolution (Figure 1C). A repeat CD4 count which was repeated after 6 months of initial count was 240 cells/mm<sup>3</sup>.

### 3. Discussion

Osteomyelitis is primarily caused by haematogenous spread or direct inoculation of microorganisms (1). In this patient, the initial infection was likely due to haematogenous spread, considering his immunodeficient status and no prior history of trauma. The latter infections may have been nosocomial. The diagnosis of osteomyelitis is primarily dependent on radiological modalities. Although, Magnetic Resonance Imaging (MRI) and Bone scan have high sensitivity and specificity for diagnosis, the most accurate method for diagnosing and excluding osteomyelitis is FDG PET (6,7). The treatment of long bone osteomyelitis can be staged according to the Cierny Mader system (8). According to this system, our patient was categorized as Stage 4 diffuse osteomyelitis. The treatment for stage 4 is a combination of antibiotics and surgical debridement.

In recent years, there has been an increase in gram negative bacilli causing osteomyelitis. These are mostly associated with nosocomial transmission and are very difficult to treat because of antimicrobial resistance (9). There are very few reports of *Acinetobacter spp.* causing osteomyelitis. It has also been suggested based on animal model studies and case series that *Acinetobacter spp.* has a limited role in development of chronic osteomyelitis (10). So, in patients with non-resolving chronic osteomyelitis where *Acinetobacter spp.* grew in culture, possibility of other infections should still be entertained.

NTM is a rare cause of osteomyelitis but they are more commonly seen in immunosuppressed individuals. They should be suspected when ZN staining is positive and Gene Xpert is negative. Culture is essential for the final diagnosis. It is essential to differentiate NTM from MTB complex as it does not respond to conventional ATT. Patients with high suspicion of tuberculosis who do not respond to conventional ATT are often presumptively diagnosed as MDR TB. Comprehensive data from Indian settings are lacking but the isolation rates range from 0.7 to 34% (11-13). In a study from 1998 to 2011, 29 patients were diagnosed with musculoskeletal NTM infection (4). The treatment for NTM osteomyelitis is complicated requiring surgical debridement and long term chemotherapy (14). It is important to identify the NTM up to species level as the management is considerably different for different species. The regimen that is classically used for *M. abscessus* is clarithromycin or azithromycin, amikacin and imipenem or cefoxitin (15). Even though, according to American thoracic society, this regimen is standard for treatment of *M. abscessus*, we also added rifabutin considering the patient was sick. Rifabutin was chosen considering its bactericidal activity, cost, availability and relatively fewer side effects (16). Linezolid could also be added to the primary regimen as it has shown to have good *in-vitro* activity against *M. abscessus*

but we did not add it because long term therapy with linezolid has been associated with high frequency of side effects like myelosuppression and neuropathy. Also, considering a high prevalence of multidrug resistant tuberculosis in India, linezolid is usually kept as a reserve drug. We shifted our patient to azithromycin, when there was inadequate clinical response to the clarithromycin based regimen. *Mycobacterium abscessus* is one of the organisms where clarithromycin is known to induce resistance by the expression of erm (erythromycin resistance methylase) proteins. In comparison, azithromycin causes reduced induction of erm expression (17). There was no discernible change in the status of disease even after switching over to azithromycin. This was the reason he was planned for girdle stone arthroplasty. We used bone cement (PMMA) mixed with tobramycin. To successfully mix antibiotic with cement, the antibiotic has to be heat stable and hydrophilic. Vancomycin, gentamycin, tobramycin, erythromycin and daptomycin have been successfully tried. Using this technique, the antibiotic reaches 10-200 times of the usual bactericidal concentration. There is a sustained release of antibiotics lasting up to four weeks. Those bacteria which do not respond to high serum concentration of antibiotics may respond to this technique due to sustained high concentration of antibiotics at the target site (18). The tissue samples taken during the procedure showed aseptate hyphae. Bone mucormycosis, also a rare entity, is a progressively destructive disease with poor prognosis. The mode of infection is primarily by direct inoculation (trauma or surgery). The mucormycosis in our case was likely to be nosocomially acquired as the patient had multiple post-operative wounds. Bone mucormycosis in general, requires extensive debridement as amphotericin B alone is often not enough. Bone infections due to NTM and Mucormycetes are uncommon entities and are mostly seen in the immunodeficient population. Our patient was found to have idiopathic CD4 lymphocytopenia. Studies have shown that tuberculosis itself can cause transient CD4 lymphocytopenia (19). Such a type of lymphocytopenia would improve with anti-tubercular therapy. In our patient, although there was improvement in the CD4 count when it was repeated it did not go beyond 300 cells/uL. We hypothesized that the patient had initial infection with NTM because the patient had granulomas in the initial synovial biopsy. Also, NTM osteomyelitis is established by haematogenous route while bone infection due to *Acinetobacter spp.* and mucormycosis are mostly described in post-operative nosocomial settings. As mentioned above, *Acinetobacter spp.* has not been conclusively shown as a sole significant cause of osteomyelitis.

The patient was doing well at the follow-up with significant improvement in functional status. In a tertiary care government centre of a resource limited country with an exceedingly heavy load of poor patients, we

were only able to effectively manage the case because of the untiring efforts of the administration, who allowed us to keep the patient admitted for more than a year and provided the expensive investigations and drugs free of cost. Also, the support provided to the treating physicians by the diagnosticians who gave the clues about microbial etiology and the orthopaedicians who performed multiple surgeries was invaluable. In such intractable cases requiring a multimodality approach, active participation from different specialities is warranted for alleviation of long term morbidity and possible mortality.

To conclude, in patients with suspected immunodeficiency, possibility of rare infections should always be kept in mind. Also, multiple infections are far more common in immunodeficient individuals and therefore, they should be searched and managed aggressively. The management in these patients, especially in those with bone infections require proper teamwork between diagnosticians, surgeons and physicians.

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(Received May 24, 2017; Revised June 13, 2017; Accepted July 11, 2017)

## Rare case of ameloblastoma with pulmonary metastases

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### Summary

Ameloblastoma is a rare low-grade odontogenic tumor of epithelial origin. The World Health Organization (WHO) has defined malignant ameloblastoma (MA) as a histologically benign-appearing ameloblastoma that has metastasized. Treatment of the primary ameloblastoma usually consists of radical excision of the tumor and adjuvant radiotherapy. Chemotherapy should be used to treat metastases due to its indolent clinical course. Presented here is the case of a 43-year-old woman who was admitted to a hospital in 2006 with a large mass involving the neck and left mandible. The mass had formed over years and had been neglected. The woman was diagnosed with a primary ameloblastoma of the mandible. Surgical resection was performed, followed by adjuvant radiotherapy. In September 2016, she was admitted again, and the findings were consistent with metastases of the previously identified ameloblastoma to the lungs. The patient was evaluated for further chemotherapy with 6 cycles of cisplatin at a dose of 100 mg/m<sup>2</sup> on day 1, 5-FU at a dose of 1000 mg/m<sup>2</sup>/day on day 1-4 (3 wk), and pegylated filgrastim. The current case represents the classical course of a rare disease, which in this instance involved the common presentation of MA. This case is a valid incidence of MA based on the typical histology, findings from a lung biopsy, the immunohistochemical profile of the tumor, the typical clinical features, and a history of a previous primary disease.

**Keywords:** Ameloblastoma, ameloblastic carcinoma, metastases

### 1. Introduction

Ameloblastoma is a rare low-grade odontogenic tumor of epithelial origin. In most cases, it arises from the mandible (80%), but is also frequently found to affect the maxilla (20%). Despite being locally aggressive and highly recurrent after surgery, metastases from ameloblastoma are extremely rare and tend to appear years after treatment of the primary tumor (1). The World Health Organization has defined malignant ameloblastoma (MA) as a histologically benign-appearing ameloblastoma that has metastasized. Metastases are usually localized in the regional lymph nodes or distant organs – most commonly the lungs – and retain the benign characteristics of the primary

neoplasm. Thus, whether metastasis will occur or not cannot be predicted based on histology. That said, an ameloblastoma displaying cellular atypia is considered to be an ameloblastic carcinoma (AC), which is a highly aggressive malignant tumor that should be treated as such. Treatment of the primary ameloblastoma usually consists of radical excision of the tumor and adjuvant radiotherapy, with the aim of minimizing the chances of recurrence and metastasis or its high-grade counterpart, *i.e.* ameloblastic carcinoma (2,3). However, there is considerable debate in the literature as to whether chemotherapy should be used to treat metastases due to its indolent clinical course.

### 2. Case Report

In 2006, a 43-year-old woman was admitted to a hospital with a large mass involving the neck and left mandible. The mass had formed over years and had been neglected. Diagnostic X-rays and a biopsy were performed. The woman was diagnosed with a primary ameloblastoma of the mandible. Surgical resection was then performed, consisting of left hemi disarticulation

Released online in J-STAGE as advance publication August 7, 2017.

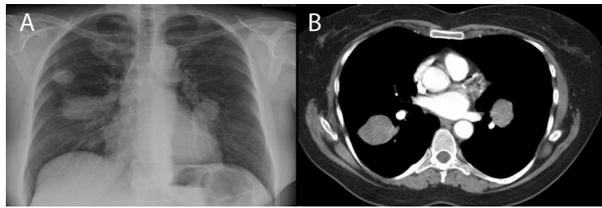
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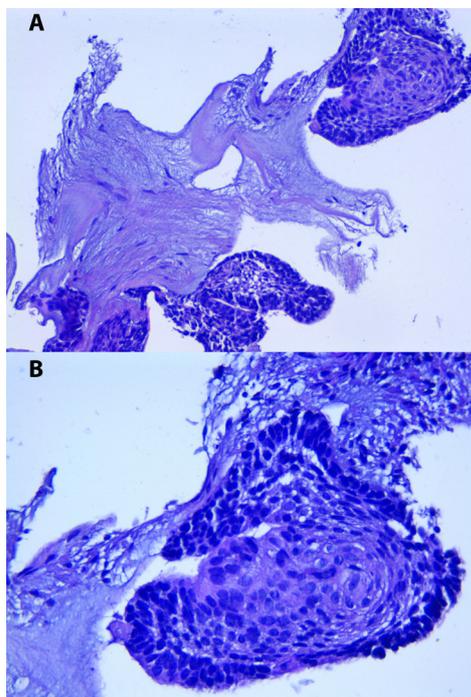
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of the lower jaw, left parotidectomy, and neck lymph node dissection, followed by adjuvant radiotherapy. In September 2016, the woman was admitted again



**Figure 1.** Lung metastases in the current case. (A), X-ray, (B), CT.

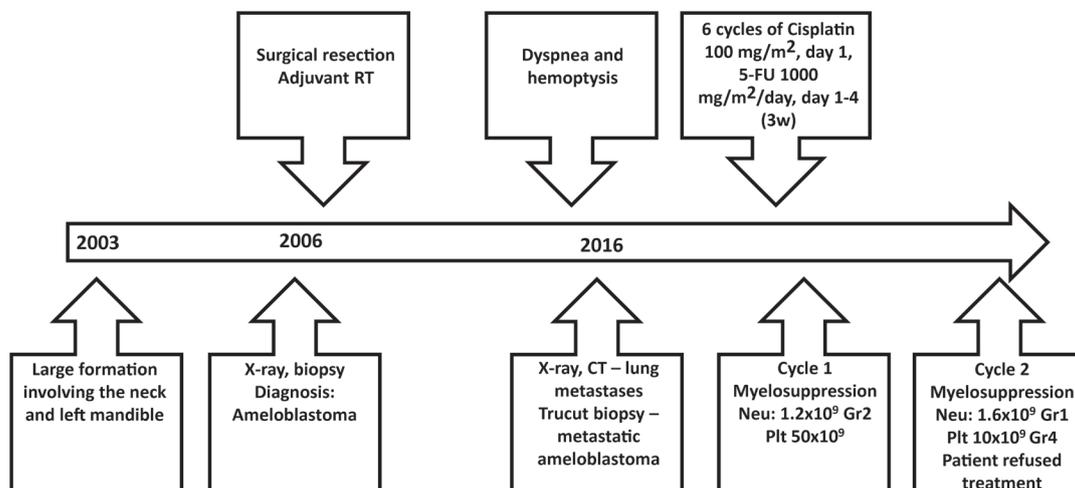


**Figure 2.** Histological slide confirming the metastasis of ameloblastoma. (A), Magnification 80x; (B), Magnification 160x.

for dyspnea and hemoptysis without any significant weight loss. A chest X-ray revealed round, relatively homogenous opacities bilaterally, which suggested lung metastases. A CT scan of the chest with contrast was performed. The scan revealed lobulated lesions – 3 were located in the right lung (the largest had a diameter of 48 mm) and 1 was located in the left lung (with a diameter of 31 mm). All of lesions were contrast-enhanced. No significantly enlarged lymph nodes were detected. There were no signs of other metastases (Figure 1). To confirm the diagnosis, a fine-needle aspiration (FNA) biopsy was performed and it revealed malignant cytological material that suggested small-cell lung carcinoma (the cells were small, ovoid, and devoid of cytoplasm with large, oval, hyperchromatic nuclei). A subsequent Trucut biopsy revealed small nests of fusiform cells with oval hyperchromatic nuclei with a peripheral palisade. Squamous cell metaplasia was noted in the center of the nests – the findings were consistent with metastases of the previously identified ameloblastoma (Figure 2). Immunohistochemistry revealed the expression of CK5/6 and CK19 with no expression of TTF1. The patient was then evaluated for further chemotherapy with 6 cycles of cisplatin at a dose of 100 mg/m<sup>2</sup> on day 1, 5-FU at a dose of 1000 mg/m<sup>2</sup>/day on day 1-4 (3 wk), and pegylated filgrastim. The patient underwent only two cycles of CT because she developed severe hematological toxicity (Figure 3).

**3. Discussion**

In 2005, the WHO defined MA as a histologically benign-appearing ameloblastoma that has metastasized. The histological sample from the current case represents the classical form of ameloblastoma as has been described previously (4). Thus, whether metastasis will occur or not is impossible to predict based on histology. Consequently, MA was diagnosed retrospectively. Before ameloblastoma was defined as mentioned



**Figure 3.** Timeline of the course of disease and the diagnostic and treatment strategy in the current case.

earlier, there were no clear diagnostic criteria for the condition. It was defined as a heterogeneous clinicopathological entity that consists of ameloblastomas with different histological and clinical behaviors – from very aggressive to highly indolent. MA has also been previously reported under different names such as "metastatic ameloblastoma" and "ameloblastoma with metastasis." Because cytological atypia is not excluded from the early diagnostic criteria for MA, many reported cases may be malignant entities other than MA (5). In contrast, AC is now an entirely different entity, which is characterized by the presence of cellular atypia. Ameloblastoma with cellular atypia is defined as carcinoma, even if metastases are absent. The distinction between these two terms is confusing and a distinction must be made.

Emmura was the first to report MA in 1923, and since then about 100 cases have been reported (6,7). MA represents 1.6-2.2% of all odontogenic tumors (8). There is one report, by Rizzitelli *et al.* in 2014, on the incidence of MA and its survival rate (9). Unfortunately, that study did not calculate the rate of MA and AC separately; instead, they were calculated as a single percentage. According to the authors, the overall incidence of MA, including both the benign-appearing metastatic form and the cancerous form, is 1.7 per 10 million people. The overall survival rate is 17.6 years – despite being metastatic, the disease has an indolent course. The peak incidence is between the third and fifth decade (9).

The most frequent site for metastases of MA is the lungs, which is where the first metastases manifested in the current patient. The frequency of metastases is estimated to be as high as 75-88% (7).

The disease-free interval in MA is usually quite long – ranging from 2 months to 45 years (mean: 14-18 years) (10) – and that interval was 10 years in the current case. Three mechanisms of tumor metastases are proposed: hematogenic, lymphatic, and *via* aspiration. Tumor development has been observed in the bronchi and bronchioli (11), which supports the aspiration mechanism. The literature contains no information regarding a predilection for one (right or left) or both lungs.

The immunohistochemical profile of ameloblastoma usually resembles that of normal odontogenic tissue. A number of cytokeratin (CK) subtypes are expressed, namely CK5, CK6, and CK19. In the current case, immunohistochemistry yielded similar results. CK13 and CK14 are found to be expressed in ameloblastoma (7,8,12). Thyroid transcription factor-1 (TTF-1) is a marker used to distinguish a primary lung adenocarcinoma from a lung metastasis. This is due to the fact that it is expressed only in lung adenocarcinoma and thyroid carcinoma. However, TTF-1 is not detected with adenocarcinomas arising from other sites (13). Thus, the tumor in the current patient was metastatic and

of ameloblastic origin, a conjecture that was confirmed by additional immunohistochemistry.

The treatment for MA still remains controversial. For lung metastases, lung surgery with radical resection remains the main treatment option, and the role of chemotherapy and radiotherapy has yet to be specified. Based on data from case series and small-scale studies, chemotherapy is the treatment of choice when radical resection cannot be achieved. There is no standard chemotherapy regimen that can be recommended for patients with unresectable metastases.

About 50 cases of cytotoxic treatment of MA have been reported, though their outcomes varied (14). Therapy with cyclophosphamide, methotrexate, and 5-FU yielded passable results in a patient with lung metastases that recurred nine years after initial therapy. Gall *et al.* noted clinical improvement for long periods of time but no objective response (15). Another study noted partial response after 13 cycles of a combination of cisplatin and cyclophosphamide to treat lung metastases (11). Other drugs also had an effect (reducing the tumor size and alleviating symptoms), including vinblastine, bleomycin, paclitaxel, topotecan, and carboplatin (10,15-18). Based on the tumor spread in the current case and the desire for a more aggressive approach, a cisplatin-containing doublet as has been described in other studies (16,19) was chosen. Unfortunately, the current patient was unable to complete a regimen of 4 cycles with cisplatin and 5-FU due to severe myelosuppression, thus hampering adequate treatment and evaluation of its outcomes.

In conclusion, the current case represents the classical course of a rare disease, which in this instance involved the common presentation of MA. This case is a valid incidence of MA based on the typical histology, findings from a lung biopsy, the immunohistochemical profile of the tumor, the typical clinical features, and a history of a previous primary disease. This is important since most reports describing what was thought to be MA have in fact identified it as some other malignant entity instead.

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(Received June 2, 2017; Revised July 6, 2017; Accepted July 11, 2017)

# Filarial huge splenomegaly dramatically regressed by anti-filarial medication: A rare clinical scenario

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## Summary

Lymphatic filariasis is caused by nematodes *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Lymphatic filariasis is a spectrum of illness and can manifest as, asymptomatic microfilaraemia, acute lymphatic filariasis (lymphangitis and lymphoedema), chronic lymphoedema, elephantiasis, hydrocele, tropical pulmonary eosinophilia and some systemic manifestations which involves joint, heart, kidney, nerve, etc. We here present a case of huge splenomegaly caused by lymphatic filariasis which is a rare presentation and only few cases had been reported in the world literature so far. After treatment of filariasis spleen size was reduced dramatically and patient is doing well even after 6 months of follow up after therapy.

**Keywords:** Filariasis, lymphatic, splenomegaly

## 1. Introduction

Lymphatic filariasis is one of the oldest & neglected tropical disease. Nearly 1.4 billion people worldwide are threatened by lymphatic filariasis and over 120 million people are currently infected. After leprosy, lymphatic filariasis is the second most common cause of long term disability (1). India, China & Indonesia jointly bear the burden of 2/3rd of all caseloads (2). In India lymphatic filariasis is endemic in seventeen states & six union territories affecting almost thirty one million people (3). It is endemic in Orissa, where its prevalence is very high in the coastal district of Cuttack. Although a common disease, there are very few reports in the world literature indicating possible involvement of the spleen in lymphatic filariasis. However, there are several case reports of incidental detection of microfilariae in the bone marrow without any other clinical findings of lymphatic filariasis (4-11).

## 2. Case Report

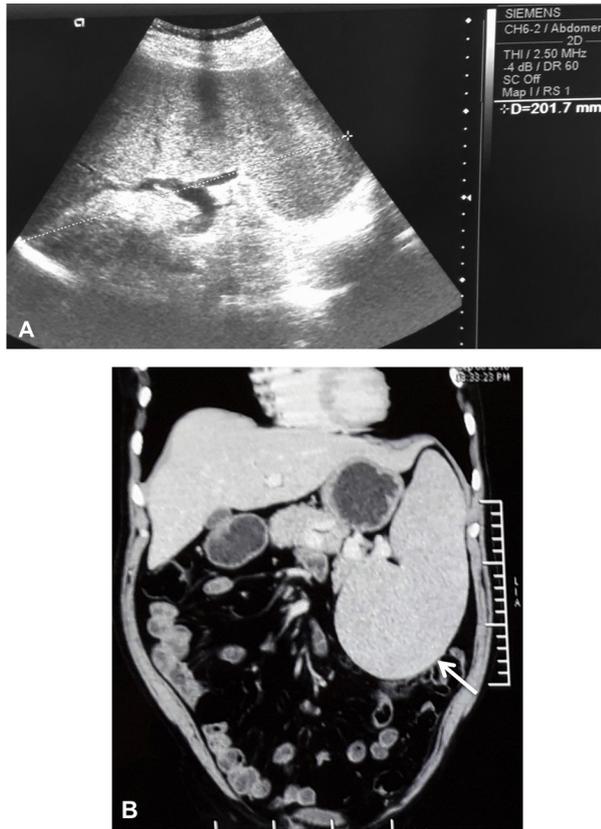
62 years old non-alcoholic, non-smoker male patient

resident of east Champaran district of Bihar presented to our outpatient department with slowly progressive massive splenomegaly and easy fatigability for 8 months. He had a past history of peripheral blood eosinophilia (25%) for which he was given tablet Di Ethyl Carbamazine Citrate (DEC) for inadequate dose and duration (100 mg tid for 7 days) by a local physician with some reduction of eosinophilia but without any reduction of size of spleen. He had past history of occasional intermittent fever for few days 6 months ago but without any weight loss, jaundice, ascites, haematemesis, melaena, cough, wheeze, haemoptysis, lymphangitis, lymphadenopathy or hydrocele. He had no past history of tuberculosis, recurrent malaria, diabetes or blood transfusion. On examination only mild pallor and 14 cm firm splenomegaly were found without any hepatomegaly, lymphadenopathy or bony tenderness. Other systemic examinations were unremarkable. Investigations showed pancytopenia with Haemoglobin (Hb) 9.4 gm/dL, Total Leucocyte Count (TLC) 3000/cmm (Differential leucocyte count ie DLC showed-Neutrophil-59%, Lymphocyte-22%, Eosinophil-10.4%, Monocyte-8%, Basophil-0.6%), platelet count 88000/cmm, haematocrit 28.5%, Mean Corpuscular Volume (MCV) 83.7, Mean Corpuscular Haemoglobin (MCH) 26.8, Mean Corpuscular Haemoglobin Concentration (MCHC) 32, reticulocyte count 0.8%, Direct Coombs test was negative. Liver

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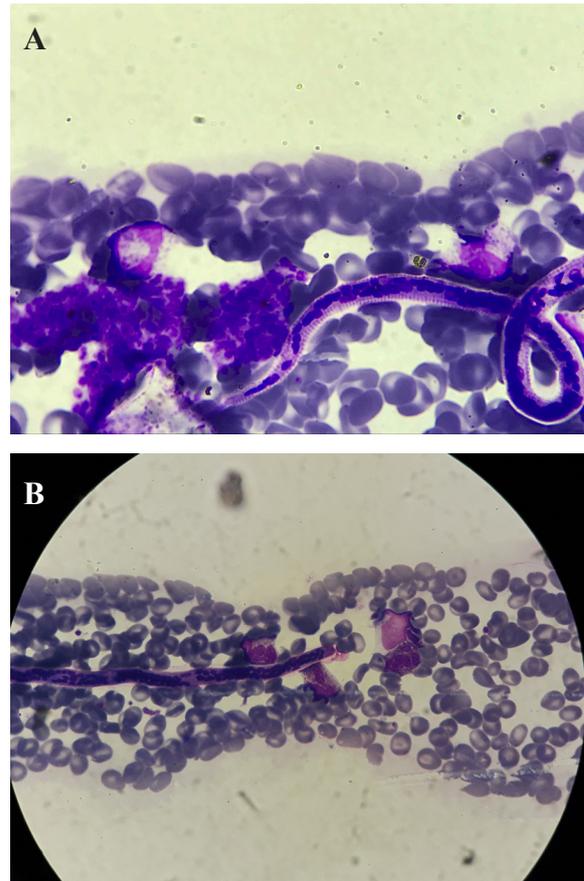
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**Figure 1.** (A) Baseline ultrasound reveals gross splenomegaly (size 20.1 cm) and (B) CECT coronal reformatted image reveals the markedly enlarged (22 cm), homogenous spleen (arrow).

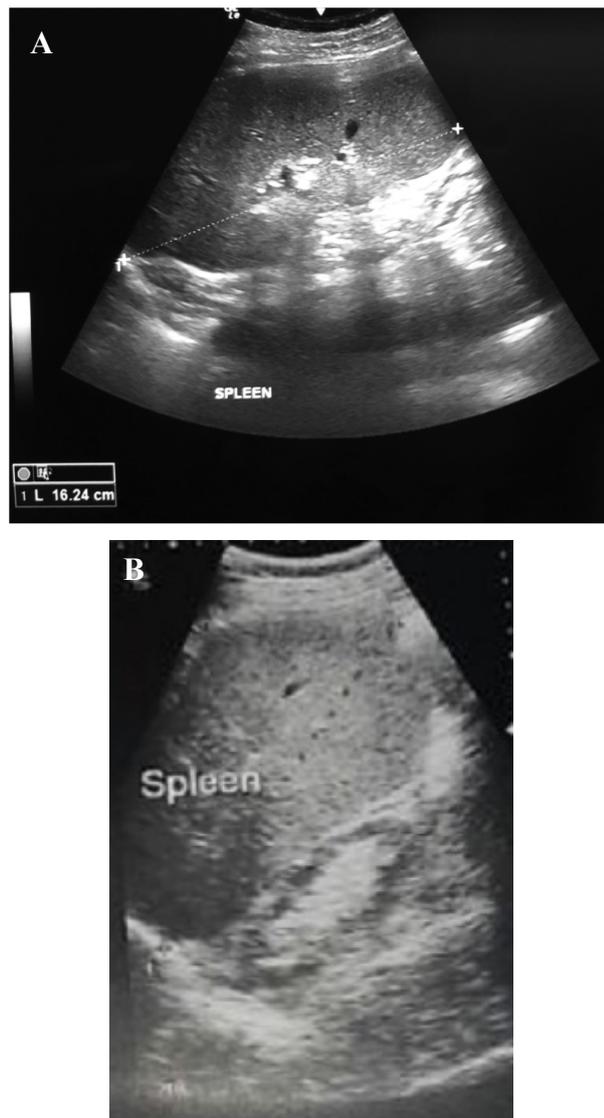
function test showed total bilirubin 0.7 mg/dL with unconjugated bilirubin 0.4, total protein 6.6gm% and albumin 4.7gm%, AST (Aspartate Aminotransferase) 21 IU/L, ALT (Alanine Aminotransferase) 11 IU/L and alkaline phosphatase 213 IU/mL, Prothrombin time 13.6 sec and INR 1.2, urea 36 and creatinine was 1.2 mg/dL, fasting blood sugar 96 mg/dL, vitamin B12 403 ng/mL, folate 6.24 ng/mL, ferritin 77.5  $\mu$ gm/mL, LDH 383, thyroid and lipid profile were normal. rK 39 strip test and anti-malarial antibody tests were negative. HBsAg (Hepatitis B surface antigen), anti HCV (Hepatitis C Virus) antibody and HIV serology were non-reactive. Examination of stool did not reveal any ova parasite or cyst. Ultrasonography (USG) and computed tomography of abdomen showed huge splenomegaly (20 cm and 22 cm respectively) (Figure 1A and 1B) without any evidence of portal hypertension, ascites, hepatomegaly or intra-abdominal lymphadenopathy. Chest X ray and upper gastro intestinal endoscopy were normal. Immunohistochemistry study showed normal banding. 18F FDG (Fluoro Deoxy Glucose) whole body Positron Emitted Tomography Computed Tomography (PET CT) study revealed metabolically active mediastinal lymph nodes (largest being 2.1  $\times$  1.5 cm) with fibrotic changes in the right lung apex but FNAC (Fine Needle Aspiration Cytology) could not



**Figure 2.** Microscopic finding. (A & B) Microfilariae of *Wuchereria bancrofti* in bone marrow.

be done due to the location of lymph node near blood vessels. Bone marrow aspirate showed cellular reactive bone marrow with all haematopoietic cells and adequate megakaryocytes. Myeloid cells showed prominence of eosinophils. Erythroid cells showed normoblastic maturation. Few histiocytes were seen. Peripheral smear showed normal total leucocyte count and adequate platelets with 60% neutrophils. No atypical cells were seen. There was occasional microfilariae of *Wuchereria bancrofti* seen (Figure 2A and 2B). Biopsy report showed adequate bone marrow with normal marrow cellularity and normal marrow components. Occasional well defined small mature lymphoid cell aggregates were seen.

Suspecting it was a case of extra lymphatic manifestation of filariasis we performed circulating filarial antigen test and it became positive. Though quantitative buffy coat test even after single 100 mg of tab DEC challenge did not reveal any microfilariae or malaria parasite. So we started tablet DEC at a dose of 150 mg thrice daily (6 mg/kg/day, patient's body weight was 71 kg) and tablet doxycycline 100 mg twice daily for a total duration of 21 days. Five days after therapy his spleen size was regressed to 9 cm per abdominal palpation and 16.2 cm on ultrasonography (Figure 3A). Ten days after therapy his spleen size reduced to



**Figure 3.** Ultrasonography done at 5<sup>th</sup> day (A) and end of the treatment (B) showed reduction of splenomegaly (16.24 cm and 12.5 cm respectively).

4 cm per abdominal palpation (USG- 15.3 cm) and his complete blood count improved (Hb 12.3, TLC 4200, N 61, L 28, E 6, M 4, B 1, platelet count 90,000). After completion of treatment patient was advised to take monthly single dose of 300 mg tablets of DEC and single 400 mg tablet of Albendazole for 6 months and then yearly thereafter for 5 years. Patient is symptomatically well with improvement of blood parameters and total regression of splenomegaly (Figure 3B) after 6 months of follow up (see Table 1 and Table 2).

### 3. Discussion

*Wuchereria bancrofti*, *Brugia malayi*, or *B. timori* affect the lymphatic vessels structure and function as well as some extralymphatic organs. Diagnosing extralymphatic filariasis poses a real challenge as symptoms and signs mimic other non-filarial diseases. There were several case reports of incidental detection of microfilariae in the bone marrow. In 1976, Pradhan *et al.* reported the first case of microfilariae in bone marrow aspirate (4). They showed *W. bancrofti* in peripheral blood and bone marrow in 7 cases but none had aplastic anaemia. However many authors have reported the coexistence of aplastic anaemia and microfilariae of *W. bancrofti* (5-7). Microfilariae of *W. bancrofti* have been isolated from various cytological smears, like fine needle aspiration cytology, body fluids, vaginal and endometrial smears, ovarian cyst, laryngeal, pharyngeal and bronchial brushings, breast and joint aspirates (12,13). In the lymphatic system and lymph node adult parasite of *W. bancrofti* settles down and produce microfilariae which enters circulation through thoracic duct and gets lodged in various organs like lungs, liver, spleen, lymph nodes, and bone marrow. Uzma Zafar *et al.* (8) reported an interesting case of low back pain and destruction of first lumbar vertebra by the microfilariae residing in the

**Table 1.** Improvements of haematological parameters after start of therapy

Investigations	At presentation	10 days after treatment	At the end of treatment	1 month after end of treatment
Hb (gm/dL)	9.4	12.3	12.8	13
TLC (per cmm)	3,000	4,200	4,700	5,100
DLC(N,L,E,M,B)	N-59, L-22, E-10.4, M-8, B-0.6	N-61, L-28, E-6, M-4, B-1	N-61, L-34, E-3, M-2, B-0	N-60, L-32, E-3, M-4, B-1
Haematocrit (%)	28.5	37.8	36.8	36.2
Platelet (per cmm)	88,000	90,000	98,000	120,000

**Table 2.** Regression of size of spleen after treatment

Spleen size	At presentation	5 days after treatment	10 days after treatment	At the end of treatment	1 month after end of treatment
Per abdominal palpation (in cm from left costal margin)	14	9	4	Just palpable	Not palpable
Ultrasonography (in cm)	22	16.24	15.3	12.5	11.6

bone marrow. There were several case reports of huge splenomegaly in leukaemia patients with incidental detection of bone marrow microfilariae (9-11). Possible explanation for splenomegaly in those cases were due to leukaemia not filariasis because spleen is usually not involved in filariasis except in some experimentally infected animals although Schuurkamp GJ *et al.* (14) showed occurrence of filarial splenomegaly in their articles.

But our case was unique because here splenomegaly was solely due to filariasis. It was further proved by regression of size of the spleen by antifilarial medications. A study conducted by Schuurkamp GJ *et al.* showed that splenomegaly in the Ok Tedi region of Papua New Guinea was solely due to Bancroftian filariasis, and splenomegaly due to filariasis could be controlled or even reduced by mass drug administration of DEC. This was observed in the 5 villages participating in the initial program. A significant reduction in splenomegaly by DEC was reported in another 7 villages in the expanded program during Phase 2; Enlarged spleen rates were reduced from 50% (1986) to 32% (1990) and from 76% (1988) to 48% (1990), respectively (14).

In conclusion, patients presenting from filaria endemic area with splenomegaly and with either symptoms or initial investigations suggestive, filaria should be given a serious thought with other well-known causes of splenomegaly. Because a simple regimen of DEC can make patient get rid of cumbersome splenomegaly, which is not commonly mentioned or known in the world literature.

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(Received July 19, 2017; Revised August 25, 2017; Accepted August 26, 2017)

## Sigmoid volvulus in young patients: A new twist on an old diagnosis

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### Summary

According to the literature, sigmoid volvulus typically develops in patients of an older age with co-morbidities such as a psychiatric illness or a bed-bound chronic illness. Recent reports suggest that it should also be considered in young individuals without any preceding medical history. Abdominal roentgenography is a cheap and effective diagnostic modality that can avoid a delay in diagnosis. The treatment of colonic volvulus remains controversial and relies upon the procedure selected and the most appropriate therapeutic approach in terms of the clinical status of the patient, the location of the problem, the suspected existence or identification of peritonitis, bowel viability, and the expertise of the surgical team. Presented here are four cases of young male patients with sigmoid volvulus. All of the patients were diagnosed radiologically prior to surgical intervention. Two of the patients initially underwent an endoscopic procedure that succeeded in one and that failed in the other. Three of the patients underwent a laparotomy.

**Keywords:** Ischemia, laparotomy, obstipation, roentgenogram, sigmoid volvulus, strangulation, torsion

### 1. Introduction

Large bowel volvulus is an uncommon cause of bowel obstruction in the industrialized world (1). However, it is four times more common in the Middle East than in Western countries (2). It constitutes the third leading cause of large bowel obstruction in Western countries after carcinoma and diverticulitis (3-5). India, Iran, and Russia have one of the higher incidences of sigmoid volvulus (6). Sigmoid volvulus occurs when the sigmoid colon twists axially around a narrow base of mesentery, leading to a close loop of bowel that is at risk of strangulation, ischemia, gangrene, and perforation (7,8).

Sigmoid volvulus is the most common cause of strangulation of the colon (4). It constitutes less than 10% of bowel obstructions in industrialized countries, compared with a rate as high as 25% in the developing regions of the world (9). Sigmoid volvulus, which often

develops during the 7<sup>th</sup> and 8<sup>th</sup> decades of life, has been reported in young people as well (10) Oren *et al.* (11) found that the mean age of its incidence was 58 years. Liang *et al.* (12) corroborated that finding, and Liang *et al.* noted the highest prevalence of sigmoid volvulus in the 7<sup>th</sup> decade of life. Slidell *et al.* (13) reported three teenagers ages 17-19 with sigmoid volvulus. A 19-year-old male with the same signs and symptoms was encountered by Salinas *et al.* (7). Reported here are four male patients with a mean age of 27.5 years who had abdominal pain and obstipation and who were diagnosed with acute sigmoid volvulus.

### 2. Case Report

#### 2.1. Case 1

A 25-year-old Indian laborer presented to the Emergency Department with abdominal pain and absolute constipation for three days. The man occasionally vomited as well. The pain was colicky in nature. The man had no past medical history. He was afebrile with a distended abdomen and mild generalized tenderness. Bowel sounds were exaggerated. A rectal digital examination revealed an empty rectum. Abdominal

Released online in J-STAGE as advance publication August 7, 2017.

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**Figure 1. Classic coffee-bean sign on plain abdominal radiograph in Case No. 1.**

roentgenography revealed a classic sign of sigmoid volvulus (coffee-bean sign) (3) (Figure 1). The man's WBC count was  $18,000/\text{mm}^3$  ( $4-11 \text{ mm}^3$ ) with a neutrophil percentage 80%. Serum electrolytes were: sodium 130 mmol/L (134-145 mmol/L), potassium 3.0 mmol/L (3-5 mmol/L), urea 5.5 mmol/L (2.9-9.3 mmol/L), and creatinine 110 micromol/L (80-115 micromol/L). Detorsion was attempted endoscopically but unsuccessful. A laparotomy was performed, revealing sigmoid volvulus.

#### 2.2. Case 2

A 25-year-old male of Arab origin presented to the Emergency Department with colicky abdominal pain. The man had no past medical history. He vomited twice during the last couple of hours he was seen. The man had not moved his bowels for one week. His abdomen was moderately distended and globally tender. Bowel sounds were audible. A digital rectal examination revealed that the rectum was empty. The man had previously experienced three episodes of abdominal pain. Those episodes were of lesser severity and responded to analgesics. Analgesics failed to relieve the man's pain, so he sought medical attention.

#### 2.3. Case 3

A 25-year-old male from the Indian sub-continent

presented to the Emergency Department after two days of central abdominal pain along with nausea and obstipation. Two years ago, the man suffered the same problem, but it was fortunately relieved by oral analgesics. When he was seen by the Emergency Department, he was afebrile. An abdominal examination revealed a distension with tympanitic percussion. Bowel sounds were present. Plain abdominal radiography revealed the coffee-bean sign indicative of sigmoid volvulus (3). Colonoscopy was attempted but was unsuccessful. A laparotomy was ultimately performed.

#### 2.4. Case 4

A 35-year-old male from the Indian sub-continent was seen by the Emergency Department after multiple episodes of vomiting (yellow-colored) along with generalized abdominal pain lasting three days. The man had no past medical history. Clinically, he was sickly looking. His WBC count was  $20,000/\text{mm}^3$  with a neutrophil percentage of 84%. Serum electrolytes were: sodium 128 mmol/L, potassium 2.8 mmol/L, urea 10.5 mmol/L, and creatinine 115 micromol/L. His abdomen was distended with tympanitic percussion. Bowel sounds were hyperactive. Initial plain abdominal radiography suggested sigmoid volvulus. A plain abdominal CT scan revealed the same features (Figure 2 and Figure 3). Colonoscopy revealed an obstruction 45 cm from the anal verge.

### 3. Discussion

Large bowel volvulus is more prevalent in the developing world, where it constitutes nearly 50% of all bowel obstructions as compared to only 5% in the developed world. In the Middle East alone, sigmoid volvulus is four times as common as it is in the Western world (1,2). There are a few contributing factors that are assumed to lead to large bowel volvulus in the Gulf region. The first is the dietary habits of the local population. People consume more junk food, less fiber (vegetables and fruits), and even less water. In addition, hot and humid weather leads to greater water loss from the body. Above all, a sedentary lifestyle is a leading factor for the development of sigmoid volvulus. Sigmoid Volvulus is more common in older patients, and especially those with a psychiatric co-morbidity (14,15). Sigmoid volvulus is also seen in patients suffering from Parkinson's disease, Alzheimer's disease, pseudobulbar palsy, and chronic schizophrenia (3). Oren *et al.* (11) reported a large series of 827 patients with a mean age of 57.9 years. Tiah *et al.* (16) studied 28 patients with an average age of 74 years and Liang *et al.* (12) studied 14 patients with an average age of  $68.4 \pm 12.2$  years. In a study of 32 patients by Heis *et al.* (17), only two were under the age of 30; out of 30 cases of acute sigmoid volvulus, Sule *et al.* (18) found that 4 patients



Figure 2. CT image of sigmoid volvulus in Case No. 4.

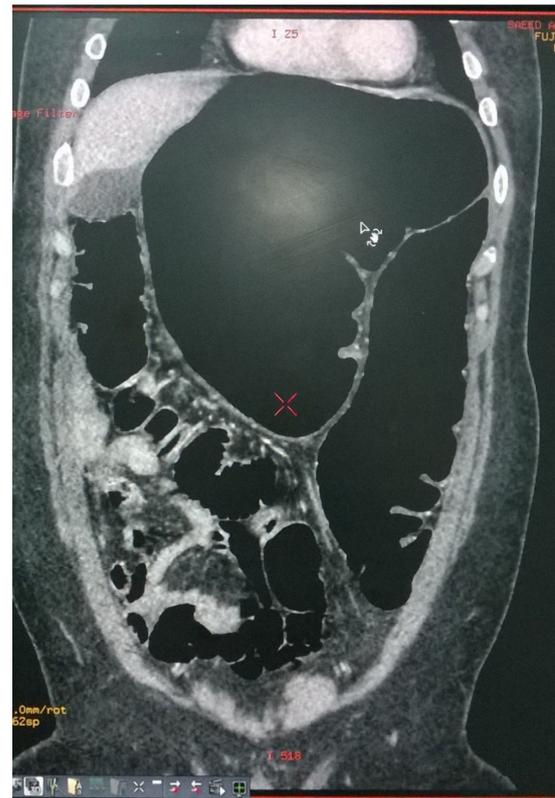


Figure 3. CT image of sigmoid volvulus in Case No. 4.

Table 1. Comparison of the number, gender, and mean age of patients in different studies

No.	Lead author of the study	Number of patients			Mean age (yrs)
		Male	Female	Total	
1	Sarfaraz M (2017)	4	0	4	27.5
2	Chalya PL (2015)	122	24	146	48
3	Salinas NL (2007)	1	0	1	19
4	Oren D (2007)	688	139	827	57.9
5	Tiah L (2006)	16	12	28	74
6	Liang JT (2006)	10	4	14	68.4 ± 12.2
7	Agaoglu N (2005)	21	11	32	73.5 ± 8.38
8	Slidell M (2004)	3	0	3	18
9	Raveenthiran V (2004)	42	39	81	50
10	Turan M (2003)	61	20	81	65

were between the ages of 20-30 years. Slidell *et al.* (13) reported three teenagers with sigmoid volvulus. Salinas *et al.* (7) encountered a 19-year-old male with the same condition. In a study of 28 patients with acute sigmoid volvulus by Lou *et al.* (19), 19 were male and 9 were female with a mean age of  $63.1 \pm 22.9$  years. All of the current patients were male. Three were 25 years old and one was 35 years old, representing a mean age of 27.5 years. All of the current patients were otherwise healthy with no noticeable medical illness (Table 1). Three of the current patients (75%) presented after 2-3 days while one was brought to the Emergency Department within a few hours of the onset of abdominal pain and vomiting.

Classically, sigmoid volvulus can be divided into two subtypes on clinical grounds, as described by

Hinshaw and Carter (20). Acute fulminating volvulus, caused by complete obstruction, clinically presents as sudden onset of central abdominal pain accompanied by emesis and constipation. Gangrene and perforation are common early complications of this type of volvulus. With subacute progressive volvulus, patients have only a partial obstruction and more insidious onset. Older patients frequently have the subacute form (21). The patients encountered by Heis *et al.* (17) presented with abdominal pain (96%), distension (84%), vomiting (72%), and constipation (63%). Kuzu *et al.* (22) noted abdominal distension in 89% of patients and vomiting in 64%. All of the current patients had abdominal pain, 75% vomited, and one only had nausea. Fifty percent of the current patients had constipation and 50% had

obstipation. All were afebrile.

Plain abdominal X-rays are a rapid and useful diagnostic tool (4,23). The classic coffee-bean sign on plain abdominal radiographs was found in 68.7% of patients encountered by Heis *et al.* (17) and in 68.5% of patients encountered by Khanna *et al.* (24). Javors *et al.* (25) found that sigmoid volvulus was diagnosed with X-rays in 87% of patients while Welch and Anderson (14) found that it was similarly diagnosed in 83% of patients. Tiah *et al.* (16) reported that sigmoid volvulus was diagnosed with plain abdominal radiography alone in 34.6% of cases. In suspected cases, a barium enema is recommended both as a diagnostic and therapeutic tool (26). CT provides the additional advantage of excluding other causes of intestinal obstruction in uncertain cases and also facilitating diagnosis of complications, such as a perforation (27). Early diagnosis can prevent ischemia or perforation (25) particularly in younger patients, in whom the chances of a misdiagnosis or a delayed diagnosis are higher (13).

A redundant segment of colon with a short mesentery and close proximity to the point of fixation to the segment are predisposing factors for the development of volvulus (2). Torsion of the sigmoid colon occurs along its mesenteric axis and axial torsion occurs around the axis of the bowel, leading to volvulus (28). The ileo-sigmoid knot is a rare but serious abdominal emergency in which the ileum and sigmoid entangle each other to form a knot; this can lead to vascular compromise and gangrene of both the ileum and sigmoid colon (21). Venous congestion compromises the colonic blood supply, which occasionally alleviates venous infarction and gangrene. Although less common, involvement of the arterial blood supply can expedite colonic ischemia.

The treatment of sigmoid volvulus remains controversial. It depends on the procedure selected in light of the clinical status of the patient, the location of the problem, the suspected existence or identification of peritonitis, bowel viability and, last but not least, the expertise of the surgical team (21). Surgeons generally have two surgical options. First, a single-stage procedure in which, initially, endoscopic derotation is followed by semi-elective sigmoidectomy and primary anastomosis (29). The second option is available when decompression fails and there are signs of colonic gangrene. This surgery is in two stages. In the first, a sigmoid resection and Hartman's procedure or a double-barreled colostomy is performed in order to avoid a high rate mortality with primary anastomosis in that situation. In the second stage 6-8 weeks later, Hartman's procedure is reversed or the colostomy is closed (30,31).

Presented here are unconventional cases of four young patients with a median age of 27.5 years. All of those patients had cramping abdominal pain, usually of several days' duration. One of the patients experienced pain a few hours prior. Common symptoms among the patients were absolute constipation for a couple of days

with occasional vomiting. The diagnostic tool Used was simple abdominal radiography. Three patients underwent Hartman's procedure and one was managed with sigmoidoscopic decompression and semi-elective single-stage sigmoid resection with primary anastomosis.

#### 4. Conclusion

Acute sigmoid volvulus is a common differential diagnosis in older patients who are bed-bound or who have a psychiatric co-morbidity. This condition should also be included in the differential diagnosis of young patients with colicky abdominal pain and absolute constipation. The chances of a misdiagnosis or a delayed diagnosis are greater when the symptoms are mild and recurrent. Plain abdominal radiography is a simple, inexpensive, and widely available diagnostic tool that should be used to screen for this rare but serious condition.

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(Received June 3, 2017; Revised July 14, 2017; Accepted July 20, 2017)

# Metastatic neuroendocrine tumor of the esophagus with features of medullary thyroid carcinoma

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## Summary

A 41-year-old female presented with a pedunculated mass in the upper esophagus and bilateral lymphadenopathy. Biopsies suggested a neuroendocrine tumor, possibly carcinoid, and ensuing imaging revealed cervical lymph node metastases. The esophageal mass was removed endoscopically and discovered by pathologists to closely resemble medullary thyroid carcinoma (MTC) on immunohistochemistry staining. Following surgery, further work up demonstrated very high serum calcitonin levels, suggestive of medullary thyroid carcinoma, however the thyroid gland was normal on ultrasound. The patient underwent a neck dissection to remove the lymph node metastases and subsequently her calcitonin levels dropped to 0 ng/mL, indicating remission. It appears that the primary tumor was not in the thyroid, but in the cervical esophagus. The thyroid has appeared normal on multiple ultrasounds without any detectable nodules or masses. This is quite a unique case because this patient presented with a tumor resembling medullary carcinoma of the thyroid that presented as a pedunculated mass in the cervical esophagus. The actual final diagnosis of this mass in the cervical esophagus was neuroendocrine tumor (NET), consistent with a carcinoid tumor, not ectopic MTC. This case report highlights that calcitonin-secreting tumors outside the thyroid should not lead to erroneous recommendations for thyroidectomy.

**Keywords:** Esophageal neuroendocrine carcinoma, esophageal carcinoid, neuroendocrine tumors, esophageal neoplasm, calcitonin

## 1. Introduction

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are a rare heterogeneous group of tumors that can involve any part of the gastrointestinal tract (1). Esophageal neuroendocrine tumors are exceedingly rare and are the rarest of the GEP-NETs, representing approximately 1% of all neuroendocrine tumors (1).

GEP-NETs originate from diffuse neuroendocrine cells which are unique in their ability to synthesize and secrete neuropeptides and hormones, the most common of which are synaptophysin, chromogranin and CD56 (1). Esophageal neuroendocrine tumors are found more commonly in men, in the sixth decade of life (2,3). Our research objective in presenting this case study was to describe a unique esophageal neuroendocrine tumor that was found in a young, female patient that secreted calcitonin, in addition to the neuropeptides chromogranin and synaptophysin that are commonly found in esophageal neuroendocrine tumors.

Released online in J-STAGE as advance publication August 19, 2017.

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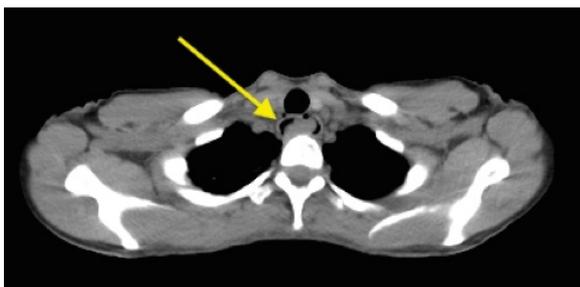
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## 2. Case Report

A 41-year old African American female with a chief

complaint of fatigue was being evaluated for chronic iron deficiency anemia. Past medical history was significant for iron deficiency anemia and menorrhagia due to uterine leiomyomas. She had a surgical history of inguinal herniorrhaphy and her only medications included iron supplementation and naproxen. On a review of symptoms, she was asymptomatic except for mild dysphagia with an occasional feeling of food sticking while swallowing. She denied weight loss, odynophagia, hemoptysis, and hematemesis. There were no focal changes and no shortness of breath. She denied any history of smoking, excessive alcohol use, or any radiation exposure. Her family history was significant for two first-degree relatives with colon cancer and one first-degree relative with breast cancer. Due to the strong family history of colon cancer, she underwent screening with upper and lower endoscopy as a preventive measure. Colonoscopy was unremarkable but upper endoscopy revealed a large pedunculated polyp at the pharyngoesophageal junction. A biopsy was taken of the polyp and was interpreted as a well-differentiated and low-grade NET, consistent with a carcinoid tumor. The discovery of this rare GI tumor and the strong family history of multiple GI tumors prompted a referral to a medical genetics specialist, however the evaluation was non-contributory. Further evaluation of the esophagus was necessary to find the primary site and the first echelon lymphatic basins in the mediastinum and neck. Ensuing work-up included CT scans of the abdomen and pelvis, which were negative for distant disease. Chest CT revealed the pedunculated mass on the anterior wall

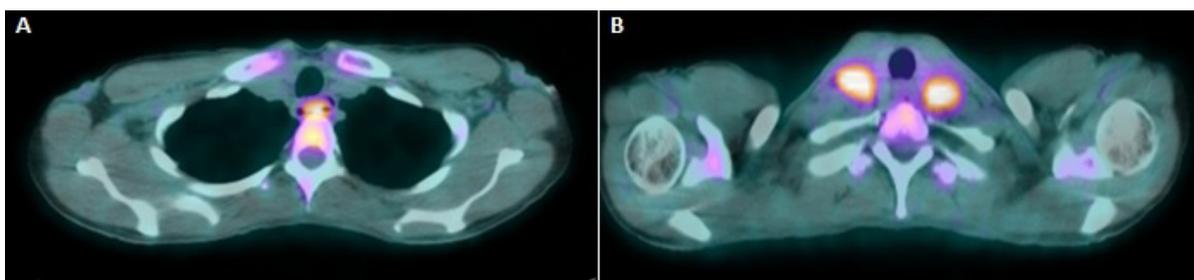


**Figure 1.** Chest CT showing the mass on the anterior wall of the cervical esophagus.

of the cervical esophagus (Figure 1). Octreotide scan was non-contributory, however the fludeoxyglucose (FDG) PET/CT displayed the metabolically active esophageal lesion as well as two active bilateral neck lymph nodes (Figure 2A and 2B).

Follow-up included endoscopic evaluation and possible removal. Pre-operative metabolic panel revealed mild hypocalcemia at 8.0 mg/dL but no contraindications to surgery. During the surgery, the tumor was visualized endoscopically which showed a very mobile pedunculated mass on a stalk that was pedicled within the anterior esophagus, close to the tracheal party wall just left of the midline. The mass was excised transorally and sent for permanent histology (Figure 3A and 3B). Additional deep tissue and mucosal biopsies were taken which showed negative margins. An additional biopsy of the lymph node on the left side of the neck was taken for permanent histology. Post-operatively, the patient recovered well with no complications.

Shortly after, we received the pathology interpretation of immunohistochemistry staining, which became a turning point in our observations. We previously thought we were managing an esophageal carcinoid tumor, a rarity itself, however the pathology results informed us that the tumor actually had features of medullary thyroid carcinoma (MTC) since calcitonin staining was positive. This peculiar finding incited further evaluation. Supportive of the pathologist's interpretation, serum calcitonin levels were in fact markedly elevated at 570 pg/mL (Normal is < 5.0 pg/mL), which implied metastatic disease especially since this was measured two months post-operatively. At this point, the possibility of completely missing a primary medullary carcinoma from the thyroid was considered, however, the ensuing thyroid and neck ultrasound did not show any thyroid nodules but did show three level II nodes on the right and two level II nodes on the left. Thyroid hormone levels were also normal. Serum chromogranin A and serotonin were normal, which disfavors carcinoids, and the 24-hour urine metanephrines were negative, ruling out a concurrent pheochromocytoma. Serum CEA levels were negative as well, which are usually positive in MTC. A PET scan showed mild uptake in bilateral neck lymph nodes



**Figure 2.** PET/CT displaying. (A) metabolically active mass in the upper cervical esophagus (2.5 × 1 cm with standardized uptake value (SUV) of 3.23); (B) bilateral cervical lymph node metastases.

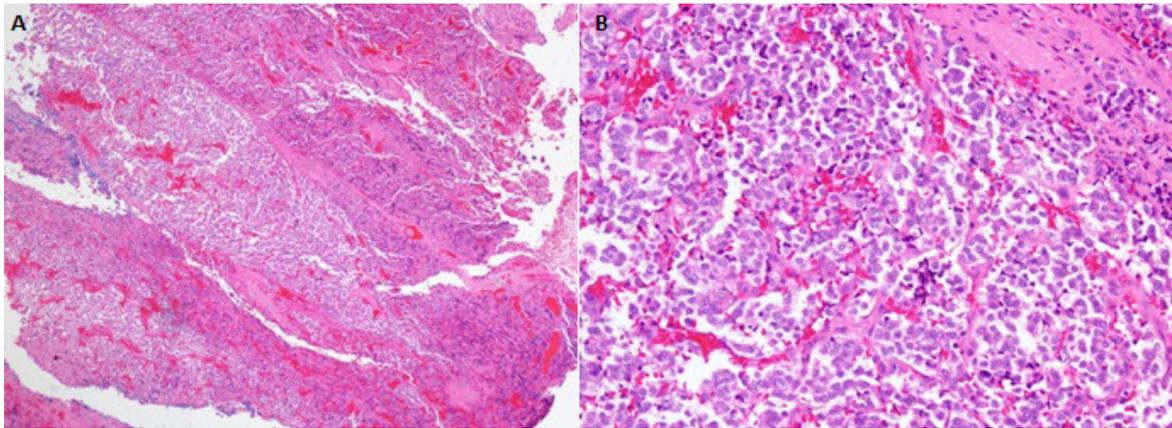


Figure 3. Pedunculated esophageal mass at (A) 4×, (B) 20×.

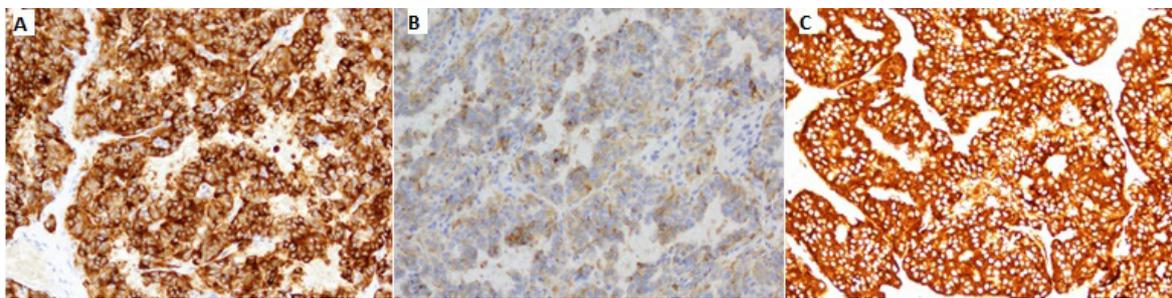


Figure 4. Left neck dissection lymph node at 20× showing positive immunohistochemistry staining for (A) Calcitonin; (B) Chromogranin; and (C) Synaptophysin.

and subsequent fine needle aspiration on the right was interpreted as neuroendocrine carcinoma, which was identical on the left. However, no lesions were present in the thyroid. The patient was still doing very well and had no symptoms except for mild bilateral lymphadenopathy on physical exam. At this point, the next step in management was puzzling because high calcitonin levels usually advocates for removing the thyroid gland, yet this patient had a normal thyroid on imaging. The only reasonable explanation was that the primary tumor was from the esophagus.

The patient had surgery for the second time where she underwent a bilateral neck dissection to remove the metastatic spread to the lymph nodes. The thyroid gland was left untouched. 3/30 excised nodes demonstrated the previous diagnosis of metastatic well-differentiated neuroendocrine carcinoma with features of medullary thyroid carcinoma. Immunohistochemistry was positive for calcitonin (Figure 4A), chromogranin (Figure 4B) and synaptophysin (Figure 4C), which are suggestive of medullary thyroid carcinoma, and negative for CEA, serotonin, and P53. Ki-67 stain showed 3% of cells in a proliferative phase. The patient did very well post-operatively without any complications. Although the diagnosis was labeled as neuroendocrine tumor, the pathologists continued to be suspicious for medullary thyroid carcinoma. Thyroid ultrasounds and PET scans failed to reveal any thyroid disease. Following the second surgery, serum calcitonin levels normalized

which proves that the cancer was fully resected and that the primary was most likely from the esophagus. It was decided that adjuvant radiation therapy was not needed.

The patient's disease was indolent and caught early. She has remained healthy without evidence of recurrence. After two years of follow-up, the patient's only complaints have been acid reflux and occasional fatigue. Since she presented with low-neck metastases, she has received surveillance chest CTs watching for interval changes, which have all been negative. Since part of the differential diagnosis indicated possible medullary thyroid carcinoma based on pathology, the patient has had regularly scheduled thyroid ultrasounds and calcitonin levels which all have remained negative. Due to the very unusual nature of this case, we will continue to follow-up with this patient indefinitely to screen for recurrence and occult MTC.

### 3. Discussion

Neuroendocrine tumors (NETs) consist of a broad spectrum of heterogeneous tumors originating from the diffuse endocrine system. They share certain features such as similar appearance, presence of secretory granules, and production of various hormones through an amine precursor uptake and decarboxylation (APUD) mechanism (4). Despite these similarities, there are substantial differences in clinical behavior, often attributed to the site of origin. NETs most commonly

arise in the small intestine but can develop in the lung, pancreas, and other areas, each with different incidences and presentations. NETs are challenging to diagnose and the lack of inclusive knowledge has led to periodic changes in classification systems (4). One of the difficulties is that the current understanding of NETs has accumulated from different directions, causing use of extraneous terminology. In an effort to simplify this, NETs are currently classified under two groupings, the pancreatic NETs and the carcinoids (5).

The determination of NETs is made by the morphology and the presence of neuroendocrine biomarkers. Biomarker workup is useful for screening and diagnosis, but since there is no standardized panel established, the serum markers to investigate should be tailored to the patient's clinical presentation. The general immunohistochemical markers indicating neuroendocrine differentiation are synaptophysin, chromogranin and CD56 (6). In the current World Health Organization (WHO) classification system, NETs are graded using the Ki-67 proliferative index and mitotic rate. There is not a standard staging criterion for TMN classification, but use of either the AJCC or ENETs systems is acceptable. NETs presenting with mixed or unusual histotypes are treated with focusing on the most aggressive component that is present. Since medullary thyroid carcinoma was in our differential diagnosis, it was obligatory to rule out this aggressive malignancy. Another challenge with NETs is that patients' symptoms are rarely linked to the correct diagnosis initially, which leads to a delay in proper treatment. For example, many gastrointestinal carcinoids are initially misdiagnosed as irritable bowel syndrome, lactose intolerance, or celiac sprue (4). In addition, many NETs do not become symptomatic until late in the disease course when there is advanced metastases, therefore prognosis can be poor (5). Our patient was fortunate that her NET was discovered incidentally on screening upper endoscopy, especially since she already had early metastatic disease at the time of diagnosis.

Since the neuroendocrine system is not as well developed in the esophagus, it is rare for NETs to grow in this region (4). NETs in the cervical portion of the esophagus are especially rare and its analysis can be difficult. Generally, most primary esophageal cancers occur in the lower two thirds of the esophagus. While there are many advanced secondary cancers of thyroid and trachea that can extend into the cervical esophagus, isolated malignancies in this region are rare. Some authors group cervical esophageal cancer along with hypopharyngeal cancer, which is fitting due to overlapping embryology and anatomy (7). Most primary tumors of the cervical esophagus include SCC and adenocarcinoma. Post-cricoid cancers tend to arise in females and are associated with Plummer-Vinson Syndrome. These patients also present with iron-deficiency anemia and severe GERD. NETs in

the esophagus may represent as little as 0.05-2.4% of all esophageal cancers (8). Esophageal NETs have a poor prognosis because they are aggressive and often present at advanced stages with metastases. Of the few esophageal NETs reported, most occurred in the distal third of the esophagus in the presence of concurrent Barrett's esophagus (9).

Since our patient's cervical tumor stained positive for calcitonin on immunohistochemistry, the possibility of metastatic medullary thyroid carcinoma (MTC) was considered. MTC is the NET of the thyroid gland, which arises from neoplastic parafollicular C cells and is responsible for 5-10% of all thyroid malignancies (10). It presents usually in the fourth decade and has a female predominance. Approximately 80% of cases are sporadic and 20% of cases are hereditary, which are part of the multiple endocrine neoplasia (MEN) syndromes due to a mutation of the RET proto-oncogene. An elevated serum calcitonin level is the classic marker for MTC. Calcitonin is useful for detection, staging, post-op management, and prognosis (11). Higher levels of calcitonin are associated with greater likelihood of MTC, with values > 100 pg/mL correlating extremely high for MTC, as seen in our patient (12). Calcitonin levels > 150 ng/mL are associated with distant metastases. Therefore, systemic imaging of the thorax, liver, and bones is indicated (13). However, since our patient's thyroid gland was normal on neck ultrasound, metastatic disease originating from the thyroid gland was very unlikely. This concerning finding raised questions in management because if we were dealing with MTC, then early surgical removal would be necessary, especially since this patient had lymph node metastases, which is already associated with a significantly poorer prognosis (14). Normally, survival is dependent upon the adequacy of the initial surgical procedure, accomplished by total thyroidectomy and bilateral lymph node dissection (13). Serum calcitonin alone has a high false positive rate corresponding with a low positive predictive value that may lead to unnecessary thyroid surgery (15). Serum CEA is a useful marker in clinically evident MTC and the doubling time is useful in postoperative surveillance (16). Our patient's CEA was negative in both serum and immunohistochemistry staining, but CEA may have low sensitivity in less aggressive or occult disease, especially in the absence of a thyroid nodule (15). Currently, the most accurate method of measuring serum calcitonin is with immunoassays. Calcitonin is somewhat of a heterogeneous hormone and this test can accurately distinguish the epitope associated with MTC. Currently, RET mutation analysis is being used to identify individuals at risk and determine the timing of prophylactic thyroidectomy (16). Because our patient's thyroid gland was normal appearing and she did not have a hereditary syndrome, there was no clear indication for thyroidectomy, so the decision was made to preserve it. At this point, the differential diagnosis of

metastatic calcitonin-secreting NETs in the esophagus was debatable.

While there are well known cases of ectopic follicular and papillary thyroid carcinomas, ectopic medullary thyroid carcinoma is exceptionally rare and the mechanism has yet to be described. Ectopic medullary thyroid carcinoma has only been reported twice in the literature, once in a lingual thyroid (17) and another in the submandibular region (18). Medullary thyroid carcinoma develops from neoplastic parafollicular C cells, which have a separate lineage from the thyroid follicles. Most ectopic thyroid tissue is negative for calcitonin staining on immunohistochemistry, indicating a failure of colonization by the c-cells from neural crest (19). Although hypercalcitoninemia is highly indicative of MTC, it is not pathognomonic. It is important to identify other possible etiologies of hypercalcitoninemia. Causes of elevated serum calcitonin include conditions such as hypercalcemias, hypergastrinemias, renal insufficiency, other thyroid disorders, and other NETs. Elevated serum calcitonin can also be observed with prolonged use of certain treatments, such as omeprazole, beta-blockers, glucocorticoids, and potential secretagogues (20). Cancers such as bronchogenic carcinoma and small cell lung cancer (SCLC) may secrete high levels of calcitonin. SCLC, in particular, may grow up into the neck making it indistinguishable from primary MTC that has spread down into the mediastinum. This distinction is especially important since SCLC treatment involves chemotherapy (21). There are several case reports of NETs in the larynx associated with elevated serum calcitonin levels, closely resembling medullary thyroid carcinoma. These lesions were often supraglottic or glottic. Similar to our case, these were polypoid lesions that arose in the mucosa and submucosa. It was suggested that these calcitonin-secreting laryngeal NETs represented ectopic medullary thyroid carcinomas, but this is uncertain and probably difficult to ascertain because they were classified using different systems (22). At any rate, the evidence points to a NET of the esophagus as the diagnosis in this case, as opposed to ectopic MTC, as normal thyroid tissue was not present on histopathology.

#### 4. Conclusion

There are several causes of hypercalcitoninemia, few of which are due to head and neck tumors. This case report underlines that calcitonin-secreting tumors outside of the thyroid should not lead to erroneous recommendations for thyroidectomy. Calcitonin immunoassays are available and should be utilized more frequently when the diagnosis is questionable. This case report further elucidates the need for more comprehensive clarification of neuroendocrine tumors, which can be challenging and diagnosed late. Knowledge of the embryological development of C cell migration or factors promoting

differentiation of calcitonin-secreting cells may be key in understanding how anomalies appear clinically and how they should be managed. Certain signals and factors cause neuroendocrine cells to differentiate into the calcitonin secreting cells of the thyroid. Perhaps rest cells of neuroendocrine origin in the esophagus lining led to a calcitonin-secreting tumor, much like that of medullary thyroid carcinoma.

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*(Received June 23, 2017; Revised August 5, 2017; Accepted August 16, 2017)*

# A case of leprosy, erythema nodosum leprosum, and hemophagocytic syndrome: A continuum of manifestations of same agent-host interactions

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## Summary

A young adult man with 4-years history of lepromatous leprosy (received irregularly multidrug therapy) presented with two and half years history of symptoms suggestive of chronic erythema nodosum leprosum (ENL), initially responded to steroids and thalidomide, but later on failed. During the last 2-months, he developed fever, vomiting, and subsequently altered sensorium. On evaluation, he had hepatosplenomegaly, hyponatremia, hyperferritinemia, hemophagocytosis in bone marrow aspiration, lobular panniculitis in skin biopsy, and multiple parenchymal nodules in chest imaging. Hence diagnosis of ENL with hemophagocytic lymphohistiocytic (HLH) syndrome was established and treatment with dexamethasone (10 mg/m<sup>2</sup>) started. During hospitalization, he developed sinus bradycardia, QT prolongations, recurrent ventricular tachycardia, and moderate systolic dysfunction. The cardiac complications recovered using a temporary pacemaker and were presumed to be due to micronodular cardiac deposition of ENL. This case iterates that ENL can present with varied presentations like asymptomatic lung nodules and storming cardiac complications. More importantly leprosy, ENL, and HLH are a continuum of manifestations of the same agent-host interactions.

**Keywords:** Cardiac arrhythmia, hemophagocytic lymphohistiocytic syndrome, lepromatous leprosy, pulmonary nodule, type 2 lepra reaction

## 1. Introduction

In the era of leprosy elimination, a greater concern is the lepra reaction [erythema nodosum leprosum (ENL, type 2 reaction), reversal reaction (type 1 reaction), or Lucio's phenomena. It represents an acute episode that interrupts slow, insidious, and chronic evolution of leprosy. The ENL, characterized by painful erythematous nodulations that grows in apparently normal skin, is seen mainly in the lepromatous type of leprosy patients (1). Although there is a consensus of decreasing cases of ENL after the inclusion of

clofazimine containing multidrug therapy (MDT), however, the greatest concerns are associated with systemic manifestations, morbidity, and mortality (2).

Hereby, we report a typical case of chronic ENL presenting with hemophagocytic syndrome, cardiac dysfunction, and asymptomatic lung nodules.

## 2. Case Report

A 26-year-old man was diagnosed as a case of Hansen's disease (multibacillary type as per slit smear) in February 2013. After continuous 6-months of treatment (MDT - rifampicin, clofazimine, and dapsone), he discontinued the treatment of his own when symptoms resolved. In October 2014, he developed multiple skin nodules over the body, fever, and joint pain. He was diagnosed as ENL and treated with prednisolone and

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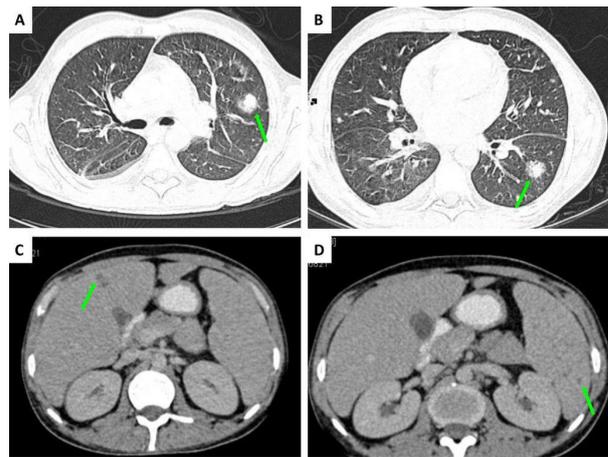
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restarted with MDT. During the tapering of steroids, he again developed similar symptoms and thalidomide was added and later on prednisolone was stopped. Symptoms improved. He also received MDT again, continued for 12-months. In April 2016, during the tapering of thalidomide, he again developed ENL symptoms; the dose of thalidomide was increased but symptoms didn't improve; and prednisolone was restarted along with MDT. But symptoms didn't improve and further worsened.

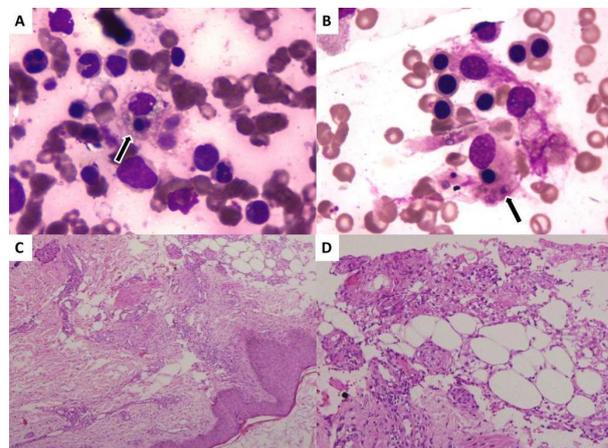
In September 2016, he presented to our hospital with above ENL symptoms. Along with these symptoms he had upper abdominal pain, vomiting, decreased oral intake, and leg swelling for 2-months and altered sensorium for 3-days.

On examination, he was drowsy and pale, and had pedal edema. Hemodynamically he was stable. Spleen and liver were enlarged. There was swelling and tenderness over both ankles and knees with multiple red nodular skin lesions over both shins. Other systemic examinations were unremarkable except for drowsiness. On day of presentation, his sensorium worsened without any focal neurological deficits and he developed respiratory distress. Mechanical ventilation was started and emergency investigations showed hyponatremia ( $\text{Na}^+$  125 meq/L). After correcting sodium imbalance, his sensorium improved and he was extubated early. The hyponatremia was considered due to either long standing vomiting after use of multiple drugs (steroids, thalidomide, and MDT) or a part of the disease process. All previous ongoing drugs were stopped temporarily except steroids.

Routine hemogram showed Hb, 5.7mg/dL; WBC,  $16.5 \times 10^9/\text{L}$ ; platelet count,  $136 \times 10^9/\text{L}$ ; and ESR, 72mm/hr. Test for malaria, dengue, chikungunya, hepatitis B and C, and HIV were negative. CSF examination was also unremarkable. Routine cultures were sterile. Autoimmune tests including RA factor and ANA were negative. CECT chest and abdomen was performed that showed hepatosplenomegaly with multiple hypodense lesions in the liver and spleen, and multiple lung nodules (Figure 1) suggesting a possible disseminated fungal infection or ENL. Furthermore, bronchoalveolar lavage was performed that was negative for bacterial and fungal infections including a galactomannan study. However, antibacterials (cefoperazone and levofloxacin) and antifungal treatment (voriconazole) were started for possible superadded bacterial/fungal infections because of his prolonged immunosuppressed state, but symptoms didn't improve. Serum ferritin was high (2,000 ng/dL). Hence, secondary hemophagocytic lymphohistiocytosis (HLH) syndrome was suspected considering fever and hepatosplenomegaly. Bone marrow (BM) examination confirmed hemophagocytosis (Figures 2A and 2B). His serum triglyceride level was 98 mg/dL. NK cell activity was not done. HScore, an online software to determine the probability of the syndrome, was calculated, and

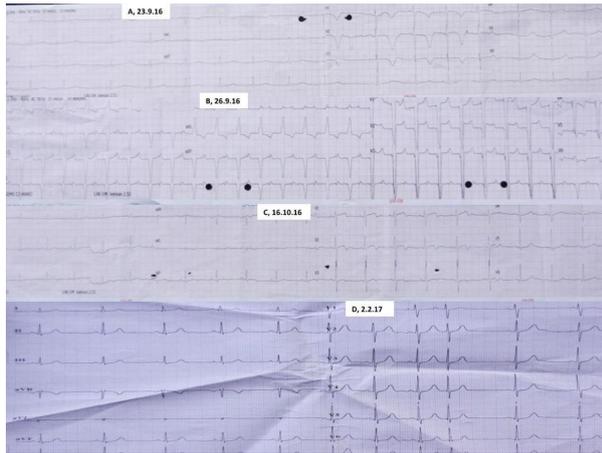


**Figure 1. Contrast-enhanced computed tomography of chest and abdomen.** Axial images of lung windows (A/B) revealing multiple parenchymal nodules (arrows) in both lungs without any cavitation and of abdomen (C/D) showing focal hepatic and splenic hypodense lesions (arrows).



**Figure 2. Photomicrographs of Bone marrow aspiration and Skin biopsy.** Giemsa Stain of Bone marrow aspiration (A  $\times 1,000$ , B  $\times 1,000$ ) showing multiple histiocytes with erythrophagocytosis. Hematoxylin and Eosin stain of Subcutis (C  $\times 100$ , D  $\times 400$ ) showing septal thickening, fibrosis, and inflammation comprised of lymphocytes, neutrophils, and histiocytes with spill over into the fat lobules, suggesting lobular panniculitis, possibly ENL. Modified Fite stain was negative.

found to be 194 (probability of HLH, 84%). Since both ENL and HLH as inflammatory conditions require steroids, he was started on injection dexamethasone ( $10 \text{ mg/m}^2$ ) and his symptoms dramatically responded in few days. To know the primary cause of HLH, USG guided fine needle aspiration cytology of the liver was performed, which showed inconclusive results (repetition could not be performed because of major cardiac issues as described below). Skin biopsy of the nodular lesions was performed suggesting a lobular panniculitis and features were compatible with ENL with negative Fite stained bacilli (Figures 2C and 2D). A slit smear also was negative. Thalidomide (100 mg OD) was continued. Considering viable lepra persists as triggers for both inflammatory conditions, especially HLH, he was



**Figure 3. Electrocardiographs (ECG).** On first day of cardiac problem, ECG (A) showing sinus bradycardia (HR, 55), inverted P waves (inferior leads), deep T wave inversions (anteroseptal leads), and prolonged QT intervals (QTc, 516 ms). Post-pacemaker ECG (B) showing persistence of prolongation of QT intervals. However, one month later after removal of pacemaker, ECG (C) showing normalization of QT interval but persistent sinus bradycardia. On recent follow up, ECG (D) showing persistent sinus bradycardia with variable block.

restarted with MDT intensively with dapsone (100 mg OD) and rifampicin (600 mg monthly).

However, during the hospital course, there were continuous brainstorming cardiac events that needed detailed description. Before biopsy results were obtained, one early morning, he developed retrosternal chest pain, typical of angina, with hypotension (BP, 80/60 mm/Hg). ECG showed anteroseptal deep T wave inversions with sinus bradycardia, inverted P waves, and QT prolongations (QTc, 516 ms), suggesting right ventricular myocardial infarction (Figure 3A), confirmed by screening 2D-Echo. He was given IV fluids and dual antiplatelets. Although symptoms improved except for persistent bradycardia and QT prolongation, in a few hours, he developed ventricular tachycardia (VT). He received defibrillation and VT resolved. Suspecting drug related arrhythmia, voriconazole and levofloxacin were stopped. However, again VT recurred and a temporary pacemaker was inserted and cardiac rhythm stabilized (Figure 3B). There were no electrolyte disturbances. Routine 2D-Echo showed moderate systolic dysfunction (EF-40%). After 3-weeks as the patient improved, the pacemaker was removed, however, sinus bradycardia was persistent with resolving T wave inversion (Figure 3C). To determine the local cause of these cardiac problems, cardiac-MRI was performed, which was normal, although it could not preclude small ENL granuloma causing these cardiac abnormalities, since the patient had already received high dose steroids for more than 3-weeks. Antiplatelets were stopped. The patient was discharged in hemodynamically stable condition with MDT, thalidomide, and a tapering steroid dose as per HLH protocol. Finally he was diagnosed as a case of chronic ENL with HLH syndrome and cardiopulmonary

involvement.

In a recent follow up, he was completely symptom free and ECG showed persistent sinus bradycardia with a variable block (Figure 3D). Repeat CT-chest and abdomen revealed disappearance of nodules in lungs, liver, and spleen. Treatment plan is to continue intensive MDT [rifampicin for 5-years, dapsone for life (to inhibit viable persisters)] and thalidomide for 6-months at least.

### 3. Discussion

Type 2 lepra reaction *i.e.* ENL is an immune-complex mediated disease. It is usually precipitated by lepromatous leprosy or borderline lepromatous disease with high bacterial load, pregnancy, lactation, puberty, intercurrent infection, vaccination, stress and use of interferon-gamma; however, sometimes it occurs spontaneously similar to our case (3).

ENL can affect any part of the body unpredictably during any time in the course of leprosy. Only active vigilance in society can help in detection of various manifestations of it. Hematological involvement in it are mainly chronic disease anemia, hemolytic anemia, leukocytosis, hyperfibrinogenemia, and sometimes a prothrombotic state (4). HLH is a very rare manifestation. There are only two reported cases of HLH in ENL with the simultaneous presence of multibacilli leprosy (5-6). Furthermore, lepra bacilli is a known trigger for HLH even without ENL. Even isolated hemophagocytosis have been reported without HLH syndrome in leprosy (3). Our case represents the first described HLH case in association with ENL without evident lepra bacilli.

Triggers for HLH in our case are yet to be answered. As we know lepra bacilli can be dormant for lifelong as viable persisters. At any point in time under a favorable environment (*e.g.* stoppage of MDT treatment), the bacilli or their antigens can act as a trigger for an inflammatory state like ENL or HLH or both in a continuum like our case. In this regard, our case represents a possibility of leprosy, ENL, and HLH as progressive stages of manifestations based on host-antigen interactions. To make matters more intriguing, ENL presents with a similar clinical picture of dapsone hypersensitivity syndrome. In such a scenario, BM evaluation can be of great help to differentiate these entities. In our case, skin biopsy suggested ENL and BM revealed HLH. HSscore, often used for diagnosing HLH syndrome (<http://saintantoine.aphp.fr/score>), was higher suggesting an 84% chance of the possibility though we could not perform all diagnosed criteria (*e.g.* NK cell activity, soluble CD25) of the HLH-2004 protocol because of technical constraints.

Cardiac involvement has not been reported in ENL but is a known complication of leprosy, especially lepromatous type (8).

The cardiac conduction system is mainly affected in leprosy in the form of variable heart rate, ST and T

wave changes, bundle branch block, extrasystoles, and prolongation of QT interval (9). Sudden cardiac death has also been reported. In our case, there is a possibility of granulomatous/nodular deposition in the cardiac tissue resulting in various rhythm disturbances and structural dysfunction. This possibility is considered after: ruling out HLH as a cause of cardiac problems since myocarditis is the usual manifestation and conduction abnormality has not been reported yet in this condition; and ruling out drug induced QT prolongation (voriconazole and levofloxacin) since after stopping the drugs it was not seen and structural dysfunction was not seen either.

Respiratory involvement has been described scarcely in ENL in contrast to frequent nasal and laryngeal involvement in leprosy patients. Only three cases of ENL in a radiological survey in 1976 have been reported to have lung parenchymal nodules that disappear subsequently with time (10). These are thought to be an allergic reaction to lepra antigen similar to a generalized immunological reaction. Our case describes a nodular lung involvement, possibly due to granuloma of ENL similar to their presence in the liver and spleen. This is another uniqueness to our case, however, a biopsy could not be done because of underlying cardiac issues. Interestingly, all nodules disappeared after treatment as was evident on a recent CT-scan.

In conclusion, the present case represents few rare manifestations of ENL including HLH, storming cardiac complication, and asymptomatic lung nodules. Furthermore, one should understand that leprosy, ENL, and HLH are a continuum of manifestation of the same agent-host interactions seen in the course of the illness. Hence, one should take precautions in withdrawing MDT and immunosuppressants in chronic/recurrent ENL patients.

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(Received August 5, 2017; Revised August 23, 2017; Accepted August 26, 2017)

**Correction**

DOI: 10.5582/irdr.2017.E1

**Correction: Novel *SLC16A2* mutations in patients with Allan-Herndon-Dudley syndrome**

This corrects the article "Novel *SLC16A2* mutations in patients with Allan-Herndon-Dudley syndrome" in volume 5 on page 214-215.

**Keiko Shimojima, Koichi Maruyama, Masahiro Kikuchi, Ayako Imai, Ken Inoue, and Toshiyuki Yamamoto** (2016). Novel *SLC16A2* mutations in patients with Allan-Herndon-Dudley syndrome. *Intractable Rare Dis Res.* **5**:214-217.

On page 214, abstract line 10, p.(Gly221Ser) should be p.(Gly147Arg). On page 215, 2.2. *Genetic examination* lines 12-13, c.661G>A [p.(Gly221Ser)] should be c.439G>A [p.(Gly147Arg)].

## Guide for Authors

### 1. Scope of Articles

Intractable & Rare Diseases Research is an international peer-reviewed journal. Intractable & Rare Diseases Research devotes to publishing the latest and most significant research in intractable and rare diseases. Articles cover all aspects of intractable and rare diseases research such as molecular biology, genetics, clinical diagnosis, prevention and treatment, epidemiology, health economics, health management, medical care system, and social science in order to encourage cooperation and exchange among scientists and clinical researchers.

### 2. Submission Types

**Original Articles** should be well-documented, novel, and significant to the field as a whole. An Original Article should be arranged into the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References. Original articles should not exceed 5,000 words in length (excluding references) and should be limited to a maximum of 50 references. Articles may contain a maximum of 10 figures and/or tables.

**Brief Reports** definitively documenting either experimental results or informative clinical observations will be considered for publication in this category. Brief Reports are not intended for publication of incomplete or preliminary findings. Brief Reports should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 4 figures and/or tables and 30 references. A Brief Report contains the same sections as an Original Article, but the Results and Discussion sections should be combined.

**Reviews** should present a full and up-to-date account of recent developments within an area of research. Normally, reviews should not exceed 8,000 words in length (excluding references) and should be limited to a maximum of 100 references. Mini reviews are also accepted.

**Policy Forum** articles discuss research and policy issues in areas related to life science such as public health, the medical care system, and social science and may address governmental issues at district, national, and international levels of discourse. Policy Forum articles should not exceed 2,000 words in length (excluding references).

**Case Reports** should be detailed reports of the symptoms, signs, diagnosis, treatment, and follow-up of an individual patient. Case reports may contain a demographic profile of the patient but usually describe an unusual

or novel occurrence. Unreported or unusual side effects or adverse interactions involving medications will also be considered. Case Reports should not exceed 3,000 words in length (excluding references).

**News** articles should report the latest events in health sciences and medical research from around the world. News should not exceed 500 words in length.

**Letters** should present considered opinions in response to articles published in Intractable & Rare Diseases Research in the last 6 months or issues of general interest. Letters should not exceed 800 words in length and may contain a maximum of 10 references.

### 3. Editorial Policies

**Ethics:** Intractable & Rare Diseases Research requires that authors of reports of investigations in humans or animals indicate that those studies were formally approved by a relevant ethics committee or review board.

**Conflict of Interest:** All authors are required to disclose any actual or potential conflict of interest including financial interests or relationships with other people or organizations that might raise questions of bias in the work reported. If no conflict of interest exists for each author, please state "There is no conflict of interest to disclose".

**Submission Declaration:** When a manuscript is considered for submission to Intractable & Rare Diseases Research, the authors should confirm that 1) no part of this manuscript is currently under consideration for publication elsewhere; 2) this manuscript does not contain the same information in whole or in part as manuscripts that have been published, accepted, or are under review elsewhere, except in the form of an abstract, a letter to the editor, or part of a published lecture or academic thesis; 3) authorization for publication has been obtained from the authors' employer or institution; and 4) all contributing authors have agreed to submit this manuscript.

**Cover Letter:** The manuscript must be accompanied by a cover letter signed by the corresponding author on behalf of all authors. The letter should indicate the basic findings of the work and their significance. The letter should also include a statement affirming that all authors concur with the submission and that the material submitted for publication has not been published previously or is not under consideration for publication elsewhere. The cover letter should be submitted in PDF format. For example of Cover Letter, please visit <http://www.irdrjournal.com/downcentre.php> (Download Centre).

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**Suggested Reviewers:** A list of up to 3 reviewers who are qualified to assess the scientific merit of the study is welcomed. Reviewer information including names, affiliations, addresses, and e-mail should be provided at the same time the manuscript is submitted online. Please do not suggest reviewers with known conflicts of interest, including participants or anyone with a stake in the proposed research; anyone from the same institution; former students, advisors, or research collaborators (within the last three years); or close personal contacts. Please note that the Editor-in-Chief may accept one or more of the proposed reviewers or may request a review by other qualified persons.

**Language Editing:** Manuscripts prepared by authors whose native language is not English should have their work proofread by a native English speaker before submission. If not, this might delay the publication of your manuscript in Intractable & Rare Diseases Research.

The Editing Support Organization can provide English proofreading, Japanese-English translation, and Chinese-English translation services to authors who want to publish in Intractable & Rare Diseases Research and need assistance before submitting a manuscript. Authors can visit this organization directly at <http://www.iacmhr.com/iac-eso/support.php?lang=en>. IAC-ESO was established to facilitate manuscript preparation by researchers whose native language is not English and to help edit works intended for international academic journals.

### 4. Manuscript Preparation

Manuscripts should be written in clear, grammatically correct English and submitted as a Microsoft Word file in a single-column format. Manuscripts must be paginated and typed in 12-point Times New Roman font with 24-point line spacing. Please do not embed figures in the text. Abbreviations should be used as little as possible and should be explained at first mention unless the term is a well-known abbreviation (e.g. DNA). Single words should not be abbreviated.

**Title page:** The title page must include 1) the title of the paper (Please note the title should be short, informative, and contain the major key words); 2) full name(s) and affiliation(s) of the author(s), 3) abbreviated names of the author(s), 4) full name, mailing address, telephone/fax numbers, and e-mail address of the corresponding author; and 5) conflicts of interest (if you have an actual or potential conflict of interest to disclose, it must be included as a footnote on the title page of the

manuscript; if no conflict of interest exists for each author, please state "There is no conflict of interest to disclose"). Please visit [Download Centre](#) and refer to the title page of the manuscript sample.

**Abstract:** The abstract should briefly state the purpose of the study, methods, main findings, and conclusions. For article types including Original Article, Brief Report, Review, Policy Forum, and Case Report, a one-paragraph abstract consisting of no more than 250 words must be included in the manuscript. For News and Letters, a brief summary of main content in 150 words or fewer should be included in the manuscript. Abbreviations must be kept to a minimum and non-standard abbreviations explained in brackets at first mention. References should be avoided in the abstract. Key words or phrases that do not occur in the title should be included in the Abstract page.

**Introduction:** The introduction should be a concise statement of the basis for the study and its scientific context.

**Materials and Methods:** The description should be brief but with sufficient detail to enable others to reproduce the experiments. Procedures that have been published previously should not be described in detail but appropriate references should simply be cited. Only new and significant modifications of previously published procedures require complete description. Names of products and manufacturers with their locations (city and state/country) should be given and sources of animals and cell lines should always be indicated. All clinical investigations must have been conducted in accordance with Declaration of Helsinki principles. All human and animal studies must have been approved by the appropriate institutional review board(s) and a specific declaration of approval must be made within this section.

**Results:** The description of the experimental results should be succinct but in sufficient detail to allow the experiments to be analyzed and interpreted by an independent reader. If necessary, subheadings may be used for an orderly presentation. All figures and tables must be referred to in the text.

**Discussion:** The data should be interpreted concisely without repeating material already presented in the Results section. Speculation is permissible, but it must be well-founded, and discussion of the wider implications of the findings is encouraged. Conclusions derived from the study should be included in this section.

**Acknowledgments:** All funding sources should be credited in the Acknowledgments section. In addition, people who contributed to the work but who do not meet the criteria for authors should be listed along with their contributions.

**References:** References should be numbered in the order in which they appear in the text. Citing of unpublished results, personal

communications, conference abstracts, and theses in the reference list is not recommended but these sources may be mentioned in the text. In the reference list, cite the names of all authors when there are fifteen or fewer authors; if there are sixteen or more authors, list the first three followed by *et al.* Names of journals should be abbreviated in the style used in PubMed. Authors are responsible for the accuracy of the references. Examples are given below:

*Example 1 (Sample journal reference):*  
Inagaki Y, Tang W, Zhang L, Du GH, Xu WF, Kokudo N. Novel aminopeptidase N (APN/CD13) inhibitor 24F can suppress invasion of hepatocellular carcinoma cells as well as angiogenesis. *Biosci Trends*. 2010; 4:56-60.

*Example 2 (Sample journal reference with more than 15 authors):*  
Darby S, Hill D, Auvinen A, *et al.* Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. *BMJ*. 2005; 330:223.

*Example 3 (Sample book reference):*  
Shalev AY. Post-traumatic stress disorder: Diagnosis, history and life course. In: *Post-traumatic Stress Disorder, Diagnosis, Management and Treatment* (Nutt DJ, Davidson JR, Zohar J, eds.). Martin Dunitz, London, UK, 2000; pp. 1-15.

*Example 4 (Sample web page reference):*  
World Health Organization. The World Health Report 2008 – primary health care: Now more than ever. [http://www.who.int/whr/2008/whr08\\_en.pdf](http://www.who.int/whr/2008/whr08_en.pdf) (accessed September 23, 2010).

**Tables:** All tables should be prepared in Microsoft Word or Excel and should be arranged at the end of the manuscript after the References section. Please note that tables should not in image format. All tables should have a concise title and should be numbered consecutively with Arabic numerals. If necessary, additional information should be given below the table.

**Figure Legend:** The figure legend should be typed on a separate page of the main manuscript and should include a short title and explanation. The legend should be concise but comprehensive and should be understood without referring to the text. Symbols used in figures must be explained.

**Figure Preparation:** All figures should be clear and cited in numerical order in the text. Figures must fit a one- or two-column format on the journal page: 8.3 cm (3.3 in.) wide for a single column, 17.3 cm (6.8 in.) wide for a double column; maximum height: 24.0 cm (9.5 in.). Please make sure that artwork files are in an acceptable format (TIFF or JPEG) at minimum resolution (600 dpi for illustrations, graphs, and annotated artwork, and 300 dpi for micrographs and photographs). Please provide all figures as separate files. Please note that low-resolution images are one of the leading causes of article resubmission and schedule delays. All color figures will be reproduced in full color in the online edition of the journal at no cost to authors.

**Units and Symbols:** Units and symbols conforming to the International System of Units (SI) should be used for physicochemical quantities. Solidus notation (*e.g.* mg/kg, mg/mL, mol/mm<sup>2</sup>/min) should be used. Please refer to the SI Guide [www.bipm.org/en/si/](http://www.bipm.org/en/si/) for standard units.

**Supplemental data:** Supplemental data might be useful for supporting and enhancing your scientific research and Intractable & Rare Diseases Research accepts the submission of these materials which will be only published online alongside the electronic version of your article. Supplemental files (figures, tables, and other text materials) should be prepared according to the above guidelines, numbered in Arabic numerals (*e.g.*, Figure S1, Figure S2, and Table S1, Table S2) and referred to in the text. All figures and tables should have titles and legends. All figure legends, tables and supplemental text materials should be placed at the end of the paper. Please note all of these supplemental data should be provided at the time of initial submission and note that the editors reserve the right to limit the size and length of Supplemental Data.

## 5. Submission Checklist

The Submission Checklist will be useful during the final checking of a manuscript prior to sending it to Intractable & Rare Diseases Research for review. Please visit [Download Centre](#) and download the Submission Checklist file.

## 6. Online submission

Manuscripts should be submitted to Intractable & Rare Diseases Research online at <http://www.irdjournal.com>. The manuscript file should be smaller than 5 MB in size. If for any reason you are unable to submit a file online, please contact the Editorial Office by e-mail at [office@irdjournal.com](mailto:office@irdjournal.com)

## 7. Accepted manuscripts

**Proofs:** Galley proofs in PDF format will be sent to the corresponding author *via* e-mail. Corrections must be returned to the editor ([office@irdjournal.com](mailto:office@irdjournal.com)) within 3 working days.

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(As of February 2013)

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## Intractable & Rare Diseases Research



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- 2) The article has not been published previously and is not currently under consideration for publication elsewhere. If accepted by Intractable & Rare Diseases Research, the article will not be submitted for publication to any other journal.
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